



People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
Abdelhamid Ibn Badis University of Mostaganem



Faculty of natural and life sciences

Biological Sciences branch

Department of Biology

Speciality: Animal Biology and Physiology

Option: Animal physiology

THESIS

Submitted for the doctoral degree 3rd cycle LMD

Presented by:

Mrs. Sendous Wadjila SOUIDI

Effects of diabetes and phyto-therapeutic treatment on male reproductive function in the Wistar rat.

Defended on: 28/06/2026.

Board of Examiners:

Chairman: Pr. BENAKRICHE Ben Mehel

University of Mostaganem

Supervisor: Pr. BELHOCINE Mansouria

University of Mostaganem

Examiner: Dr. DOUICHENE Salima

University of Mostaganem

Examiner: Pr. BENDAHMANE Malika

University of Sidi Bel Abbès

Guest: Pr. KAHLOULA Khaled

University of Saida

Academic Year: 2025/2026

Animal Production Sciences and Techniques Laboratory

Abstract

The negative impact of diabetes mellitus on male fertility highlights the need to find effective treatments. Historically, sage, whose Latin name "salvia", derived from the word "salvare" (to save), underscores its long-standing reputation for medicinal properties. Thus, our study aimed to assess the effects of *Salvia balansae* Noë ex Coss flower aqueous extract (FAE) on the reproductive function of diabetic rats based on behavioral, hormonal, cytological (spermogram), and histological approaches.

Total phenolic and flavonoid content, quantification of phytochemicals by ultrahigh performance liquid chromatography (UHPLC)–electrospray ionization (ESI)–tandem mass spectrometry (MS/MS) method, and in vitro antioxidant capacity, in vivo anti-inflammatory property and acute toxicity of FAE extract were elucidated. For the *in vivo* study, male Wistar rats were divided into four groups: a normoglycemic control and treated group with FAE (200 mg/kg b.w. by gavage), and two different groups were given a single dose of streptozotocin (40 mg/kg b.w. by intraperitoneal injection). These groups were divided into: hyperglycemic control and hyperglycemic + 200 mg/kg b.w/day of FAE by gavage for 12 weeks. This study was based on different analyses: behavioral (elevated plus maze (EPM), sexual incentive motivation test (SIM), and a mating behavior test), hormonal (serum testosterone levels), cytological examination of spermatic parameters (count, motility, morphology, and viability) and histological investigation of the testicular structure.

Our study indicates that FAE contained a large amount of phenolic compounds and flavonoids, thirteen constituents were quantified; cynaroside (3,8 mg analyte/g extract) was the main compound, followed by rosmarinic acid (3,145 mg analyte/g extract), thus confirming its high antioxidant capacity, reflected by the DPPH IC₅₀ values of 31.06 ± 0.12 µg/mL, along with FRAP A_{0.5} values of 27.93 ± 1.04 µg/mL.

Acute toxicity tests of FAE demonstrated a substantial safety margin, as no toxicity was observed at doses up to 2 g/kg. Additionally, a significant anti-inflammatory effect was shown through carrageenan-induced plantar edema tests in rats, with a maximum inhibition of 88.06% observed at 6 hours. When administered at a dose of 200 mg/kg to diabetic rats, FAE significantly improved blood glucose levels ($P < 0.001$). There were also notable increases in body and testicular weights ($P < 0.001$). Furthermore, anxiety levels were reduced ($P < 0.01$), and sexual motivation was enhanced ($P < 0.001$). The treatment also led to improved sexual interactions, evidenced by an increased frequency of male-female interactions ($P < 0.001$) and

decreased latency times ($P < 0.001$). Additionally, testicular histological changes (degeneration of the germinal epithelium, presence of cellular debris, reduction in the size and number of germ cells, decrease in sperm density, and apoptosis), abnormal sperm and morphometric parameters ($P < 0.001$), and a significant drop in serum testosterone levels ($P < 0.001$) were noted in diabetic rats. All these altered parameters were restored and normalized by our FAE at a dose of 200 mg/kg ($P < 0.001$).

The present work demonstrates the therapeutic potential of *Salvia balansae* flowers in enhancing reproductive function in diabetic rats, potentially through its antioxidant, anti-inflammatory, antihyperglycemic, and aphrodisiac properties.

Keywords: *Salvia balansae*, antioxidant, diabetes mellitus, sexual behavior, testosterone, testis.

المخلص

يسلط التأثير السلبي لمرض السكري على خصوبة الرجال الضوء على الحاجة إلى إيجاد علاجات فعالة. تاريخياً، تؤكد المريمية، التي اشتق اسمها اللاتيني "سالفيا" من كلمة "salvare" (إنقاذ)، على سمعتها العريقة في الخصائص الطبية. لذلك كان الهدف من دراستنا هو تقييم آثار مستخلص الزهرة المائي (FAE) من نبات السالفيا بالاناساي نوي إكس كوس على الوظيفة التكاثرية للجرذان المصابة بالسكري باستخدام مناهج سلوكية وهرمونية وخلوية (فحص الحيوانات المنوية) ونسجية.

تم توضيح المحتوى الكلي للفينول والفلافونويد، والقياس الكمي للمواد الكيميائية النباتية عن طريق الكروماتوغرافيا السائلة فائقة الأداء (UHPLC) وقياس الطيف الكتلي الترادفي (MS/MS)، وكذلك القدرة المضادة للأكسدة في المختبر، والخصائص المضادة للالتهابات في الجسم الحي والسمية الحادة لمستخلص FAE. بالنسبة للدراسة في الجسم الحي، تم تقسيم ذكور جرذان ويستار إلى أربع مجموعات: مجموعة مراقبة عادية لسكر الدم ومجموعة معالجة ب FAE (200 ملغم/كغم من وزن الجسم عن طريق تغذية فموية جبرية)، وتلقّت مجموعتان أخرى جرعة واحدة من الستربتوزوتوسين (40 ملغم/كغم من وزن الجسم عن طريق الحقن داخل الصفاق). تم تقسيم هذه المجموعات إلى: مجموعة فرط سكر الدم، ومجموعة فرط سكر الدم + 200 مجم/كغم من FAE يومياً عن طريق التغذية الفموية لمدة 12 أسبوعاً. استندت هذه الدراسة على تحاليل مختلفة: سلوكية (مناهة متقاطعة مرتفعة واختبار الدافع الجنسي واختبار سلوك التزاوج)، وهرمونية (مستوى هرمون التستوستيرون في الدم)، وفحص خلوي لمعايير الحيوانات المنوية (العدد، والحركة، والمورفولوجيا، والحيوية) والفحص النسيجي لبنية الخصية.

تشير دراستنا إلى احتواء المستخلص على كمية كبيرة من المركبات الفينولية والفلافونويد، حيث تم تحديد ثلاثة عشر مكوناً كيميائياً؛ وكان السيناروزيد (3,8 ملغم من المادة المحللة/غرام من المستخلص) هو المركب الرئيسي، يليه حمض الروزمارينيك (3,145 ملغم من المادة المحللة/غرام من المستخلص)، مما يؤكد قدرته العالية المضادة للأكسدة، وهو ما تعكسه قيم DPPH IC_{50} التي بلغت 0.12 ± 31.06 ميكروغرام/غرام، إلى جانب قيم $FRAP A_{0.5}$ التي بلغت 1.04 ± 27.93 ميكروغرام/غرام.

أظهرت اختبارات السمية الحادة FAE هامش أمان كبير، مع عدم ملاحظة أي سمية عند جرعات تصل إلى 2 جم/كجم. بالإضافة إلى ذلك، تم إثبات تأثير كبير مضاد للالتهابات في اختبارات الوذمة الأخصية المستحثة بالكاراجينان في الجرذان، حيث أظهر تنبيهاً أقصى بنسبة 88.06% بعد 6 ساعات. عند إعطاء جرعة قدرها 200 مجم/كجم للفئران المصابة بالسكري، حسن مستخلص FAE مستويات الجلوكوز في الدم بشكل ملحوظ ($P < 0.001$). كما لوحظت زيادات كبيرة في وزن الجسم والخصيتين ($P < 0.001$). بالإضافة إلى ذلك، انخفضت مستويات القلق ($P < 0.01$) وتعزز الدافع الجنسي ($P < 0.001$). كما حسنت العلاجات أيضاً التفاعلات الجنسية، كما يتضح من زيادة تواتر التفاعلات بين الذكور والإناث ($P < 0.001$) وانخفاض في أوقات الكمون ($P < 0.001$). وبالإضافة إلى ذلك، لوحظت تغيرات نسيجية في الخصية (ضمور في الأنسجة الطلائية الجنينية، ووجود بقايا خلوية، وانخفاض في حجم وعدد الخلايا الجنينية، وانخفاض في كثافة الحيوانات المنوية، وموت الخلايا المبرمج)، وتغيرات غير طبيعية في الحيوانات المنوية والمورفومترية ($P < 0.001$)، وانخفاض كبير في مستويات هرمون التستوستيرون في الدم ($P < 0.001$) في الجرذان المصابة بالسكري. تم استعادة جميع هذه المتغيرات وتعديلها من خلال العلاج بجرعة 200 مجم/كجم من FAE ($P < 0.001$).

يوضح هذا العمل الإمكانيات العلاجية لزهور سالفيا بالانساي في تحسين الوظيفة التكاثرية لدى الجرذان المصابة بالسكري، وذلك من خلال خصائصها المضادة للأكسدة ومضادات الالتهاب ومضادات فرط سكر الدم والمنشطات الجنسية.

الكلمات المفتاحية: سالفيا بالانساي، مضادات الأكسدة، داء السكري، السلوك الجنسي، التستوستيرون، الخصية.

Résumé

L'impact négatif du diabète sucré sur la fertilité masculine souligne la nécessité de trouver des traitements efficaces. Historiquement, la sauge, dont le nom latin « *salvia* », dérivé du mot « *salvare* » (sauver), souligne sa réputation de longue date pour ses propriétés médicinales. Le but de notre étude était donc d'évaluer les effets de l'extrait aqueux de fleur (FAE) de *Salvia balansae* Noë ex Coss sur la fonction reproductrice de rats diabétiques en se basant sur des approches comportementales, hormonales, cytologiques (spermogramme) et histologiques.

La teneur totale en composés phénoliques et en flavonoïdes, la quantification des composés phytochimiques par chromatographie liquide à ultra-haute performance (CLUHP) et par spectrométrie de masse en tandem (MS/MS), ainsi que la capacité antioxydante *in vitro*, les propriétés anti-inflammatoires *in vivo* et la toxicité aiguë de l'extrait FAE ont été élucidées. Pour l'étude *in vivo*, des rats Wistar mâles ont été divisés en quatre groupes : un groupe témoin normoglycémique et un groupe traité avec FAE (200 mg/kg b.w. par gavage), et deux autres groupes ont reçu une dose unique de streptozotocine (40 mg/kg b.w. par injection intrapéritonéale). Ces groupes ont été divisés en : contrôle hyperglycémique, et hyperglycémique + 200 mg/kg b.w./jour d'FAE par gavage pendant 12 semaines. Cette étude était basée sur différentes analyses : comportementales (labyrinthe en croix surélevé, test de motivation sexuelle et test de comportement d'accouplement), hormonales (taux de testostérone sérique), examen cytologique des paramètres spermatiques (numération, motilité, morphologie et viabilité) et examen histologique de la structure testiculaire.

Notre étude indique que le FAE contient une grande quantité de composés phénoliques et de flavonoïdes. Le cynaroside (3,8 mg d'analyte/g d'extrait) était le composé principal, suivi par l'acide rosmarinique (3,145 mg d'analyte/g d'extrait), confirmant ainsi sa capacité antioxydante élevée, reflétée par les valeurs DPPH IC₅₀ de 31,06 ± 0,12 µg/mL, ainsi que par les valeurs FRAP A_{0,5} de 27,93 ± 1,04 µg/mL. Le test de toxicité aiguë de FAE a montré une marge de sécurité substantielle, aucune toxicité n'ayant été observée à des doses allant jusqu'à 2 g/kg. En outre, un effet anti-inflammatoire significatif a été démontré par des tests d'œdème plantaire induit par la carragénine chez le rat, montrant une inhibition maximale de 88,06 % après 6 heures. Administré à une dose de 200 mg/kg à des rats diabétiques, le FAE a entraîné une amélioration significative de la glycémie ($P < 0,001$). Des augmentations notables du poids du corps et des testicules ont également été observées ($P < 0,001$). En outre, les niveaux d'anxiété ont été réduits ($P < 0,01$) et la motivation sexuelle a été renforcée ($P < 0,001$). Le traitement a

également permis d'améliorer les interactions sexuelles, comme en témoignent l'augmentation de la fréquence des interactions entre mâles et femelles ($P < 0,001$) et la diminution des temps de latence ($P < 0,001$). En outre, des changements histologiques testiculaires (dégénérescence de l'épithélium germinal, présence de débris cellulaires, réduction de la taille et du nombre de cellules germinales, diminution de la densité des spermatozoïdes et apoptose), des paramètres spermatiques et morphométriques anormaux ($P < 0,001$) et une baisse significative du taux de testostérone sérique ($P < 0,001$) ont été observés chez les rats diabétiques. Tous ces paramètres altérés ont été restaurés et normalisés par notre FAE à une dose de 200 mg/kg ($P < 0,001$).

Le présent travail démontre le potentiel thérapeutique des fleurs de *Salvia balansae* dans l'amélioration de la fonction reproductive chez les rats diabétiques, potentiellement grâce à leurs propriétés antioxydantes, anti-inflammatoires, antihyperglycémiques et aphrodisiaques.

Mots-clés : *Salvia balansae*, antioxydant, diabète sucré, comportement sexuel, testostérone, testicule.

Table of contents

Abstract

الملخص

Résumé

Tables list

Figures list

Abbreviations list

INTRODUCTION	1
PART I: REVIEW OF THE LITERATURE	4
CHAPTER I	5
OVERVIEW OF MALE REPRODUCTIVE FUNCTION IN RATS	5
<i>I.1. Anatomophysiology of the male reproductive system:</i>	<i>6</i>
I.1.1. Testes and seminiferous tubules:	6
I.1.2. Extra-testicular ducts: (rete testis, efferent ducts, epididymis, vas deferens and urethra)	7
I.1.3. Accessory sexual glands:	8
I.1.4. Penis:	10
<i>I.2. Hypothalamic-Pituitary-Gonadal Axis:</i>	<i>10</i>
I.2.1. Steroidogenesis and synthesis of testosterone:	12
I.2.2. Spermatogenesis:	13
I.2.2.1. Stages of spermatogenesis development:	13
I.2.2.2. Spermatozoon:	14
I.2.3. Sexual behavior of the male rat:	15
<i>I.3. Neurobiology of sexual behavior:</i>	<i>15</i>
I.3.1. Brain regions involved in sexual behavior:	15
I.3.1.1. Olfactory system:	15
I.3.1.2. Medial amygdala (MeA):	16
I.3.1.3. Bed nucleus of the stria terminalis (BNST):	16
I.3.1.4. Hypothalamus:	16
I.3.1.5. Median preoptic area (mPOA):	16
I.3.1.6. Prefrontal cortex:	16
I.3.1.7. Ventral tegmental area (VTA) and nucleus accumbens:	16
I.3.2. Spinal regions involved in sexual behavior	17
I.3.2.1. Spinal bulbocavernosus nucleus (SBN)	17
I.3.3. Neurotransmitters involved in sexual behavior	17
I.3.3.1. Dopamine	17
I.3.3.2. Serotonin	17
I.3.3.3. Oxytocin	17
I.3.3.4. Nitric oxide (NO)	18
I.3.4. Detailed description of male rat sexual behavior	18
I.3.4.1 Anticipatory phase	18
I.3.4.2. Courtship phase	18
I.3.4.3. Copulating	19
I.3.4.4. Post-copulating and satiety after ejaculation	20
<i>I.4. Infertility</i>	<i>21</i>
CHAPTER II	22

DIABETES MELLITUS AND THE IMPAIRMENT OF MALE REPRODUCTIVE FUNCTION	22
II.1. Glimpse of Diabetes mellitus (DM)	23
II.1.1 Diabetes Mellitus Prevalence	24
II.1.1.1. In the world	24
II.1.1.2. Diabetes Mellitus Prevalence in the Middle East and North Africa (MENA)	24
II.1.2. Diabetes mellitus classification	25
II.1.2.1 Type 1 Diabetes mellitus (T1DM)	25
II.1.2.2. Type 2 Diabetes mellitus (T2DM)	25
II.1.2.3. Type 3 Diabetes mellitus (T3DM)	26
II.1.2.4. Other types of diabetes	26
II.1.3. Animal experimental model of diabetes	27
II.1.3.1. Streptozotocin (STZ)	27
II.2. Diabetes mellitus and male infertility	27
II.2.1. Pre-testicular effects of diabetes mellitus (hypothalamic-pituitary-gonadal axis)	28
II.2.2. Testicular effects of diabetes mellitus	29
II.2.2.1. Diabetes and testis tissue damage	29
II.2.2.2. Diabetes and hormone disorder	30
II.2.2.3. Diabetes and spermatogenesis	30
II.2.3. Post-testicular effects of diabetes mellitus (Sexual behavior and fertility outcome)	31
II.2.3.1. Libido	31
II.2.3.2. Erectile dysfunction (ED)	32
II.2.3.3. Ejaculatory dysfunction	33
CHAPTER III	35
PHYTOTHERAPY AND TREATMENT OF MALE INFERTILITY CAUSED BY DIABETES.	35
III.1. Treatment of male infertility caused by diabetes	36
III.1.1. Natural components and diabetes mellitus blood glucose control	36
III.1.2. Natural compounds and DM-induced decline of male reproductive function	36
III.1.3. Medicinal plants	36
III.2. Overview of the plant	37
III.2.1. Geographical distribution of <i>Salvia balansæ</i>	37
III.2.2. Taxonomy of <i>Salvia balansæ</i>	38
III.2.3. Botanical description of <i>Salvia balansæ</i>	38
III.2.4. Morphological characteristics of <i>Salvia balansæ</i>	39
III.3. Main Biologically Active Compounds of <i>Salvia balansæ</i>	40
III.4. Biological activities of <i>Salvia balansæ</i>	41
III.4.1. Antioxidant Assays	41
III.4.2. Antimicrobial Activity	42
III.4.3. Antidiabetic Activity	42
III.4.4. Effects on Reproductive System	42
III.5. Toxicity and Safety of <i>Salvia</i>	42
PART II: EXPERIMENTAL STUDY	44
CHAPTER IV	45
MATERIEL AND METHODS	45
IV.1 Ethical Consideration	45
IV.2 Objectives	45
IV.3 Place of Study	45
IV.4. Plant material	46
IV.4.1. Plant authentication and collection	46

IV.4.2. Preparation of extracts	47
IV.4.3. Determination of polyphenol and flavonoid contents of the extract	48
IV.4.3.1. Total phenolic content (TPC)	48
IV.4.3.2. Total flavonoid content (TFC)	48
IV.4.4 Ultra-High Performance Liquid Chromatography (UHPLC)-Electrospray Ionization (ESI)-Tandem Mass Spectrometry (MS/MS)	48
IV.4.5. In vitro biological activity	50
IV.4.5.1. In vitro antioxidant activity:	50
IV.4.5.1.1. DPPH free radical-scavenging assay	50
IV.4.5.1.2. ABTS scavenging assay	50
IV.4.5.1.3. Ferric reducing antioxidant power assay (FRAP)	50
IV.4.6. In vivo biological activity	51
IV.4.6.1. In vivo anti-inflammatory activity: Carrageenan-induced plantar edema test	51
IV.4.6.2 Acute Oral Toxicity of FAE: Up-and-Down Procedure	52
IV.5. <i>Animal</i>	52
IV.5.1. Experimental animals	52
IV.5.2. Male rats' preselection and female rats' receptivity	52
IV.5.3. Rat model for type I diabetes and batch allocation	52
IV.5.4. Assessment of body weight and blood glucose	54
IV.6. <i>Behavioral test battery</i>	54
IV.6.1. Elevated plus maze (EPM)	54
IV.6.2. Study of sexual behavior	55
IV.6.2.1. Sexual incentive motivation test (SIM)	55
IV.6.2.2. Assessment of mating behavior	56
IV.7. <i>Necropsy and organ sampling</i>	57
IV.7.1. Testosterone assay	57
IV.7.2. Sperm quality assessment	57
IV.7.2.1. Sperm epididymis counts	58
IV.7.2.2. Sperm motility	58
IV.7.2.3. Sperm morphology	59
IV.7.2.4. Sperm viability	59
IV.8. <i>Histopathological analysis</i>	59
IV.8.1. Fixation	59
IV.8.2. Dehydration and paraffin impregnation	60
IV.8.3. Paraffin inclusion	60
IV.8.4. Cutting and spreading	60
IV.8.5. Slide staining and mounting	61
IV.8.6. Microscopic observation	61
IV.9. <i>Statistical analysis</i>	61
CHAPTER V	62
RESULTS AND DISCUSSION	62
V.1. Extraction yield, total phenolic content, and flavonoid content, of <i>S. balansae</i>	63
V.2. Phytochemical compounds of <i>S. balansae</i>	64
V.3. Assessment of biological activities	68
V.3.1. In vitro antioxidant activity	68
V.3.2. In vivo anti-inflammatory activity	70
V.3.3. Acute Oral Toxicity of FAE	73
V.4. Blood glucose, body and organ weights	74
V.5. Elevated plus maze	76
V.6. Sexual Incentive Motivation Test (SIM)	79

V.7. Assessment of mating behavior	81
V.8. Correlation analysis of blood glucose levels and sexual behavior parameters	87
V.9. Testosterone assay	88
V.10. Sperm quality assessment	89
V.11. Histopathological Investigation	93
CONCLUSION AND PROSPECTS	100
REFERENCES	102
ANNEXES	123

Acknowledgements

First and foremost, all praise and gratitude are due to Allah, the Most Gracious and the Most Merciful, for granting me the strength, health, and perseverance to carry out and complete this work.

Indeed, [سورة يوسف – الآية 76] «وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ»

I would like to extend my deepest appreciation to my supervisor, **Pr. Mansouria BELHOCINE**, for her support, guidance, and encouragement throughout this research. Her advice and availability were a great help during every stage of this thesis.

I am deeply grateful to **Pr. Ben Mehel BENAKRICHE** for the honour of his chairmanship of the jury for my work, despite his heavy workload.

My sincere thanks are extended to **Pr. Malika BENDAHMANE**, from Dr. Djillali Liabes University of Sidi Bel Abbès, as well as **Dr. Salima DOUICHEN**, from Abdelhamid Ibn Badis University of Mostaganem, for graciously accepting to examine this modest work. To whom I would like to express my deepest respect.

I would like to express my deepest gratitude to **Pr. Khaled KAHLOULA**, from Dr. Moulay Tahar University of Saida, whose mentorship has been a guiding force throughout my academic journey. Just as the Papez circuit integrates memory and emotion in the brain, your support has shaped my intellectual growth and emotional resilience. More than a professor, you have been a visionary mentor, instilling in me the passion, discipline, and curiosity essential to scientific research. Your influence remains deeply embedded in my hippocampus, continuously guiding my path. I am truly grateful to have you as an invited member of my thesis committee.

My sincere thanks to the following research laboratories that provided invaluable support:

- Laboratory of Biotoxicology, Pharmacognosy and Biological Valorization of Plants (LBPVBP), Dr. Moulay Tahar University of Saida, Algeria, initially under the direction of **Pr. Khaled KAHLOULA** and subsequently under the leadership of **Pr. Tayeb SITAYEB**.
- Laboratory of Sciences and Techniques of Animal Production (LSTPA), University Abdelhamid Ibn Badis of Mostaganem, Algeria, under the direction of **Pr. Abdelkader E. DAHOU**.
- Laboratories 05 and 06, affiliated with the Biotechnology Research Center (CRBT), and working within team 01: Natural Substances, Applied Pharmacology and Toxicology, under the scientific supervision of **Pr. Chawki BENSOUICI**, Director of the Laboratory, and under the institutional direction of **Pr. Ammar AZIOUNE**, Director of the Research Center.
- Pathological Anatomy laboratory of the Ahmed Medaghri Hospital, Wilaya of Saida, under the direction of **Dr. Bakhta BOUZOUIRA**, and laboratory of the veterinary institute at the Ibn Khaldoun University of Tiaret under the direction of **Pr. Hemida HOUARI**.

I extend my sincere appreciation to **Mr. Hmed HAMADI** for his continuous technical support, dedication, and professionalism throughout my research journey.

A special thanks to **Pr. Mustafa Abdullah YILMAZ** from the Science and Technology Research and Application Center (DUBTAM), Dicle University of Diyarbakir, TURKIYE, for his supervision during my internships in 2024 and 2025. His expert guidance in the LC-MS/MS technique and continuous support enriched my academic development. Beyond his scientific guidance, his generosity and encouragement left a lasting impression. It was a true honor to learn from a distinguished researcher ranked among the top 2% of scientists worldwide by Stanford University.

I would like to express my heartfelt gratitude to **Mr. Massinissa AROUN**, a neurobiologist from Ali Ait Idir Neurosurgery Hospital, who has been my silent strength throughout this journey. His insightful reflections, constant encouragement, and careful proofreading have accompanied me every step of the way. His support has been like a well-timed neural signal—reliable and essential—and his presence, a quiet surge of motivation, similar to dopamine in moments of doubt. For reasons encoded far beyond words, I remain sincerely grateful

Special thanks to **Mr. Laurence John BEHRENS**, a veterinary doctor at the Goddard Veterinary Group in the United Kingdom, for his kindness, patience, and unwavering support. His help with the English language and his thoughtful corrections have meant a great deal to me.

I would like to sincerely thank my friend **Helin SULIMAN**, a chemistry graduate from Damascus University in Syria, for being such an outstanding friend. She warmly welcomed me into her home with her family and offered invaluable support during both of my internships in Diyarbakir, Turkey. Her guidance and help exceeded professional expectations, and I am truly grateful for her generosity and friendship.

My heartfelt thanks to **Dr. Karima BENTAIBA** for her professionalism and availability. Her expert advice and support in specimen collection and histology interpretation were of immeasurable value.

I am deeply grateful to my colleague **Dr. Ines FEKNOUS**, Doctor in Veterinary Medicine and PhD Student in Nutrition and Food Sciences at Mostaganem University, for her valuable assistance with the in vitro experiments, her continuous support in proofreading and refining my English writing, and, above all, her constant encouragement.

I would also like to express my sincere thanks to all the PhD students of the LBPVBP laboratory for the friendly working atmosphere they helped create. A special mention goes to the one who shared a twin-like bond with me, making each day lighter and more joyful, and to the one whose determination and energy were a true source of motivation throughout this journey. Finally, to the person who is reading this and waiting for her turn, I have not forgotten you. Thank you for being there at every stage of my career.

I would also like to thank the professors in Saida University's Biology Department who provided me with the tools I needed to succeed in my studies. Thank you for motivating me from my first day at university to this day.

Lastly, I am deeply thankful to my parents, whose love and constant support have been my foundation throughout this journey. To my sisters, my brother and his family, your encouragement, understanding, and love have been a consistent source of strength. I am deeply thankful for your presence, for the sacrifices you have made, and for always being there when I needed you most.

I dedicate this work to the one who trusted my journey before it began — my grandfather,
forever in my heart.

Tables list

Table 01: Location of <i>Salvia balansæ</i> .	38
Table 02: Standard copulatory parameters recorded during the sexual interaction test	56
Table 03: Extraction yield, total phenolic content, and total flavonoid content, of the FAE.	63
Table 04: LC–tandem mass spectrometry (MS/MS) data of compounds detected in <i>Salvia balansæ</i> flower aqueous extract (FAE).	65
Table 05: The antioxidant activities of the flower aqueous extract (FAE).	69
Table 06: Effect of <i>S. balansæ</i> FAE on carrageenan-induced plantar edema in rats.	70
Table 07: Body, organ weights and blood glucose levels of the different experimental groups.	75
Table 08: Epididymis sperm parameter of the different experimental groups	91

Figures list

Figure 01: Male reproductive tract.	06
Figure 02: The male hypothalamic-pituitary-gonadal axis.	12
Figure 03: Overview of steroidogenesis and synthesis of testosterone.	13
Figure 04: Schematic representation of spermatogenesis in the rat, BTB: Blood-Testicular Barrier.	14
Figure 05: Schematic representation of a rat spermatozoon.	15
Figure 06: Three views of Sexual behavior of rats mount, intromission, and ejaculation.	20
Figure 07: Number of people with diabetes worldwide and per IDF Region in 2021–2045 (20–79 years).	24
Figure 08: Summary of the effect of DM on the male reproductive system.	28
Figure 09: a) <i>Salvia balansae</i> De, b) <i>Salvia balansae</i> Noë collected in Algeria by Naturalis Biodiversity Center.	39
Figure 10: <i>Salvia balansae</i> de Noë: a, habit $\times 3\frac{1}{2}$; b, corolla $\times 1$; c, lower lip of corolla $\times 1\frac{1}{3}$; d, stamen $\times 1\frac{1}{3}$; e, fruiting calyx $\times 1\frac{1}{2}$; f, nutlet $\times 7$.	40
Figure 11: a) Geographical location of the <i>Salvia balansae</i> sampling area (Google Earth), b) Aerial parts of <i>S. balansae</i> , Mostaganem, 15 April 2023.	46
Figure 12: Dried and ground <i>Salvia balansae</i> flowers.	47
Figure 13: Extraction procedure.	47
Figure 14: 96-well microplate reader (Perkin Elmer, Enspire).	48
Figure 15: Shimadzu-Nexera model Ultra-High Performance Liquid Chromatography (UHPLC)-Electrospray Ionization (ESI)-Tandem Mass Spectrometry (MS/MS).	49
Figure 16: Flowchart of (1) Oral administration of treatments; (2) Subplantar injection of carrageenan and (3) Measurement of leg thickness using a digital caliper.	51
Figure 17: Flow chart of experimental design.	53
Figure 18: Elevated plus maze test apparatus.	54
Figure 19: Sexual incentive motivation test apparatus.	55
Figure 20: Sexual interaction test. A) Mount, B) Intromission, C) Ejaculation, D) Penile licking.	57
Figure 21: LC-MS/MS TIC chromatograms of <i>S. balansae</i> and standards phytochemicals.	67
Figure 22: Percentage of increased oedema induced by carrageenan over time (h) in rats treated with aqueous extract of <i>S. balansae</i> flowers and diclofenac.	71

Figure 23: Inhibition percentage of oedema induced by carrageenan over time (h) in rats treated with aqueous extract of <i>S. balansae</i> flowers and diclofenac.	72
Figure 24: Blood glucose levels of the different experimental groups before, after diabetes induction, and after 12 weeks of treatment.	74
Figure 25: Number of entries and time spent in the open arms of the different experimental groups.	77
Figure 26: Number of visits to FIZ and MIZ zones of the different experimental groups.	79
Figure 27: Time spent in seconds in the FIZ and MIZ zones of the different experimental groups.	80
Figure 28: Preference score of the different experimental groups.	80
Figure 29: The mount frequency and latency expressed in seconds of the different experimental groups.	82
Figure 30: The intromission frequency and latency expressed in seconds of the different experimental groups.	83
Figure 31: The ejaculation frequency and latency expressed in seconds of the different experimental groups.	83
Figure 32: The post-ejaculation interval expressed in seconds and copulatory rate of the different experimental groups.	84
Figure 33: Linear correlation between blood glucose levels and sexual behavioral parameters in each group.	88
Figure 34: Testosterone serum level (ng/ml) of the different experimental groups.	89
Figure 35: Microscopic observation of sperm morphology magnification (G × 400).	92
Figure 36: Microscopic observation of a sperm smear showing a pink-stained spermatozoon (dead) and a colorless spermatozoon (alive) G × 1000.	92
Figure 37: Microscopic observation of the histological structure of the testes from the control and FAE groups. H&E staining. A and C (G × 100) & B and D (G × 400).	95
Figure 38: Microscopic observation of the histological structure of the testes from the diabetic group. H&E staining. A and C (G × 100), B (G × 400) and D (G × 1000).	96
Figure 39: Microscopic observation of the histological structure of the testes from the diabetic treated with FAE group. H&E staining. A (G × 100), B (G × 400).	97

Abbreviations list

17 β -HSD: 17 β -hydroxysteroid dehydrogenase

3 β -HSD: 3 β -hydroxysteroid dehydrogenase

ABP: Androgen-binding protein

AD: Alzheimer's disease

AGEs: Advanced Glycation End-products

ALB: Anxiety-like behaviors

AMPK: Adenosine monophosphate protein kinase

ARs: Androgen receptors

BBB: Blood-brain barrier

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

BNST: Bed nucleus of the stria terminalis

BTB: Blood-Testicular Barrier

CAT: Catalase

CCL2: C-C motif chemokine ligand 2

cGMP: Cyclic guanosine monophosphate

CNS: Central nervous system

COX: Cyclooxygenases

COX-2: Cyclooxygenase-2

Cr: Copulatory rate

CYP11A1: Cytochrome P450 enzyme

DHT: Dihydrotestosterone

DM: Diabetes mellitus

DPPH: Radical 2,2-diphenyl-1-picrylhydrazyl

DW: Dry weight

ED: Erectile dysfunction

EF: Ejaculation frequency

EL: Ejaculation latency

EPM: Elevated plus maze

ER: Endoplasmic reticulum

FAE: Flower Aqueous Extract

FIZ: Female incentive zone

FRAP: Reducing antioxidant power

FSH: Follicle-stimulating hormone

GAE: Gallic acid equivalents

GAPDH: Glyceraldehyde phosphate dehydrogenase

GC-MS: Gas Chromatography-Mass Spectrometry

GDM: Gestational Diabetes Mellitus

GLUT1: Glucose transport protein 1

GLUT2: Glucose transport protein 2

GnRH: Gonadotropin-releasing hormone

GPx: Glutathione peroxidase

HPG: Hypothalamic-Pituitary-Gonadal

HPLC-DAD: High Performance Liquid Chromatography with Diode-Array Detection

HPLC-MS: High Performance Liquid Chromatography-Mass Spectrometry

IC₅₀: 50% inhibitory concentration

IDF: International Diabetes Federation

IF: Intromission frequency

IL: Intromission latency

IL-1 β : Interleukin-1 beta

iNOS: Inducible nitric oxide synthase

IS: Interstitial space

LD₅₀: median lethal dose

LH: Luteinizing hormone

MDA: Malondialdehyde

MeA: Medial amygdala

MENA: Middle East and North Africa

MetS: Metabolic syndrome

MF: Mount frequency

miR-34a: MicroRNA 34a

MIZ: Male incentive zone

ML: Mount latency

MODY: Maturity-Onset Diabetes of the Young

mPOA: Median preoptic area

NCDs: Non-communicable diseases

NO: Nitric oxide

NOEL: No observed effect level.

NOS: Nitric oxide synthase

OECD: Organization for Economic Co-operation and Development

PEI: Post-ejaculatory interval

PKC: Protein kinase C

POS: Primary olfactory system

QE: Quercetin equivalents

RE: Retrograde ejaculation

ROS: Reactive oxygen species

SAP: Stretched-attend posture

SBN: Spinal bulbocavernosus nucleus

Sc: Spermatoocytes

SD: Sexual dysfunction

Sd: Spermatids

SD: Standard deviation

SEM: Erreur standard mean

Sg: Spermatogonia

SIM: Sexual incentive motivation

SIRT1: Silent mating type Information Regulator Two homolog 1

SOA: Accessory olfactory system

SOD: Superoxide dismutase

ST: Seminiferous tubules

StAR: Steroidogenic Acute Regulatory protein

STIs: Sexually transmitted infections

STZ: Streptozotocin

Sz: Spermatozoa

T1DM: Type 1 Diabetes Mellitus

T2DM: Type 2 Diabetes mellitus

T3DM: Type 3 Diabetes mellitus

TCA: Trichloroacetic acid

TFC: Total flavonoid content

TNF- α : Tumor necrosis factor-alpha

TPC: Total phenolic content

UHPLC-ESI-MS/MS: Ultra-High Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry

VTa: Ventral tegmental area

WHO: World Health Organization

Introduction

One of the numerous challenges confronting the modern healthcare system is diabetes mellitus (DM) (Albayrak et al., 2024). According to the latest findings from the 10th edition of the International Diabetes Federation (IDF), diabetes is one of the fastest-growing global health emergencies of the 21st century, with 537 million people estimated to have diabetes in 2021, rising to 643 million by 2030 and 783 million by 2045 (IDF Diabetes Atlas, 2021; Minas et al., 2024). DM, recognized by high blood glucose levels due to impaired insulin secretion and/or action, has been identified as one of the most common endocrine metabolic disorders (Yeniçeri et al., 2024). The disease is associated with many complications such as retinopathy, nephropathy, and cardiovascular morbidity (Bakaç et al., 2023; Güven et al., 2024). Beyond these direct impacts, DM also exerts considerable effects on all endocrine functions, inducing fertility disorders in men (Minas et al., 2024).

The World Health Organization (WHO) defines infertility as a health condition in which couples cannot achieve a clinical pregnancy after 12 months of unprotected intercourse (World Health Organization, 2023). Infertility has emerged as a significant global health concern, affecting approximately 15% of reproductive-age couples, which translates to 48.5 million couples worldwide. Male infertility is implicated in nearly 50% of these cases, with 20% attributed to unknown causes (Minas et al., 2024). Research has shown that DM affects male fertility in several ways, disrupting pre-testicular, testicular, and post-testicular functions (Lekmine et al., 2023; Nna et al., 2017), underscoring the urgent need for treatments that address both the physiological and psychological dimensions of the disorder.

Maintaining normal reproductive health depends largely on the balance of intricate interactions among the hormonal, neurological, vascular, and psychological systems (Karagecili et al., 2023; Saikia et al., 2024). Male sexual behavior depends on the presence of sufficient circulating gonadal hormones, mainly testosterone and its metabolites (Jennings and de Lecea, 2020). Sexual stimuli, whether auditory, olfactory, visual, tactile, or even imagined in humans, trigger the activation of neural circuits (Gaytán-Tocavén et al., 2023). These circuits convey sexual data from the brain's higher regions (hypothalamus and its nuclei) to the male genitalia through the spinal cord and the autonomic nervous system, facilitating penile erection and thereby enabling coitus (Melis et al., 2022). The impairment of sexual behavior, and erection, along with ejaculation and histological changes in the testes, including alterations

in spermatogenesis and tissue structure, involves the disruption of these interconnected systems **(Gaytán-Tocavén et al., 2023; Melis et al., 2022)**. DM disrupts this harmony, leaving individuals vulnerable to fertility disorders **(R. Huang et al., 2024)**.

As conventional pharmacological treatments have their limitations and can be accompanied by undesirable side effects, the search for herbal remedies offers a promising alternative **(Kanedi et al., 2019)**. The use of plants, such as sage, has become an area of growing interest in managing diabetes and its multifaceted complications **(R. Huang et al., 2024)**. Sage (*Salvia*) is the largest genus of herbaceous perennial plants in the Lamiaceae family and has been valued for its healing properties since ancient times **(G. Yilmaz et al., 2023)**. The name "*Salvia*" is derived from the Latin word "salvare," which means, "to save," highlighting its long-standing medicinal reputation **(Balkır et al., 2023; Uysal et al., 2023)**. Originating from the Mediterranean basin and eastern and southwestern Asia, the Sage genus comprises around 1,000 species worldwide **(Onder et al., 2022; Yaris et al., 2021)**. Algeria is home to a significant diversity of about 30 identified species **(Achir et al., 2021)**. The Mostaganem region in Algeria is renowned for its diverse array of endemic and rare plant species, prominently featuring the perennial medicinal plant *Salvia balansae* Noë ex Coss **(Mostari et al., 2020)**. *S. balansae*, commonly known as "hchichet koul blia," translates to "the plant that cures all ailments." This sage is utilized in traditional medicine to treat a wide variety of illnesses within the local community. Its distribution is limited to the Cheliff Valley, located in the two communes of Sidi Belattar and Ain Boudinar, in the coastal Oranie region, as well as the Aurès Mountains **(Bendrihem et al., 2024; Mostari et al., 2016)**. As a result, this species is strictly endemic to Algeria. Variations in climate contribute to differences in flower color and leaf shape among the populations found in the Aurès Mountains **(Bendrihem et al., 2024)**. The genus *Salvia* is rich in polyphenolic compounds such as phenolic acids, flavonoids, and anthocyanins, which are major classes of bioactive phytoconstituents **(M. A. Yilmaz et al., 2024)**. They exert a wide range of biological and pharmacological activities including antioxidant **(Fidan et al., 2021; Jažo et al., 2023)**, antimicrobial, antitumor, anti-inflammatory **(Zhumaliyeva et al., 2023)**, and anti-enzymatic activities such as anticholinesterase, anti-tyrosinase **(Uysal et al., 2023)**. They also exert antipyretic, analgesic, and cytotoxic activities **(Brindisi et al., 2021; G. Yilmaz et al., 2023)**, as well as their efficacy in modulating metabolic imbalances and combating oxidative stress **(Alchalabi et al., 2016; Zhumaliyeva et al., 2023)**, and restoring heavy metal-induced testicular intoxication **(Basal et al., 2023; Omar et al., 2024)**. In addition, ethnobotanical studies suggest that sage has traditionally been used in the

Mediterranean region to regulate blood sugar and increase male fertility. Indigenous people from tribes living in Algeria use species of *Salvia* for these purposes (**Hamza et al., 2019**).

While previous studies have examined the biological properties, there is limited research investigating the comprehensive effects of *S. balansae* on diabetic health. Despite its therapeutic potential, the effects of *S. balansae* on diabetes-induced sexual dysfunction, particularly in male reproductive health, remain underexplored. Only three papers to date have specifically addressed *S. balansae* from Mostaganem. **Mekki et al. (2023)** examined its leaves, showing antioxidant and antidiabetic effects, including improved metabolic parameters, testosterone levels, and testicular structure in a high-fat diet-induced diabetic model, and other studies examined the *in vitro* biological properties (**Amirat et al., 2023; Mahdjoub et al., 2023**). An evaluation of the photoprotective properties of the same *Salvia* species found in the Aures Mountains has also been investigated by **Bendrihem et al. (2024)**.

Based on these findings, this study uniquely evaluates the therapeutic potential of flowers of the currently studied *S. balansae* cultivar in a streptozotocin (STZ)-induced diabetic model, providing a different perspective from the high-fat diet-induced diabetic model investigated by **Mekki et al. (2023)**. Our research seeks to evaluate, for the first time, the efficacy of this plant part in mitigating diabetes-related alterations in male reproductive health. The study employs a methodological approach that is divided into two distinct parts:

- The first part is an *in vitro* study to characterize the plant using phytochemical assays and chromatographic analyses and to optimize its antioxidant and anti-inflammatory properties and acute oral toxicity.
- The second part is devoted to the *in vivo* study, of the Wistar rat, this part follows a rigorous methodological sequence, starting with the observation of sexual behavior, which is the first visible manifestation of the disruptions induced by diabetes. Simultaneously, blood glucose levels are measured to quantify the severity of the metabolic disorder and to evaluate its correlation with reproductive outcomes. Subsequent phases involve evaluating body and testicular weight variations. This is followed by a precise measurement of testosterone levels to identify hormonal imbalances resulting from the diabetic condition. The final phase of the study involves analyzing sperm quality. Ultimately, a comprehensive histological analysis of the testes is conducted to confirm structural alterations at the cellular level, providing direct evidence of the impact of diabetes on reproductive tissues.

Part I: Review of the literature

Chapter I

Overview of male reproductive function in rats

Reproduction, a fundamental biological process, plays a crucial role in the perpetuation of species across generations. This importance transcends simple genetic transmission, and is deeply rooted in the social dynamics and survival of mammals, especially rats. As a privileged model for biomedical research, the rat offers valuable insights into biological and behavioral mechanisms that are shared with other mammals, including humans.

The complexity of reproductive function involves the interaction of multiple systems, where the nervous system, in particular, assumes a central role. By orchestrating hormone release, responding to environmental stimuli, and regulating sexual behavior, the nervous system ensures precise coordination between the brain and the endocrine system. This sophisticated interaction adjusts the production of sex hormones, such as testosterone, which are essential for spermatogenesis and sexual behavior. Thus, reproduction in rats, as in other mammals, reveals the extraordinary complexity and interdependence of the biological systems that support not only individual and species survival, but also their adaptation and evolution over time.

This chapter aims to reveal the complexity and interconnecting aspects of the biological and behavioral systems that orchestrate reproduction in the rat, an essential model for biomedical research. By exploring the anatomical structure, physiology, and behavior associated with reproductive function, we seek to understand not only the fundamental mechanisms that support fertility and sexual behavior but also how these processes are regulated by a dynamic interplay between the nervous and endocrine systems.

I.1. Anatomophysiology of the male reproductive system:

The rat is one of the most commonly used research animals in reproductive physiology. The male reproductive system of the rat consists of two testes, extratesticular ducts that include the epididymis, efferent ducts and vas deferens, as well as sexual glands that include the seminal vesicles, prostate, ampullary glands, bulbourethral glands, preputial glands and, besides all this, the penis and urethra (**Knoblauch et al., 2018**). Ampullary glands and preputial glands are only found in rodents, whereas they are not present in humans (**Nagarajan et al., 2021**).

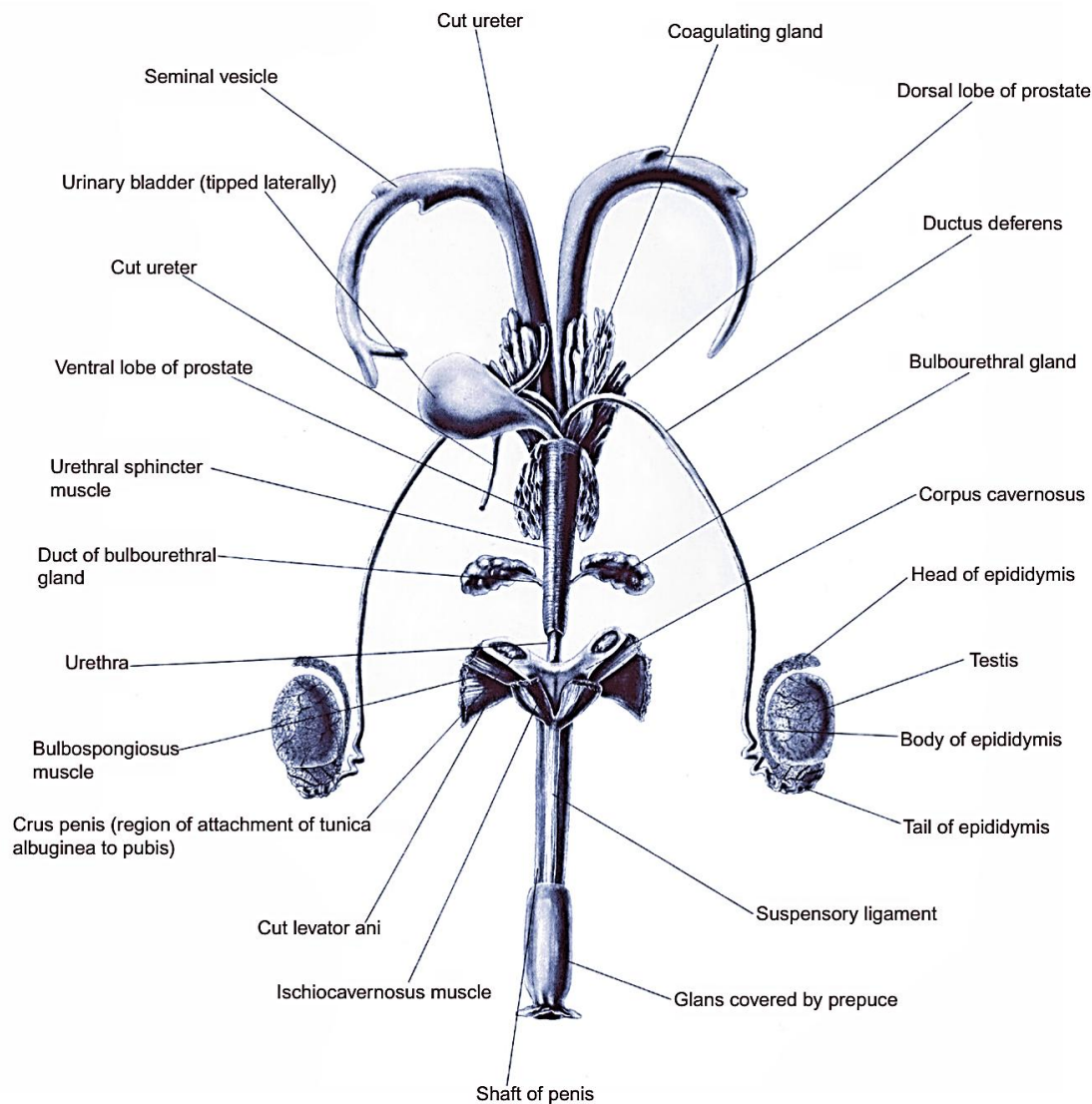


Figure 01: Male reproductive tract (Hargaden and Singer, 2012).

I.1.1. Testes and seminiferous tubules:

In both rodents and humans, the paired **testes** are situated within the scrotum, encased in a thick fibrous shell known as the tunica albuginea, and wrapped in a dual layer of mesothelium,

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

the tunica vaginalis, which additionally lines the scrotum's inner surface (**Nagarajan et al., 2021; Whitney and Suttie, 2018**). They lie between the prepuce and the anus. In rats, each testis weighs ~1.7 g and measures ~2.4 cm × 1.3 cm × 0.9 cm (**Knoblauch et al., 2018**). Rodents are particular in that they retain open inguinal ducts for life, connecting their testes to the abdominal cavity (**Nagarajan et al., 2021**). These ducts enclose the epididymal fat pad when the testes are in the scrotum, and this fat pad envelops each testicle outside the scrotum. They are the ones responsible for producing and releasing male gametes, called spermatozoa (**Knoblauch et al., 2018**).

Each testis is mainly composed of complex networks of **seminiferous tubules** and an interstitium containing somatic cells (Sertoli cells, Leydig cells, and myoid cells) and germ cells (spermatogonia, spermatocytes, and spermatids) (**Maynard and Downes, 2019**). These tubules, essential for sperm development and transport, adopt a hairpin shape and connect to the rete testis (**Maynard and Downes, 2019**). Their epithelium is characterized by a basal layer of Sertoli cells overlaid by layers of germ cells. The seminiferous tubules are composed of three cell types that can be quantified: myoid or peritubular cells, Sertoli cells, and germ cells (spermatogonia, spermatocytes, and spermatids), underscoring their crucial role in the sperm germination and maturation processes (**Nagarajan et al., 2021**). Moreover, the tight junctions between Sertoli cells form the "blood-testis barrier", essential for regulating exchanges between the blood and the testicle (**Knoblauch et al., 2018**).

I.1.2. Extra-testicular ducts: (rete testis, efferent ducts, epididymis, vas deferens and urethra)

The system of excretory or extra-testicular ducts includes the rete testis, efferent ducts, epididymis, vas deferens, and urethra.

The rete testis, located at the upper pole of the testis just beneath the tunica albuginea, appears as a saccular structure covered by a flat epithelium (**Whitney and Suttie, 2018**). It often extends outside the testis, establishing a connection with the efferent ducts and lying close to the testicular artery (**Knoblauch et al., 2018**).

Efferent ducts, immersed in the adipose tissue of the epididymis according to **Whitney et Suttie. (2018)**, usually vary between six and nine in rats, according to **Knoblauch et al. (2018)**, their main function is the collection, storage, and transport of spermatozoa from the rete testis to the head of the epididymis.

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

The epididymis, forming a pair of elongated tubular structures attached to the dorsal surface of each testicle, are distinguished by their singularity per testicle (**Maynard et Downes, 2019**). Morphologically, the epididymis is divided into three segments: the epididymal caput, which is the enlarged section at the proximal end of the testicle; the isthmus, or body of the epididymis, extending along the dorsomedial surface of the testicle; and the cauda, located at the distal extremity (**Crisler et al., 2019**). However, depending on specific functions, the epididymis may be segmented into several parts. Its essential function lies in the accumulation, final maturation and storage of mature spermatozoa, underlining its crucial role beyond that of a simple conduit for spermatozoa, contributing significantly to the formation of fully mature and viable spermatozoa (**Crisler et al., 2019; Knoblaugh et al., 2018**).

The vas deferens, a bilateral structure, reaches a length of 56 cm. Starting from the epididymal cauda within the spermatic cord, leading to the urethra, it crosses the prostate (**Maynard et Downes, 2019**). The deferential arteries irrigate it (**Crisler et al., 2019**). Its wall structure is made up of three layers of smooth muscle, facilitating the transport and ejection of spermatozoa from the epididymal cauda to the urethra during ejaculation (**Knoblaugh et al., 2018; Maynard and Downes, 2019**).

The urethra, a singular duct, extends from the bladder to the external urethral opening, performing the function of evacuating both semen and urine (**Knoblaugh et al., 2018**). It is segmented into two regions: the prostatic/membranous urethra, or pelvic urethra, and the penile urethra (**Pradidarcheep et al., 2019**). More than a simple conduit, the urethra is dorsoventrally flattened, with dorsolateral and ventrolateral projections, and incorporates small urethral glands (**Maynard and Downes, 2019**).

I.1.3. Accessory sexual glands:

In the rat, there are five pairs of accessory sex glands distributed in the pelvis and around the bladder, including the ampullary glands of the vas deferens, a pair of seminal vesicles, a pair of coagulating glands (part of the prostatic structure), a pair of bulbourethral glands (also known as Cowper's glands) and the preputial glands located on the penis (**Crisler et al., 2019; Maynard and Downes, 2019**). These are essential for generating most of the liquid volume of the ejaculate, which coagulates to form a vaginal plug, ensuring the closure of the female reproductive tract post-copulation (**Whitney and Suttie, 2018**).

Originating from the bulbs of the vas deferens, **the ampullary glands** almost envelop the duct (**Crisler et al., 2019**). They are bilateral, androgen-dependent accessory structures located

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

on either side of the proximal segment of the vas deferens (**Knoblauch et al., 2018**). Present in rodents but absent in humans, secretions from these glands play a key role in sperm composition (**Knoblauch et al., 2018**).

The seminal vesicles, shared by rodents and humans, are sexual accessory glands (**Knoblauch et al., 2018**). They appear as elongated, paired structures, positioned dorso-laterally to the bladder and in juxtaposition with the ventral surface of the colon, measuring between 17 and 25 mm in length (**Whitney and Suttie, 2018**). In the rat, the seminal vesicles and coagulating glands play a crucial role in fertility. They produce fluids essential to the creation of an effective vaginal plug, which seals off the female reproductive tract after mating (**Crisler et al., 2019**). In addition, the secretions of these vesicles, together with those of the coagulating gland and contributions from the bulbourethral gland, constitute the seminal fluid (**Knoblauch et al., 2018**).

The coagulating glands, equivalent to the anterior prostate in rodents, are located on either side of the minor curvature of the seminal vesicles (**Knoblauch et al., 2018**). They reach a length of around 36 mm and consist of five to six tubules converging into a single duct that opens into the urethra (**Whitney and Suttie, 2018**).

Bulbourethral glands, similar to Cowper's glands in humans, are distinguished by their pear shape and structure, consisting of a main body, a tail and a secretory duct (**Knoblauch et al., 2018**). Positioned further downstream along the urethra, near the base of the penis, they are segmented into lobes by connective tissue partitions and function under the influence of androgens. Their secretion, a mixture of mucus and seromucus, plays a role in lubrication (**Whitney and Suttie, 2018**).

Preputial glands, located near the base of the penis, in rodents are enlarged, yellowish sebaceous structures (**Maynard and Downes, 2019**). Measuring up to 20 mm long by 5 to 8 mm wide, and weighing between 70 and 150 mg, they release pheromonal secretions into the space between the prepuce and the glans (**Maynard and Downes, 2019**).

The prostate in the rat is divided into three pairs of lobes: the anterior or dorsocranial lobe (coagulating glands), the ventral lobe, and the dorsolateral lobe, each with specific anatomical and histological characteristics. Its function and development depend on androgens (**Crisler et al., 2019; Whitney and Suttie, 2018**).

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

- **The ventral prostate** located along the ventrolateral side of the bladder, the ventral prostate is approximately 14 to 17 mm long and 69 mm in diameter, indicating its significant size (**Whitney and Suttie, 2018**).

- **The dorsolateral prostate** characterized by its division into two lobes, dorsal and lateral, each histologically distinct. The dorsal lobe shares histological similarities with the coagulating gland, while the lateral lobe displays features closer to those of the ventral prostate, denoting structural and functional diversity within the same gland (**Knoblauch et al., 2018; Whitney and Suttie, 2018**).

I.1.4. Penis:

The rat penis, measuring between 20 and 28 mm in length, 3.6 mm in width, and 2.8 mm in height, is characterized by a distinctive caudal curvature (**Maynard and Downes, 2019**). Comprising a base, body, and skin-covered glans, it is enveloped by a flexible preputial sheath (**Knoblauch et al., 2018**). Notably, the rat's penile anatomy is similar to that of man but is distinguished by the presence of a penile bone, or baculum (**Crisler et al., 2019; Maynard et Downes, 2019**). The vascular system of the penis consists of paired arteries and a single median vein located dorsally, accompanied by lymphatic vessels, illustrating the complexity of its blood and lymphatic supply (**Maynard and Downes, 2019; Whitney and Suttie, 2018**).

In the field of reproductive biology, the regulation of reproductive function is a fascinating chronicle of the interaction between the endocrine, nervous, and reproductive systems.

The reproductive system in males has three functional levels: pre-testicular (neuroendocrine regulation), testicular (spermatogenesis), and post-testicular (ejaculation) (**Barkabi-Zanjani et al., 2020**).

I.2. Hypothalamic-Pituitary-Gonadal Axis:

The regulation of sexual behavior in male rats is part of the Hypothalamic-Pituitary-Gonadal (HPG) axis, indispensable for sperm production (fertility) and testosterone secretion (virility) (**Llahana et al., 2019; Son et al., 2022**). In humans and other higher vertebrates, this reproductive function is essential to the survival of the species, and is regulated by the coordinated endocrine action of the HPG axis (**Llahana et al., 2019**). Gonadotropin-releasing hormone (GnRH) is recognized as the only hypothalamic neuropeptide regulating reproduction, and is released pulsatile from a small population of specialized hypothalamic parvocellular

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

secretory neurons (<2000) into the bloodstream, where it is transported to the pituitary and initiates the neuroendocrine activity of the HPG axis (**Llahana et al., 2019**).

Recent research has identified signals "upstream" of GnRH (**Abreu and Kaiser, 2016; Boehm et al., 2015**). Yet GnRH has been considered the "pilot light" of reproduction (**Balasubramanian et al., 2010**). It then binds to the gonadotropin receptor GnRH-R to stimulate the production and pulsatile release of the heterodimeric glycoprotein gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (**Llahana et al., 2019; Mitchell et al., 2017; Ramaswamy and Weinbauer, 2014**).

After release into the systemic circulation and transport to the testis, both gonadotropins act via specific transmembrane receptors, FSH-R and LH-R, respectively (**Mitchell et al., 2017; Shulman and Spritzer, 2014**). FSH-R is primarily expressed in Sertoli cells, while LH-R is expressed in interstitial Leydig cells (**Ramaswamy and Weinbauer, 2014**). On the one hand, LH will stimulate testosterone steroidogenesis in Leydig cells (**Mitchell et al., 2017**). Testosterone in turn acts via androgen receptors (ARs) on Sertoli cells and other testicular cells to activate signaling pathways that are required to support spermatogenesis (**Shulman and Spritzer, 2014**). On the other hand, FSH plays a key role in testicular development, controlling Sertoli cell proliferation (**Mitchell et al., 2017**).

Another protein, androgen-binding protein (ABP) produced by Sertoli cells (**Robaire and Hinton, 2015**), binds specifically to testosterone or dihydrotestosterone, making them less lipophilic and more concentrated in seminiferous tubules to enable spermatogenesis and sperm maturation, it also promotes germ cell differentiation (**Y. Ma et al., 2015**).

Testicular factors are known to negatively regulate the production and release of gonadotropins from the pituitary gland (**Mitchell et al., 2017**). Retro control of the HPG axis is provided by testicular factors, notably the testicular hormone inhibin B, which is a glycoprotein produced by Sertoli cells (**Mitchell et al., 2017**), which can inhibit FSH secretion by the pituitary gland (**Ramaswamy and Weinbauer, 2014**). Testosterone released into the bloodstream also sends a signal to the brain to modulate LH release in negative feedback, it can act alone or after its aromatization into estradiol (**Mitchell et al., 2017**).

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

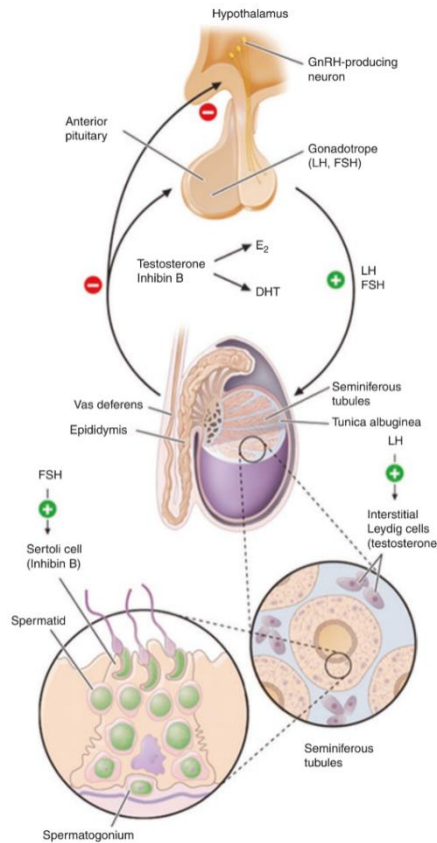


Figure 02: The male hypothalamic-pituitary-gonadal axis, pulsatile secretion of GnRH from hypothalamic neurons triggers the release of LH and FSH from the pituitary and into the peripheral circulation. FSH supports spermatogenesis via the stimulatory effect on the Sertoli cells in the seminiferous tubules. LH stimulates the Leydig cells in the interstitium to produce testosterone. Positive and negative feedback regulate the axis function (Llahana et al., 2019).

I.2.1. Steroidogenesis and synthesis of testosterone:

Androgens, a family of steroid hormones in which testosterone and dihydrotestosterone (DHT) stand out as the main components, play a crucial role in the development and maintenance of male characteristics (Shea et al., 2014). Synthesized mainly in the testes in men and to a lesser extent in the ovaries in women, as well as in the adrenal glands for both sexes, these hormones are all derived from cholesterol via a chain of specific enzymatic reactions (Figure 03) (Shea et al., 2014). Testosterone and DHT considered the most active, exert their effects on various tissues, including the prostate and hair follicles, and play an essential role in spermatogenesis via stimulation of the Leydig cells in the testes (Dušková et Pospíšilová, 2011; Swerdloff et al., 2017). This production is finely regulated by the HPG axis, where the pulsatile release of the hormone GnRH by the hypothalamus induces secretion of LH and FSH by the pituitary, in turn stimulating androgen synthesis by Leydig cells. A negative feedback mechanism, whereby increased testosterone inhibits GnRH and LH production, maintains

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

hormonal balance. Adrenal androgens, although largely inactive, serve as important precursors to testosterone and DHT, underlining the complex and multifaceted role of these hormones in the body (Shea et al., 2014).

Male rats release testosterone reflexively when they sense (anticipatory release) or mate (ejaculatory release) with a new receptive female, in addition to the spontaneous releases that occur throughout the day (Shulman and Spritzer, 2014).

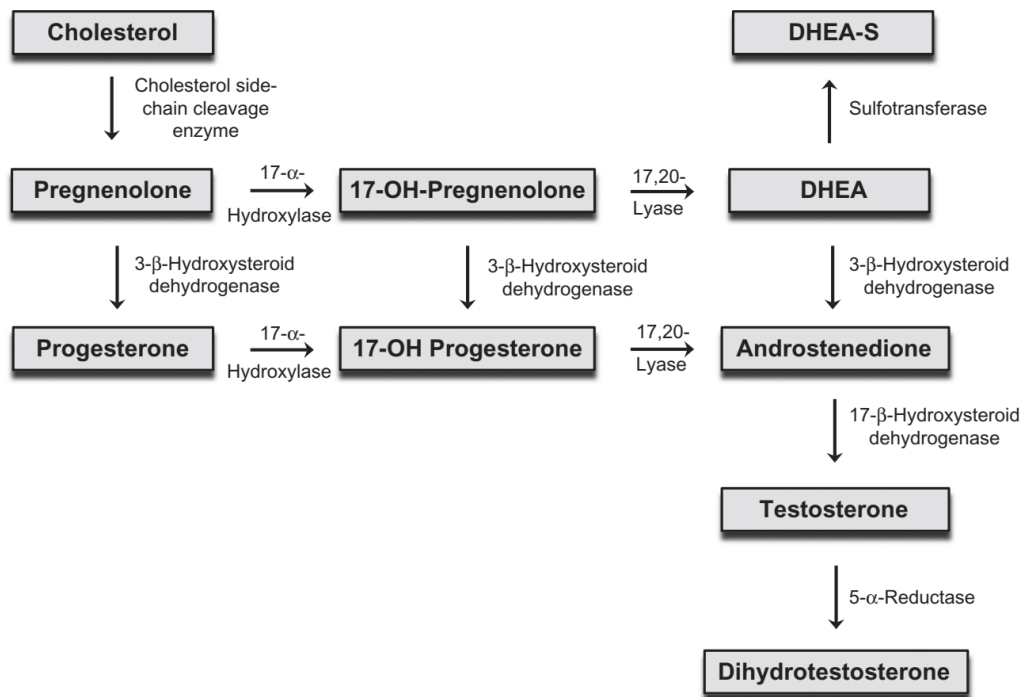


Figure 03: Overview of steroidogenesis and synthesis of testosterone (Shea et al., 2014).

I.2.2. Spermatogenesis:

Spermatogenesis is a prime example of the regulatory precision of the HPG axis. It takes place in several stages: from the transformation of spermatogonia into spermatocytes, then into spermatids, and finally into mature spermatozoa involving three successive phases of proliferation, meiosis and spermiogenesis (Maynard and Downes, 2019; Whitney and Suttie, 2018).

I.2.2.1. Stages of spermatogenesis development:

During the process of spermatogenesis, the initial germ cells, known as spermatogonia, localize to the periphery of the seminiferous tubules and begin maturation (Knoblaugh et al., 2018; Mitchell et al., 2017) These cells then migrate to the tubule lumen, where they divide by

mitosis into type A and type B spermatogonia, the latter evolving into spermatocytes. The process of meiosis, which takes place in two distinct phases, enables spermatocytes to divide first into secondary spermatocytes, then into spermatids during the second division. The spermatids, without undergoing further division, mature into spermatozoa through a process of elongation, the creation of a dense acrosome and the condensation of their nucleus (**Maynard and Downes, 2019; Whitney and Suttie, 2018**). Once formed, sperm undergo further maturation in the epididymis, where they undertake the morphological, biochemical and physiological changes essential for achieving motility and ovule fertilization capacity (**Knoblaugh et al., 2018; Mitchell et al., 2017**).

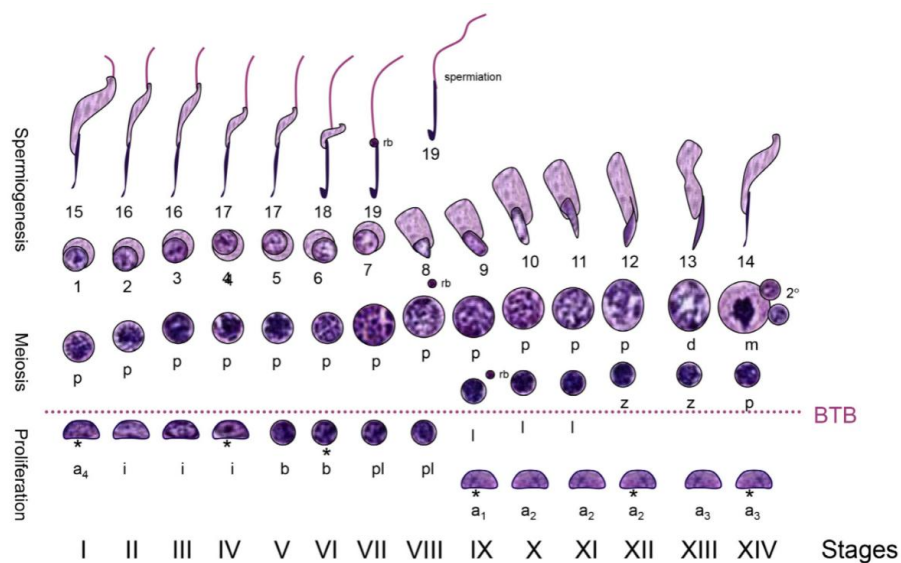


Figure 04: Schematic representation of spermatogenesis in the rat, BTB: Blood-Testicular Barrier (Whitney and Suttie, 2018).

I.2.2.2. Spermatozoon:

Rat spermatozoa are considerably longer than human spermatozoa, measuring around 190 μm (**Maynard and Downes, 2019; Whitney and Suttie, 2018**). Their structure consists of a head and a tail (**figure 00**). The falciform head is made up of four main parts: the nucleus, the acrosome, the perinuclear theca, and the plasma membrane (**Maynard and Downes, 2019**). The nucleus, which contains the haploid genome, occupies most of the volume of the head (**Y. W. Lin et al., 2013**). As for the tail, it is divided into a central connecting piece surrounded by the mitochondrial spiral, a long main piece and a shorter caudal piece (**Maynard and Downes, 2019; Whitney and Suttie, 2018**). The sperm pathway begins in the testis, then passes through the epididymis and vas deferens to the urethra, and finally, during copulation, is transported via the penis to the female rat (**Maynard and Downes, 2019**).

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

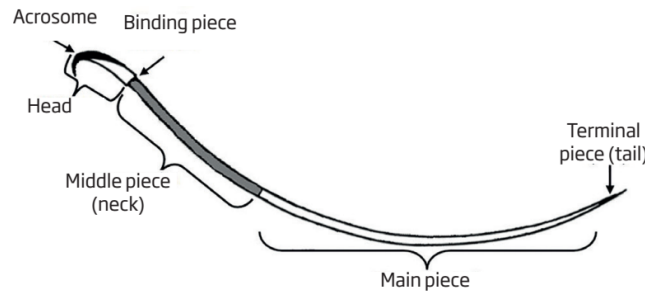


Figure 05: Schematic representation of a rat spermatozoon (Ríos and Mascorro, 2019).

I.2.3. Sexual behavior of the male rat:

The HPG axis represents the main control system in the regulation of sexual behavior, acting as a bridge between neural signals and hormonal responses. The secretion of GnRH by the hypothalamus triggers a cascade of hormonal events, stimulating the release of LH and FSH by the adenohypophysis, which in turn regulates testosterone and estrogen production by the gonads. These steroid hormones exert retroactive effects on the brain, modulating sexual behavior (Acevedo-Rodriguez et al., 2018; Llahana et al., 2019).

I.3. Neurobiology of sexual behavior:

I.3.1. Brain regions involved in sexual behavior:

The coordination of sexual behavior in male rats involves several brain regions, each playing a specific role in the mating process. These brain regions process sensory information, generate hormonal and motor responses, and facilitate the social and emotional behaviors associated with reproduction (Gaytán-Tocavén et al., 2023).

I.3.1.1. Olfactory system:

Pheromones secreted by both partners play a key role in regulating sexual behavior in rats. Indeed, chemosensory signals are essential for initiating sexual behavior (Jennings and de Lecea, 2020). Olfactory afferents then converge on hypothalamic GnRH neurons, enabling modulation of endocrine responses (Jennings and de Lecea, 2020). Olfactory information is detected by two anatomically distinct groups of chemosensory neurons which are: (Brunert and Rothermel, 2021; Gaytán-Tocavén et al., 2023)

- **The primary olfactory system (POS):** is sensitive to odors known as volatiles, such as food, litter that can be smelled at great distances.
- **The accessory olfactory system (SOA):** is sensitive to non-volatile odors, such as pheromones in urine, which must be sniffed; it plays an essential role in sexual identification.

I.3.1.2. Medial amygdala (MeA):

The medial amygdala integrates information relayed by the accessory olfactory system and by stimuli from the genitalia, this region is responsible for sexual motivation and performance, as well as erectile responses (**Gaytán-Tocavén et al., 2023; Iovino et al., 2019**).

I.3.1.3. Bed nucleus of the stria terminalis (BNST):

The bed nucleus of the stria terminalis receives afferents from the anterior MeA but also from the accessory olfactory system. It is involved in the expression of sexual behavior and the release of LH (**Baum and Bakker, 2013**).

I.3.1.4. Hypothalamus:

The hypothalamus plays a key role in regulating sexual motivation and triggering mating behavior. It integrates hormonal and environmental signals to modulate sexual activity (**Iovino et al., 2019**).

I.3.1.5. Median preoptic area (mPOA):

This brain region, located in the hypothalamus, is predominantly involved in regulating the copulatory behavior and sexual motivation in male rats. The mPOA contains a high density of dendritic spines, indicating high synaptic plasticity (**Jennings and de Lecea, 2020**).

I.3.1.6. Prefrontal cortex:

The prefrontal cortex is involved in decision-making and the evaluation of sexual partners, playing a role in sexual choice and preference. It helps modulate behavior according to social consequences and experiences (**Calabrò et al., 2019; Spinella, 2007**).

I.3.1.7. Ventral tegmental area (VTA) and nucleus accumbens:

These regions are essential to the reward system and are activated in response to satisfying sexual stimuli (**Jennings and de Lecea, 2020**). They play a role in reinforcing sexual behavior through the release of dopamine, thus increasing motivation to continue mating (**Gaytán-Tocavén et al., 2023; Matsumoto et al., 2012**).

I.3.2. Spinal regions involved in sexual behavior:

I.3.2.1. Spinal bulbocavernosus nucleus (SBN):

The SBN plays a crucial role in controlling erectile responses and ejaculation. The erectile response can be triggered by various stimuli such as olfactory, visual, or tactile cues. During ejaculation, information is first processed within limbic brain regions like the amygdala and hypothalamus before being relayed to the spinal cord through specific nerve pathways **(Dean and Lue, 2005; Sakamoto, 2010)**. This process involves both autonomic pathways, which innervate the penis to concentrate seminal fluids, and somatic pathways that control the activity of striated muscles, forming a ring at the base of the penis. These coordinated actions enable the emission of ejaculate and the maintenance of an erection, highlighting the intricate neural control involved in sexual responses and reproductive functions **(Dean and Lue, 2005; Sakamoto, 2010)**.

I.3.3. Neurotransmitters involved in sexual behavior:

I.3.3.1. Dopamine:

Dopamine is a neurotransmitter that plays many key roles in motivating and rewarding behavior, and is associated with desire in particular. Moreover, dopamine levels are elevated during copulation, and it plays a major role in the responses of arousal, ejaculation and penile erection **(Karabaşoğlu and Erbas, 2021)**.

I.3.3.2. Serotonin:

Serotonin (5-HT) is a neurotransmitter that plays an important role in the regulation of various psychological processes and behaviors, such as mood regulation, reward and learning. The serotonin system affects sexual behavior. In general, a decrease in 5-HT facilitates sexual behavior **(Karabaşoğlu and Erbas, 2021)**.

I.3.3.3. Oxytocin:

Oxytocin is a neurotransmitter that plays a role in social bonding, learning and milk production, as well as in sexual activities in rats, such as stimulation of the reproductive organs. The release of oxytocin increases sexual motivation, induces penile erection and reduces the post-ejaculatory period **(Karabaşoğlu and Erbas, 2021)**.

I.3.3.4. Nitric oxide (NO):

Nitric oxide acts as a neurotransmitter for its role in facilitating erection. It induces relaxation of the smooth muscles of the cavernous corpora of the penis, enabling the blood flow that leads to erection **(Davies, 2015)**.

I.3.4. Detailed description of male rat sexual behavior:

Male rat sexual behavior is a complex, multi-step process that can be described through four main phases: Anticipatory, courtship, copulating and post-copulating. Each of these phases is characterized by specific behaviors, regulated by interactions between environmental stimuli, hormonal signals and neurological mechanisms **(Bialy et al., 2019)**.

I.3.4.1 Anticipatory phase:

This phase initiates the reproductive process. Guided by visual, auditory, olfactory, tactile and in humans even imaginative stimuli, the male actively seeks out potential sexual partners **(Melis et al., 2022)**. Female pheromones play a crucial role in this behavior, triggering the male's motivation to explore his environment. Olfaction is therefore the primary sense at this stage, enabling the male rat to detect the presence of an estrous female from a distance **(Hoglen and Manoli, 2022)**.

I.3.4.2. Courtship phase:

Sexual motivation is related to paracopulatory behaviors **(Karabaşoğlu and Erbas, 2021)**. Once a potential female partner has been located, the male rat begins a series of courtship behaviors designed to seduce the female and gain her cooperation in mating. These behaviors include courtship displays, specific ultrasonic vocalizations, and sometimes self-presentation, such as grooming or displaying certain postures **(Hull and Dominguez, 2015)**. In female rats, paracopulatory behavior included ear wagging, characterized by rapid anteroposterior vibration of the ears, a short run where female rats suddenly stop and present their posteriors to male rats (darting), and a short jump with stiff legs followed by immobility and presentation (hopping) **(Boublata et al., 2021)**.

This phase is essential to establish a positive interaction between the partners and reflects an appetitive sexual behavior, as it involves actions that increase the motivation (which can be described by the sexual incentive motivation test arena) and the probability of mating, thus increasing the chances of successful mating **(Jennings and de Lecea, 2020)**.

I.3.4.3. Copulating:

Initiation of copulation is highly linked to sexual motivation and is strongly inhibited by high levels of anxiety (**Bialy et al., 2019**). Copulating is the pivotal stage of sexual behavior, and takes place in several stages: mounting, intromission and ejaculation (**Figure 06**). It occurs more frequently during dark periods than light ones, and more frequently during the latter part of the dark cycle than the former (**Otto et al., 2015**). This phase reflects consummatory sexual behavior, since it involves the act of mating itself (**Jennings and de Lecea, 2020**).

During mating, rats engage in social activities such as sniffing and looking for the anogenital zone (**Karabaşoğlu and Erbas, 2021**). Then the receptive females generally adopt an immobile posture, allowing the male to approach, raise his front body above the female's hindquarters, grasp her flanks with his front paws, and begin a series of rapid, shallow anteroposterior movements of the pelvis, otherwise known as **mounting** (**Bialy et al., 2019; Sanna et al., 2020**). These preliminary movements facilitate genital contact and increase the probability of intromission. The female can then assume a characteristic lordosis posture (measured as % displaying lordosis) (**Bialy et al., 2019**), with her back flat or concave and her tail deviated to the side, thus facilitating mating (**Hull and Dominguez, 2015**).

The male, now engaged in the mating process, performs a series of rapid, superficial thrusts, with his penis (**Bialy et al., 2019; Hull and Dominguez, 2015**). When the glans penis locates the vagina, the male can achieve a rapid erection and triggers a deeper intravaginal thrust, followed by rapid dismount with a vigorous backward thrust, characteristic of the **intromission** in rats and many other rodents (**Bialy et al., 2019; Hull and Dominguez, 2015; Sanna et al., 2020**). These repeated movements are not just mechanical; they play a crucial role in sperm transport and increasing the chances of fertilization. Multiple intromissions (5-15), each lasting 0.3 to 0.6 s and accompanied by two to nine pelvic thrusts, precede the first ejaculation (**Otto et al., 2015**). This initial sequence of intromissions, which lasts for around ten minutes, is typically referred to as ejaculatory latency (**Otto et al., 2015**).

Ejaculation marks the end of mating, occurring after multiple intromissions, is characterized by a deeper, prolonged thrust, followed by a slow, relaxed dismount without any backward thrust (**Sanna et al., 2020**). Basically, seminal emission refers to the transfer of semen into the proximal urethra, while ejaculation refers to the forced expulsion of semen through the distal urethra (**Hull and Dominguez, 2015**). This key moment in mating is accompanied by rhythmic contractions of the skeletal and striated perineal muscles, notably the

Part I: Review of the literature
Chapter I: Overview of male reproductive function in rats

bulbospongiosus, ischiocavernosus and anal sphincter, a phenomenon also observed in humans and associated with orgasm (**Hull and Dominguez, 2015**).

I.3.4.4. Post-copulating and satiety after ejaculation:

After ejaculation, the male grooms himself and enters a phase of sexual quiescence (**Hull and Dominguez, 2015**). The Post-ejaculatory interval is a process that lasts between ejaculation and a new sexual attempt with mount (**Karabaşoğlu and Erbas, 2021**), and can vary considerably from one species to another, ranging from a few seconds to several hours or days. In rats, this period lasts from five to ten minutes and allows the male to recover and prepare for future mating (**Hull and Dominguez, 2015**). Around two minutes after the first ejaculation (and even longer after the second), the male rats start to move and explore their environment even when they are in the post ejaculatory phase (**Bialy et al., 2019**). These movements show that they are becoming more alert and curious, although not because of any renewed interest in sex. In the experiments, when they get close to a female, they tend to move away from her and try to escape, which shows that they are not looking for sexual contact (**Bialy et al., 2019**). After seven to eight ejaculations, males reach satiety, which prevents them from copulating for one to three days (**Bialy et al., 2019; Hull and Dominguez, 2015**). Sexual intercourse in rats can be verified by identifying spermatozoa on a vaginal smear, observing a vaginal plug (a pale coagulum formed by the seminiferous fluid visible in the vagina), or directly observing sexual activity (**Otto et al., 2015**).

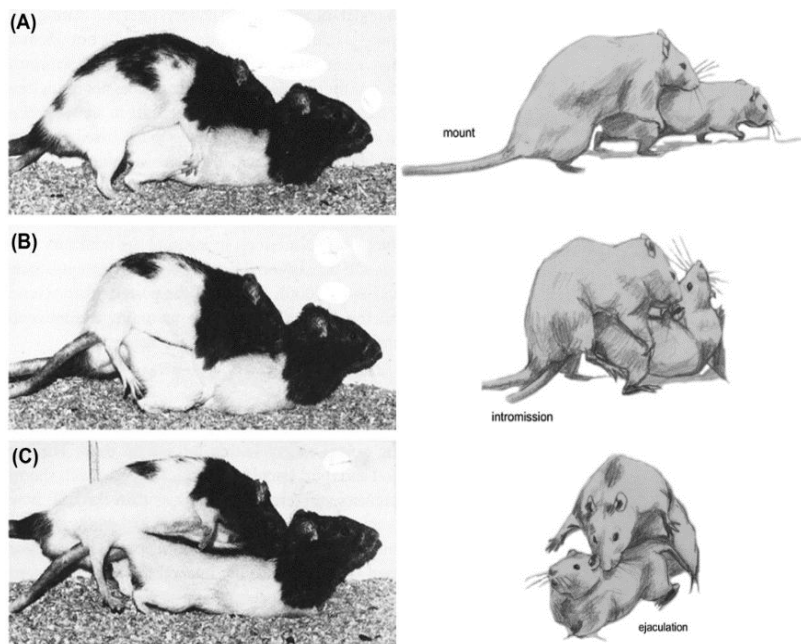


Figure 06: Three views of Sexual behavior of male rats: mount, intromission, and ejaculation (**Hull and Dominguez, 2015; Snoeren et al., 2014**).

I.4. Infertility:

Infertility, a disease of the reproductive system, has become an integral part of global health as it affects, according to statistics, 60 to 80 million couples, 50% directly attributed to a male factor (**Omolaoye and Du Plessis, 2021**). The causes of male infertility can vary widely, including problems with sperm quality and quantity, obstructions in the seminal tract, erectile and ejaculatory dysfunction, and hormonal imbalances (**Abdul-Razzaq, 2023**).

Lifestyle factors, genetic conditions, infections, exposure to toxins, and diseases such as diabetes, sexually transmitted infections (STIs) and autoimmune disorders can also contribute to male infertility. Understanding and addressing these various factors is crucial in diagnosing and treating male infertility (**Grande and Foresta, 2023; Jafari et al., 2021**).

The complexity of the male reproductive system, with its numerous regulatory mechanisms and crucial role in fertility, underlines the importance of preserving its function against external aggression. However, systemic diseases such as diabetes can disrupt this delicate balance. This leads to the question: how exactly does diabetes affect the male reproductive system, and what are the implications of these effects for understanding and treating diabetes-associated male infertility?

Chapter II

Diabetes mellitus and the impairment of male reproductive function

Diabetes mellitus is one of the most common endocrine metabolic disorders, characterized by hyperglycemia due to defects in insulin secretion and/or action. This disease is an incarnation of various metabolic pathologies (**Shi et al., 2017**).

In response to the question previously outlined, this chapter will look at the multifactorial effects of diabetes on the male reproductive system. By examining the links between glycemic dysregulation and disturbances in reproductive function, we will explore how diabetes can become a key factor in male infertility, highlighting the underlying pathophysiological mechanisms.

II.1. Glimpse of Diabetes mellitus (DM):

Diabetes mellitus, commonly known as diabetes, is a serious and persistent disease, classified as chronic, characterized by increased blood glucose levels. This results from the body's inability to produce enough insulin, a vital hormone made by the pancreas, or to use it effectively (**IDF, 2021**). Insulin plays a crucial role in facilitating the entry of glucose into the body's cells for conversion into energy or storage. It is also essential for protein and lipid metabolism (**IDF, 2021**). When the body lacks insulin, or when cells fail to respond adequately to this hormone, glucose accumulates in the blood, leading to hyperglycemia, the most obvious sign of diabetes (**Shi et al., 2017**). This disorder presents a major challenge due to its numerous complications, including retinopathy, nephropathy, and cardiovascular disease (**Barkabi-Zanjani et al., 2020**). Beyond these direct impacts, DM also exerts considerable effects on all endocrine functions, inducing fertility disorders in men (**Kotian et al., 2019**).

Diabetes emerges through diverse pathological mechanisms, including autoimmune destruction of pancreatic β -cells, leading to insulin deficiency, and dysfunctions that cause insulin resistance. This results in a critical disruption of glucose balance, characterized by hyperglycemia, due to inadequate glucose uptake and utilization by glucose-dependent cells (**Omolaoye and Du Plessis, 2018**). In addition, diabetes is exacerbated by a variety of pathophysiological conditions, including hypertension, dyslipidemia, chronic inflammation, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress (**R. Huang et al., 2024**). These factors contribute to a cascade of complications such as vascular disease, microangiopathy, and neuropathy, which, in turn, can lead to extensive organ and tissue damage, dysfunction, and risk of failure of body systems (**R. Huang et al., 2024**).

Diabetes mellitus presents a range of symptoms, including high blood sugar, polyuria (frequent micturition), polydipsia (excessive thirst), hunger, fatigue, blurred vision, difficulty concentrating, weight loss, slow-healing wounds, pain, heartburn, shortness of breath, swelling, slurred speech, confusion, memory problems, weakness and drowsiness/sleepiness, Candidiasis (yeast infections) (**Baynest, 2015; Brady et al., 2022**). These symptoms may be indicative of type 1 or type 2 diabetes, with type 2 diabetes being more prevalent and often associated with insulin resistance and metabolic abnormalities (**Chaudhari et al., 2023**).

II.1.1 Diabetes Mellitus Prevalence:

II.1.1.1. In the world:

Among the challenges facing the modern healthcare system, DM, one of the fastest growing global health emergencies of the 21st century (**IDF, 2021**). It is estimated that 537 million people have diabetes in 2021, and this number is projected to reach 643 million by 2030, and 783 million by 2045 (**Figure 07**) (**IDF, 2021**).

Diabetes ranks among the leading 10 causes of death worldwide, and together with the three other principal non-communicable diseases (NCDs)—cardiovascular disease, cancer, and respiratory disease—it contributes to over 80% of all premature NCD deaths (**IDF, 2021**).

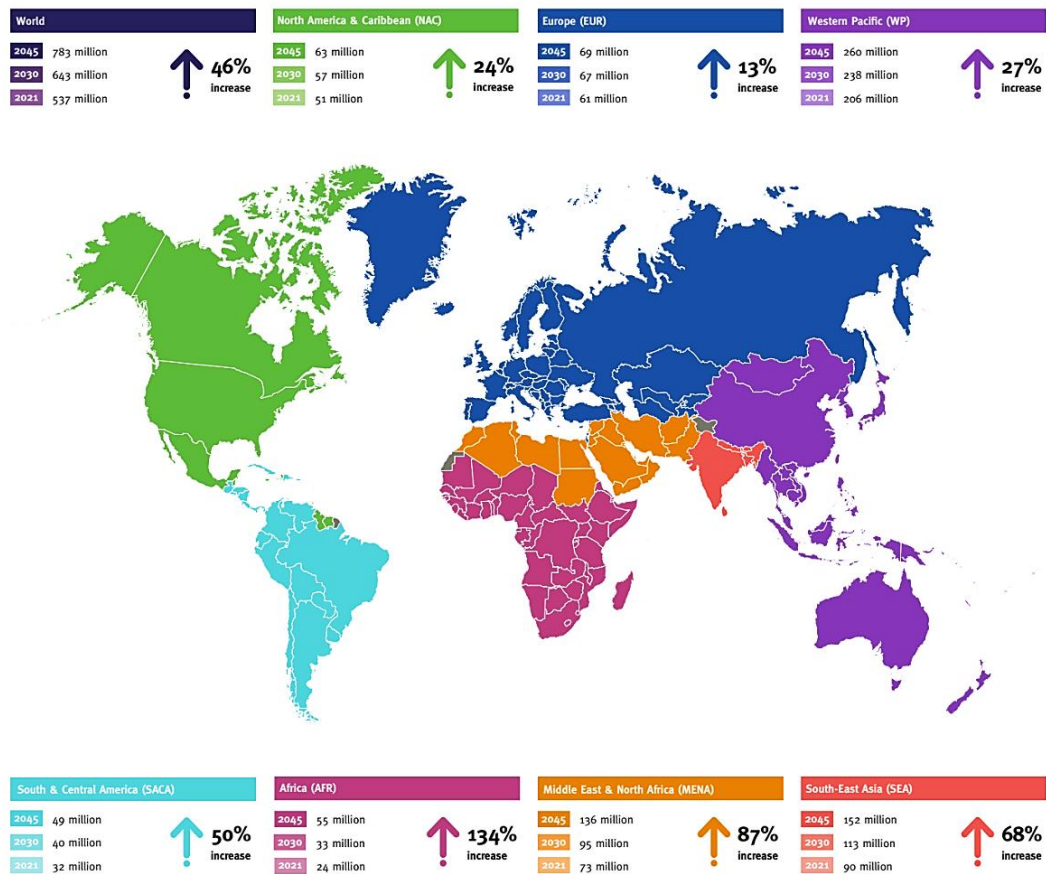


Figure 07: Number of people with diabetes worldwide and per IDF Region in 2021–2045 (20–79 years) (**IDF, 2021**).

II.1.1.2. Diabetes Mellitus Prevalence in the Middle East and North Africa (MENA):

In 2021, The MENA Region has the highest comparative prevalence of diabetes (18.1%) in people aged 20–79 years. This estimate is set to rise, with the MENA region continuing to show the highest comparative prevalence in 2045 (20.4%) (**IDF, 2021**). Approximately 72.7 million

people, or 16.2% of adults aged 20-79 years are living with diabetes in MENA in 2021 and about 37.6% of these are undiagnosed. It is estimated that the number of people with diabetes in the region will increase to 135.7 million people, or 19.3 % by 2045 (**IDF, 2021**).

II.1.2. Diabetes mellitus classification:

There are several types of diabetes, including type 1, type 2, gestational diabetes, a form known as diabetes type 3, linked to Alzheimer's disease and other specific types of diabetes.

II.1.2.1 Type 1 Diabetes mellitus (T1DM):

Type 1 Diabetes Mellitus (T1DM) is characterized by autoimmune destruction of pancreatic beta cells, leading to insulin deficiency and hyperglycemia, necessitating life-long insulin therapy to prevent diabetic ketoacidosis, coma, and death (**Vallianou et al., 2021**). Among the 422 million people worldwide affected by diabetes, 10% suffer from type 1. Nearly 85% of those affected are diagnosed before the age of 20, while 15% of diagnoses occur in adults aged 30 or over (**Omolaoye and Du Plessis, 2018**). Factors such as genetics, ethnicity, age, and gender are among the risk factors influencing the cells' vulnerability to damage. Disease progression can be rapid, manifesting itself over days or weeks, and is characterized by reduced insulin production, increased dependence on lipids as an energy source, and elevated cholesterol production by the liver, in addition to a decline in body proteins (**Omolaoye and Du Plessis, 2018**).

II.1.2.2. Type 2 Diabetes mellitus (T2DM):

The rapidly increasing prevalence and incidence of type 2 diabetes worldwide account for 90-95% of all diabetes cases (**IDF, 2021**). Conventionally considered an old-age condition, type 2 is now seeing a rise in incidence in younger populations, signaling a potential future increase in cases (**Omolaoye and Du Plessis, 2018**). This condition is mainly due to a reduction in tissue sensitivity to insulin. Often referred to as the disease of the contemporary age, type 2 diabetes is strongly influenced by lifestyle, including diet and obesity. Ethnic origin, environmental conditions, and socio-economic status are among the notable risk factors. Type 2 often manifests as hyperinsulinemia, the result of a compensatory response by pancreatic β -cells to the reduced sensitivity of tissues to the effects of insulin. This reduced sensitivity impedes the assimilation and storage of carbohydrates, raising blood sugar levels and stimulating increased insulin production to compensate (**Omolaoye and Du Plessis, 2018**).

II.1.2.3. Type 3 Diabetes mellitus (T3DM):

Alzheimer's disease (AD) increasingly recognized for its link with insulin resistance in the brain, has been labelled "type 3 diabetes". This association is based on the common pathophysiological underpinnings that AD shares with diabetes, in particular T2DM (**Hamzé et al., 2022**). The Rotterdam study, carried out in the 1990s, was the first to highlight this link, showing that the risk of dementia was doubled in people with T2DM (**Hamzé et al., 2022; Ott et al., 1999**). Subsequent studies have reinforced the evidence of a strong correlation between T2DM and AD, suggesting a likely bidirectional relationship (**Hamzé et al., 2022**). The impaired glucose metabolism, insulin signalling and subsequent neuronal loss observed in AD correspond to the insulin resistance observed in T2DM (**Yoon et al., 2023**). Moreover, this dysfunction plays an important role in the progression of Alzheimer's disease, as does the amyloid cascade involving the beta-amyloid and tau proteins (**Tekin et al., 2023**). The term "type 3 diabetes" underlines the crucial impact of insulin dysregulation on brain energy deficit and neurodegeneration, highlighting the complex interplay of oxidative stress, inflammation and metabolic alterations in the link between diabetes and Alzheimer's disease (**Hamzé et al., 2022**).

II.1.2.4. Other types of diabetes:

First introduced by **O'Sullivan in 1961**, the term Gestational Diabetes Mellitus (GDM) refers to a disorder of carbohydrate metabolism characterized by hyperglycemia detected during pregnancy (**Moreno-Castilla et al., 2013; Zygmunt-Siembida et al., 2023**). Increasingly common in pregnant women, GDM results from impaired tissue sensitivity to insulin produced by pancreatic beta cells, a problem aggravated by hormones secreted by the placenta that antagonize the action of insulin (**Zygmunt-Siembida et al., 2023**). This form of diabetes represents one of the most common complications of pregnancy, posing significant risks for both mother and child, and has implications for their long-term health (**Baynest, 2015**). Gestational diabetes serves as a classification for women who develop diabetes mellitus during gestation, including those who develop type 1 diabetes during pregnancy and those in whom asymptomatic type 2 diabetes is detected and usually appears in the third trimester (**Baynest, 2015**).

The classification known as "Other Specific Types" encompasses various etiologies of diabetes mellitus, grouping together individuals with genetic defects affecting beta-cell function, such as Maturity-Onset Diabetes of the Young (MODY) (**Baynest, 2015; Reis et al.,**

2023). In addition to MODY, this classification includes individuals with insulin action defects, diseases of the exocrine pancreas like pancreatitis or cystic fibrosis, dysfunction due to other endocrinopathies (e.g., acromegaly), or pancreatic dysfunction induced by drugs, chemicals, or infections. Together, these cases account for less than 10% of all diabetes mellitus cases **(Baynest, 2015).**

II.1.3. Animal experimental model of diabetes:

Various animal models, including type 1 diabetic rats (induced by streptozotocin (STZ), alloxan, or through BioBreeding (BB) rats) and type 2 diabetic rats (created with nicotinamide and STZ, a combination of STZ and a high-fat diet, or Goto-Kakizaki (GK) rats), have been pivotal in exploring the effects of DM on male reproductive health **(Nna et al., 2017).**

II.1.3.1. Streptozotocin (STZ):

Streptozotocin, discovered by **Vavra et al. in 1959**, is a compound with antibiotic and anticancer properties, produced by *Streptomyces achromogens* **(Omolaoye et al., 2018)**. Since its diabetogenic properties were first reported in 1963, extensive research has revealed its mechanism: STZ's structure, rich in deoxyglucose molecules, targets GLUT2 receptors on pancreatic β -cells **(Lenzen, 2008; Omolaoye et al., 2018)**. Its highly reactive methylnitrosourea group causes cytotoxic effects, damaging these cells and leading to altered insulin and blood glucose levels **(Omolaoye et al., 2018)**. STZ's efficacy as a diabetes inducer was underlined, its rapid elimination by the liver was highlighted, and the lesions observed were linked mainly to its hyperglycemic impact **(Omolaoye et al., 2018)**.

II.2. Diabetes mellitus and male infertility:

DM affects male reproductive system at pre-testicular, testicular and post- testicular levels **(Figure 08) (Nna et al., 2017).**

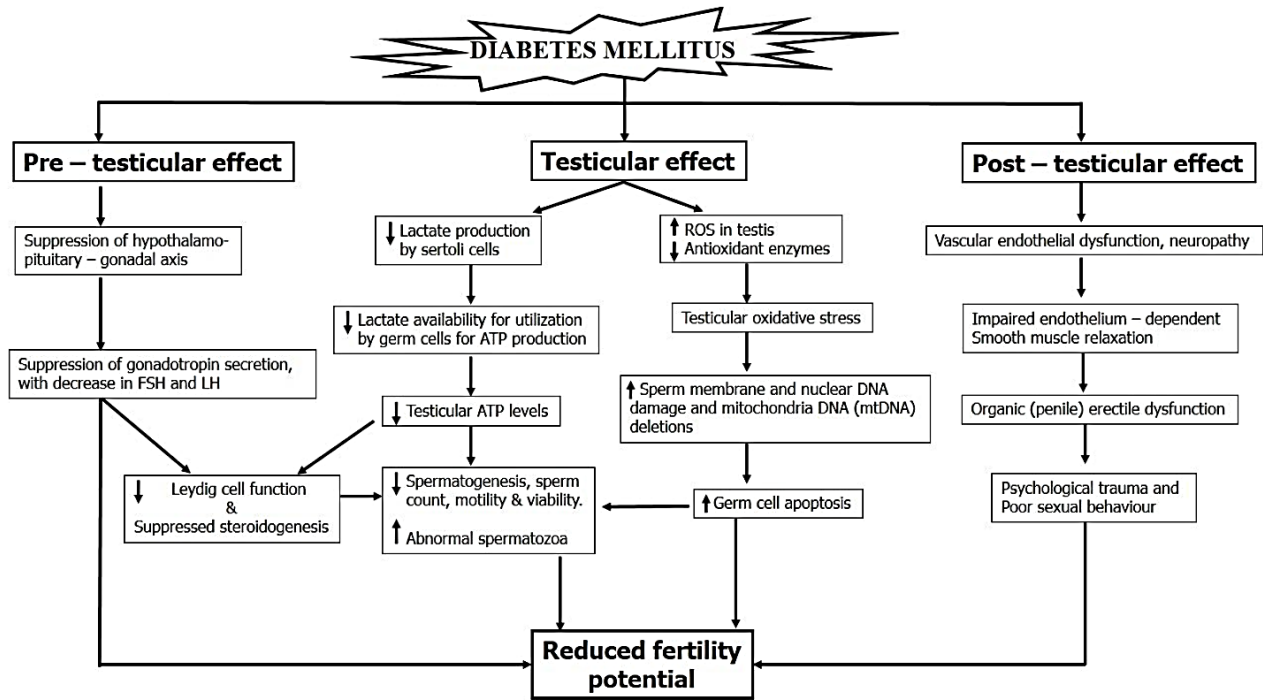


Figure 08: Summary of the effect of DM on the male reproductive system (Nna et al., 2017).

II.2.1. Pre-testicular effects of diabetes mellitus (hypothalamic-pituitary-gonadal axis):

The efficiency of the HPG axis is crucial for initiating puberty, generating androgens, and ensuring fertility. It relies on neuroendocrine systems that process internal and external signals to manage reproductive function. GnRH, a key regulator, orchestrates the production of gonadotropins that directly affect the testes. Optimal activity of this axis ensures healthy sexuality and reproduction, with a positive impact on overall health (Condorelli et al., 2018).

The physiological changes that adversely affect the male reproductive endocrine system have been identified as being primarily mediated by the HPG axis (R. Huang et al., 2024). Several studies show that diabetes mellitus disrupts the activity of the HPG axis, affecting the levels of certain important reproductive hormones, such as testosterone, FSH, and LH in men. In T1DM, this disruption results in a reduction in FSH and LH production in response to GnRH. In addition, the effects of insulin on this axis are regulated via its receptor and corresponding signaling pathways. In T2DM, decreased Leydig cell activity, leading to reduced testosterone secretion, is associated with abnormalities in insulin signaling (Nna et al., 2017).

II.2.2. Testicular effects of diabetes mellitus:

The testes in males perform two main physiological functions: testosterone production and sperm generation (**Ayuob et al., 2015**). Preserving the morphological integrity of the testes is essential for their proper functioning. Testes weight, testosterone levels, and sperm count are key indicators of male reproductive health (**Shi et al., 2017**).

DM can affect male reproductive function in a multitude of ways, including altering sperm quality, modifying the process of spermatogenesis, causing structural changes in the testes, disrupting glucose metabolism at the blood-testis barrier of Sertoli cells, and reducing testosterone levels (**Jain and Jangir, 2014**).

II.2.2.1. Diabetes and testis tissue damage:

In animal models with diabetes induced by STZ, significant abnormalities in testicular structure and function were observed. The gonadosomatic index and the relative weights of the testis, epididymis, and seminal vesicles were notably reduced compared to other experimental groups, indicating severe testicular damage (**Adedara et al., 2015**). Histopathological changes were evident through Hematoxylin and eosin staining, which revealed decreased size of seminiferous tubules and degeneration within spermatogonia, spermatocytes, and spermatids, accompanied by widespread vacuolation and varying degrees of spermatogenetic arrest (**Shi et al., 2017**). This damage is likely exacerbated by oxidative stress, a common feature of DM, which promotes morphological distortions in the testicular tissue (**Nna et al., 2017**). Specifically, increased mitochondrial glucose oxidation due to hyperglycemia releases a substantial amount of superoxide and other free radicals, damaging germ cells and contributing to testicular atrophy (**Xu et al., 2023**).

Antioxidants within the testis play a crucial role in safeguarding key processes such as steroidogenesis and spermatogenesis from the detrimental effects of oxidative stress (**Nna et al., 2017**). Research involving both Type 1 and Type 2 diabetes animal models has consistently demonstrated significant reductions in the levels of key antioxidant enzymes within the testis including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), alongside notable increases in lipid peroxidation indicators through elevated levels of malondialdehyde (MDA) (**ElBanna et al., 2023**). Furthermore, various studies have consistently noted reduced spermatogenesis, evidenced by lower Johnsen's scores, and diminished populations of Sertoli, Leydig, and germ cells in diabetic models (**Xu et al., 2023**). The occurrence of oxidative stress and consequent increase in apoptotic germ cells further

underscores the deleterious impact of DM on testicular health and function (**Buhur et al., 2023**).

II.2.2.2. Diabetes and hormone disorder:

Hormonal regulation, particularly the regulation of sexual hormones by the male reproductive endocrine system, is crucial for maintaining normal reproductive functions (**Ayuob et al., 2015**). In diabetic rats, studies have observed a significant reduction in serum testosterone levels, which impacts spermatogenesis in the testes' seminiferous tubules (**Shi et al., 2017**). This hormonal deficiency is further exemplified by reduced serum concentrations of testosterone, LH, and FSH, affecting both libido and spermatogenesis as evidenced by numerous studies (**Shi et al., 2017**). Steroid hormones, including testosterone, are produced from cholesterol through a series of well-controlled steps. Leydig cells in the testes are responsible for the secretion of testosterone, which supports spermatogenesis. However, research has reported an increased testicular cholesterol level in an STZ-induced T1DM model (**Nna et al., 2017**). This rise may be due to the down-regulation of the cholesterol transport protein, Steroidogenic Acute Regulatory protein (StAR), leading to cholesterol accumulation (**Reddy et al., 2016**). Moreover, there is a significant decrease in the activity of key steroidogenesis enzymes such as 3 β -hydroxysteroid dehydrogenase (3 β -HSD) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD), as well as reduced expressions of StAR and cytochrome P450 enzyme (CYP11A1) (**Andlib et al., 2023**). These findings suggest that the reduction in serum testosterone levels in diabetic conditions, despite high testicular cholesterol levels, could be attributed to the down-regulation of steroidogenic transport proteins and marker enzymes (**Reddy et al., 2016; Saumya and Basha, 2017**). These alterations may significantly contribute to the reproductive disturbances observed in diabetes cases (**Nna et al., 2017**).

II.2.2.3. Diabetes and spermatogenesis:

Diabetes mellitus, markedly affects sperm production and quality. Research using STZ-induced T1DM in animal models has shown decreases in sperm production, motility, and count, alongside an increase in sperm with abnormal morphology (**Carvalho et al., 2021**). Additionally, diabetic neuropathy can cause muscle tension loss in the seminal vesicles, bladder, and urethra, which lowers the volume of ejaculated sperm (**H. Ghasemi et al., 2016**). Globally, diabetes is linked to infertility in approximately 15% of couples, with 40% to 50% of these cases resulting from poor sperm quality and compromised spermatogenesis (**Shi et al., 2017**). In T1DM, the disruption in spermatogenesis is primarily due to changes in FSH levels.

Insulin deficiency indirectly affects the epithelium of seminiferous tubules by altering serum FSH levels, leading to fewer FSH receptors on the tubules and thus a diminished epithelial response to FSH stimulation (**Ballester et al., 2004; Shi et al., 2017**).

Glucose also plays a crucial role in spermatogenesis and the acrosomal response. The GLUT8 class 3 transporter, expressed mainly in the testis, facilitates the transport of glucose required for energy production in spermatogenesis and sperm motility (**Gawlik et al., 2008; Gómez et al., 2006**). Decreased GLUT8 activity, due to decreased insulin, led to reduced sperm motility and compromised fertility, possibly due to a reduced gonadotropic response to gonadotropin-releasing hormone in diabetics (**Omolaoye and Du Plessis, 2018; Sengupta et al., 2022**).

Oxidative stress significantly affects sperm function through direct mechanisms. Researchers highlight that oxidative stress disrupts the cell membrane's fluidity by attacking the polyunsaturated fatty acids in the lipid bilayer, leading to lipid peroxidation. This degradation compromises the membrane's integrity, affecting sperm functions such as material transport, energy flow, and immune defense, potentially causing male infertility (**Gualtieri et al., 2021; Mannucci et al., 2022**). Moreover, oxidative stress damages DNA within the sperm nucleus and mitochondria, a major contributor to decreased DNA integrity, which can prompt sperm apoptosis, decrease sperm count, and deteriorate semen quality. Studies also show that infertile males have higher DNA oxidation and apoptosis levels in sperm than fertile males, suggesting that excessive reactive oxygen species (ROS) exacerbate sperm damage and accelerate germ cell apoptosis (**Chianese and Pierantoni, 2021; Hosseinipour et al., 2022**).

II.2.3. Post-testicular effects of diabetes mellitus (Sexual behavior and fertility outcome):

Historically, the link between diabetes and sexual dysfunction (SD) has been recognized since the 10th century, when Avicenna, the Father of Modern Medicine, reported “collapse of sexual function” as a specific complication of diabetes (**Shi et al., 2017**). The underlying mechanisms probably include vascular insufficiency and neuropathic damage, which contribute to complications such as erectile dysfunction, ejaculation disorders, and reduced libido (**Shi et al., 2017**).

II.2.3.1. Libido:

Diabetes significantly affects libido, notably through various neurophysiological and neuropathic mechanisms. In men, a crucial aspect of this influence is the reduction in libido

caused primarily by defects in the HPG axis, resulting in hypogonadotropic hypogonadism (**Andlib et al., 2023**). This condition is frequently associated with a decline in testosterone levels, exacerbating other sexual disorders such as erectile dysfunction and premature ejaculation (**Andlib et al., 2023**).

From a neurophysiological perspective, research indicates that testosterone plays an essential role in the regulation of dopamine, a key neurotransmitter for sexual motivation (**Melis et al., 2022**). Dopamine released in different brain regions, such as the nigrostriatal area, the mesolimbic area, and the MPOA, promotes the motor and motivational components of copulation (**Melis et al., 2022**). In particular, testosterone facilitates increased dopamine release in the MPOA in response to sexual stimuli and increases nitric oxide synthase (NOS) activity in the same area, thereby enhancing dopamine release associated with sexual activity (**Dick et al., 2020**). Diabetes, by inducing peripheral and central neuropathies, causes morphological and neurochemical alterations in the brain, affecting neurons that play a direct role in reproductive behavior (**Mohamadi et al., 2015**). Lesions in key regions such as the MPOA, Me, and BNST can diminish or suppress copulatory behaviors and impair the perception of odors of the opposite sex, which is crucial for normal sexual interaction in rodents. These areas also contain glucose-sensitive neurons that respond to changes in blood glucose levels, suggesting that diabetes can directly impact these neural circuits and thus disrupt reproductive functions (**Mohamadi et al., 2015**).

II.2.3.2. Erectile dysfunction (ED):

Research has consistently shown that diabetes mellitus can adversely affect erectile function, resulting in reduced sexual desire and performance. This condition results from the impact of diabetes on key physiological processes (**Nna et al., 2017**). Diabetic conditions are known to disrupt the production and function of cyclic guanosine monophosphate (cGMP) in the penis, which plays a crucial role in erectile physiology by regulating blood flow and muscle relaxation (**Al-Roujeaie et al., 2017**). This disruption contributes to longer durations for mounts, intromissions, and ejaculations, as well as a reduction in the frequency of these sexual activities. These effects are often associated with a reduction in testosterone production by Leydig cells, essential for maintaining sexual behavior (**Al-Roujeaie et al., 2017**). In addition, normal erectile function relies on the release of neurotransmitters, notably nitric oxide, from the penile nerve endings or endothelium to relax the cavernous arteries and smooth muscles, thereby improving penile blood flow. However, diabetes can impair endothelium-dependent smooth muscle and autonomic mechanisms essential to this relaxation process, leading to

erectile dysfunction (ED) (**Omolaoye and Du Plessis, 2018**). This alteration is often due to endothelial dysfunction, characterized by reduced NO synthesis and activity, which are crucial for initiating and maintaining an erection (**R. Huang et al., 2024**). In addition, hyperglycemia-induced oxidative stress can exacerbate endothelial dysfunction, leading to decreased NO production through the inhibition of Endothelial nitric oxide synthase (eNOS) activity and thus, increasing the risk of thrombosis and vasoconstriction, all contributing to the pathophysiology of ED. Poor glycemic control can also lower serum testosterone levels, further aggravating ED (**R. Huang et al., 2024**). Furthermore, advanced Glycation End-products (AGEs) bind covalently with vascular collagen, contributing to vascular thickening, loss of elasticity, endothelial dysfunction, and atherosclerosis. High concentrations of AGE products have been found in the corpus cavernosum of diabetic patients, suggesting a specific tissue impact of AGEs (**Barkabi-Zanjani et al., 2020; Omolaoye and Du Plessis, 2018**). Moreover, AGEs are linked to ED as they may increase oxidative cell damage and diminish NO levels, leading to reduced cGMP and impaired relaxation of cavernosal smooth muscle (**Barkabi-Zanjani et al., 2020**). To achieve a normal erection, an integral neurological system is required. In the case of diabetes, oxidative stress and hyperglycemia damage direct nerve fibers and lead to sensory and motor changes (**Gaunay et al., 2013**). Autonomic nervous system dysfunction is primarily affected by decreased parasympathetic input, leading to erectile dysfunction in diabetic men (**Andlib et al., 2023**).

II.2.3.3. Ejaculatory dysfunction:

Ejaculation disorders are the main cause of infertility in diabetic men. It includes various ejaculation-related dysfunctions, such as delayed ejaculation, premature ejaculation, anejaculation, and retrograde ejaculation (RE) (**Andlib et al., 2023**). Fertilization requires normal ejaculation. Anejaculation and retrograde ejaculation are associated with diabetes and are more complicated for fertility than other ejaculatory diseases (**Gaunay et al., 2013**). Retrograde ejaculation is characterized by the reflux of semen into the bladder rather than its normal ejection through the urethra. This phenomenon is often caused by a failure of nerve reflexes due to autonomic neuropathy, a frequent consequence of diabetes (**Shen et al., 2017**). In parallel, premature ejaculation is also recurrent in diabetic patients. It is defined by an onset before vaginal penetration, often linked to damage to the nerves involved in ejaculation mechanisms. Damage to nerve pathways, potentially exacerbated by diabetes, is considered a major contributor to this type of dysfunction (**Omolaoye and Du Plessis, 2018**).

Hyperglycemia plays a key role in the pathogenesis of various diabetic complications, including male fertility disorders. It affects different organs through three main mechanisms: the flux of the polyol pathway, the formation of AGEs and the activation of protein kinase C (PKC) isoforms (**Barkabi-Zanjani et al., 2020**). These mechanisms lead to overproduction of superoxide, which inhibits the enzyme glyceraldehyde phosphate dehydrogenase (GAPDH), thus contributing to organ damage (**Réus et al., 2019**). Studies indicate that increased AGEs in diabetic conditions are associated with deterioration in testicular function and sperm quality (**Omolaoye and Du Plessis, 2018**). Finally, diabetic autonomic dysfunction also affects the peristalsis of reproductive ducts, such as the lining of the vassal and seminal vesicle, preventing ejaculation from reaching the urethra properly (**Andlib et al., 2023; Gaunay et al., 2013**).

Diabetes is thus emerging as a significant disruptor of male reproductive health, with profound implications for fertility. However, this awareness of the challenges poses a crucial question: How can we alleviate or reverse these deleterious effects of diabetes on reproductive function? Do medicinal plants, used for millennia for their therapeutic properties, offer a viable and effective solution?

Chapter III

Phytotherapy and treatment of male infertility caused by diabetes.

Phytotherapy and the use of natural products have recently gained in popularity, often due to the advantages they offer over traditional pharmaceuticals. This trend can be attributed to the diversity of phytochemical compounds they contain, which provide various therapeutic activities to treat specific disorders. Many natural remedies have been used effectively in the management of diabetes mellitus and its complications (Nna et al., 2017).

This chapter addresses the question posed at the end of the previous chapter, exploring the potential of medicinal plants as a therapeutic strategy against the effects of diabetes on male reproduction. By analyzing the available scientific data, we will seek to understand whether and how phytotherapeutics interventions can contribute to restoring reproductive function compromised by diabetes.

III.1. Treatment of male infertility caused by diabetes:

Phytotherapy and natural products have been in high demand for a while now, likely due to their perceived effectiveness compared to contemporary medications. This phenomenon can be attributed to the plentiful presence of diverse phytochemical substances, which possess several targets for intervention in the therapy of the specific ailment. A range of natural products has been utilized for the treatment and control of diabetes and its associated problems (Nna et al., 2017).

After diagnosing fertility issues as a result of diabetic complications, treatment should focus on addressing the disease itself by ameliorating the underlying cause and then addressing the associated complications (Omolaoye and Du Plessis, 2018).

III.1.1. Natural components and diabetes mellitus blood glucose control:

A higher proportion of the natural products studied effectively lowers fasting blood glucose levels in diabetic rats compared to the untreated diabetic rats. The finding is in addition to the notable positive impacts on male reproductive function (Nna et al., 2017). Multiple studies have demonstrated that natural substances promote the regeneration of pancreatic β -cells and enhance the release and survivability of insulin (Giribabu et al., 2014). In addition, natural products, which typically contain flavonoids and phenolic components, have antioxidant properties that can help lower the oxidative stress associated with DM (Giribabu et al., 2014; Ogunyinka et al., 2017).

III.1.2. Natural compounds and DM-induced decline of male reproductive function:

A wide range of herbal medicines is used with considerable success to control fertilization in laboratory animals. Recently, research has highlighted the potential benefits of these plants for reproductive health, without significant secondary effects. Various phytoconstituents such as alkaloids, flavonoids, tannins, xanthenes, triterpenes, and quinones have been studied to regulate reproductive activity. The main reason plants have been used in the management of diabetes is their anti-hyperglycemic action or their ability to scavenge oxygen radicals via various mechanisms (Shi et al., 2017).

III.1.3. Medicinal plants:

In pharmacogenomics research, the regulation of disease networks is critically important for the development of therapeutics for various conditions (Andlib et al., 2023). A range of

phytochemicals extracted from plants has been evaluated for their effectiveness against several disorders, including infertility (**Andlib et al., 2023**). These phytochemicals influence male reproductive dysfunction at three distinct levels. At the pre-testicular level, some enhance testosterone levels, which aids in the production of healthy and viable sperm. At the testicular level, a reduction in LDH activity has been noted. Moreover, increases in the expression of StAR and 17 β -HSD proteins have been recorded. Enhanced expression of the StAR protein boosts the transport of cholesterol to the Leydig cell mitochondria, aiding in testosterone production (**Nah et al., 2012**). Furthermore, certain natural compounds have been found to improve sexual performance and address erectile dysfunction (**Andlib et al., 2023**).

Sage, in particular, has been highlighted for its significant phytochemical content that contributes to these effects. Its active components are known to modulate hormonal balance and improve reproductive health, making it a focus of ongoing research in natural treatments for reproductive issues.

III.2. Overview of the plant:

Sage (*Salvia*) is the largest genus of herbaceous perennials in the Lamiaceae family and occupies a place of choice, recognized since antiquity for its curative virtues. Its Latin name, *Salvia*, derived from "salvage", refers to its historic role as a "savior", underlining its reputation as a quasi-magical plant with salvific properties (**Achir et al., 2021**). *Salvia* species are a mixture of annuals, biennials, or perennials, as well as semi-woody subshrubs. Their flowers emerge in tight or loose groups, often creating an attractive scene. The shades of these flowers vary considerably, presenting mainly shades of blue to red, while white and yellow are relatively rare (**Askari et al., 2021**).

Originally from the Mediterranean basin, eastern and southwestern Asia, and belonging to the Lamiaceae family (**Uysal et al., 2023**), sage has around 1000 species worldwide (**Jash et al., 2016**), with particular diversity in Algeria, where some 30 species have been identified (**Achir et al., 2021**). The region of Mostaganem in Algeria boasts a wealth of endemic and rare *Salvia* species, including *Salvia balansae* (**Mostari et al., 2020**).

III.2.1. Geographical distribution of *Salvia balansae*:

Salvia balansae De Noé ex Coss, a sage whose common name is "hchichet koul blia: حشيشة كل بلية", a plant that cures all ills and is used in traditional medicine. Its range is limited to the Cheliff Valley, in the two communes of Sidi Belattar and Ain Boudinar, in the coastal Oranie

region and therefore *Salvia balansae* in the strict sense is endemic to Mostaganem (**Mostari et al., 2016**). Two flowering periods, the first from February to April and the second in November characterize it.

Table 01: Location of *Salvia balansae* (**Bijmoer et al., 2023**):

Continent	Africa
Country	Algeria
Decimal latitude	35.933333
Decimal longitude	0.083333
Geodetic datum	WGS84
Locality	Algérie. Mostaganem
State province	Mostaganem
Habitat	Calcareous fields

Specimens are deposited in the herbarium of the University of Montpellier (MPU010315, MPU01016) and that of the Natural History Museum, Vienna - Herbarium W (1889-0099212).

III.2.2. Taxonomy of *Salvia balansae*:

- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Class:** Magnoliopsida
- **Order:** Lamiales
- **Family:** Lamiaceae
- **Genus:** *Salvia*
- **Species:** *balansae*
- **Generic name:** *Salvia*
- **Scientific name:** *Salvia balansae* Noë
- **Common name in Arabic:** hchichet koul blia (حشيشة كل بلية)

III.2.3. Botanical description of *Salvia balansae*:

Suffruticose herb up to 70 cm. **Stems** numerous, little branched, arachnoid-eglandular below, above with short glandular and eglandular hairs. **Leaves** simple, narrow linear or oblong-linear, up to 7.5 × 0.7 cm, revolute, crenulate, bullate ± white lanate below with oil globules, above with scattered eglandular hairs; lowermost leaves narrowed into a short petiole,

Part I: Review of the literature

Chapter III: Phytotherapy and treatment of male infertility caused by diabetes

upper sessile. **Verticils** up to 8, 6-flowered, lowermost up to 4.5 cm apart, approximating above. **Floral leaves** broad-ovate, acuminate, up to 11×7 mm, reflexed in fruit; bracts absent. **Pedicels** erect-spreading up to 4.5 mm. **Calyx** campanulate, up to 13 mm, slightly enlarging in fruit and with a concave upper lip, 13-veined, with an indumentum of glandular and eglandular hairs; upper lip with short incurving teeth, outer 0.5 mm and median 0.3 mm; lower lip with two 2.3 mm lanceolate, acuminate teeth. **Corolla** white or bluish, up to 24 mm; upper lip clearly falcate, longer than lower lip with a large suborbicular median lobe; tube glabrous within, widening near throat, invaginated near base. **Staminal connectives** 16 mm; filaments 3 mm; lower thecae dolabriform, sterile, adhering. **Nutlets** round-trigonus, russet-brown, 3×2 mm, mucilaginous on wetting (**Hedge, 1974**) (**Figure 09**).



Figure 09: a) *Salvia balansae* De Noë (**Battandier and Trabut, 1886**), b) *Salvia balansae* De Noë collected in Algeria by Naturalis Biodiversity Center (**Bijmoer et al., 2023**).

III.2.4. Morphological characteristics of *Salvia balansae*:

Salvia balansae. – De Noë, plant from the base, shrubby, with many stems, square stems, very bristle stems, especially at the tips of very flowering branches. Linear leaves, rolled at the edges, smooth on top, very woolly below, the upper ones almost stemless, sessile. Bracts ovate-

acuminate, then reflexed, shorter than the calyx. Verticillasters have distinct, sub-floral, pedicels longer than the bracts. Calyx with the lower lip divided into two capitate padded parts. Viscous, bell-shaped, up to the middle bilabiate with the upper lip bifold at the tip, rounded, tridentate with tiny denticles. Corolla glandular, large, candid, much longer than the calyx, with an inflated throat; upper lip falcon-shaped, pressed, indented; lower lip three-lobed, the side lobes oblong, lying on the bottom, middle lobe from the base abruptly dilated, somewhat doubled, edged, dentate, suborbicular (**Battandier and Trabut, 1886**) (**Figure 10**).



Figure 10: *Salvia balansae* de Noé: a, habit $\times 3\frac{1}{2}$; b, corolla $\times 1$; c, lower lip of corolla $\times 1\frac{1}{3}$; d, stamen $\times 1\frac{1}{3}$; e, fruiting calyx $\times 1\frac{1}{2}$; f, nutlet $\times 7$. (Davis 51768) (**Hedge, 1974**).

III.3. Main Biologically Active Compounds of *Salvia balansae*:

The chemical composition of *Salvia balansae*, as detailed in the study of **Amirat et al. (2023)**, includes a variety of phenolic compounds and fatty acids.

The methanolic extracts of *Salvia balansae*'s aerial parts were analyzed using High Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) and High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS), revealing the presence of Flavonoids, hydroxycinnamic acids, and phenolic acid derivatives, including luteolin, ferulic acid, vanillic acid, and kaempferol. The main phenolic compounds identified were: Catechin (72.5% of total phenolics) and Myricetin (21.7% of total phenolics). Other compounds like epicatechin and butylated hydroxyanisole were present in lower amounts (**Amirat et al., 2023**).

For the fatty Acid Composition, the petroleum ether extract of *Salvia balansae* was characterized by Gas Chromatography-Mass Spectrometry (GC-MS), identifying: Saturated and unsaturated fatty acids: Predominantly palmitic acid (C16:0), linoleic acid (C18:2), and oleic acid (C18:1) and other notable fatty acids as Myristic acid (C14:0), Stearic acid (C18:0) and Eicosanoic acid (C20:0) (**Amirat et al., 2023**).

Mahdjoub et al. (2023) revealed in their study that the chemical composition of *Salvia balansae*, as analyzed using HPLC-DAD, comprises a variety of phenolic compounds extracted via ultrasound-assisted methodology. Significant constituents identified include ascorbic acid, gallic acid, tannic acid, catechin hydrate, caffeine, vanillin, coumaric acids, benzoic acid, myrecetin, quercetin, and hydroxyflavon. Tannic acid and benzoic acid were found in the highest concentrations.

III.4. Biological activities of *Salvia balansae*:

III.4.1. Antioxidant Assays:

Several assays were employed to measure the antioxidant properties of *Salvia balansae* leaves extract, including ABTS and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, iron (Fe^{2+}) chelating ability, and the ferric reducing antioxidant power (FRAP) test. These methods are used to assess how effectively the plant extract can neutralize free radicals and chelate metal ions, which are crucial functions in preventing oxidative stress linked to various chronic diseases (**Amirat et al., 2023**). The results indicated that the extract possesses considerable antioxidant activity, with the ABTS and DPPH assays showing significant radical scavenging capacities that increased with the concentration of the extract (**Amirat et al., 2023; Mekki et al., 2023**). Similarly, the iron chelating and reducing power assays demonstrated that *Salvia balansae* could effectively reduce iron and exhibit a strong reducing power, further underscoring its potential as a natural antioxidant source for therapeutic and pharmacological uses (**Mahdjoub et al., 2023**).

III.4.2. Antimicrobial Activity:

The antimicrobial activity of *Salvia balansae* was evaluated using methanolic extracts against a variety of bacterial and fungal strains. The results demonstrated that the methanolic extract exhibited substantial antimicrobial properties. Notably, the extract showed significant inhibition against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, with inhibition zones measuring 14 mm and 12 mm, respectively. The extract also displayed moderate activity against Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*, with inhibition zones of 10 mm and 8 mm, respectively (Amirat et al., 2023). Furthermore, the methanolic extract exhibited antifungal activity against *Candida albicans*, with an inhibition zone of 6 mm (Amirat et al., 2023).

III.4.3. Antidiabetic Activity:

In Vivo efficacy: In studies with Wistar rats, the aqueous extract of *Salvia balansae* ameliorated symptoms of diabetes induced by a high-fat diet. This included reductions in blood glucose levels and improvements in insulin sensitivity (Mekki et al., 2023).

The antidiabetic effects of the plant are further corroborated by its capacity to hinder crucial enzymes responsible for carbohydrate breakdown, like α -amylase and α -glucosidase, consequently delaying the conversion of carbohydrates into glucose. The aqueous extract of *Salvia balansae* demonstrates modest anti-diabetic activity (103.64 ± 2.04 μ gAE/mL), albeit weaker than the anti-amylase standard, Acarbose (91.76 ± 0.07 μ g/mL) (Mekki et al., 2023).

III.4.4. Effects on Reproductive System:

Salvia balansae extracts have been shown to protect against testicular damage and dysfunction caused by oxidative stress in rats fed a high-fat diet. This includes improvements in the structural integrity of testicular tissue, increased testosterone levels, and enhanced sperm production (Mekki et al., 2023). The protective effects on the reproductive system are largely attributed to the antioxidant properties of the plant's extracts. By mitigating oxidative stress, the extracts help preserve the function of reproductive organs and improve overall reproductive health (Davoodi et al., 2020).

III.5. Toxicity and Safety of Salvia:

While *Salvia* is widely recognized for its therapeutic benefits, it is crucial to understand that like many medicinal plants, it can pose risks if not used properly. The safety of *Salvia* depends

on several factors, including the dosage, the specific species of *Salvia*, the method of administration, and the duration of use. Studies indicate that *Salvia* contains active chemical compounds such as phenolics and diterpenes, which may have toxic effects on healthy cell lines at high doses (**Jažo et al., 2023**).

Salvia or sage demonstrates varying levels of toxicity depending on the type of exposure and dosage. Acute dermal toxicity studies revealed that the "median lethal dose" (LD₅₀) for sage leaf extract is greater than 2000 mg/kg in mice and for sage oil, it is greater than 5000 mg/kg in rabbits, indicating low dermal toxicity. Acute oral toxicity studies showed a very high LD₅₀ of 44,760 mg/kg for hydroalcoholic sage extract in mice, but a significantly lower LD₅₀ of 2600 mg/kg for sage flower/leaf/stem water (the aqueous solution of the steam distillate obtained from the flowers, leaves, and stems) in rats, suggesting higher oral toxicity for the latter. In short-term oral toxicity studies, a no observed effect level (NOAEL) of 250 mg/kg body weight per day was determined for sage oil in rats, with higher doses causing convulsions and mortality. Genotoxicity studies indicated that sage extracts and oils are not genotoxic at typical exposure levels. Dermal and ocular irritation studies found that undiluted and 10% sage leaf extracts were non-irritating to rabbit skin, while sage oil was moderately irritating. Sensitization tests revealed no sensitization at an 8% concentration of sage oil in petrolatum, and sage flower/leaf/stem water was not an ocular irritant (**Cherian et al., 2021**).

Part II: Experimental Study

Chapter IV

Materiel and Methods

IV.1 Ethical Consideration:

All the experimental protocols were conducted with reference to the Guide for the Care and Use of Laboratory Animals. All animals received humane care according to this guide, of the Institute for Laboratory Animal Research, Eighth edition (National Research Council, 2011).

IV.2 Objectives:

This study assesses, for the first time, the protective effects of this specific sage species on restoring impaired sexual behavior, enhancing sex hormone levels, particularly testosterone, improving testis histopathological lesions, spermatogenesis, and sperm parameters in STZ-induced diabetic male rats.

IV.3 Place of Study:

The *in vitro* study, notably the preparation of extract, was conducted initially at the level of the Laboratory of Sciences and Techniques of Animal Production (LSTPA) and Laboratory of Plant Protection, University Abdelhamid Ibn Badis, Mostaganem, Algeria, and then all biological activities were performed at the Biotechnology Research Center (CRBT), laboratories 05 and 06, Constantine, Algeria. Phytochemical identification of the extract was performed at Dicle University, Science and Technology Application and Research Center (DUBTAM), Diyarbakir, TURKIYE.

The *in vivo* study was conducted at the level of the Laboratory of Biototoxicology, Pharmacognosy and Biological Valorization of Plants (LBPVBP), University Dr. Moulay Tahar, Saida, Algeria. Rats were housed and sacrificed and Sperm parameters were analyzed at the LBPVBP laboratory. Hormonal assays were performed in a private laboratory of Dr Bouziane Rachid in Mostaganem, Algeria. The histological study was carried out in the laboratory of the veterinary institute at the Ibn Khaldoun University in Tiaret, the Pathological Anatomy laboratory of the Ahmed Medaghri Hospital, Wilaya of Saida, and the pathological anatomy and cytology laboratory of Dr BENSADA, Ain Defla.

IV.4. Plant material:

IV.4.1. Plant authentication and collection:

Salvia balansae De Noé ex Coss, a sage whose common name is "hchichet koul blia: حشيشة كل بلية", a plant that cures all ills and is used in traditional medicine. Its range is limited to the Cheliff valley, in the two communes of Sidi Belattar and Ain Boudinar, in the Oranie littoral region, with a first flowering in November and a second from February to April. *Salvia balansae* in the strict sense is endemic to Mostaganem, forming a facies on the left river bank only, according to the vegetation map of Algeria established by **S. Santa and P. Daumas in 1958** on a scale of 1/250000 (**Mostari et al., 2016**). The research was conducted using the flowers of *Salvia balansae* De Noé ex Coss (sage) harvested in spring, April 2023, at its peak flowering stage (**Figure 11**), in Sidi Belattar, located at coordinates 36.056300° N, 0.256869° E (WGS84) in the Cheliff Valley in the wilaya of Mostaganem. The botanical identification was performed by Dr. MOSTARI A, from the Department of Biology – Mostaganem University, and a reference specimen is preserved in their herbarium.

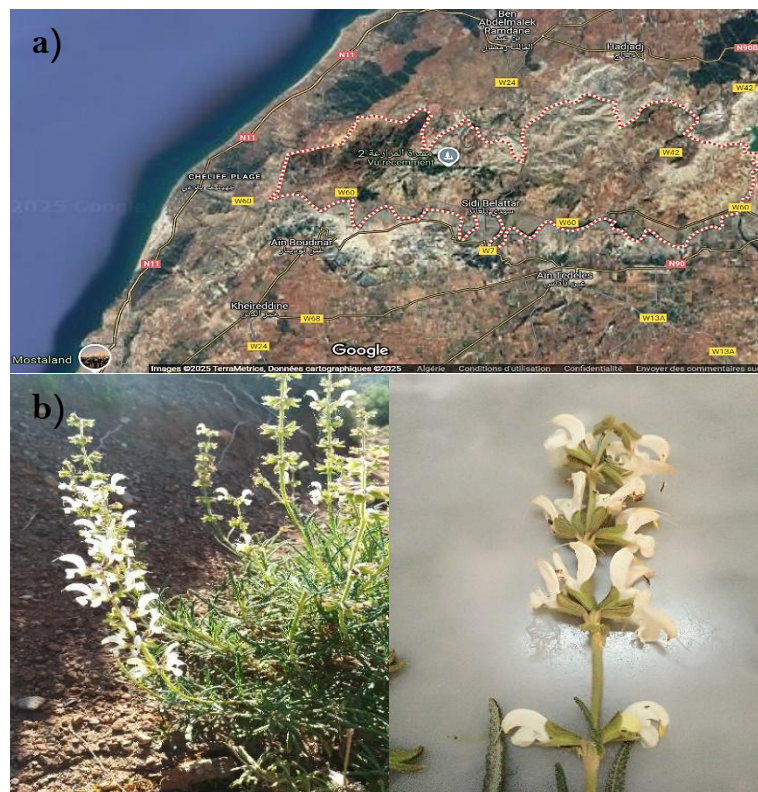


Figure 11: a) Geographical location of the *Salvia balansae* sampling area (Google Earth), **b)** Aerial parts of *S. balansae*, Mostaganem, 15 April 2023, *Photo credit: SOUIDI Sendous Wadjila*

Part II: Experimental Study
Chapter IV: Materiel and methods

Fresh stems with leaves and flowers were collected at random. The flowers were carefully separated, cleaned, and then dried in the shade at room temperature (25-30°C) for 20 days (**Figure 12**). After the weight of the samples stabilized, they were ground using a domestic electric grinder, employing 2-minute grinding cycles followed by 1-minute pauses until a fine powder was achieved. This powder was then stored in a hermetically sealed glass jar to maintain its quality and prevent oxidation and degradation of the secondary metabolites.



Figure 12: Dried and ground *Salvia balansae* flowers.

IV.4.2. Preparation of extracts:

The extraction was performed as described by **Kowalczyk et al. (2020)**, with some modifications (**Figure 13**). Briefly, 100 g of the flowers' powder was added to 1 l distilled water, and heated for 1 hour in a 90°C water bath, under continuous stirring, to obtain a 10% decoction. The mixture was then cooled, vacuum filtered using a Wattman filter No. 1, and lyophilized using a freeze-dryer Alpha 1–2 LD plus, for later use. To determine the extraction yield of the Flower Aqueous Extract (FAE), we have used the following formula:

Yield (%) = $m/M \times 100$. With: m: the dry extract mass and M: the dry plant powder mass.

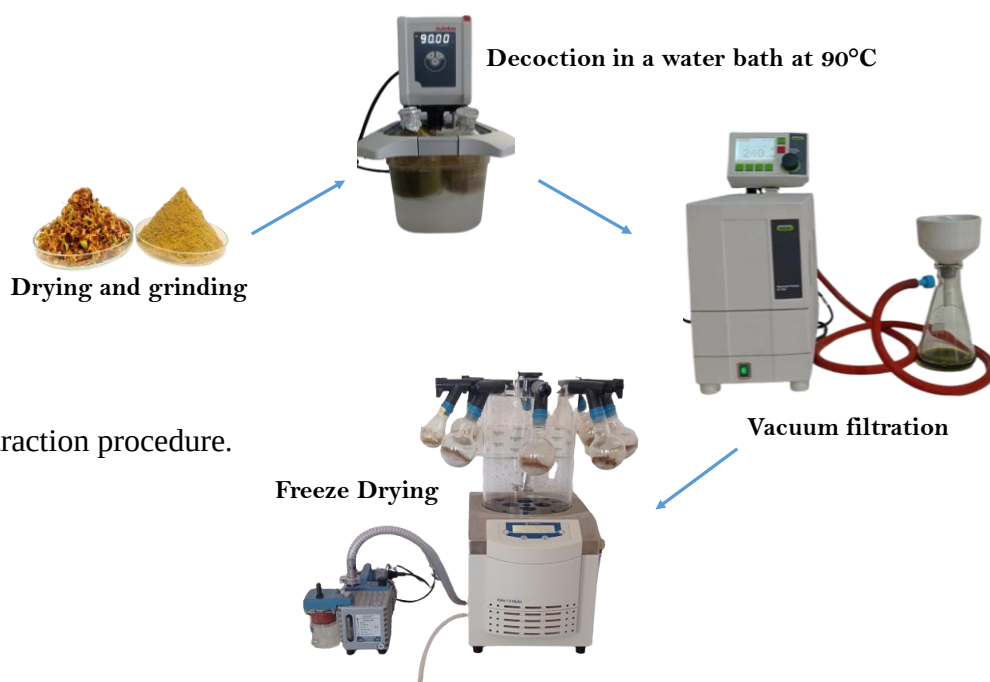


Figure 13: Extraction procedure.

IV.4.3. Determination of polyphenol and flavonoid contents of the extract:

IV.4.3.1. Total phenolic content (TPC):

Using the Folin-Ciocalteu reagent, the total phenolic content of FAE was determined. The microplate assay method described by **Müller et al. (2010)** was followed. The procedure involved mixing 20 µl of the extract solution (1 mg/ml) with 100 µl of Folin-Ciocalteu reagent and 80 µl of sodium carbonate (7.5%) sequentially. The solution was kept at room temperature in the absence of light for 2 hours. Using a Perkin Elmer Enspire 96-well microplate reader, the absorbance was measured at 765 nm. Following the calibration equation below, the results were reported as µg of Gallic acid equivalents per milligram of extract (µg GAE/mg). $R^2 = 0.984$ and $y = 0.0032x + 0.2363$

IV.4.3.2. Total flavonoid content (TFC):

Flavonoid determination in the extract is based on forming a complex between flavonoids and Al^{+3} . The procedure demonstrated by **Bouzana et al. (2023)** was used, with a slight modification for determination on 96-well microplates. Briefly, 50 µl of plant extract was mixed with 130 µl of distilled water and 10 µl of potassium acetate (CH_3COOK), then 10 µl of aluminum nitrate ($Al(NO_3)_3 \cdot 9H_2O$). The mixture was left to incubate for 40 min, and the absorbance was measured at 415 nm using a 96-well microplate reader (Perkin Elmer, Enspire). Results were expressed as µg of quercetin equivalents per mg of extract (µg QE/mg) using the calibration equation: $y=0.0048X$, with $R^2=0.997$.



Figure 14: 96-well microplate reader (Perkin Elmer, Enspire), CRBT laboratory [Original].

IV.4.4 Ultra-High Performance Liquid Chromatography (UHPLC)-Electrospray Ionization (ESI)-Tandem Mass Spectrometry (MS/MS):

A Shimadzu-Nexera model ultrahigh performance liquid chromatograph (UHPLC) coupled with a tandem mass spectrometer was used to accomplish quantitative evaluation of our extracts and 53 phytochemicals. The reversed-phase UHPLC was equipped with an auto sampler (SIL-

Part II: Experimental Study
Chapter IV: Materiel and methods

30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU- 20A3R model). The chromatographic conditions were optimized in order to achieve optimum separation for 53 phytochemicals and overcome the suppression effects. The chromatographic separation was performed on a reversed phase Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm) analytical column. The column temperature was set to 40°C. The elution gradient was composed of eluent A (water+5 mM ammonium formate+0.1% formic acid) and eluent B (methanol+5 mM ammonium formate+0.1% formic acid). The following gradient elution profile was used: 20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min) **(M. A. Yilmaz, 2020)**. Furthermore, the solvent flow rate and injection volume were settled as 0.5 mL/min and 5 µL, respectively.

The mass spectrometric detection was carried out using a Shimadzu LCMS-8040 model tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes. LC-ESI-MS/MS data were acquired and processed by LabSolutions software (Shimadzu). The MRM (multiple reaction monitoring) mode was used for the quantification of the phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on screening specified precursor phytochemical-to-fragment ion transitions. The collision energies (CE) were optimized to generate optimal phytochemical fragmentation and maximal transmission of the desired product ions. The MS operating conditions were applied as: drying gas (N₂) flow, 15 L/min; nebulizing gas (N₂) flow, 3 L/min; DL temperature, 250°C; heat block temperature, 400°C, and interface temperature, 350°C **(M. A. Yilmaz, 2020)**. See the Supporting Information table for the analytical method validation parameters that belong to the LC–MS/MS method in the annexes section.



Figure 15: Shimadzu-Nexera model Ultra-High Performance Liquid Chromatography (UHPLC)-Electrospray Ionization (ESI)-Tandem Mass Spectrometry (MS/MS), DUBTAM laboratory **[Original]**.

IV.4.5. In vitro biological activity:

IV.4.5.1. In vitro antioxidant activity:

IV.4.5.1.1. DPPH free radical-scavenging assay:

The assessment of the scavenging activity of free radicals was conducted according to the method described by **Bouzana et al. (2023)**. A 40 µl of FAE (produced at various doses) was combined with 160 µl of DPPH solution. The solution was kept at ambient temperature in the absence of light for 30 minutes. Absorbance was measured using a 96-well microplate reader at 517 nm. The findings were compared to the conventional antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The comparison was based on their 50% inhibitory concentration (IC₅₀), which was represented in micrograms per milliliter (µg/mL)

IV.4.5.1.2. ABTS scavenging assay:

The ABTS scavenging activity was assessed according to the methodology described in detail elsewhere by **Bouzana et al. (2023)**. A 40 µl volume of FAE (with different concentrations) was combined with 160 µl of ABTS solution. The solution was kept at an ambient temperature in the absence of light for 10 minutes. Absorbance was measured using a 96-well microplate reader at 734 nm. The results were compared with common antioxidants, BHA and BHT, and expressed as an IC₅₀ value, which represents the amount in micrograms per milliliter that inhibits 50% of activity.

IV.4.5.1.3. Ferric reducing antioxidant power assay (FRAP):

The reducing power activity was assessed based on the procedure described by **Bursal et al. (2021)**, requiring a few microplate reader adjustments. After mixing 10 µl of FAE (prepared in various concentrations) with 50 µl of potassium ferricyanide K₃Fe (CN)₆ (1%) and 40 µl of phosphate buffer (0.2 M, pH 6.6), the plate was allowed to incubate at 50°C for 20 minutes. Subsequently, 40 µl of distilled water, 50 µl of trichloroacetic acid TCA (10%), and 10 µl of ferric chloride FeCl₃ were added. Using a 96-well microplate reader, the absorbance of the mixture was measured at 700 nm. The results were compared to antioxidant standards (BHA and BHT) and expressed as absorbance A_{0.5} µg/mL, representing the concentration resulting in 0.5 absorbance.

IV.4.6. In vivo biological activity:

IV.4.6.1. In vivo anti-inflammatory activity: Carrageenan-induced plantar edema test

The anti-inflammatory activity of the extract was assessed using the method described by **Winter et al. (1962)**. Rats of both sexes were fasted for 16 hours before experimentation, with free access to water. They were divided into 3 groups of 6 animals each. Group I (control) received 10 ml/kg of 1% Tween 80. Group II (standard) received 10 mg/kg diclofenac (a reference anti-inflammatory), while group III (experimental) received 200 mg/kg FAE. Thirty minutes after oral treatments, edema was induced by injection of 50 μ l of carrageenan (1% w/v) into the plantar arch of the right hind paw (**Abdelli, 2017**).

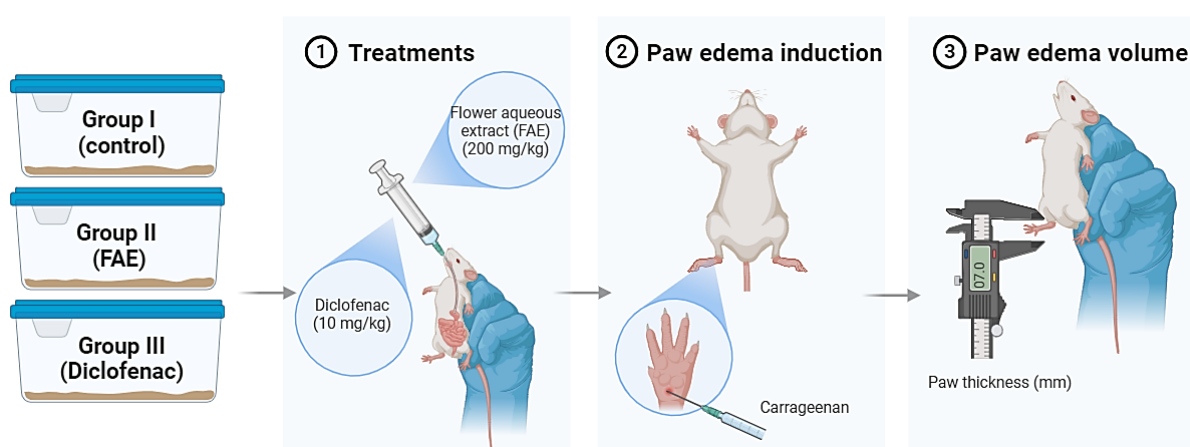


Figure 16: Flowchart of (1) Oral administration of treatments; (2) Subplantar injection of carrageenan and (3) Measurement of leg thickness using a digital caliper [**Original**].

Paw thickness (mm) was measured with a caliper before injection (V_0) and every 60 min for 06 h after induction of inflammation (V_t).

- **The percentage of edema increase** was calculated according to the following formula:

$$\% \text{ edema increase} = (V_t - V_0) / V_0 \times 100$$

- **The percentage of edema inhibition** was calculated according to the following formula:

$$\% \text{ edema inhibition} = [(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}] / (V_t - V_0)_{\text{control}} \times 100$$

We consider:

V_0 : Volume of the paw (before injection of carrageenan); V_t : paw volume at a given time interval after carrageenan injection; Treated: groups treated with diclofenac and FAE.

IV.4.6.2 Acute Oral Toxicity of FAE: Up-and-Down Procedure

The acute toxicity testing of FAE was conducted following the guidelines enumerated by the Organization for Economic Co-operation and Development (**OECD, 2022**), utilizing an up-and-down procedure. A total of 24 rats were divided into 04 batches of 06 rats each were fasted for an overnight period, during which only water was provided, and then received oral doses of FAE at varying concentrations (0.2, 0.6, 1, and 2 g/kg body weight). Subsequently, the rats had limitless access to water and food. They were monitored for toxicological and mortality indicators for an initial period of 0.5 h, followed by periodic assessments for the subsequent 24 h, and subsequently each day for a total of 14 days.

IV.5. Animal:

IV.5.1. Experimental animals:

Twenty-eight sexually active, two-month old male Wistar rats, weighing 220 ± 5.00 g, were procured from the Department of Biology of the Faculty of Natural and Life Sciences, University of Saïda. An equal number of female rats was also obtained. The rats were housed in polypropylene cages in an animal house with adequate ventilation, under conventional laboratory conditions. These conditions encompassed a 12-h light/dark cycle at $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

IV.5.2. Male rats' preselection and female rats' receptivity:

The research focused on the sexual behavior of experienced male subjects, who were first trained to mate with receptive females. This training was conducted before the induction of diabetes, following the methodology outlined by **Olvera-Roldán et al. (2023)**, and only sexually active rats were selected for the experiment.

To make female rodents artificially enter estrus, a 10 µg/rate estradiol benzoate injection was used to increase their receptivity. This was followed by a dose of 2 mg/rate progesterone four to six hours before the experiment to make mating easier (**Kashif et al., 2019**). Female rats in the estrous phase demonstrate their willingness to engage in coitus by assuming paracopulatory behavior including ear wagging, darting, hopping, and the lordosis posture, which allows for intromission (**Boublata et al., 2021**).

IV.5.3. Rat model for type I diabetes and batch allocation:

Rats were rendered diabetic (excluding rats in the control group and the FAE 200 mg/kg group) with one single intraperitoneal injection of STZ (40 mg/kg) in freshly prepared cold

Part II: Experimental Study
Chapter IV: Materiel and methods

sodium citrate buffer (0.01M) (pH = 4.5) following an overnight fast. Glucose (5%) was added to the drinking water for 48 hours after induction to prevent STZ-induced hypoglycemia. The control normoglycemic rats received an intraperitoneal injection of citrate buffer alone. A glucometer (Check 3) was used to test a drop of blood from the rodents' tail vein 72 hours after the STZ injection to confirm that the rats had diabetes. Rats with fasting glucose levels over 2,5 g/L were included in the present study (Kotian et al., 2019; Mohamed et al., 2017). The rats were randomly allocated to one of four groups (n = 14 each; 7 males and 7 females) and were treated as follows:

Group I (control): The control group consisted of rats that were not subjected to any treatment and were provided ad libitum with water and food. These rats were considered normoglycemic.

Group II (FAE): Rats were administered 200 mg/kg of FAE via gavage for 12 weeks.

Group III (STZ): Represented by diabetic rats and is considered a hyperglycemic control.

Group IV (STZ-FAE): Rats with diabetes were treated with FAE at a dose of 200 mg/kg for 12 weeks via gavage. We have selected one-tenth of the maximum tolerated dose as the therapeutic dose, which is 200 mg/kg, ensuring both safety and therapeutic efficacy.

On a daily basis, the male rats were administered their respective designated treatments. Meanwhile, the female rats received only estradiol benzoate and progesterone, respectively, at 24 and 4 hours prior to the designated mating time.

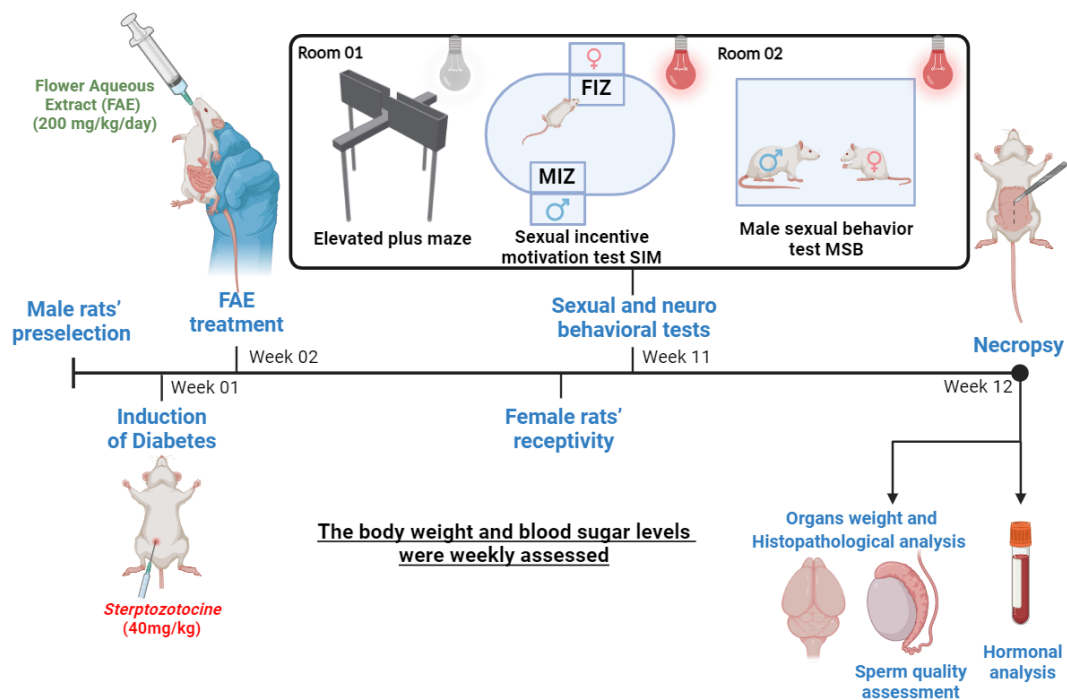


Figure 17: Flow chart of experimental design [Original].

IV.5.4. Assessment of body weight and blood glucose:

Blood glucose and initial and final body weights of each rat were registered. The percentage change in body weight was computed based on the formula provided by **Faddladdeen (2022)** and was calculated as final body weight minus initial body weight divided by initial body weight multiplied by 100.

IV.6. Behavioral test battery:

IV.6.1. Elevated plus maze (EPM):

The device consists of an elevated wooden maze in the shape of a cross with two open arms and two closed arms ($L = 50\text{ cm}$ and $l = 10\text{ cm}$) that communicate through a central area ($10 \times 10\text{ cm}$). The whole is placed 53 cm above the ground and illuminated by a halogen lamp with an intensity of 40 lux (**Figure 18**). The animal was placed in the central zone, facing a closed arm. A camera was used to record the entire test, allowing the number of entries and the time spent in each compartment to be measured over 5 minutes. This test highlights the anxiolytic or anxiogenic properties of pharmacological compounds or lesions, as the time spent in the open arms increases with a decrease in the animal's anxiety (**Kahloula, 2010; Orzelska-Górka et al., 2023**). After evaluation, the apparatus was cleaned with 70% ethanol and dried before the next rat.

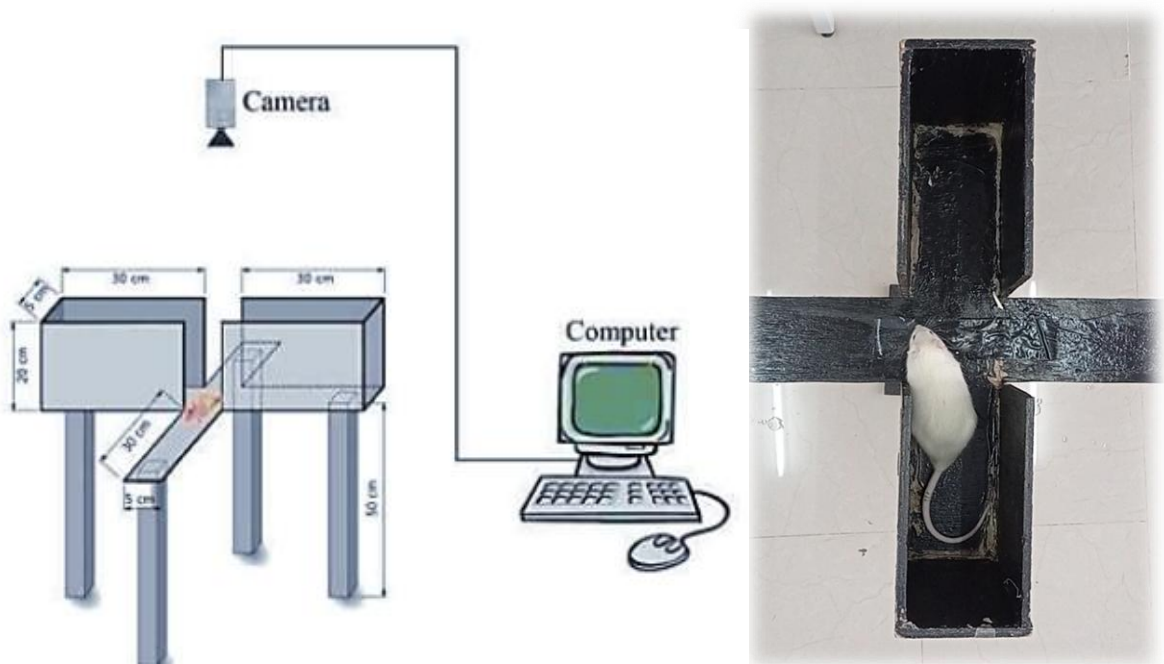


Figure 18: Elevated plus maze test apparatus [Original].

IV.6.2. Study of sexual behavior:

IV.6.2.1. Sexual incentive motivation test (SIM):

Moreira et al. (2017) described in detail the testing arena and the procedure in which sexual motivation was demonstrated. It occurs when a male rat is subjected to a social incentive (male) and a sexual incentive (receptive female). Male rats can choose to maintain contact with the sexual incentive (**Lehtimäki et al., 2021**). The maze consisted of a circular arena (80 cm in diameter) surrounded by a wall with two openings covered with double-wire mesh. An incentive animal was positioned behind the wire mesh, unreachable to the rat in the arena. In the present experiment, the incentive animals were a sexually receptive female and an intact male. There was no physical contact between the animals and the subject, but instead they were able to hear, smell and see each other. A black area was marked out in front of the cages on the arena floor: the male incentive zone (MIZ), the female incentive zone (FIZ), and the neutral zone, defined by the rest area of the arena (**Figure 19**) (**Chu et al., 2021**). The labyrinth was situated in a silent chamber with red lighting. Rats were initially acclimated to the testing environment for five minutes each, without the presence of the incentive animals. The duration of the test was 20 minutes. The variables to be quantified were: number of visits to FIZ and MIZ (with the rat's four legs), time in seconds spent in FIZ and MIZ, preference score = $\text{time in FIZ} / (\text{time in FIZ} + \text{time in MIZ})$ (**Moreira et al., 2020**).

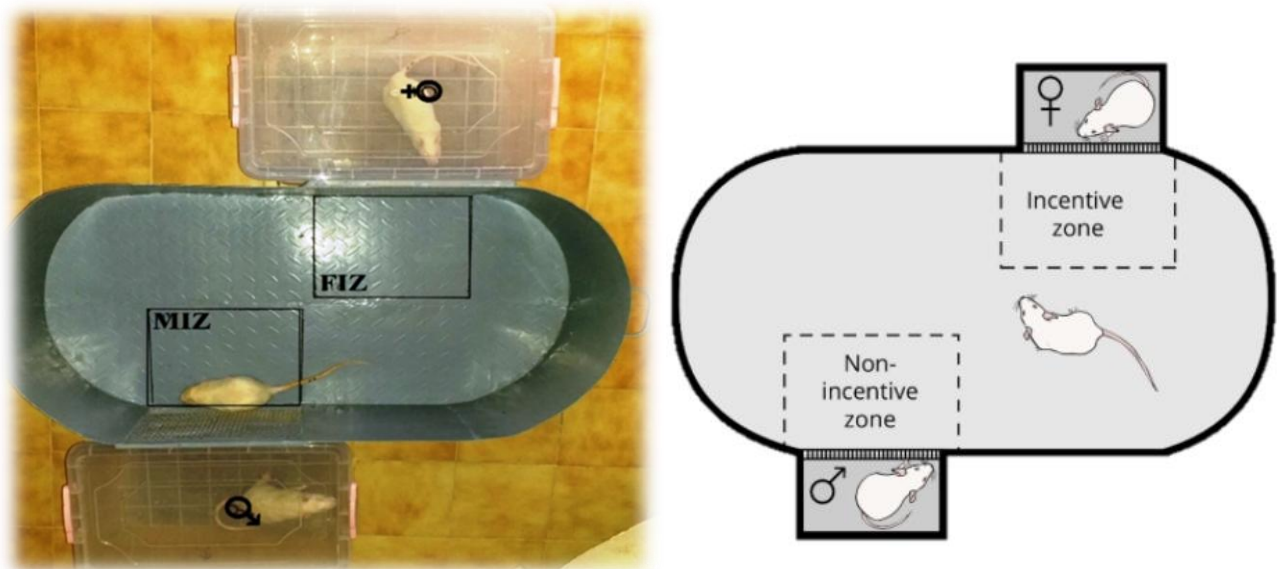


Figure 19: Sexual incentive motivation test apparatus [Original].

IV.6.2.2. Assessment of mating behavior:

Following the sexual incentive test, the experimental rats were promptly relocated to another maze for the purpose of observing sexual behavior. As previously mentioned by **Olvera-Roldán et al. (2023)**, the assessment of the copulatory behavior tests was carried out within the first three hours of the dark period in a silent room with dim red illumination. Initially, every male rat was placed in a transparent Plexiglas box with sawdust on the floor for 5 minutes in order to acclimatize to the test box. A receptive female was then introduced into the box with the male to allow them to interact sexually for 30 minutes (**Figure 20**). Various copulatory parameters were observed and recorded during this time (**Table 02**) (**Moreira et al., 2020**). Rats that did not engage in sexual behavior during training, even after ten minutes of pairing, were excluded from the study. At the end of the test, we examined each female rat's vaginal smear using an optical microscope to detect the presence of spermatozoa, which would be evidence of mating.

Table 02: Standard copulatory parameters recorded during the sexual interaction test.

Parameters	Description
Mount latency (ML)	Time in seconds from entry of receptive female to first mounting. It is a measure of sexual motivation and should be less than 1 minute.
Intromission latency (IL)	Time in seconds between female introduction and first intromission.
Ejaculation latency (EL)	Time in seconds between first intromission and behavioral signs of ejaculation.
Mount frequency (MF)	The appearance of a mount with pelvic thrusting, but without any behavioral signal of vaginal penetration. The number of mounts reflects sexual motivation.
Intromission frequency (IF)	The occurrence of mount with vaginal penetration to the pelvic thrust and behavioral signs of vaginal penetration. Intromission requires an erection of the penis and coordinated activity of the corpus striatum muscles, reflecting the penile erection potency required to induce ejaculation.
Ejaculation frequency (EF)	The onset of a mount with vaginal penetration and deep forward thrust. Most male rats are capable of achieving between 5 and 8 ejaculations.
Post-ejaculatory interval (PEI)	Time from the first ejaculation up to the next intromission by the male rat. It usually takes between 4 and 7 minutes.
Copulatory rate	(number of intromissions/ number of mount + intromissions)



Figure 20: Sexual interaction test. A) Mount, B) Intromission, C) Ejaculation, D) Penile licking [Original].

IV.7. Necropsy and organ sampling:

At the end of the experiment, in the morning after a 12-hour fast, blood was collected for testosterone assay, and then the rats were euthanized. The epididymis was carefully collected, rinsed with physiological saline solution (NaCl 0.9%), weighed, and preserved for a spermogram study. Brains and testes were also removed, rinsed with physiological saline solution, and weighed before being fixed for further study.

IV.7.1. Testosterone assay:

Following sacrifice, blood samples were collected in non-heparinized tubes, centrifuged to obtain serum, and then kept at -20 °C for subsequent analysis. Serum levels of total testosterone were assayed by the electrochemiluminescence (Elecsys method).

IV.7.2 Sperm quality assessment:

The spermogram is an essential test for determining the cause of infertility in rats. The sperm sample is subjected to various analyses to quantify and assess sperm quality. Sperm suspensions were prepared using the technique as demonstrated by **Boudou et al. (2020)** and **Wang (2003)**. After removing the testes from each rat, the cauda epididymis was transferred to

Part II: Experimental Study
Chapter IV: Materiel and methods

a Petri dish sliced with scissors, homogenized in 10 ml of physiological saline solution (0.9%) at 35-37 ° C, and incubated for 15 minutes to allow the spermatozoa to move outwards and form the sperm suspension.

IV.7.2.1. Sperm epididymis counts:

Generally, the epididymis is divided into three parts: the head, the corpus (body), and the tail. It is the tail of the epididymis that is widely used to perform sperm counts (**Y. Wang, 2003**). A 0.5 ml aliquot of the previously prepared sperm suspension was added to 2 ml of the dilution solution (5 g sodium bicarbonate, 1 ml formaldehyde, and 99 ml distilled water) prepared according to the method described by **Srinivasulu and Changamma (2017)**. The purpose of this method is to immobilize the sperm from movement. 10 µL of diluted sperm was deposited on a Malassez cell, and the sample was left to stand in a humid place for 10 minutes. Sperm were counted in the appropriate Malassez cell squares under an optical microscope at 400x magnification. The number of sperm per ml of epididymal suspension was calculated according to **Y. Wang (2003)**.

Total sperm = mean count × dilution factor

Where:

$$\text{Mean count} = (\text{count 1} + \text{count 2})/2$$

In addition:

$$\text{Dilution factor} = \frac{\text{total vol. phsio solution in dish}}{\text{transferred vol.}} \times \frac{\text{total vol. test tube}}{\text{vol. secondary square} \times \text{no. squares}}$$

IV.7.2.2. Sperm motility:

Percentage sperm motility was assessed using the method described by **Soliman et al. (2019)**. A 20 µl aliquot of sperm suspension was deposited on the Malassez cell. Sperm motility was determined after 10 microscopic observations at magnification 40. We have assessed the percentage of motility by counting immobile spermatozoa on the bottom of the slide versus motile spermatozoa moving alongside.

The percentage of sperm motility was defined by:

$$\text{Sperm mobility \%} = \left(\frac{\text{mobile sperm count}}{\text{total count}} \right) \times 100$$

IV.7.2.3. Sperm morphology:

To assess sperm morphology, 20 µL of the previous suspension was deposited on a slide. Once air-dried, spermatozoa were fixed with 95° ethanol for 5 minutes, stained with Gentian Violet for 3 minutes, then rinsed with distilled water. A minimum of 200 spermatozoa were examined under an optical microscope at 400 magnification. As soon as an abnormality was identified, the spermatozoa were directly classified as "abnormal" (**Menkveld et al., 2011; Srivastava and Pande, 2017**).

IV.7.2.4. Sperm viability:

The eosin-nigrosine viability test is a technique based on the principle of the permeability of dead cells to certain stains, such as eosin (damaged plasma membranes). It enables us to assess the percentage of living spermatozoa (**Olvera-Roldán et al., 2023**). We have placed a drop of sperm suspension on a slide and added a drop of 1% eosin. After 30 seconds, an equal volume of nigrosine was added to this mixture. Thin smears were prepared and left to dry on the slides. They were then observed under an optical microscope at magnification $\times 100$. Viable spermatozoa whose plasma membrane was impermeable to stains remain colorless, while dead spermatozoa become permeable and stained pink (**Soliman et al., 2019**).

IV.8. Histopathological analysis:

This technique allowed us to obtain thin slices of organs, facilitating the detection of any lesions. Organs were subjected to a series of successive steps followed by examination under an optical microscope.

IV.8.1. Fixation:

The purpose was to immobilize the cellular constituents in a state close to the *in vivo* state. It was carried out directly after removal to prevent putrefaction (microbial alteration), autolysis (tissue destruction by enzymatic constituents), and, at the same time, hardening of the organ (to hold any tissue formations in place) and protection against distortion and retraction. Furthermore, it preserves cellular and tissue morphology by enhancing the consistency of the tissue, which not only makes cutting easier but also improves the absorption of coloring substances. It was carried out directly after the sacrifice. First, the samples were immediately rinsed with 0.9% NaCl and then fixed in 10% formalin for at least 24 hours at room temperature, which may last for several days or even weeks. After this step, the organs were placed in previously marked histo-cassettes and undergoing a dehydration process.

IV.8.2. Dehydration and paraffin impregnation:

In this process, the tissue was transferred from formalin to paraffin to obtain blocks, then ribbons, which were then smeared onto glass slides. The aim of dehydration was to eliminate intracellular and intra-tissue water and replace it with paraffin by immersing the pieces in alcohol (ethanol or acetone) of increasing concentration: 50°, 70°, 90° and 100° in order to perform a section without losing the cellular structure as the plasma membrane ruptures.

In order for the paraffin to penetrate the tissue, the dehydrated fragment must be clarified using toluene or xylene. As paraffin is not soluble in alcohol, it is necessary to go through the clarification step with toluene or xylene to remove the alcohol.

Histological samples were dehydrated in the machine with 12 baths:

First bath: Fixation, contains 10% formalin to ensure good fixation (1 hour).

2nd to 5th baths: Dehydration, the pieces were immersed in ethanol at 50°, 70°, 90°, and 96°, respectively (1 hour in each bath) to replace the water with ethanol.

From the 6th to the 9th bath: Clarification, the samples were immersed in toluene (1 hour in each bath) to replace the ethanol with toluene. Because of the immiscibility of paraffin in ethanol.

10th to 12th baths: Impregnation, the samples were immersed in melted paraffin at 56°C (1 hour in the first 2 baths and 10 hours in the last bath) to remove the toluene and replace it with the melted paraffin.

IV.8.3. Paraffin inclusion:

Paraffin inclusion consists of placing impregnated fragments in blocks so that they are ready for slicing. The histological samples were placed in metal inclusion molds, and then the melted paraffin was poured over the fragments. The molds were then placed in the freezer to cool.

IV.8.4. Cutting and spreading:

Once the Paraffin blocks had solidified, each block was fixed to the block holder in front of the microtome blade (Leica RM 2235) and oriented to produce 3-µm-thick sections forming a pleated ribbon. These ribbons were then spread out on the surface of a water bath (Leica HI 1210) at 48°C so that the section acquired a certain flatness and the sections of interest were placed on glass slides (previously identified with a diamond pencil). Drying and dewaxing were

Part II: Experimental Study
Chapter IV: Materiel and methods

then carried out in the Drying Oven TX-11 (56°C) in a few minutes, after which the slides were placed in a rack and immersed in a vat of xylene to begin the coloring process.

IV.8.5. Slide staining and mounting:

Haematoxylin-Eosin topographical staining is the most frequently used, enabling observation of the various tissue structures: hematoxylin, a basic substance, stains nuclei blue-violet, while eosin (an acidic substance) stains cytoplasm and other cellular elements pink-red, depending on their acidity. In practical terms, this staining was carried out as follows:

The slides were successively dipped in the automat baths (Leica st 4040) for simultaneous hydration, staining, and dehydration. Histological sections were deparaffinized in two 5-minute xylene baths, hydrated in alcohol baths of decreasing concentration (100°, 96°, 70°, 50°), then rinsed with distilled water. Slides were then immersed in a hematoxylin bath for 3 minutes, rinsed with running water, and placed in an eosin bath for 10 minutes. After rinsing with water, the sections were dehydrated in alcohol baths of increasing concentration (50°, 70°, 69°, 100°), followed by two xylene baths.

The colored slides were then mounted by placing a few drops of *Eukitt* on the sections and covering them with coverslips so that the resin used coats the entire section (with no air bubbles), accelerating the polymerization of the biological glue by placing the slides in an oven (56°C) for a few minutes, followed by air drying. Slides prepared in this way can be stored without risk of deterioration for an unlimited period.

IV.8.6. Microscopic observation:

Finally, the slides were ready for observation under an optical microscope at several magnifications (x10 then x40), and photos were taken for comparative purposes.

IV.9. Statistical analysis:

The quantitative data were presented as the mean $\pm$ SD for *in vitro* results and as the mean $\pm$ SEM for *in vivo* results. The data were subjected to a two-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls post-hoc test. Pearson correlation coefficients were employed to assess the correlation between blood glucose levels and sexual behavioral parameters. A *P* value of less than 0.05 was considered to indicate a statistically significant result, with $*P \leq 0.05$ indicating a statistically significant difference, $**P < 0.01$ indicating a very significant difference, and $***P < 0.001$ indicating a highly significant difference. All of the aforementioned analyses were conducted using Sigma Stat version 3.5

Chapter V

Results and discussion

This chapter presents the results of our experimental study. The first part was devoted to characterizing the plant using UHPLC-ESI-MS/MS, examining its antioxidant and anti-inflammatory properties, and assessing the acute oral toxicity of our extract. The second part was devoted to the *in vivo* experimental study on the Wistar rat, which included an assessment of body weight and blood glucose levels, a sexual behavior study, including both the motivational aspect and mating behavior, a study of testosterone levels and sperm quality, and finally a histological analysis of the testes to confirm structural alterations at the cellular level in diabetic and diabetic treated rats with our *S. balansae* extract.

V.1. Extraction yield, total phenolic content, and flavonoid content, of *S. balansae*:

The findings shown in table 03 indicate the extraction yield, total phenolic content, and total flavonoid content, of *S. balansae* flowers aqueous extract (FAE).

Table 03: Extraction yield, total phenolic content, and total flavonoid content, of the FAE.

	Yield %	TPC ($\mu\text{gGAE}/\text{mg Extract}$) ¹	TFC ($\mu\text{gQE}/\text{mg Extract}$) ²
FAE	32.59%	290.17 $\pm$ 1.34	114.72 $\pm$ 0.44

Note: Values were expressed as means $\pm$ SD of three parallel measurements.

¹ Total phenolic contents were expressed as μg Gallic acid equivalent/mg of extract ($\mu\text{g GAE}/\text{mg}$).

² Total Flavonoid contents were expressed as μg quercetin equivalent/mg of extract ($\mu\text{g QE}/\text{mg}$).

As stated herein, we have obtained an aqueous extract from *S. balansae* flowers growing in Mostaganem by a 10 % decoction and the extraction yield was 32.59% relative to 100 g of the initial mass of dry plant matter. However, this result differs from that obtained by **Brindisi et al. (2021)**, who reported obtaining a *S. officinalis* flower extract with a yield of 8.9% after maceration, using methanol as a solvent. The same result accords with that obtained recently for the same species in the Aures Mountains. The hydroalcoholic extract prepared from the flowers by maceration in ethanol and methanol at 70% and 80% respectively showed an extraction yield very close to our value for the methanolic extract, while the ethanolic extract showed a slightly higher value (**Bendrihem et al., 2024**). This difference in yield can be attributed to several factors, including geographical origin, species, harvesting period, drying conditions, extraction technique and the polarity of the solvent used.

The medicinal effects of our FAE may arise from the presence of a variety of secondary metabolites, such as flavonoids and phenols. Based on our findings, the concentrations of TPC in the FAE averaged between (290.17 $\pm$ 1.34 $\mu\text{g GAE}/\text{mg extract}$). These results are superior to those reported by **Mahdjoub et al. (2023)** and **Mekki et al. (2023)** for leaves of the same species subjected to ultrasound-assisted extraction (49.63 $\pm$ 6.31mg GAE/g DW) and simple aqueous infusion (134.91 $\pm$ 0.230 mg GAE/g extract), respectively. Similar low values for TPC were obtained by **Bendrihem et al. (2024)** in the 70% ethanolic and 80% methanolic extracts of flowers of the same species collected in June 2019 (end of the development and flowering season) in 'Ichmoul', a commune in the wilaya of Batna, the values are (83.56 $\pm$ 0.10 $\mu\text{g GAE}/\text{mg extract}$) and (147.87 $\pm$ 0.21 $\mu\text{g GAE}/\text{mg extract}$) respectively. This quantitative difference in total polyphenol content is probably due to geographical location, climatic and

environmental conditions, the harvesting period, the stage of development of the plant and the extraction method used, including solvents and extraction time.

The TFC concentration of our extract averaged between ($114.72 \pm 0.44 \mu\text{g QE/mg extract}$). Different flavonoid contents have been found in extracts of *S. balansae* leaves prepared in various organic solvents and methods. The highest values were recorded in the 70% ethanolic leaves extract ($61.79 \pm 0.63 \mu\text{g QE/mg extract}$) followed by the 80% methanolic leaves extracts ($60.22 \pm 0.40 \mu\text{g QE/mg extract}$) (**Bendrihem et al., 2024**), ultrasound-assisted extraction ($8.14 \pm 0.39 \text{ mg QE/g DW}$) and simple aqueous infusion ($5.96 \pm 0.004 \text{ mg QE/g extract}$) (**Mahdjoub et al., 2023; Mekki et al., 2023**). Moreover **Bendrihem et al. (2024)** have recorded a relatively low quantity of TFC in there 70% ethanolic and 80% methanolic extracts of *S. balansae* flowers ($34.70 \pm 0.06 \text{ mg QE/g extract}$; $55.47 \pm 0.24 \text{ mg QE/g extract}$ respectively), these quantities are much lower than ours. The levels of TPC and TFC are strongly affected by the extraction method and the solvents used. Solvents such as methanol, ethanol, acetone, ethyl acetate and mixtures are widely used to extract phenolic and flavonoids compounds from plants in general. Furthermore, methods using water as the extraction solvent are particularly promising as water is a cheap, safe and abundant solvent compared with the use of toxic organic solvents (**Boudou, 2022**).

V.2. Phytochemical compounds of *S. balansae*:

As outlined in **table 04**, the detailed phytochemical profiles of the extract were obtained through the utilisation of UHPLC-ESI-MS/MS analysis. This method involved the use of 56 standard compounds, with the resulting chromatograms for both the standards and the identified compounds presented in **figure 21**. Phenolic compounds and flavonoids are important plant secondary metabolites displaying multiple biological activities, including antioxidant, antibacterial, cardioprotective, anticancer, and anti-inflammatory properties, which are of great value regarding human health and prevention against various diseases (**Sun et Shahrajabian, 2023; Yilmaz et al., 2023**). To the best of our knowledge, this is the first report on the LC-ESI-MS/MS analysis of the major primary and secondary metabolites in the flowers of *S. balansae* from Chelf Valley, Mostaganem. The LC-ESI-MS/MS analysis of the aqueous extract of the plant has shown a complex profile of its constituents, where thirteen compounds were quantified: six phenolic acids, six flavonoids, and one phenolic aldehyde. These include cynaroside, with a concentration of $3.8 \text{ mg analyte/g extract}$; rosmarinic acid, with a concentration of $3.145 \text{ mg analyte/g extract}$; caffeic acid at $0.803 \text{ mg analyte/g extract}$; and luteolin at $0.488 \text{ mg analyte/g extract}$. The other major constituents present in it are quinic acid,

Part II: Experimental Study
Chapter V: Results and Discussion

fumaric acid, protocatechuic acid, protocatechuic aldehyde, salicylic acid, cosmosiin, kaempferol, apigenin, and acacetin.

Table 04: LC–tandem mass spectrometry (MS/MS) data of compounds detected in *Salvia balansae* flower aqueous extract (FAE)

No	Analytes	RT	M.I. (m/z)	F.I. (m/z)	mg analyte/g extract
1	Quinic acid	3.0	190.8	93.0	0.398
2	Fumaric acid	3.9	115.2	40.9	0.295
3	Aconitic acid	4.0	172.8	129.0	N.D.
4	Gallic acid	4.4	168.8	79.0	N.D.
5	Epigallocatechin	6.7	304.8	219.0	N.D.
6	Protocatechuic acid	6.8	152.8	108.0	0.034
7	Catechin	7.4	288.8	203.1	N.D.
8	Gentisic acid	8.3	152.8	109.0	N.D.
9	Chlorogenic acid	8.4	353.0	85.0	N.D.
10	Protocatechuic aldehyde	8.5	137.2	92.0	0.02
11	Tannic acid	9.2	182.8	78.0	N.D.
12	Epigallocatechin gallate	9.4	457.0	305.1	N.D.
13	1,5-dicaffeoylquinic acid	9.8	515.0	191.0	N.D.
14	4-OH Benzoic acid	10.5	137.2	65.0	N.D.
15	Epicatechin	11.6	289.0	203.0	N.D.
16	Vanillic acid	11.8	166.8	108.0	N.D.
17	Caffeic acid	12.1	179.0	134.0	0.803
18	Syringic acid	12.6	196.8	166.9	N.D.
19	Vanillin	13.9	153.1	125.0	N.D.
20	Syringic aldehyde	14.6	181.0	151.1	N.D.
21	Daidzin	15.2	417.1	199.0	N.D.
22	Epicatechin gallate	15.5	441.0	289.0	N.D.
23	Piceid	17.2	391.0	135/106.9	N.D.
24	<i>p</i> -Coumaric acid	17.8	163.0	93.0	N.D.
25	Ferulic acid-D3-IS ^h	18.8	196.2	152.1	N.A.
26	Ferulic acid	18.8	192.8	149.0	N.D.

Part II: Experimental Study
Chapter V: Results and Discussion

27	Sinapic acid	18.9	222.8	193.0	N.D.
28	Coumarin	20.9	146.9	103.1	N.D.
29	Salicylic acid	21.8	137.2	65.0	0.015
30	Cynaroside	23.7	447.0	284.0	3.8
31	Miquelianin	24.1	477.0	150.9	N.D.
32	Rutin-D3-IS ^h	25.5	612.2	304.1	N.A.
33	Rutin	25.6	608.9	301.0	N.D.
34	Isoquercitrin	25.6	463.0	271.0	N.D.
35	Hesperidin	25.8	611.2	449.0	N.D.
36	o-Coumaric acid	26.1	162.8	93.0	N.D.
37	Genistin	26.3	431.0	239.0	N.D.
38	Rosmarinic acid	26.6	359.0	197.0	3.145
39	Ellagic acid	27.6	301.0	284.0	N.D.
40	Cosmosiin	28.2	431.0	269.0	0.342
41	Quercitrin	29.8	447.0	301.0	N.D.
42	Astragalin	30.4	447.0	255.0	N.D.
43	Nicotiflorin	30.6	592.9	255.0/284.0	N.D.
44	Fisetin	30.6	285.0	163.0	N.D.
45	Daidzein	34.0	253.0	223.0	N.D.
46	Quercetin-D3-IS ^h	35.6	304.0	275.9	N.A.
47	Quercetin	35.7	301.0	272.9	N.D.
48	Naringenin	35.9	270.9	119.0	N.D.
49	Hesperetin	36.7	301.0	136.0/286.0	N.D.
50	Luteolin	36.7	284.8	151.0/175.0	0.488
51	Genistein	36.9	269.0	135.0	N.D.
52	Kaempferol	37.9	285.0	239.0	0.02
53	Apigenin	38.2	268.8	151.0/149.0	0.019
54	Amentoflavone	39.7	537.0	417.0	N.D.
55	Chrysin	40.5	252.8	145.0/119.0	N.D.
56	Acacetin	40.7	283.0	239.0	0.073

R.T., retention time; FI (m/z), fragment ions; MI (m/z), molecular ions of the standard analytes (m/z ratio); IS^h, internal standard; N.A., not applicable; N.D., not detected.

Part II: Experimental Study
Chapter V: Results and Discussion

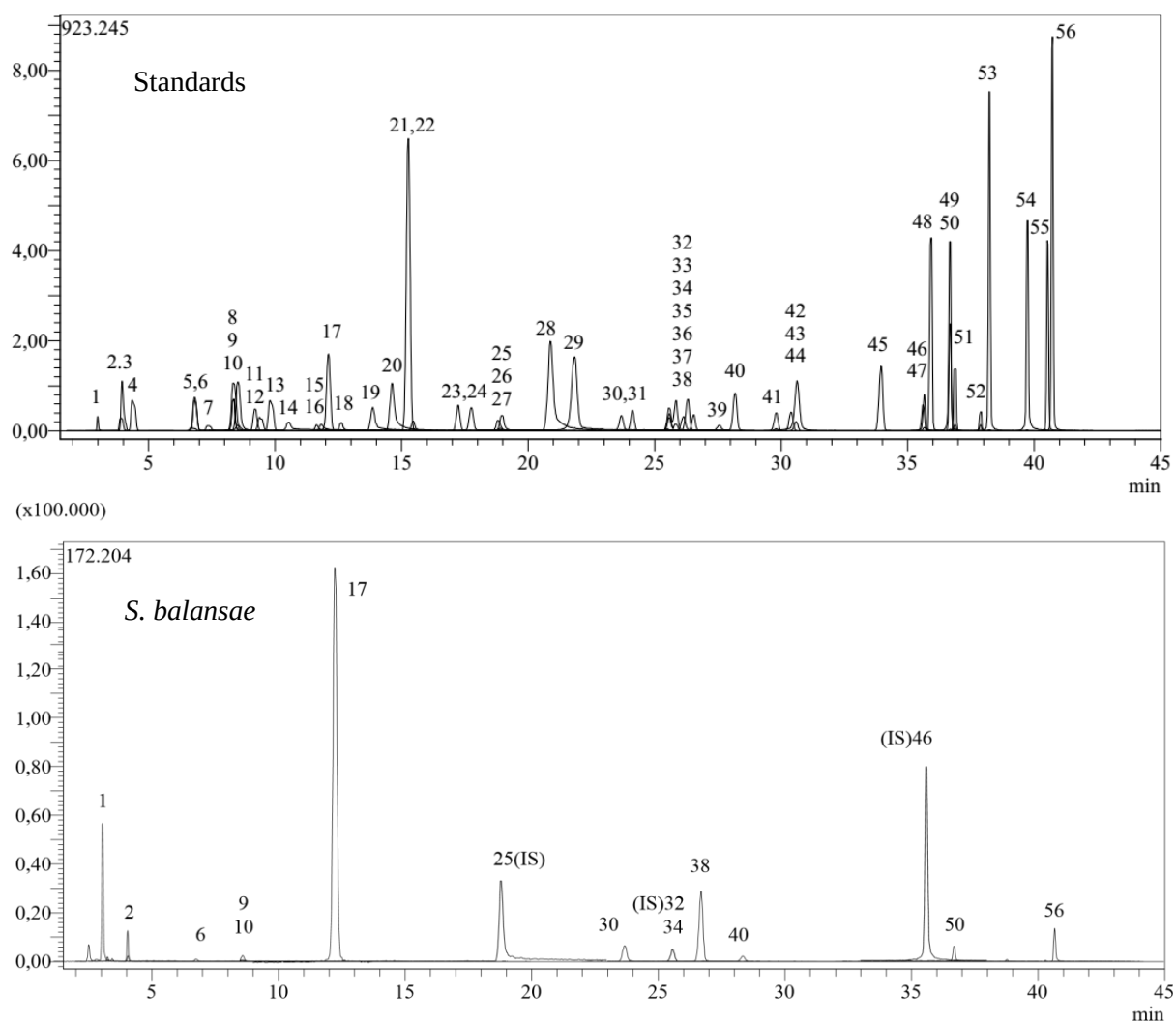


Figure 21: LC-MS/MS TIC chromatograms of *S. balansae* and standard phytochemicals.

Regarding the *S. balansae* collected in the Aures Mountains, which is probably a different species from our samples, as confirmed by our results, there is a distinction between these two species in not only flower colour and leaf morphology but also the phenolic compounds. To our knowledge, only one study has been conducted to screen phenolic compounds in extracts of *S. balansae* flowers collected from the Aures Mountains using LC/MS. A total of 14 phenolic compounds were identified, in both 70% ethanolic and 80% methanolic extracts and 15 in 60% acetone, with different amounts (**Bendrihem et al., 2024**). Quinic acid (47.509 ppm in flower ethanol extract) and rosmarinic acid (32.914 ppm in flower methanol extract) were classified as the most abundant phenolic acids. Three distinct flavonoid compounds were identified as the major compounds: luteolin-7-o-glucoside (44.917 ppm in flowers, acetone extract), apigenin-7-o-glucoside (68.507 ppm in flowers, ethanol extract), and cirsiol (3.81 ppm in flowers methanol extract) (**Bendrihem et al., 2024**). All these compounds, along with the other identified components such as gallic acid, protocatchuic acid, caffeic acid, p-coumaric acid,

trans-frulic acid, hyperoside, naringin, apegenin, acacetin, are commonly found in the genus *Salvia* (**Bendrihem et al., 2024**). However, for the *S. balansae* from Chelf Valley in Mostaganem only two recent studies investigated the qualitative and quantitative analysis of phenolic compounds in the leaves extract of *S. balansae* and not flowers (**Amirat et al., 2023; Mahdjoub et al., 2023**). According to **Amirat et al. (2023)**, nine phenolic components were quantified using HPLC-MS, including catechin as the major compound at 72.5%, followed by myricin at 21.7%, epicatechin at 1.3%, and butylated hydroxyanisole at 1.1% in lower concentrations. **Mahdjoub et al. (2023)** highlighted the significant presence of two main classes, phenolic acids and flavonoids, characterised by significant amounts of tannic acid, benzoic acid, gallic acid and ascorbic acid. Among the minor chemicals found are the flavonoids myricetin and catechin, as well as their derivatives (**Mahdjoub et al., 2023**).

More importantly, phytochemical composition can vary as a function of several aspects related to the used plant part, harvesting period, and laboratory test conditions (temperature, solvent, and extraction method) (**Benabdallah et al., 2022**). The presence of such compounds underlines the potential pharmacological benefits of the extract involving antioxidant, anti-inflammatory, and anti-diabetic activities. This comprehensive phytochemical profile confirmed the traditional use of *S. balansae* in medicine and suggested a promising application within modern therapeutic practices.

V.3. Assessment of biological activities:

V.3.1. In vitro antioxidant activity:

The antioxidant capacity of *S. balansae* FAE was evaluated using three techniques, expressed as IC_{50} ($\mu\text{g/mL}$) and $A_{0.5}$ ($\mu\text{g/mL}$), and compared to the standards BHA, BHT, Ascorbic acid and α -Tocopherol.

As **table 05** below shows, the DPPH, ABTS and reducing power assays of the FAE showed promising results of 31.06 ± 0.12 , 19.11 ± 0.14 and 27.93 ± 1.04 , respectively. Nevertheless, they remained below the standards BHA, BHT and Ascorbic acid. In addition, the results obtained for the reducing power assay revealed an $A_{0.5}$ ($\mu\text{g/mL}$) equal to 27.93 ± 1.04 , a higher value than the α -Tocopherol standard with an $A_{0.5}$ ($\mu\text{g/mL}$) of 34.93 ± 2.38 (**Table 05**). Typically, when comparing the IC_{50} values of the extract to the reference standards BHA, BHT and α -tocopherol, the extract exhibited lower potency in all antioxidant assays. However, this does not inherently indicate that our extract has weak antioxidant activity. It is crucial to recognize

Part II: Experimental Study
Chapter V: Results and Discussion

that the extract is composed of numerous components. Unlike the pure reference standards, our extract' effectiveness can still be considered significant.

Table 05: The antioxidant activities of the flower aqueous extract (FAE).

Samples	Antioxidant Activity		
	DPPH assay	ABTS assay	Ferric reducing power assay
	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)	A _{0.5} (µg/mL)
FAE	31.06±0.12	19.11±0.14	27.93±1.04
BHA	5.73±0.41	1.03±0.00	9.29± 0.22
BHT	12.99 ± 0.41	1.59±0.03	NT
Ascorbic acid	4.39±0.01	3.04±0.05	6.77±1.15
α-Tocopherol	13.02 ± 5.17	NT	34.93±2.38

Note: Values expressed are means ± SD of three parallel measurements ($P < 0.05$).

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; NT, not tested.

The high phenolic compound content of an extract indicates its high antioxidant capacity (**Gülçin et al., 2019**), as is the case with sage infusion (**Sotiropoulou et al., 2020**). Our results showed greater scavenging capacity than the different IC₅₀ values recorded for the hydroethanolic extract from *S. balansae* leaves (545.03 ± 3.267 and 328.95 ± 5.29 µg/mL for DPPH and ABTS, respectively) and the methanolic extract of *S. balansae* (242.7 ± 7.44 and 124.1 ± 9.70 µg/mL for DPPH and ABTS, respectively) (**Amirat et al., 2023; Mahdjoub et al., 2023**). Furthermore, the reducing power assay results showed an A_{0.5} (µg/mL) of 27.93 ± 1.04 , which was greater than the α-Tocopherol standard's A_{0.5} (µg/mL) of 34.93 ± 2.38 and the hydroethanolic extract from *S. balansae* leaves of 689.40 ± 0.86 µg/mL (**Mahdjoub et al., 2023**). On the other hand, **Bendrihem et al. (2024)** found a capacity to neutralize DPPH radicals close to our own in the methanolic extract from *S. balansae* followed by acetone extracts, particularly in the flower extracts, depicting an IC₅₀ of 50.27 to 55.62 µg/ml, respectively. Besides DPPH activity, the IC₅₀ of *S. balansae* recorded variable amounts evaluated by ABTS assay ranging from 33.68 to 59.61 µg/ml, with the flower methanol extract found to be the most efficient in quenching free radicals, followed by the flower ethanol extract (**Bendrihem et al., 2024**). Our results for antioxidant activity by DPPH and ABTS methods are relatively similar to those recorded for *S. balansae* collected from the Aures Mountains.

V.3.2. In vivo anti-inflammatory activity:

The anti-inflammatory activity of our extract was assessed using carrageenan, a sulfated polysaccharide extracted from seaweed (**Widyarini et al., 2023**), which induces plantar edema in rats. The results obtained are presented in **tables 06** and **figure 22** and **23**.

The data in **table 06** indicate a progressive increase in the volume of plantar edema following injection of the phlogogenic agent, reaching its maximum threshold at 3 hours in the control group (5.50 ± 0.032 mm). Compared with the latter, the groups treated with FAE and diclofenac showed a highly significant ($P < 0.001$) reduction in edema from 3 hours post-injection (4.82 ± 0.037 mm and 4.78 ± 0.060 mm respectively). However, the treatment with FAE prevented the development of inflammation to a highly significant extent ($P < 0.001$) than that with diclofenac. Indeed, 6 hours after induction of inflammation, FAE reduced the thickness of the paw to 3.66 ± 0.040 mm, while the diclofenac reduced it to 4.04 ± 0.081 mm. This suggests that our extract of *S. balansae* flowers is significantly more effective than diclofenac in preventing the progression of inflammation.

Table 06: Effect of *S. balansae* FAE on carrageenan-induced plantar edema in rats.

Hours	Leg diameter in millimetres		
	Control	Diclofenac	FAE
00h	3.10 ± 0.081	3.40 ± 0.058	3.42 ± 0.117
01h	4.66 ± 0.075	4.78 ± 0.058	4.64 ± 0.037
02h	5.26 ± 0.075	5.08 ± 0.080	5.08 ± 0.037
03h	5.50 ± 0.032	$4.78 \pm 0.060\mathbf{a}^{***}$	$4.82 \pm 0.037\mathbf{a}^{***}$
04h	5.48 ± 0.049	$4.36 \pm 0.084\mathbf{a}^{***}$	$4.32 \pm 0.037\mathbf{a}^{***}$
05h	5.30 ± 0.089	$4.10 \pm 0.084\mathbf{a}^{***}$	$4.04 \pm 0.024\mathbf{a}^{***}$
06h	5.08 ± 0.058	$4.04 \pm 0.081\mathbf{a}^{***}$	$3.66 \pm 0.040\mathbf{a}^{***}\mathbf{b}^{***}$

Note: Values expressed as Mean $\pm$ S.E.M (n = 6); Control: vehicle (1% Tween 80); Standard: Diclofenac; FAE (200 mg/kg); $P < 0.05$; $** < 0.01$; $*** < 0.001$ statistically significant differences compared to control group (a), standard group (b).

Injection of carrageenan caused a time-dependent increase in paw volume in all three experimental groups, with a greater increase in the control group, with a maximum of 77.68%

Part II: Experimental Study
Chapter V: Results and Discussion

(Figure 22) at the third hour. Compared with the diclofenac-treated group, which showed a considerable reduction ($P < 0.001$) in oedema volume from the third hour onwards, with a further reduction of 19.02% at the sixth hour, the FAE group also showed a very significant reduction in oedema of 7.15% compared with the control and standard ($P < 0.001$, $P < 0.05$ respectively).

The results showed that the FAE effectively inhibited oedema formation from the first hour (22.32%), whereas diclofenac inhibited oedema formation from the second hour (21.38%) (Figure 23). Preventive administration of FAE progressively reduced oedema ($P < 0.001$), reaching a maximum inhibition percentage of 88.06% at 6 h. This was significantly greater ($P < 0.01$) than the 67.24% inhibition of diclofenac.

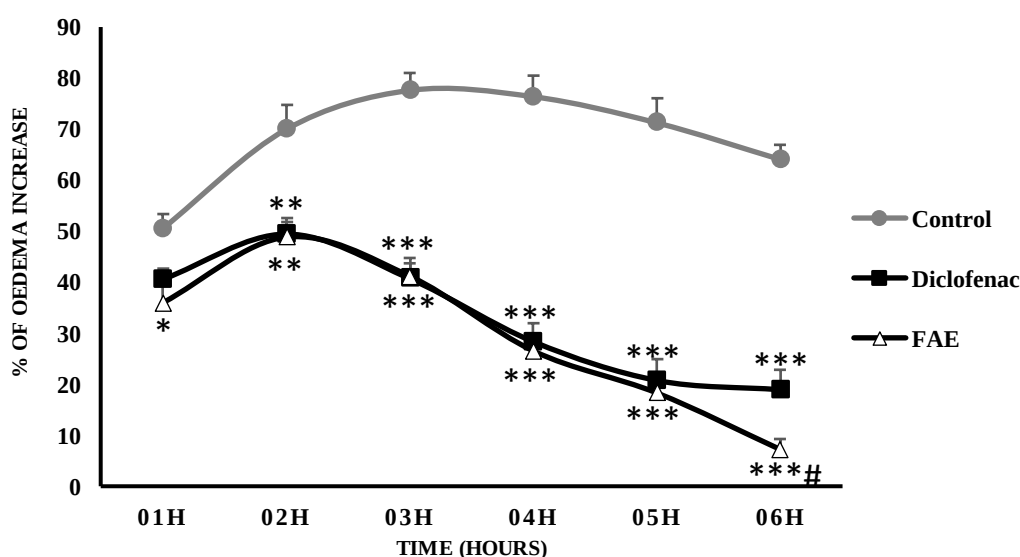


Figure 22: Percentage of increased oedema induced by carrageenan over time (h) in rats treated with aqueous extract of *S. balansae* flowers and diclofenac. Statistically significant differences $P^* < 0.05$; $P^{**} < 0.01$; $P^{***} < 0.001$ compared to control group, $P^{\#} < 0.05$ compared to standard group.

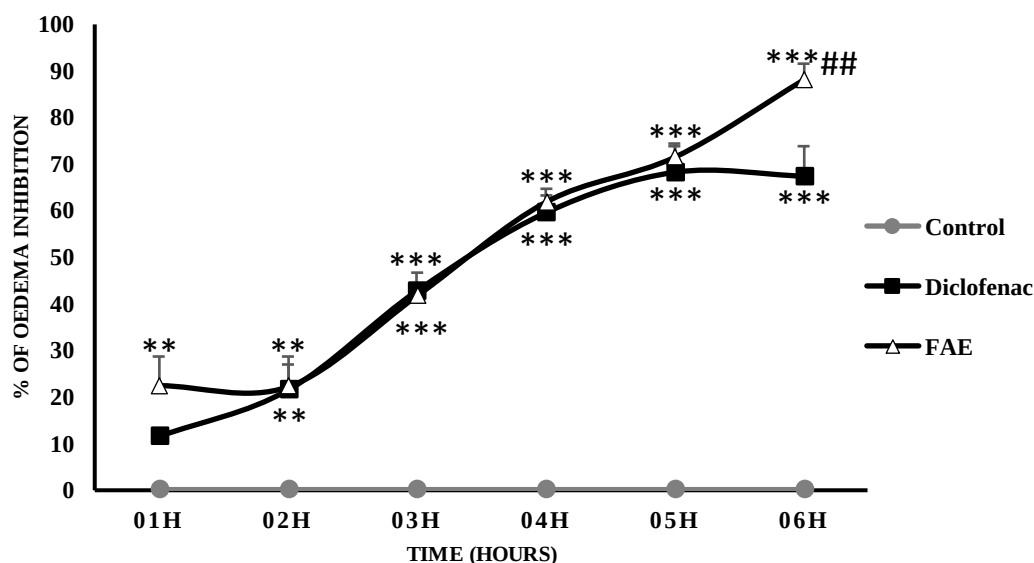


Figure 23: Inhibition percentage of oedema induced by carrageenan over time (h) in rats treated with aqueous extract of *S. balansae* flowers and diclofenac. Statistically significant differences $P < 0.05$; $** < 0.01$; $*** < 0.001$ compared to control group, $P \# < 0.01$ compared to standard group.

The physiology of oedema, particularly in the context of carrageenan-induced inflammation, reveals a distinct progression characterized by a peak in oedema development around three hours post-injection, followed by a stabilization phase (Vazquez et al., 2015).

The initial response to carrageenan injection involves the rapid release of inflammatory mediators such as histamine, serotonin, and bradykinin (Mansouri et al., 2015). This phase typically peaks within the first hour post-injection and is primarily responsible for the immediate swelling observed in the affected area. Following the early phase, a secondary phase occurs, generally peaking around 4-6 hours post-injection. This phase is associated with the continued release of prostaglandins and the infiltration of neutrophils, which contribute to the sustained inflammatory response, hence the peak at the third hour (Annamalai et Thangam, 2017; El awady et al., 2024; Kuedo et al., 2016).

In the present study, diclofenac 2-[2-(2,6-dichloroanilino)phenyl]acetic acid, a highly effective non-steroidal anti-inflammatory, was tested as a reference for comparison with our plant extract. Studies by Grohs et al. (2023) and Hasan et al. (2023) have already reported that the reference molecule used (diclofenac) acts in secondary phase, mainly by inhibiting the synthesis of prostaglandins, substances involved in inflammatory and painful processes. Its mechanism of action is based on the inhibition of cyclooxygenases (COX), in particular COX-2, which is responsible for the production of prostaglandins in response to inflammatory stimuli.

The aqueous extract of *S. balansae* flowers reduced oedema from the first and second hour compared to the control ($P < 0.01$). At the sixth hour, there was a significant difference ($P < 0.001$), even exceeding that of the standard diclofenac ($P < 0.01$). The anti-inflammatory activity of our plant can be attributed to its composition of secondary metabolites. The aqueous extract of *S. balansae* flowers is rich in total phenolics and flavonoids, which may be involved in inhibiting the deferential phases of inflammation. Flavonoids exert their anti-inflammatory effects through multiple mechanisms, including the inhibition of key pro-inflammatory enzymes like COX-2, lipoxygenase, and inducible nitric oxide synthase (iNOS), which results in a decrease in pro-inflammatory mediators such as prostaglandins and nitric oxide (Al-Khayri et al., 2022; Maleki et al., 2019). Additionally, they regulate cytokine production by reducing the expression of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6 (Al-Khayri et al., 2022; González-Gallego et al., 2010). Flavonoids also inhibit the activation of the NF- κ B pathway, a crucial regulator of inflammation, leading to a decrease in the expression of inflammatory genes (Al-Khayri et al., 2022; Maleki et al., 2019). Their strong antioxidant properties help mitigate oxidative stress, reducing tissue damage, while their membrane-stabilizing effect prevents the release of inflammatory mediators from damaged cells, collectively contributing to their overall anti-inflammatory action (Al-Khayri et al., 2022; Derouich et al., 2020). Luteolin, present in our extract, is a flavonoid that exhibits significant anti-inflammatory activity. It has been reported to inhibit the activation of NF- κ B and reduce the expression of inflammatory cytokines, contributing to its efficacy in reducing edema in experimental models (Mansouri et al., 2015). Similarly, other natural compounds, such as protocatechuic acid and caffeic acid, have demonstrated their effectiveness in mitigating inflammation (Albarakati, 2022; Sandhiutami et al., 2024). Protocatechuic acid has been shown to reduce inflammation in similar models, while caffeic acid specifically inhibits the production of pro-inflammatory cytokines, which play a crucial role in the inflammatory response (Albarakati, 2022; Sandhiutami et al., 2024). Additionally, rosmarinic acid suppresses pathways like NF- κ B and inhibits the synthesis of pro-inflammatory mediators, such as cytokines and enzymes, contributing to its anti-inflammatory effects (Nadeem et al., 2019).

V.3.3. Acute Oral Toxicity of FAE:

The acute toxicity of the aqueous extract of *S. balansae* flowers reveals a promising safety profile. The results show that it is non-toxic when administered orally, with no lethality observed in rats even at high doses of up to 2 g/kg, making it difficult to determine an exact LD₅₀ value. This indicates a high margin of safety for its consumption. Consequently, we have

selected one-tenth of the maximum tolerated dose as the therapeutic dose, which is 200 mg/kg, ensuring both safety and therapeutic efficacy. Its key active compounds, including rosmarinic acid and caffeic acid, which exhibit strong antioxidant and anti-inflammatory properties, support the non-toxicity of sage. These compounds present in our extract contribute to the herb's ability to protect against oxidative stress and inflammation, further affirming its safe use (**Ghorbani and Esmailizadeh, 2017**). Rosmarinic acid, in particular, has shown neuroprotective effects, while caffeic acid is known for its hepatoprotective properties, both of which are crucial in maintaining cellular integrity and preventing toxicity (**Mocan et al., 2020; Nadeem et al., 2019**).

V.4. Blood glucose, body and organ weights:

Experimental diabetes was induced in rats using STZ, a widely recognized model for T1DM due to its ability to destroy β -pancreatic cells, resulting in severe hyperglycemia (**Bikri et al., 2021**).

As depicted in **figure 24**, diabetic rats (STZ) exhibited significant hyperglycaemia with 5.47 g/L ($P < 0.001$) after STZ injection, and severe polydipsia and polyuria (data not shown) compared to control rats with 1.39 g/L. Conversely, administration of FAE to diabetic rats (STZ-FAE) significantly reduced serum glucose levels ($P < 0.001$) by the end of the treatment period to a value of 1.61 g/L, confirming the glycaemic normalisation effect.

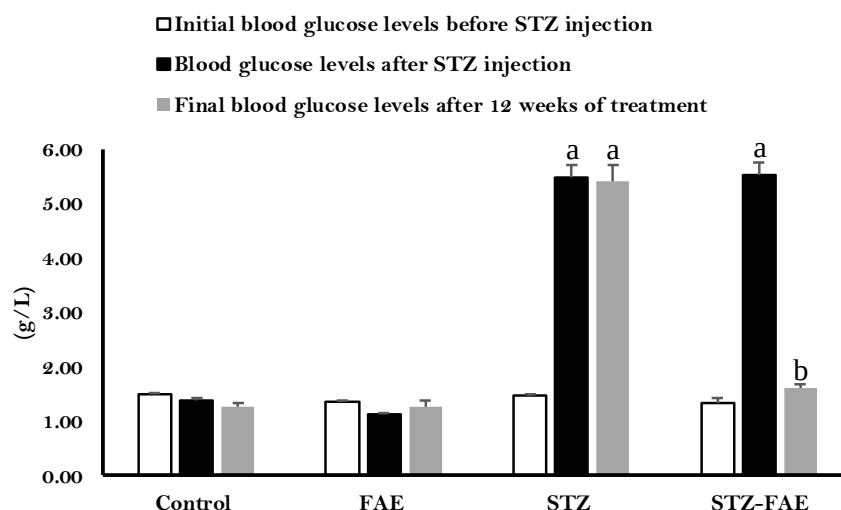


Figure 24: Blood glucose levels of the different experimental groups before, after diabetes induction, and after 12 weeks of treatment. STZ - streptozotocin. FAE – Flower Aqueous Extract. Each bar indicates the mean $\pm$ SEM ($n = 7$). $P < 0.001$ indicates a highly significant difference vs. (a) control and FAE and (b) STZ.

Part II: Experimental Study
Chapter V: Results and Discussion

The significant hyperglycemia observed in diabetic rats was associated with a noteworthy decrease in body weight ($P < 0.001$) and organ weight compared to control (C) rats (**Table 07**). These symptoms reflect the clinical manifestations of T1DM in humans, as reported in other studies (**Rebolledo-Solleiro et al., 2016**). On the other hand, treatment with FAE resulted in a highly significant increase in both body weight ($P < 0.001$) and organ weight ($P < 0.001$) in diabetic treated rats (STZ-FAE) compared to untreated diabetic rats (STZ) (**Table 07**).

Table 07: Body, organ weights, and blood glucose levels of the different experimental groups

Parameters/groups	C	FAE	STZ	STZ-FAE
Initial weight (g)	222±5.22	222±4.34	220±4.08	224±8.22
Final weight (g)	327±14.35	356±8.50	195±6.55***a	304±14.70***b
% Body weight variation	47.51±6.21	60.41±1.55*a	-9.18±2.87***a	35.87±2.97***b
Testis weight (g)	1.82±0.06	1.74±0.06	1.36±0.03***a	1.67±0.10**b
Brain weight (g)	1.93±0.03	2.02±0.04	1.74±0.02*a	1.92±0.08*b

Note: Values are expressed as mean ± SEM of seven animals in each group. * $p \leq 0.05$ indicates a significant difference; ** $p < 0.01$ indicates a very significant difference; *** $p < 0.001$ indicates a highly significant difference.

Abbreviations: FAE, flower aqueous extract; STZ, streptozotocin.

a) Significantly different from the values of the negative control rats (C).

b) Significantly different from the values of the diabetic control rats (STZ)

Streptozotocin, synthesised by *Streptomyces achromogenes*, is mainly used in cancer treatment and medical research to induce type 1 or type 2 diabetes depending on dosage (**Ghasemi et Jeddi, 2023**). It exhibits toxicity to pancreatic cells and diabetogenic properties by targeting the GLUT-2 receptor on β -cells or causing oxidative damage via NO and free radicals. Low doses cause β -cell dysfunction, while higher doses lead to necrosis (**Dagna et al., 2020**).

In the current study, adult male Wistar rats given a single intraperitoneal dose of STZ developed hyperglycemia five days later. They showed blood glucose levels significantly higher than the initial fasting level. In addition, untreated diabetic rats showed a clear decline in body weight gain. This reduction has been linked to undue tissue protein breakdown and may be due to reduced insulin release, regarded as one of the body's major anabolic hormones

(Mohamed et al., 2017). The dose of 40 mg/kg STZ was chosen for its ability to induce moderate hyperglycemia in Wistar rats without prior dietary intervention. Studies such **Benabbou et al. (2018) ; Kotian et al. (2019),** and **Mohamed et al. (2017)** have successfully used this dose to study the impact of diabetes on male reproductive function.

The results observed in our study corroborate an earlier study showing that untreated diabetic rats displayed a significant elevation in blood glucose levels alongside a dramatic reduction in body weight gain **(Adedara et al., 2019)**. Organ weight and size are linked to functional unit availability and organ integrity. The testicular weight decline recorded in this study is consistent with the results obtained by **Adedara et al. (2019)**. Yet, the administration of sage FAE in diabetic rats resulted in blood glucose levels restoration to approximately normal, as well as a notable enhancement in body weight gain and testicular weight. In fact, earlier research has indicated that flavonoids and phenolic compounds such as rosmarinic acid play an important role in glucose homeostasis by inhibiting carbohydrate digestion and glucose absorption, as well as regulating insulin release through multiple signaling pathways **(Noor et al., 2022; Sok Yen et al., 2021)**. In addition, studies suggest that cynaroside, a specific type of flavonoid found in our extract, may be used in the prevention and treatment of diabetes and metabolic syndrome **(Saldanha et al., 2021)**. This showed improved metabolism and prevented tissue loss caused by hyperglycaemia.

V.5. Elevated plus maze:

The results obtained show that during the elevated cross maze test, the number of entries and the time spent in the open arms were significantly reduced ($P < 0.001$; $P < 0.001$ respectively) in diabetic rats compared to control rats. In addition, there was a significant increase in the number of entries into the open arms in diabetic rats treated with FAE ($P < 0.01$) compared to diabetic rats **(Figure 25)**. Anxiety-like behavior is therefore likely to be more pronounced when the number of entries and duration of stay in the open arms are low. These results clearly show that diabetes, through its anxiogenic effect, greatly reduces their exploratory abilities.

Part II: Experimental Study
Chapter V: Results and Discussion

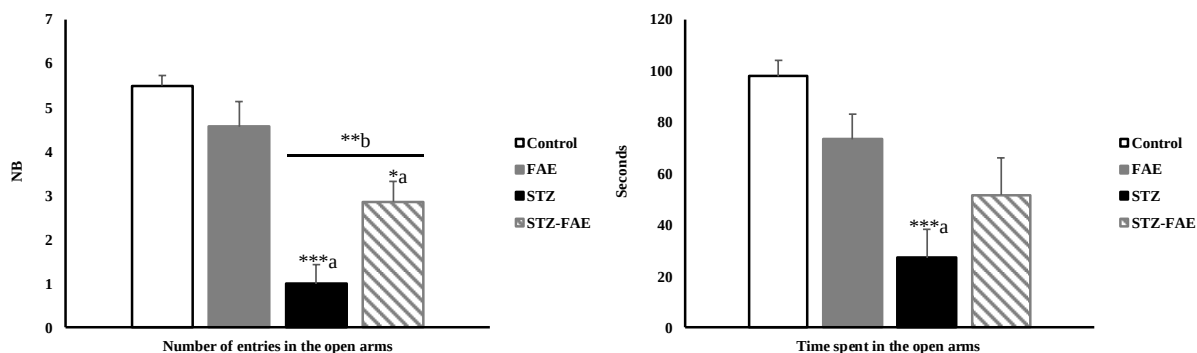


Figure 25: Number of entries and time spent in the open arms of the different experimental groups. Each bar indicates the mean $\pm$ SEM ($n=7$). $*P \leq 0.05$ indicates a significant difference; $**P < 0.01$ indicates a very significant difference; $***P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Research has shown a complex interplay between diabetes and anxiety-like behaviors in animal models, particularly rats. The elevated plus maze (EPM) is a well-known test used to assess anxiety-related behaviors in rodent subjects. Its primary purpose is to evaluate the potential anxiolytic effects of compounds (Himanshu et al., 2020). High levels of head-dipping behavior are generally interpreted as a sign of neophilia, while lower levels can indicate heightened anxiety or a lack of interest in new experiences (Brown et Nemes, 2008). In our study, we observed a decreased number of entries and time spent in the open arms of the EPM alongside some anxiety-like behaviors (ALB), including increased freezing time, decreased head-dipping, stretched-attend posture (SAP), and increased total self-grooming behavior time (data are not shown). This suggests that STZ-induced diabetes can indeed heighten anxiety-like responses (Amin et al., 2022; Caliskan et al., 2019). Further examination using EPM revealed significant differences in head-dipping behavior, number of entries, and time spent in the open arms between groups. Specifically, diabetic rats showed lower levels of the number of head dives compared with controls ($P < 0.01$) (Caliskan et al., 2019, 2020). The relationship between poor glycaemic control and oxidative stress in diabetes was associated with neurological complications, including elevated anxiety and depressive symptoms (Bikri et al., 2021).

While there is a robust connection between diabetes and anxiety, it is essential to consider the role of STZ itself. As a toxic agent, STZ interacts with glucose transporter proteins, particularly GLUT2, affecting various organs. However, it is noteworthy that STZ does not cross the blood-brain barrier (BBB), suggesting that its neurotoxic effects may not directly cause the observed behavioral changes (Caliskan et al., 2020). Still, STZ even by a single injection could indirectly influence central nervous system function by increasing blood-brain

Part II: Experimental Study
Chapter V: Results and Discussion

barrier permeability, potentially altering acid-base balance and allowing excess ketone bodies to enter the brain. Additionally, the molecular mechanisms linking diabetes and anxiety involve several key factors, including molecules related to oxidative stress (such as MDA and glutathione), inflammatory markers (like TNF- α and IL-6), neurotransmitters (such as serotonin and noradrenaline) and also stress hormones like cortisol (**Caliskan et al., 2019, 2020**). Previous studies have shown that STZ-induced diabetes is associated with elevated levels of serotonin and noradrenaline in specific brain regions, which may contribute to the anxiety-like behaviors observed (**Amin et al., 2022**). Furthermore, recent research indicates that certain compounds, including flavonoids and phenolic derivatives, can modulate the release of these neurotransmitters, potentially providing an anxiolytic effect in diabetic models (**Bikri et al., 2021**). These findings might demonstrate our extract's anxiolytic-like effect in diabetic rats.

Around 80% of people in developing countries rely on traditional medicine, according to the World Health Organization (**Abdelhalim et al., 2021**). Beneficial effects in the treatment of stress and fatigue, depression, anxiety, psychosis, cognitive dysfunction, epilepsy, and brain trauma have been reported for many species of Lamiaceae (**Abdelhalim et al., 2021**). The administration of FAE to diabetic rats allowed us to observe a significant increase in the number of entries into the open arms of the rats. These results are in agreement with those obtained by **Y. S. Lin et al. (2021)**, who demonstrated that *Salvia miltiorrhiza* has anxiolytic effects in the EPM test in mice. The ethanol extract from this plant significantly increased the percentage of open-armed entries and time spent open-armed, and reduced time spent closed-armed, which aligns with our results (**Y. S. Lin et al., 2021**). Flavonoids, major constituents of medicinal plants, are attracting increasing interest in psychopharmacology for their potential anxiolytic effects (**Machado et al., 2024**). Their ability to cross the BBB enables them to act on the central nervous system (CNS) by modulating several key neurotransmitters (GABA, serotonin, norepinephrine, dopamine), thus playing an essential role in regulating anxiety and depression (**H. Wang et al., 2023**). Studies show that certain flavonoids interact directly with GABA_A receptors, particularly via the benzodiazepine site (**Machado et al., 2024**). Apigenin, one of our FAE compounds, targets the GABA_A/benzodiazepine receptor complex and 5-HT_{1A}/5-HT_{2A} receptors and exerts anxiolytic effects (**Amin et al., 2022; Machado et al., 2024**). Polyphenols, known for their potent antioxidant properties, are being investigated for their ability to reduce markers of oxidative stress and improve the activity of antioxidant enzymes, thereby helping to reduce stress and anxiety in rodents (**Pizarro Meléndez et al., 2024**). In this regard, rosmarinic acid, one of the main compounds in FAE, has shown significant anxiolytic

effects, increasing the time spent in the open arms of the EPM and improving exploratory behavior in treated animals (Mirza et al., 2021). In addition, a reduction in the time spent in the closed arms and an increase in the ratio of entries in the open arms were observed in rats treated with rosmarinic acid, reinforcing the hypothesis of its beneficial effect on anxiety (Vilmosh et al., 2022).

V.6. Sexual Incentive Motivation Test (SIM):

The results recorded for this test indicate that diabetic rats exhibited significantly increased trips to the male incentive zone (MIZ) and decreased visits to the female incentive zone (FIZ) ($P < 0.01$, $P < 0.001$, respectively) compared to control rats, indicating low sexual motivation. Compared to diabetic rats, treated diabetic rats with FAE had considerably fewer visits in the MIZ compartment and more in the FIZ compartment ($P < 0.001$, $P < 0.05$, respectively), indicating that FAE increased their sexual drive (Figure 26).

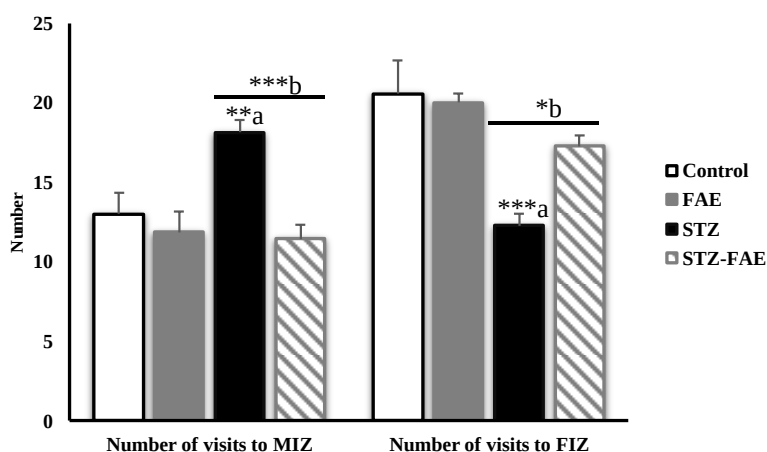


Figure 26: Number of visits to FIZ and MIZ zones of the different experimental groups. Each bar indicates the mean $\pm$ SEM ($n = 7$). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

In terms of time spent with incentives, results show that diabetic rats spend significantly less time in FIZ than control rats ($P < 0.01$) (Figure 27). Moreover, the statistical analysis of the preference score obtained in this test revealed that the diabetes group exhibited a considerably lower preference score ($P < 0.001$) compared to the control group. This indicates that the diabetic rats do not display sexual attraction towards the receptive female. Nevertheless, the treatment of diabetic rats with FAE led to a considerably ($P < 0.05$) higher preference score in comparison to untreated diabetic rats (Figure 28).

Part II: Experimental Study
Chapter V: Results and Discussion

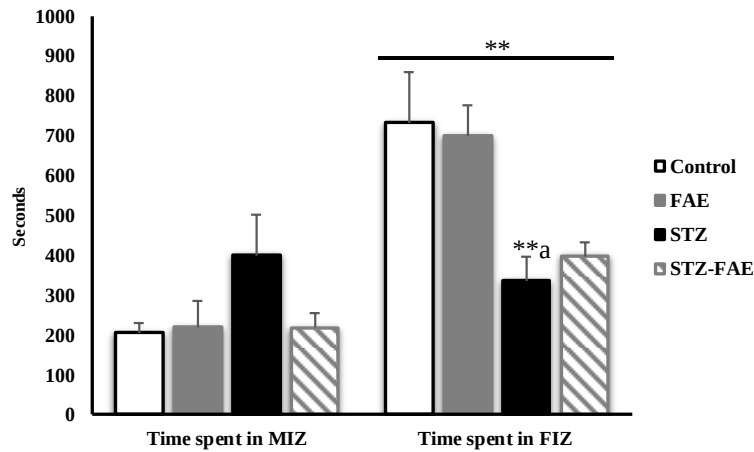


Figure 27: Time spent in seconds in the FIZ and MIZ zones of the different experimental groups. Each bar indicates the mean $\pm$ SEM (n= 7). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control.

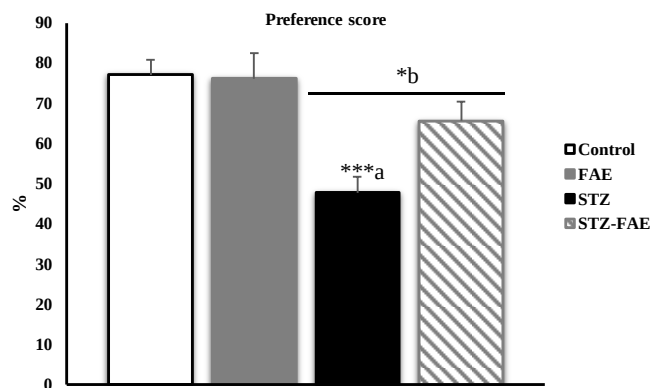


Figure 28: Preference score of the different experimental groups. Each bar indicates the mean $\pm$ SEM (n= 7). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Sexual behavior, including courtship and copulation, is primarily regulated by motivation, which is a critical psychological concept for understanding variation in responses to sexual stimuli. Motivation can be described as an internal drive that influences the intensity and direction of behavioral responses. Research by **Le Moëne et Ågmo (2019)** highlights that individuals with high sexual motivation show more pronounced responses to sexual stimuli, such as increased physiological arousal or engagement in sexual activity. Conversely, individuals with low sexual motivation may show minimal or no response to the same stimuli, reflecting lower drive or desire. These variations highlight the importance of motivation as a

determinant of sexual behavior, as it modulates the likelihood and intensity of engagement in sexual activity (**Ågmo et Laan, 2022**). In studies assessing sexual motivation, rats are exposed to both sexual and social stimuli. A male rat with high sexual motivation will typically show a preference for the sexually receptive female. A reduction in this preference is interpreted as a decline in sexual motivation (**Moreira et al., 2020**). Key indicators of motivation include the frequency of visits and the average duration spent near the stimulus. Greater time spent near a stimulus suggests higher attractiveness (**Chu et Ågmo, 2016**). To confirm an increase in sexual motivation, both the preference score and the time spent with the sexually receptive female must exhibit significant increases (**Chu et al., 2021**).

Our study found that diabetes has a detrimental impact on the sexual motivation of male rats by increasing the frequency of visits and extending the time spent in the MIZ, resulting in a decrease in the preference score. The effect of diabetes on sexual motivation in male rats is multifaceted. Studies have shown that receptive female rats stimulate male rats to release dopamine in the mesolimbic area, the medial preoptic area, the nigrostriatal system (**Dick et al., 2020**) and the medial amygdala (**Huijgens, Heijkoop, et al., 2021**). These areas promote the somatomotor component of copulation, increase motivation and focus it on sexual incentives (**Dick et al., 2020**). Therefore, diabetes could lead to impairment of the dopaminergic system in male rats and consequently their sexual motivation (**Sukhov et al., 2016**). To ensure that the effects of treatment on sexual motivation are considered functionally meaningful, it is important to record both an increase in the preference score and an increase in the time spent in the corresponding incentive zone. In fact, as the results of our study show, control-treated and diabetic-treated rats with *S. balansae* FAE at a dose of 200mg/kg bw displayed an increase in both the preference score and the time spent with the female incentive, which indicates that FAE boosts courtship behavior, resulting in sexual motivation.

V.7. Assessment of mating behavior:

During this test, when a rat is not copulating, no latency or post-ejaculatory interval can be recorded, in which case the time limit of the test is the value assigned to that rat (1800 seconds). In our study, this was the case for diabetic rats, whose sexual interaction with females differed significantly from that of control rats and diabetic rats treated with FAE.

Statistical analysis of the mating behavior test showed that the number and latency of mounts in the sexually active male rats with chronic hyperglycemia were significantly different from those in control rats. The number of mounts was significantly higher ($P < 0.01$), increasing

Part II: Experimental Study
Chapter V: Results and Discussion

from 08 mounts in control rats to 14 mounts in diabetic rats, and the latency of mounts was significantly higher in diabetic rats than in control rats ($P < 0.001$), increasing from 28 seconds in controls to 215 seconds in diabetic rats, indicating that diabetes caused intromission failure. On the other hand, we observed that administration of FAE to diabetic rats significantly reduced the number of mounts and mount latency compared to untreated diabetic rats ($P < 0.05$ and $P < 0.001$ respectively). Treatment with FAE then reduced the number of mounts from 14 in diabetic rats to 10 in treated diabetic rats and the latency from 215 seconds in diabetic rats to 51 seconds in diabetic rats to 51 seconds (**Figure 29**).

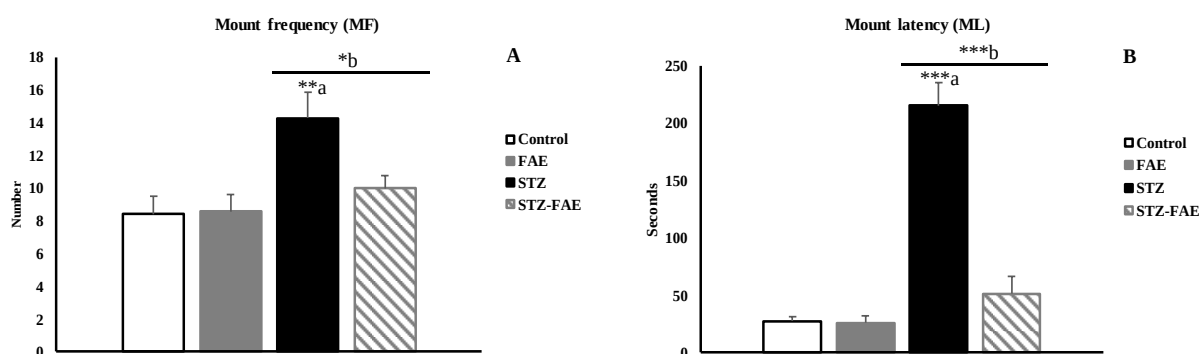


Figure 29: The mount frequency and latency expressed in seconds of the different experimental groups. Each bar indicates the mean $\pm$ SEM ($n = 7$). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Furthermore, the results obtained concerning the intromission frequency and latency measured during this test show that diabetic rats have a significantly lower intromission frequency ($P < 0.001$) compared with the control group of rats. We also note that the number of intromissions decreases from 30 intromissions in the control batch to 12 intromissions in the diabetic batch, and that the intromission latency of diabetic rats (1122 s) is significantly higher than that of control rats (38 s) and treated diabetic rats (173 s) ($P < 0.001$ and $P < 0.001$ respectively). This indicates low penile erectile potency of diabetic rats. However, statistical analysis revealed that during this test, the administration of FAE to the diabetic treated batch significantly increased the number of intromissions compared with the diabetic rats ($P < 0.001$), with the number increasing from 12 to 36 intromissions (**Figure 30**).

Part II: Experimental Study
Chapter V: Results and Discussion

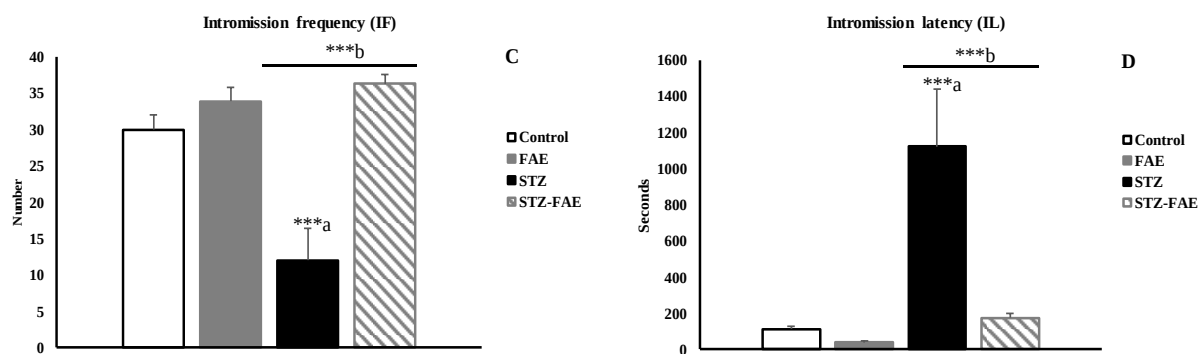


Figure 30: The intromission frequency and latency expressed in seconds of the different experimental groups. Each bar indicates the mean $\pm$ SEM (n=7). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

The results of the statistical analysis of ejaculation frequency and latency, which are indicators of copulatory performance, showed that diabetic rats differed from control rats. There was a highly significant decrease in the number of ejaculations ($P < 0.001$), with the latter decreasing from 2 in control rats to an almost complete absence of ejaculations in diabetic rats, reflecting a lower potency of penile erection required to induce ejaculation. For the ejaculation latency of diabetic rats (assay time limit: 1800 s) is significantly increased compared to that of control (660 s) and treated diabetic (480 s) rats ($P < 0.001$, $P < 0.001$, respectively). In addition, we note that the ejaculation frequency after FAE administration is significantly increased in treated diabetic rats compared to diabetic rats ($P < 0.001$), increasing from no ejaculations to 2 ejaculations. Similarly, control rats treated with FAE show a significantly increased ejaculation frequency compared to control rats ($P < 0.01$), increasing from 2 to almost 3 ejaculations in the treated diabetic batch, which may be justified by the aphrodisiac activity of FAE (**Figure 31**).

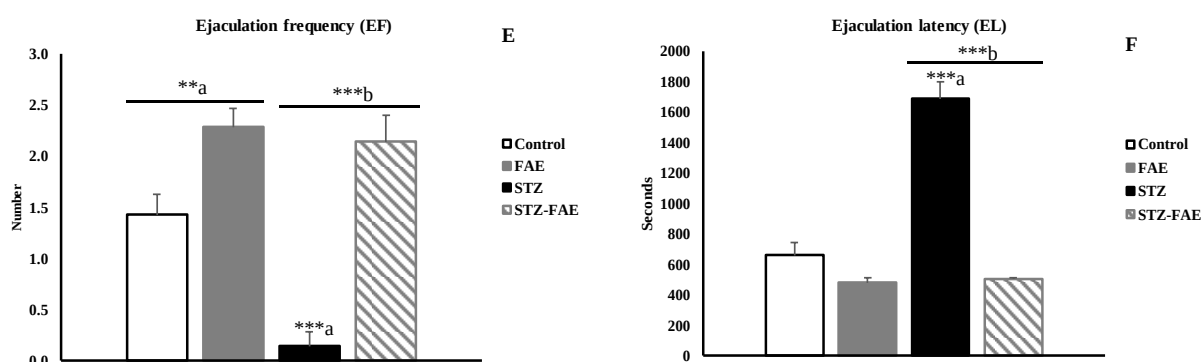


Figure 31: The ejaculation frequency and latency expressed in seconds of the different experimental groups. Each bar indicates the mean $\pm$ SEM (n= 7). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Part II: Experimental Study
Chapter V: Results and Discussion

The results for the post-ejaculation interval, a parameter used to assess the time between the first ejaculation and the next intromission, showed that there was a significant increase in that of diabetic rats compared to control and treated diabetic rats ($P < 0.001$, $P < 0.001$, respectively). This indicates that diabetic rats take a long time between the first ejaculation and the next intromission. Regarding the copulatory rate (copulatory efficiency index), there was a significant decrease ($P < 0.001$) for diabetic rats compared to control rats, indicating that diabetic rats had a decrease in copulatory speed, and there was also a significant increase ($P < 0.001$) for treated diabetic rats compared to diabetic rats (**Figure 32**).

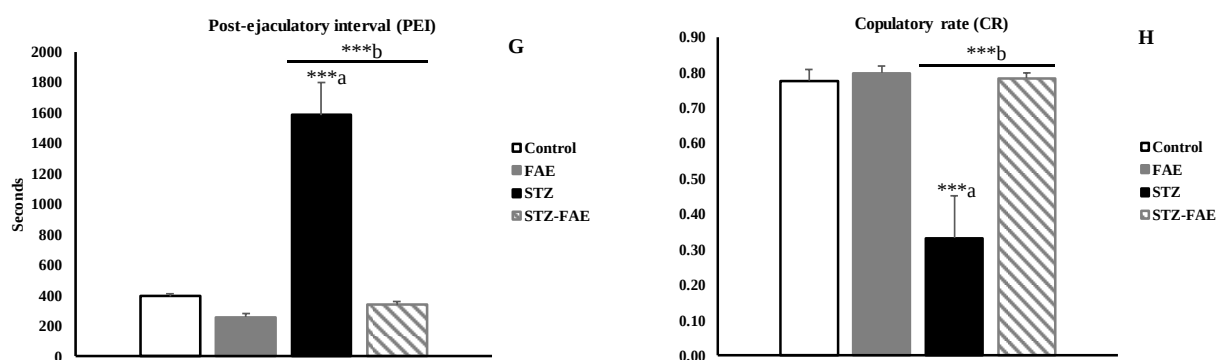


Figure 32: the post-ejaculation interval expressed in seconds and copulatory rate of the different experimental groups. Each bar indicates the mean $\pm$ SEM ($n = 7$). * $P \leq 0.05$ indicates a significant difference; ** $P < 0.01$; indicates a very significant difference; *** $P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Sexual behavior is a fundamental biological process essential for species survival and reproduction. It encompasses coordinated physiological and behavioral responses, including courtship, copulation, and post-copulatory behaviors, regulated by complex neuroendocrine and neurochemical systems (Heijkoop et al., 2017; Hull et Dominguez, 2015). In rodents, male sexual behavior is typically divided into three phases: precopulatory, copulatory, and post-ejaculatory (Heijkoop et al., 2017), each tightly regulated by hormonal and neurochemical signals, with testosterone playing a key role in maintaining sexual drive and behavior (Hull et Dominguez, 2015). The importance of studying sexual behavior lies not only in its reproductive function but also in its role as an indicator of overall health. Sexual dysfunction, whether caused by physiological conditions such as diabetes (Shi et al., 2017) or psychological factors, can significantly impact quality of life and emotional well-being (Saikia et al., 2024).

Our study confirms that diabetes disrupts copulatory behavior in male rats, significantly affecting ML, MF, IL, IF, EL, EF, and PEI. These impairments result from neuroendocrine imbalances, vascular dysfunction, oxidative stress, and hormonal disturbances involving

Part II: Experimental Study
Chapter V: Results and Discussion

testosterone, insulin, and kisspeptin signaling (**Shi et al., 2017**). The increased ML and higher MF observed in diabetic rats reflect impaired sexual arousal and difficulty in coordinating behavioral responses triggered by sexual stimuli. Our findings align with those of **Huang et al. (2022)**, who demonstrated that chronic hyperglycemia disrupts the dopaminergic system in the MPOA, thereby impairing sexual motivation and the initiation of copulatory behavior (**R. Huang et al., 2024; Minaz et al., 2019**).

Furthermore, the prolonged IL and reduced IF suggest significant penile erectile dysfunction, primarily caused by endothelial dysfunction and reduced nitric oxide (NO) synthesis, both critical for smooth muscle relaxation and penile vasodilation. These results are consistent with observations by **Olvera-Roldán et al. (2023)**. At the hormonal level, testosterone plays a central role in maintaining sexual motivation and erectile function via dopaminergic activation in the MPOA, but diabetic conditions lead to disruptions in the HPG axis), dysfunction of Leydig cells, and oxidative damage to testicular tissues collectively reduce testosterone synthesis (**Andlib et al., 2023; Sliwowska et al., 2024**). Furthermore, hyperglycemia and insulin resistance exacerbates this deficiency by impairing kisspeptin signaling, a key regulator of GnRH and LH secretion, ultimately reducing testosterone levels (**Sliwowska et al., 2024**). Kisspeptin, a neuropeptide encoded by the KISS1 gene, integrates metabolic signals, synchronizing reproductive function with energy status via pathways involving insulin and leptin (**Harter et al., 2018; Sliwowska et al., 2024**). Reduced kisspeptin expression and signaling in diabetes disrupt this integration, leading to diminished GnRH release, lower LH levels, and further testosterone decline (**Castellano et al., 2009**). Additionally, kisspeptin modulates dopaminergic activity in the MPOA, and its disruption reduces dopamine release, delaying sexual initiation and impairing sexual drive (**Iovino et al., 2019**).

The near absence of EF and significantly prolonged EL observed in diabetic rats suggest dysfunction in the spinal ejaculatory reflex arc and alterations in dopaminergic and serotonergic neurotransmission. These pathways regulate ejaculatory timing and sensitivity, and their impairment could result in delayed or absent ejaculation (**Andlib et al., 2023**). Studies by **Huijgens, Heijkoop, et al. (2021)** demonstrate that the MeA processes sensory feedback necessary for ejaculation, with disruptions leading to prolonged latency and reduced frequency. These impairments emphasize the role of the MeA-MPOA axis in coordinating copulatory performance (**R. Huang et al., 2024**). Additionally, the prolonged PEI observed in diabetic rats

reflects persistent central inhibition of dopaminergic pathways and disrupted neural signals required for resuming copulatory activity **(Huijgens, Guarraci, et al., 2021)**.

Furthermore, oxidative stress plays a significant role in these impairments, damaging testicular tissues, kisspeptin-expressing neurons, and vascular endothelial structures **(Castellano et al., 2009)**. Excessive production of ROS and reduced activity of antioxidant enzymes such as SOD and GPx aggravate neuronal and vascular damage, impairing both erectile function and ejaculatory mechanisms **(R. Huang et al., 2024; Olvera-Roldán et al., 2023)**. In summary, diabetes-induced sexual dysfunction arises from a complex interplay of hormonal imbalances, neuroendocrine disruptions, vascular dysfunction, and oxidative stress, leading to impairments across all phases of copulatory behavior.

The administration of *S. balansae* FAE to male diabetic rats resulted in an improvement in sexual interaction, as evidenced by a notable increase in the IF and EF, along with a notable reduction in MF, ML, IL, EL, and PEI, which collectively reinforce the notion that FAE possesses aphrodisiac properties. These improvements are attributed to the bioactive phytochemicals in the extract, including phenolic acids (e.g., rosmarinic acid, caffeic acid) and flavonoids (e.g., luteolin, kaempferol, cynaroside), which exert complementary effects by reducing oxidative stress, enhancing NO synthesis, and modulating neuroendocrine signaling.

Phenolic acids like Protocatechuic Acid act as potent antioxidants, reducing oxidative stress and restoring NO bioavailability via the Akt-eNOS pathway **(Festa et al., 2024)**, which is critical for endothelial function and smooth muscle relaxation, thereby improving erectile function and reducing IL and EL. Furthermore, caffeic acid and rosmarinic acid support NO synthesis **(Akomolafe, 2017; Noor et al., 2022)**. Higher NO levels can lead to an increase in dopamine in male rats when exposed to female rats, which boosts sexual desire (libido), potentially affecting motor performance and the initiation of sexual activity **(Septiani et al., 2021)**. Flavonoids such as luteolin and kaempferol contribute by mitigating oxidative damage to Leydig cells and supporting the HPG axis, thus preserving testosterone levels critical for sexual motivation and erectile responses **(Khafaji, 2023; Martin et Touaibia, 2020)**. Additionally, luteolin's role in enhancing antioxidant enzyme activities like SOD and GPx further protects against oxidative damage that disrupts reproductive signaling pathways **(B. Ma et al., 2019)**. Importantly, our findings align with the reported antioxidant and neuroprotective properties of phenolic compounds highlighted in other studies. *Salvia* species, rich in phenolic acids and flavonoids, have shown the ability to mitigate ROS and enhance enzymatic antioxidant defenses, thereby alleviating oxidative damage to critical neurovascular and

Part II: Experimental Study

Chapter V: Results and Discussion

hormonal pathways involved in sexual function (Zhumaliyeva et al., 2023). Researches on *Salvia miltiorrhiza* revealed an enhancement of SOD activity and reduced levels of ROS in the penile tissue of diabetic rats, thus improving erectile dysfunction (Y. Feng et al., 2022). This suggests a synergistic mechanism whereby *S. balansae* FAE targets oxidative stress, hormonal imbalances, and neuroendocrine disruptions, ultimately restoring normal copulatory behavior. These results underscore the therapeutic potential of *S. balansae* as a natural remedy for diabetes-induced sexual dysfunction, with its efficacy rooted in its ability to address the multifaceted impairments caused by diabetes.

V.8. Correlation analysis of blood glucose levels and sexual behavior parameters:

Statistical analysis indicates a strong negative linear correlation ($P < 0.05$) between the two variables, suggesting that as blood glucose levels decrease, IF and EF tend to increase ($r = -0.801$, $r = -0.755$, respectively), and vice versa. Meanwhile, a very strong positive correlation ($P < 0.05$) between blood glucose levels and MF, ML, IL, EL, and PEI was observed ($r = 0.666$, $r = 0.906$, $r = 0.792$, $r = 0.889$, $r = 0.841$, respectively). The high value of r suggests that as blood glucose levels decrease, MF, ML, IL, EL, and PEI tend to decrease as well and vice versa (Figure 33).

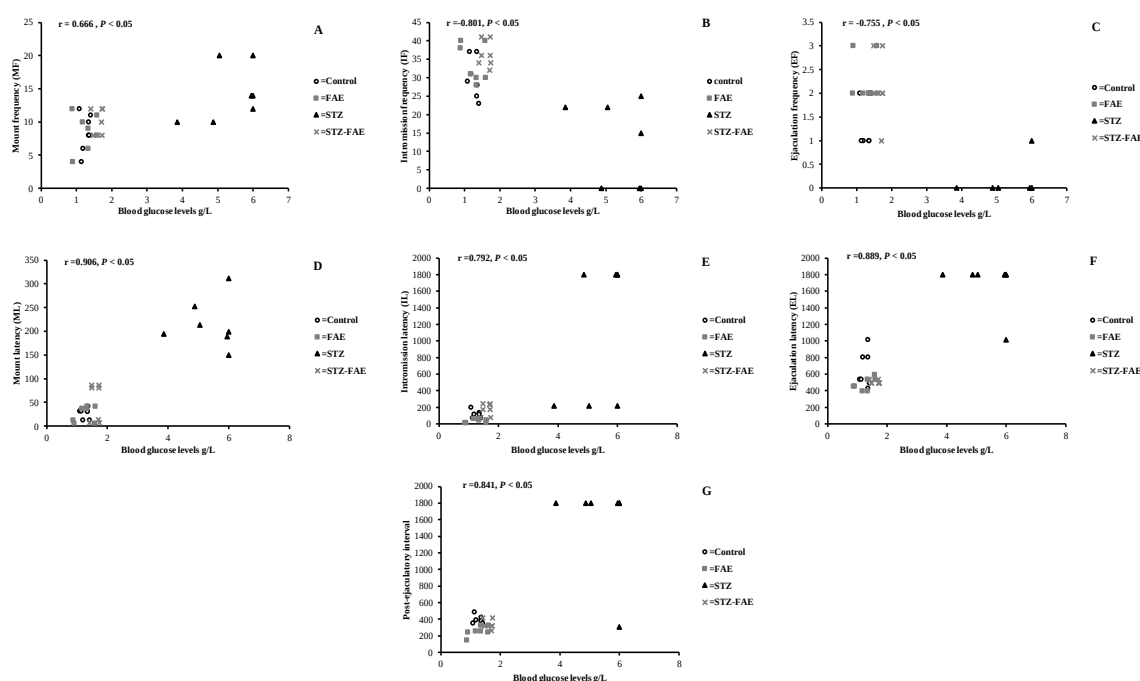


Figure 33: Linear correlation between blood glucose levels and sexual behavioral parameters in each group. **(A)** Mount frequency (MF); **(B)** Intromission frequency (IF); **(C)** Ejaculation frequency (EF); **(D)** Mount latency (ML); **(E)** Intromission latency (IL); **(F)** Ejaculation latency (EL); **(G)** Post-ejaculatory interval (PEI) in each group. $r=1$: positive correlation; $r=-1$: negative correlation; $r=0$: no linear correlation between variables.

Part II: Experimental Study
Chapter V: Results and Discussion

The results obtained enabled us to determine that *S. balansae* FAE has a beneficial impact on blood glucose levels and copulatory behavior impairments in STZ-induced diabetic rats, confirmed by a strong correlation between the amelioration of sexual function and the reduction of hyperglycemia, highlighting the dual therapeutic potential of our treatment.

V.9. Testosterone assay:

The figure 34 shows the testosterone concentration in the serum. The diabetic rats exhibited significant reductions ($P < 0.001$) in serum testosterone levels as compared to non-diabetic rats. Administration of FAE to the diabetic rats resulted in an important elevation ($P < 0.01$) in blood testosterone levels.

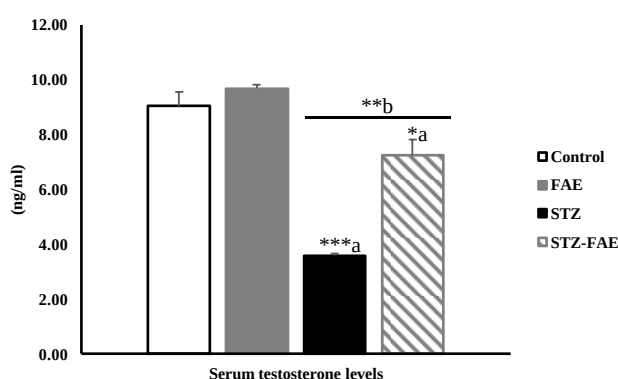


Figure 34: Testosterone serum level (ng/ml) of the different experimental groups. STZ, streptozotocin; FAE, aqueous flower extract. Each bar indicates the mean $\pm$ SEM ($n = 7$). $*P \leq 0.05$ indicates a significant difference; $**P < 0.01$; indicates a very significant difference; $***P < 0.001$ indicates a highly significant difference vs. **a** control; **b** STZ.

Our findings align with previous research on the relationship between reduced testosterone levels and decreased sexual performance in diabetic rats (Olvera-Roldán et al., 2023). The significant decrease in testosterone can be attributed to various factors, including the impact of excessive glucose on Leydig cell functionality and disrupted insulin signaling (Saeedan et al., 2021), which affects the HPG axis, in turn affecting testosterone secretion (Keyhanmanesh et al., 2018; Nna et al., 2017). Hyperglycemia plays a significant role in triggering systemic inflammation associated with diabetes. This inflammatory response can result in the release of inflammatory cytokines, which may exert a direct and independent influence on the HPG axis and testicular cells. As a consequence, chronic inflammation can lead to endocrine disruptions within the testes and impair male reproductive function (R. Huang et al., 2024). Interleukin-1 beta (IL-1 β) and C-C motif chemokine ligand 2 (CCL2) are known to impede androgen synthesis by inhibiting the activity of steroidogenic enzymes. This interference ultimately

triggers apoptosis in Leydig cells (**Jiang et al., 2020**). Additionally, it affects the vascular plexus of the corpora cavernosa, which can result in erectile dysfunction and, consequently, a reduction in reproductive function (**Oghbaei et al., 2021**).

Flavonoids, particularly luteolin and apigenin, have shown promise in enhancing androgen levels and testosterone production in Leydig cells (**Martin & Touaibia, 2020**). The administration of FAE, rich in antioxidant compounds, resulted in increased serum testosterone levels in diabetic-treated rats, potentially due to its ability to mitigate oxidative stress and stimulate the HPG axis (**Wankeu-Nya et al., 2019**). The core structure of flavonoids is similar to that of steroids and cholesterol. Such structural similarity allows flavonoids to interact with androgen receptors, including those in Sertoli cells, and enzymes of steroidogenesis, thus stimulating androgen production in Leydig cells and promoting testicular function recovery (**Laoung-On et al., 2021; Martin et Touaibia, 2020**). Flavonoids exert anti-inflammatory effects through multiple mechanisms, including regulating the production of cytokines by reducing the expression of inflammatory cytokines, including the IL-1 β , known to inhibit androgen synthesis (**Al-Khayri et al., 2022**). Moreover, flavonoids have been demonstrated to induce relaxation of the corpora cavernosa, promoting blood flow to the penis and facilitating erection (**Wankeu-Nya et al., 2014; Yaya et al., 2023**). Luteolin increases the expression of steroidogenic acute regulatory protein (StAR) in Leydig cells, crucial for cholesterol transport and testosterone production (**Martin et Touaibia, 2020**). These bioactive compounds also help maintain appropriate levels of reproductive hormones like testosterone, FSH, and LH (**Khafaji, 2023**), further elucidating the mechanisms behind the observed improvements in reproductive function and sexual performance.

V.10. Sperm quality assessment:

The data about the quality of sperm for all groups are presented in **table 08**. The presence of long-term high glucose levels demonstrated a notable decrease in the overall quality of sperm in the diabetic group as compared to the control group. The diabetic group that received FAE treatment exhibited a notable improvement in sperm quality when compared to the diabetic group without treatment.

Our results reveal a highly significant decrease in sperm count in the group of diabetic rats with a value of $7.2 \times 10^6/\text{ml}$ ($P < 0.001$) compared to control rats with a value of $17.4 \times 10^6/\text{ml}$, however treatment with FAE allowed us to observe a significant increase in sperm count in diabetic rats up to $17.1 \times 10^6/\text{ml}$.

Part II: Experimental Study
Chapter V: Results and Discussion

Whereas, the results of the percentage of sperm abnormalities indicate a significant increase ($P < 0.001$) in the number of abnormal spermatozoa in the diabetic rats with a percentage of 62.3 compared to that of the control and diabetic treated rat with FAE with values of 25.3% and 35.07% respectively.

Similarly, the results for sperm viability percentage showed that the diabetic group has the lowest values (17.8%) ($P < 0.001$) compared with the other experimental groups. As for evaluation of the spermatoc motility percentage, results showed very low, highly significant percentages ($P < 0.001$) in the diabetic group (19.61%) compared with the control, and diabetic treated groups with FAE (60.01% and 45.9%, respectively).

Table 08: Epididymis sperm parameters of the different experimental groups.

Parameters Groups	Sperm count (10 ⁶ /ml)	Morphology (abnormal %)	Viability (%)	Motility (%)
C	17.4±1.39	25.3±4.25	78.0±2.00	60.01±3.00
FAE	19.9±2.07	25.7±3.00	79.3±2.00	56.3±0.75
STZ	7.2±1.01***a	62.3±2.75***a	17.8±7.75***a	19.61±1.12***a
STZ-FAE	17.1±1.47**b	35.07±2.00***b**a	49.2±0.75***b***a	45.9±1.12***b***a

Values are expressed as Mean ± SEM of seven animals in each group.

A column with a different letter means significant at $P < 0.05$.

a: Significantly different from the values of the negative control rats (C).

b: Significantly different from the values of the diabetic control rats (STZ).

Figure 35 presents microscopic observations of sperm morphology ($G \times 400$) from diabetic rats, illustrating various diabetes-induced abnormalities in sperm structure. While image A shows a normal spermatozoon as a control reference, images B through I demonstrate the characteristic sperm defects commonly observed in diabetic conditions. These diabetes-related abnormalities include spermatozoa with twisted tails (B), angled spermatozoon (C), bent-tail formations (D), headless structures (E), coiled morphology (F), amputated flagella (G), midpiece abnormalities (H), and multiple structural defects (I).

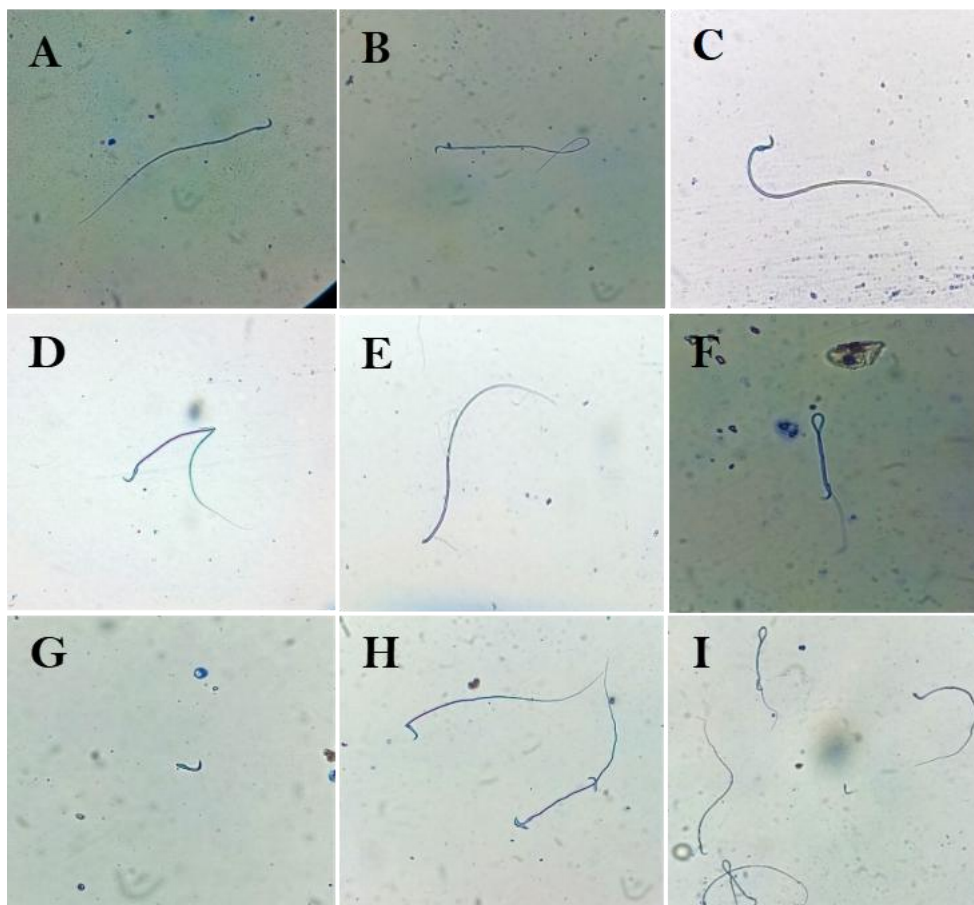


Figure 35: Microscopic observation of sperm morphology magnification ($G \times 400$) **A:** normal spermatozoon, **B:** spermatozoon with twisted tail, **C:** angled spermatozoon, **D:** bent-tail spermatozoon, **E:** headless spermatozoon, **F:** coiled spermatozoon, **G:** spermatozoon with amputated flagellum, **H:** midpiece abnormalities, **I:** abnormal spermatozoon.

Figure 36 illustrates microscopic observations of sperm viability assessment at $G \times 100$ magnification. The micrograph distinctly captures two spermatozoa with contrasting viability status: one spermatozoon appears pink due to the uptake of stain, definitively indicating cell death, while the other spermatozoon remains unstained and transparent, confirming its viability.



Figure 36: Microscopic observation of a sperm smear showing a pink-stained spermatozoon (dead) and a colorless spermatozoon (alive) $G \times 1000$.

Part II: Experimental Study
Chapter V: Results and Discussion

In the present study, sperm samples from diabetic rats showed a decrease in sperm count, viability and motility, along with an increased percentage of abnormalities including coiled tails and headless sperm. Our results are consistent with previous studies explaining how diabetes causes severe impairment in sperm through the generation of oxidative stress (**Omolaoye et Du Plessis, 2021**). Chronic high blood glucose levels cause the overproduction of ROS, resulting in structural damage to sperm cells by damaging the cell membrane, attacking lipids and proteins, impairing mitochondrial function, and decreasing energy production required for motility (**R. Huang et al., 2024; Minas et al., 2024**). Research indicates that the interaction between inflammation and diabetes exacerbates the effects of both conditions on the male reproductive system through the release of inflammatory cytokines (**Jiang et al., 2020**). Tumor necrosis factor-alpha (TNF- α) triggers the production of NO, which is essential for maintaining inflammation. This can directly and indirectly affect sperm production, damage sperm membranes, and reduce semen quality (**R. Huang et al., 2024**). Additionally, these high glucose levels induce epigenetic changes such as DNA methylation and histone modifications (**Minas et al., 2024**). **Xu et al. (2023)** showed that diabetes causes an imbalance in cell processes such as autophagy in testicular cells, leading to testicular atrophy and infertility. Furthermore, studies suggest that AGEs and their receptor RAGE increase oxidative stress, which in turn impairs sperm function (**R. Huang et al., 2024**). AGEs are formed through the covalent attachment of excess glucose to proteins; under diabetic conditions, AGEs accumulate, inducing further ROS production, DNA fragmentation, initiating cell death, and leading to further reduction in sperm count and motility (**R. Huang et al., 2024; Omolaoye et Du Plessis, 2021**).

Diabetes has significant negative effects on male fertility, but various therapeutic approaches, particularly those targeting antioxidant and anti-inflammatory mechanisms, have been shown to improve sperm quality. *S. balansae* flower extract, rich in bioactive compounds like flavonoids and phenolic acids, offers promising therapeutic potential. Besides the positive effects of *S. balansae* extract on sex hormone regulation, this plant has the potential to enhance sperm quality in diabetic-treated rats. Based on these results, it is evident that the improvement in sperm quality may be attributed to the antioxidant and anti-inflammatory properties. These effects could reduce oxidative stress in testicular tissue and decrease the release of inflammatory cytokines, ultimately leading to increased serum testosterone levels and improved sperm quality, as previously discussed (**Al-Khayri et al., 2022; Laoung-On et al., 2021; Mahanem et al., 2024**). Notable compounds such as cynaroside, rosmarinic acid, and luteolin exhibit potent antioxidant and anti-inflammatory effects, which are essential in reducing

oxidative stress in testicular tissue and improving sperm quality in diabetic rats (**Adomako-Bonsu et al., 2017; Khenifi et al., 2023; B. Ma et al., 2019**). These compounds neutralize ROS, protecting sperm cells from damage and enhancing sperm motility, viability, and morphology (**Emil et al., 2024; Palanichamy et al., 2022**). Studies have shown that rosmarinic acid activates adenosine monophosphate protein kinase (AMPK) in cells, a key signal kinase in sperm, and an essential link between metabolic energy and sperm fertilization ability. It alleviated endothelial dysfunction induced by oxidative stress by activating the AMPK/eNOS pathway (**T. Y. Feng et al., 2020**). According to **B. Ma et al. (2019)**, luteolin activates the Nrf2 signaling pathway, enhancing the expression of antioxidant enzymes like SOD and catalase, which protect cells from oxidative stress and support Sertoli cell function, essential for sperm maturation and fertility. **Alchalabi et al. (2016)** found that *Salvia officinalis* causes a significant elevation in sperm activity along with a significant reduction in sperm mortality and abnormalities in diabetic male albino rats, while **Basal et al. (2023)** have demonstrated that *Salvia officinalis* protects sperm cells from DNA damage through the elevation of glutathione peroxidase activity and reduction of MDA.

V.11. Histopathological Investigation:

Testicular tissues can present direct or indirect pathological responses (often hormonal). Histological evaluation of testicular tissue revealed significant structural changes in the groups studied.

Observation of the histological slides of the control group testes (**Figure 37 A, and B**) and those of the FAE-treated group (**Figure 37 C, and D**) shows a normal appearance, with tight seminiferous tubules (ST) with a lumen-filled with spermatozoa (Sz), as well as a cellular succession representing a stratified epithelium composed of cells from the different stages of spermatogenesis, which progresses in a centripetal manner from the wall of the tubules (basal lamina) towards its tubular lumen, demonstrating the normal evolution of spermatogenesis and spermiogenesis. At the level of the basal lamina, the spermatogonia (Sg) (stem cells) are easily visible, recognizable by their small size and small, dark-colored nuclei, followed by the larger spermatocytes (Sc), whose nuclei are voluminous and sometimes mitotic. The smaller spermatids (Sd) are located towards the inside of the seminiferous tubules. These elongate and differentiate during spermiogenesis to become spermatozoa, which detach during spermiation and are released into the lumen of the seminiferous tubule. These germ cells are held together by somatic support cells (Sertoli cells) whose nuclei can be seen close to the spermatogonia. The interstitial space (IS), which is narrow due to the proximity of the seminiferous tubules, is

Part II: Experimental Study
Chapter V: Results and Discussion

richly vascularised and composed of androgen-producing interstitial cells (Leydig cells). These are typical of a functional testicle without pathological changes confirming the safety of the treatment with FAE in the treated control group.

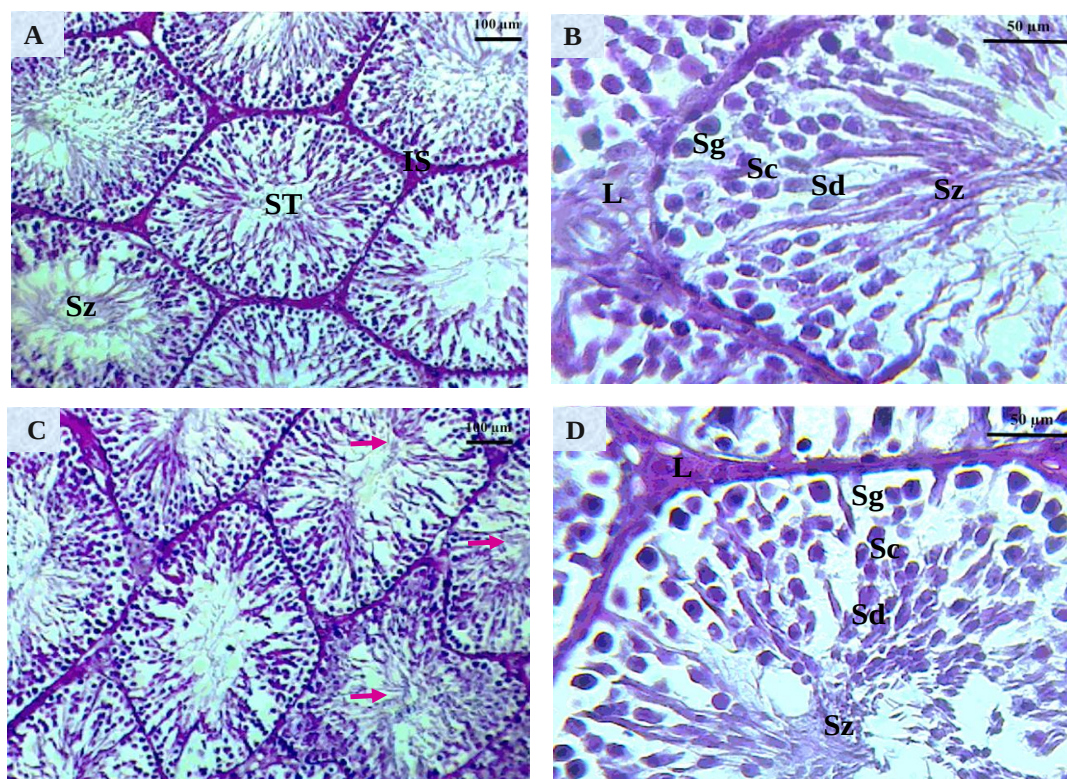


Figure 37: Microscopic observation of the histological structure of the testes from the control and FAE groups. H&E staining. **A** and **C** ($G \times 100$) & **B** and **D** ($G \times 400$).

A & C: show normal architecture of well-organised seminiferous tubules (ST), high cell density, spermatozoa (Sz) in the tubular lumen (pink arrow), and intact Leydig cells (L). **B & D:** the germinal epithelium shows a normal progression of spermatogenesis from spermatogonia (Sg) to spermatozoa (Spz) via spermatocytes (Sc) and spermatids (Sd).

On the other hand, histological sections of diabetic rat testes (**Figure 38 A and C**) show multiple abnormalities at different stages of spermatogenesis. Among the abnormalities noted are: the tubules appear dilated with a large interstitial space, suggesting a decrease in cell density, possibly due to loss of germ cells (pink arrow), and a reduction of spermatozoa in the seminiferous tubules and the presence of cellular debris (green arrow). **Figure 38 B and D** also show a decrease in the number of germ cells in the seminiferous tubules (blue arrow) and the absence of certain cell layers necessary for spermatogenesis (green arrow). We also observed the presence of cells in apoptosis (yellow arrow) and nuclear condensation, nuclear pycnosis, and chromatid fragmentation, which may be a sign of apoptosis (red arrow), and cytoplasmic vacuolation of Leydig cells (black arrow).

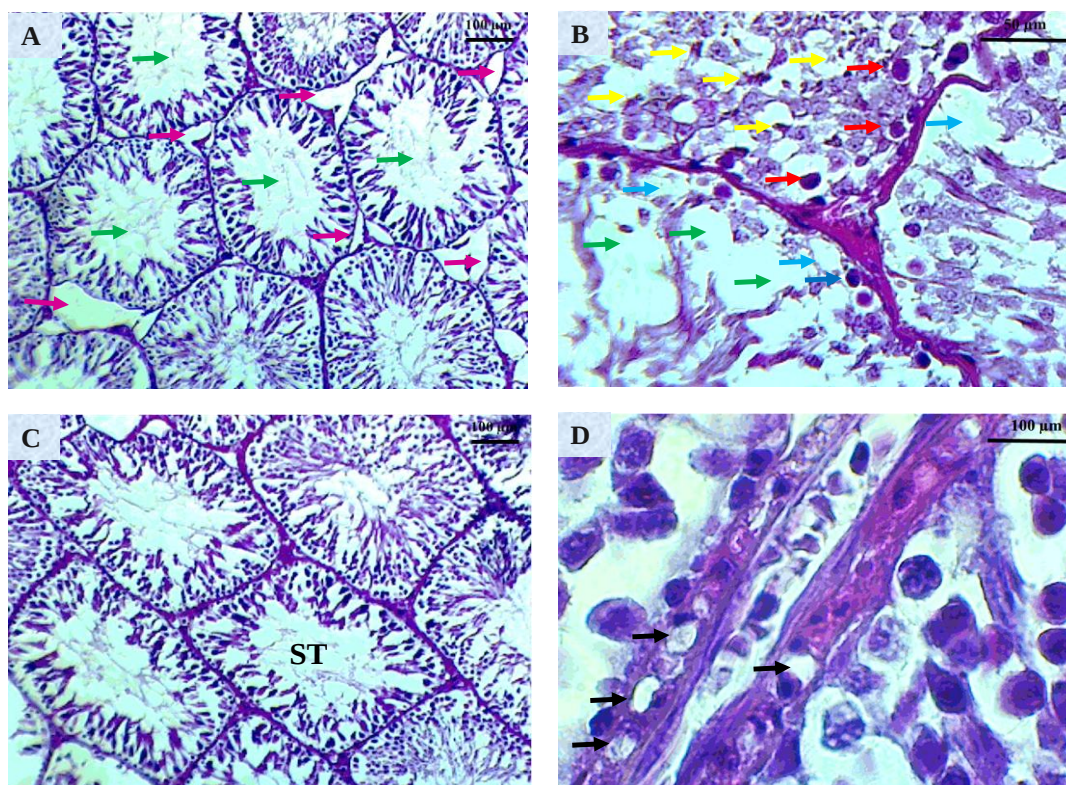


Figure 38: Microscopic observation of the histological structure of the testes from the diabetic group. H&E staining. **A** and **C** (G $\times$ 100), **B** (G $\times$ 400), and **D** (G $\times$ 1000).

A & C: show dilatation of seminiferous tubules with a large interstitial space (pink arrow) and a decrease in sperm in the seminiferous tubules (green arrow). **B & D:** Germ line depletion (blue arrow), impaired spermatogenesis (green arrow), apoptotic cells (yellow arrow), nuclear condensation and pycnosis (red arrow), and cytoplasmic vacuolisation of Leydig cells (black arrow).

However, histological analysis of testes from FAE-treated diabetic rats (**Figure 39 A, and B**) revealed a significant attenuation of most of the previously reported abnormalities, namely: reduced interstitial spaces (red arrow) with normal-sized seminiferous tubules with restored germ cell layers and sperm visible in the lumen (pink arrow), indicating that spermatogenesis was well underway. Leydig cells appeared structurally normal, and apoptotic markers were significantly reduced.

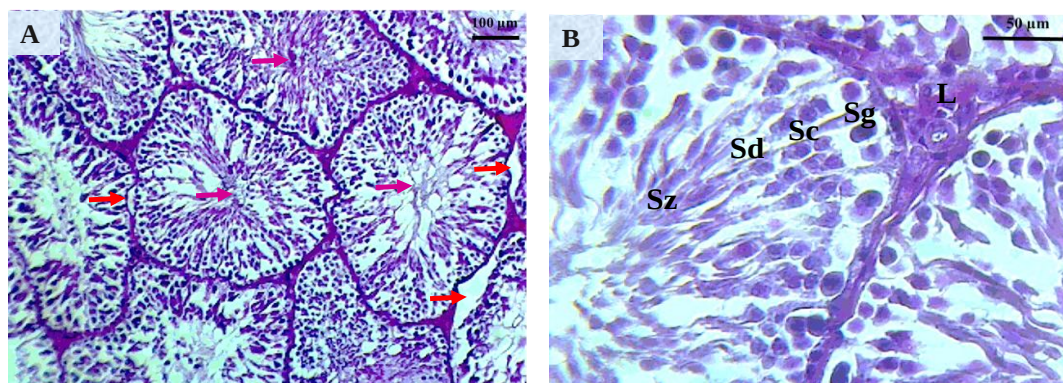


Figure 39: Microscopic observation of the histological structure of the testes from the diabetic treated with FAE group. H&E staining. **A** (G ×100), **B** (G×400).

A & B: show a high cellular density, Presence of spermatozoa in the lumen of the seminiferous tubules, and no signs of pycnosis or fragmentation, suggesting an absence of apoptotic processes.

Diabetes-induced testicular damage disrupts male reproductive health by affecting both sperm quality and the testicular microenvironment. The testes play a key role in testosterone secretion and sperm production, with indicators such as testicular weight, hormone levels, sperm count, motility, and viability serving as essential measures of reproductive function (**Zheng et al., 2024**). Diabetes can directly affect sperm quality as evidenced by our findings on sperm quality; at the same time, it damages the supportive microenvironment by affecting spermatogonia and essential cells such as Sertoli and Leydig cells (**R. Huang et al., 2024**). These effects are confirmed by histopathological analysis, which shows significant structural changes in diabetic testicular tissue (**Emil et al., 2024**).

Histopathological analysis of the testes in diabetic rats revealed significant changes affecting both the seminiferous tubules and the interstitial space. In contrast to the control group and the diabetic-treated group with FAE, where the seminiferous tubules appeared well organized and contained numerous spermatozoa, the testes of diabetic rats exhibited a marked depletion of the germinal epithelial layers. This was characterized by a loss of sperm in the lumen, the presence of cellular debris, apoptotic and exfoliated cells, as well as vacuoles and enlarged intercellular spaces. These observations are indicative of impaired spermatogenesis, similar to findings reported by **El Helew et al. (2024)**, **El Banna et al. (2023)**, and **Zheng et al. (2024)**. These lesions are a consequence of diabetes development and are unrelated to the toxic effects of STZ. STZ, a glucose analog, specifically accumulates in pancreatic beta cells through glucose transport protein 2 (GLUT2) and damages DNA via its alkylating activity. It

is not recognized by glucose transport protein 1 (GLUT1), which is present in other tissues, including the testes (**Ghasemi and Jeddi, 2023**).

The alterations observed are associated with excessive germ cell apoptosis, characterised by chromatin condensation and nuclear pycnosis as well as irregular and deformed nuclei in germ and Leydig cells (**El Helew et al., 2024**). These changes, reported by **El Helew et al. (2024)** and **Gaderpour et al. (2020)** are linked to oxidative stress-induced DNA damage and chromatin condensation in apoptotic and necrotic cells. In addition, the enlargement of the interstitial space, accompanied by vacuolation of Leydig cells, suggests an alteration in hormone production, in particular a drop in testosterone, essential for spermatogenesis (**Nna et al., 2020**). Oxidative stress, which results from an excess of free radicals generated during the auto-oxidation of glucose in diabetes, is a critical factor contributing to cellular damage. This phenomenon adversely affects the viability of spermatozoa, as their cell membranes are comprised of unsaturated fatty acids. Furthermore, oxidative stress accelerates the apoptosis of testicular cells by causing damage to their DNA structure (**Nna et al., 2020**). These abnormalities, particularly excessive apoptosis in cells, are associated with a reduction in the activity of vital antioxidant enzymes, including SOD, catalase, and GPx (**Nna et al., 2020**). This disruption of the testicular microenvironment is worsened by increased levels of MicroRNA 34a (miR-34a), which inhibits Silent mating type Information Regulator Two homolog 1 (SIRT1), a key regulator of cell survival, thus promoting p53-mediated apoptosis (the genome guardian) (**Gaderpour et al., 2020**).

Hyperglycemia caused by diabetes results in the excessive production of ROS, cytokines, growth factors, and adhesion molecules. This process activates the transcription factor NF- κ B, which is known to regulate inflammatory gene expression (**Nna et al., 2020**). This activation results in elevated levels of TNF- α , IL-1 β , and IL-6, leading to dysfunction in various organs, including the testes and adnexal glands (**ElBanna et al., 2023**). In addition, excess ROS stimulates the formation of NLRP3 inflammasomes, promoting the maturation of IL-1 β , IL-18 and IL-6, leading to caspase-1 activation and sperm cell apoptosis, contributing to male reproductive dysfunction (**Zheng et al., 2024**). Chronic inflammation associated with metabolic syndrome (MetS) leads to increased CCL2 levels, which inhibit androgen synthesis by suppressing steroidogenic enzyme activity and inducing apoptosis in Leydig cells (**Jiang et al., 2020**). This interconnection between oxidative stress and chronic inflammation creates a vicious circle that exacerbates testicular damage (**Nna et al., 2020**). Overall, diabetes

Part II: Experimental Study
Chapter V: Results and Discussion

profoundly affects testicular histology by impairing both spermatogenesis and hormone production, highlighting the importance of early treatment to prevent male infertility.

Numerous studies have demonstrated that anti-inflammatory agents, including various herbs, can enhance reproductive function. These substances achieve this by diminishing inflammation, reducing oxidative stress, and promoting increased testosterone production **(Nasiri et al., 2021)**. The findings of our study demonstrate that the administration of the aqueous extract of *Salvia balansae* flowers to diabetic rats resulted in significant regeneration of the majority of seminiferous tubules. Moreover, this treatment facilitated substantial advancement in spermatogenesis, as indicated by the presence of spermatozoa within the lumina of the seminiferous tubules and reduced apoptotic markers. In addition, the FAE improved the interstitial environment's balance, thereby attenuating the deleterious effects on Leydig cells, which play a crucial role in testosterone synthesis. The ability of our treatment with FAE to reduce ROS and modulate several inflammatory pathways, which are key factors in testicular recovery, is attributed to the phytochemical content of the extract, particularly phenolic compounds and flavonoids.

Research by **Basal et al. (2023)** and **Khodadadi et al. (2024)** has shown comparable results, indicating that the administration of *Salvia officinalis* leads to significant improvements in testicular histopathology in rats exposed to cadmium chloride. The bioactive constituents of *Salvia officinalis* extract demonstrate considerable antioxidant and radical-neutralizing activities. The predominant phenolic component of sage is rosmarinic acid **(Yilmaz and Cakir, 2024)**, which has been consistently identified in various studies and is present in high levels in our extract of *Salvia balansae*. This compound is known for its antioxidant and anti-inflammatory properties **(Albalushi et al., 2023)**. **Davoodi et al. (2020)** demonstrated that *Salvia miltiorrhiza* is a powerful antioxidant that can help reduce oxidative stress, improve sperm parameters, and minimize testicular tissue damage. In a subsequent study, they found that rosmarinic acid specifically reduced biomarkers of oxidative stress, enhanced sperm parameters, and decreased tissue damage caused by ischemia/reperfusion **(Raisi et al., 2022)**. **Al-khawaldeh et al. (2024)** investigated the effects of rosmarinic acid on testicular oxidative damage and sperm quality in STZ-induced diabetic mice. The treatment improved sperm quality, enhanced antioxidant defenses (increased SOD, CAT, GSH, and reduced lipid peroxidation). Additionally, the treatment inhibited oxidative tissue damage, increased the number of spermatogenic cells, and improved the structure of seminiferous tubules.

Part II: Experimental Study
Chapter V: Results and Discussion

Furthermore, it protected DNA fragmentation during late apoptosis, likely by preventing oxidative damage to DNA and promoting repair mechanisms **(Al-khawaldeh et al., 2024)**.

Studies has demonstrated that extracts of *S. officinalis* exhibit significant anti-inflammatory effects by inhibiting the expression of TNF- α , IL-6, and IL-1 β **(Zhumaliyeva et al., 2023)**. These extracts reduce pro-inflammatory cytokine expression in diabetes. Flavonoids have strong anti-inflammatory properties, influenced by their planar ring structure, C2–C3 unsaturation, and hydroxyl groups, especially at the B-ring's 3' and 4' positions**(Al-Khayri et al., 2022)**. Our FAE revealed the presence of flavonoids such as kaempferol, luteolin, and apigenin. Kaempferol is known to inhibit the NF- κ B signaling pathway, resulting in a significant reduction in the levels of cytokines such as IL-6, IL-1 β , TNF- α , and IL-8. Additionally, it decreases the expression of pro-inflammatory enzymes, including cyclooxygenase-2 (COX-2), iNOS, and lipoxygenase **(Salaritabar et al., 2017)**. Kaempferol has been shown to significantly reduce NO levels in the testicular environment. This effect is attributed to its ability to scavenge free radicals, facilitated by its notable antioxidant properties **(Khafaji, 2023)**. Luteolin, present in FAE, a flavone, has gained interest for its potential to enhance androgen production **(Al-Khayri et al., 2022; Martin and Touaibia, 2020)**. Due to its chemical structure similar to cholesterol and other steroids, luteolin may influence androgen production in Leydig cells by activating StAR expression, leading to increased testosterone synthesis **(Martin and Touaibia, 2020)**. Additionally, luteolin prevented blood-testis barrier (BTB) disruption and testicular damage by reducing ROS accumulation, inhibiting apoptosis, recovering BTB integrity, and repairing spermatogenesis **(B. Ma et al., 2019)**.

In conclusion, the phytochemicals present in our extract show promising potential in treating testicular injury and spermatogenesis disturbances caused by diabetes. Their antioxidant and anti-inflammatory properties, along with their ability to enhance androgen production, protect testicular structures, and promote spermatogenesis, suggest that these natural compounds could be effective in mitigating diabetes-induced reproductive issues.

Conclusion and prospects

In conclusion, diabetes mellitus represents a considerable global health challenge, significantly affecting male reproductive health through disruptions in sexual behavior, hormonal balance, and testicular function. Given that conventional treatments frequently fall short due to undesirable side effects, the investigation of herbal remedies like *S. balansae* emerges as a promising alternative. This research aimed to evaluate the effects of diabetes on sexual behavior and both endocrine and exocrine testicular function in male Wistar rats. Additionally, the study sought to demonstrate the effectiveness of flower aqueous extract of *S. balansae* (FAE) in alleviating the damage caused by this disease.

The results of the plant study indicate that the FAE of *S. balansae* possesses notable biochemical and pharmacological properties. Analysis has revealed a high concentration of polyphenols and flavonoids, which demonstrate its antioxidant potential. Thirteen bioactive compounds were identified and quantified using UHPLC-ESI-MS/MS analysis. Antioxidant assays, including DPPH, ABTS, and FRAP tests, confirmed the extract's significant antioxidant activity. Furthermore, treatment with the FAE showed a greater reduction in inflammation compared to diclofenac, highlighting its anti-inflammatory potential. Lastly, the acute toxicity study displayed a promising safety profile for the extract, as no lethality was observed in rats, indicating that the extract is safe for oral administration.

For the *in vivo* part, the study in STZ-induced diabetic Wistar rats showed that the administration of FAE significantly attenuated the deleterious effects of diabetes. The extract reduced serum glucose levels, improved body weight gain, and testicular weight. It also showed a potential anxiolytic effect, modulating anxiety-related behaviors observed in diabetic rats.

In terms of reproductive function, FAE treatment improved motivation and sexual performance in diabetic rats, as evidenced by an increase in mating frequencies and a reduction in latencies. Treatment also restored serum testosterone levels and improved sperm quality by increasing sperm count, viability, and motility while reducing morphological abnormalities.

Histopathological analysis confirmed these observations, showing an improvement in seminiferous tubule integrity and germ cell organization following the administration of FAE.

The results presented herein offer valuable insights into the potential role of *S. balansae* flowers in alleviating the metabolic and reproductive disorders associated with diabetes. These findings suggest a pathway for more extensive research in this area. It would be advisable to establish and pursue a detailed experimental protocol that explores various dimensions:

- Reinforcing the study with new diagnostic approaches, such as molecular biology and immunohistochemistry techniques, to target the regions affected by diabetes at the cellular and molecular levels.
- Assessing the disruption caused by diabetes to the cell's equilibrium and its membrane composition and integrity by measuring oxidative stress parameters, and exploring the effects of *S. balansae* in restoring this equilibrium.
- Testing new therapeutic approaches to increase the bioavailability and possibly the efficacy of bioactive compounds using encapsulation in liposome form or using nanotechnology techniques, to allow the passage of these active compounds through physiological barriers such as the blood-brain and blood-testis barriers.
- Exploring the specific interactions between the extract's bioactive compounds and their potential molecular targets, using molecular docking simulations, to provide a better understanding of the mechanisms of action involved.

References

- Abdelhalim, A., Abe, M., Akihisa, T., Anderson, L., Bassi, E. J., Bhatt, V., Boeno, S. I. da S., & Braz-Filho, R. (2021). Studies in Natural Products Chemistry. In F. Atta-Ur-Rahman (Ed.), *Studies in Natural Products Chemistry* (Vol. 29). [https://doi.org/10.1016/S1572-5995\(03\)80020-0](https://doi.org/10.1016/S1572-5995(03)80020-0)
- Abdelli, W. (2017). *Caractérisation chimique et étude de quelques activités biologiques des huiles essentielles de Juniperus phoenicea et de Thymus vulgaris*.
- Abdul-Razzaq, L. N. (2023). A Scoping Review of Male infertility Risk factors and Potential Causes. *University of Thi-Qar Journal of Science*, 10(1). <https://doi.org/10.32792/utq/utjsci/v10i1.932>
- Abreu, A. P., & Kaiser, U. B. (2016). Pubertal development and regulation. *The Lancet Diabetes and Endocrinology*, 4(3), 254–264. [https://doi.org/10.1016/S2213-8587\(15\)00418-0](https://doi.org/10.1016/S2213-8587(15)00418-0)
- Acevedo-Rodriguez, A., Kauffman, A. S., Cherrington, B. D., Borges, C. S., Roepke, T. A., & Laconi, M. (2018). Emerging insights into hypothalamic-pituitary-gonadal axis regulation and interaction with stress signalling. *Journal of Neuroendocrinology*, 30(10), 0–3. <https://doi.org/10.1111/jne.12590>
- Achir, M., Benserida, Y., Senhadji, A. A. zohor, Taibi, W., & Gaouar, S. B. S. (2021). Identification and characterization of Common Sage in the wilaya of Tlemcen. *Genetics and Biodiversity Journal Gabj*, 5(2), 12–24. <https://doi.org/10.46325/gabj.v5i2.63>
- Adedara, I. A., Awogbindin, I. O., Anamelechi, J. P., & Farombi, E. O. (2015). Garcinia kola seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*, 53(5), 695–704. <https://doi.org/10.3109/13880209.2014.937504>
- Adedara, I. A., Okpara, E. S., Busari, E. O., Omole, O., Owumi, S. E., & Farombi, E. O. (2019). Dietary protocatechuic acid abrogates male reproductive dysfunction in streptozotocin-induced diabetic rats via suppression of oxidative damage, inflammation and caspase-3 activity. In *Eur J Pharmacol*. <https://doi.org/10.1016/j.ejphar.2019.01.033>
- Adomako-Bonsu, A. G., Chan, S. L., Pratten, M., & Fry, J. R. (2017). Antioxidant activity of rosmarinic acid and its principal metabolites in chemical and cellular systems: Importance of physico-chemical characteristics. *Toxicology in Vitro*, 40, 248–255. <https://doi.org/10.1016/j.tiv.2017.01.016>
- Ågmo, A., & Laan, E. (2022). Sexual incentive motivation, sexual behavior, and general arousal: Do rats and humans tell the same story? *Neuroscience and Biobehavioral Reviews*, 135(November 2021), 104595. <https://doi.org/10.1016/j.neubiorev.2022.104595>
- Akomolafe, S. F. (2017). The effects of caffeine, caffeic acid, and their combination on acetylcholinesterase, adenosine deaminase and arginase activities linked with brain function. *Journal of Food Biochemistry*, 41(5), 1–9. <https://doi.org/10.1111/jfbc.12401>
- Al-khawaldeh, O., Al-Alami, Z. M., Althunibat, O. Y., Abuamara, T. M. M., Mihdawi, A., & Abukhalil, M. H. (2024). Rosmarinic Acid Attenuates Testicular Damage via Modulating Oxidative Stress and Apoptosis in Streptozotocin-Induced Diabetic Albino Mice. *Stresses*,

4(3), 505–517. <https://doi.org/10.3390/stresses4030032>

- Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules*, 27(9). <https://doi.org/10.3390/molecules27092901>
- Al-Roujeaie, A. S., Abuohashish, H. M., Ahmed, M. M., & Alkhamees, O. A. (2017). Effect of rutin on diabetic-induced erectile dysfunction: Possible involvement of testicular biomarkers in male rats. *Andrologia*, 49(8), 1–9. <https://doi.org/10.1111/and.12737>
- Albalushi, A., Chua, B. L., Ghanem, N., Ali, A., Tee, L. H., & Wong, W. Y. (2023). Extraction of rosmarinic acid from *Salvia officinalis* L. using tunable deep eutectic solvent and its physiochemical properties. *Asia-Pacific Journal of Chemical Engineering*, 18(6). <https://doi.org/10.1002/apj.2977>
- Albarakati, A. J. A. (2022). Protocatechuic acid counteracts oxidative stress and inflammation in carrageenan-induced paw edema in mice. *Environmental Science and Pollution Research*, 29(37), 56393–56402. <https://doi.org/10.1007/s11356-022-19688-9>
- Albayrak, S., Aksoy, A., Yilmaz, M. A., & Beyzi, E. (2024). Investigation of Phytochemical, Antioxidant and Antidiabetic Potentials of *Scabiosa* L. (Caprifoliaceae) Species with Chemometric Methods. *Chemistry and Biodiversity*, 21(2). <https://doi.org/10.1002/cbdv.202301652>
- Alchalabi, S., M Shukri, H., Mahmood, R., & B. Khalid, L. (2016). Effect of *Salvia officinalis* L. (Sage) Aqueous extract on Liver and Testicular Function of Diabetic Albino Male Rats. *Journal of Babylon University/Pure and Applied Sciences*, 24(2), 390–399.
- Amin, F., Ibrahim, M. A. A., Rizwan-ul-Hasan, S., Khaliq, S., Gabr, G. A., Muhammad, Khan, A., Sidhom, P. A., Tikmani, P., Shawky, A. M., Ahmad, S., & Abidi, S. H. (2022). Interactions of Apigenin and Safranal with the 5HT1A and 5HT2A Receptors and Behavioral Effects in Depression and Anxiety: A Molecular Docking, Lipid-Mediated Molecular Dynamics, and In Vivo Analysis. *Molecules*, 27(24). <https://doi.org/10.3390/molecules27248658>
- Amirat, M., Souhila, T., Nacéra, B., Amina, B., Alghonaim, M. I., Öztürk, M., Alsalamah, S. A., Miara, M. D., Boufahja, F., & Bendif, H. (2023). In Vitro Antibacterial, Antioxidant, Anticholinesterase, and Antidiabetic Activities and Chemical Composition of *Salvia balansae*. *Molecules*, 28(23). <https://doi.org/10.3390/molecules28237801>
- Andlib, N., Sajad, M., Kumar, R., & Thakur, S. C. (2023). Abnormalities in sex hormones and sexual dysfunction in males with diabetes mellitus: A mechanistic insight. *Acta Histochemica*, 125(1), 151974. <https://doi.org/10.1016/j.acthis.2022.151974>
- Annamalai, P., & Thangam, E. B. (2017). Local and Systemic Profiles of Inflammatory Cytokines in Carrageenan-induced Paw Inflammation in Rats. *Immunological Investigations*, 46(3), 274–283. <https://doi.org/10.1080/08820139.2016.1248562>
- Askari, S. F., Avan, R., Tayarani-Najaran, Z., Sahebkar, A., & Eghbali, S. (2021). Iranian *Salvia* species: A phytochemical and pharmacological update. *Phytochemistry*, 183(May 2020), 112619. <https://doi.org/10.1016/j.phytochem.2020.112619>
- Ayuob, N. N., Murad, H. A. S., & Ali, S. S. (2015). Impaired expression of sex hormone receptors in male reproductive organs of diabetic rat in response to oral antidiabetic drugs. *Folia Histochemica et Cytobiologica*, 53(1), 35–48.

<https://doi.org/10.5603/FHC.a2015.0005>

- Bahadori, M. B., Dinparast, L., Zengin, G., Sarikurkcu, C., Bahadori, S., Asghari, B., & Movahhedineh, N. (2017). Functional components, antidiabetic, anti-Alzheimer's disease, and antioxidant activities of *Salvia syriaca* L. *International Journal of Food Properties*, 20(8), 1761–1772. <https://doi.org/10.1080/10942912.2016.1218893>
- Bakaç, M. S., Dogan, A., Yılmaz, M. A., Altındag, F., Donmez, F., & Battal, A. (2023). Ameliorative effects of *Scutellaria Pinnatifida* subsp. *pichleri* (Stapf) Rech.f. Extract in streptozotocin-induced diabetic rats: chemical composition, biochemical and histopathological evaluation. *BMC Complementary Medicine and Therapies*, 23(1), 1–16. <https://doi.org/10.1186/s12906-023-04252-w>
- Balasubramanian, R., Dwyer, A., Seminara, S. B., Pitteloud, N., Kaiser, U. B., & Crowley, W. F. (2010). Human GnRH deficiency: A unique disease model to unravel the ontogeny of GnRH neurons. *Neuroendocrinology*, 92(2), 81–99. <https://doi.org/10.1159/000314193>
- Balkır, Ş., Hazman, Ö., Aksoy, L., Yılmaz, M. A., Cakir, O., Kara, R., & Erol, İ. (2023). Phytochemical Profile, Antioxidant and Antimicrobial Potency of Aerial Parts of *Salvia Tomentosa* Miller. *Acta Chimica Slovenica*, 70(2), 218–225. <https://doi.org/10.17344/acsi.2023.8008>
- Ballester, J., Muñoz, M. C., Domínguez, J., Rigau, T., Guinovart, J. J., & Rodríguez-Gil, J. E. (2004). Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. *Journal of Andrology*, 25(5), 706–719. <https://doi.org/10.1002/j.1939-4640.2004.tb02845.x>
- Barkabi-Zanjani, S., Ghorbanzadeh, V., Aslani, M., Ghalibafabbaghi, A., & Chodari, L. (2020). Diabetes mellitus and the impairment of male reproductive function: Possible signaling pathways. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14(5), 1307–1314. <https://doi.org/10.1016/j.dsx.2020.07.031>
- Basal, W. T., Issa, A. M., Abdelaleem, O., & Omar, A. R. (2023). *Salvia officinalis* restores semen quality and testicular functionality in cadmium-intoxicated male rats. *Scientific Reports*, 13(1), 1–11. <https://doi.org/10.1038/s41598-023-45193-1>
- Battandier, J.-A., & Trabut, L.-C. (1886). *Atlas de la Flore d'Alger* (L. I. ADOLPHE JOURDAN (ed.)). <http://www.biodiversitylibrary.org/item/143360>
- Baum, M. J., & Bakker, J. (2013). Roles of sex and gonadal steroids in mammalian pheromonal communication. *Frontiers in Neuroendocrinology*, 34(4), 268–284. <https://doi.org/10.1016/j.yfrne.2013.07.004>
- Baynest, H. W. (2015). Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *Journal of Diabetes & Metabolism*, 06(05). <https://doi.org/10.4172/2155-6156.1000541>
- Benabbou, A., Khaled, M. B., & Alchalabi, A. S. (2018). Evaluation of the Efficiency of Combined and Separated Antioxidant Supplementation of Vitamin C and E on Semen Parameters in Streptozotocin-Induced Diabetic Male Wistar Rats. *South Asian Journal of Experimental Biology*, 7(4), 166–172. [https://doi.org/10.38150/sajeb.7\(4\).p166-172](https://doi.org/10.38150/sajeb.7(4).p166-172)
- Benabdallah, A., Betina, S., Bouchentouf, S., Boumendjel, M., Bechkri, S., Bensouici, C., Nicoli, F., Vergine, M., Carmine, N., & De Bellis, L. (2022). Chemical profiling, antioxidant, enzyme inhibitory and in silico modeling of *Rosmarinus officinalis* L. and

- Artemisia herba alba Asso. essential oils from Algeria. *South African Journal of Botany*, 147, 501–510. <https://doi.org/10.1016/j.sajb.2022.02.012>
- Bendrihem, K. A., Zeraib, A., Atoki, A. V., Mihoubi, M. A., Kadi, K., Bensuici, C., Hachemi, M., Ayeb, N. E., Nagaz, K., & Messaoudi, M. (2024). Phytochemical screening, antioxidant properties, and photo-protective activities of *Salvia balansae* de Noé ex Coss. *Open Chemistry*, 22(1). <https://doi.org/10.1515/chem-2024-0024>
- Bialy, M., Bogacki-Rychlik, W., Przybylski, J., & Zera, T. (2019). The Sexual Motivation of Male Rats as a Tool in Animal Models of Human Health Disorders. *Frontiers in Behavioral Neuroscience*, 13(November), 1–10. <https://doi.org/10.3389/fnbeh.2019.00257>
- Bijmoer, Scherrenberg, & Creuwels. (2023). No Title. *Naturalis Biodiversity Center (NL) - Botany*. <https://doi.org/https://doi.org/10.15468/ib5ypt>
- Bikri, S., Aboussaleh, Y., Berrani, A., Louragli, I., Hafid, A., Chakib, S., & Ahami, A. (2021). Effects of date seeds administration on anxiety and depressive symptoms in streptozotocin-induced diabetic rats: Biochemical and behavioral evidences. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(6), 1031–1040. <https://doi.org/10.1515/jbcpp-2020-0225>
- Boehm, U., Bouloux, P. M., Dattani, M. T., De Roux, N., Dodé, C., Dunkel, L., Dwyer, A. A., Giacobini, P., Hardelin, J. P., Juul, A., Maghnie, M., Pitteloud, N., Prevot, V., Raivio, T., Tena-Sempere, M., Quinton, R., & Young, J. (2015). Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism-pathogenesis, diagnosis and treatment. *Nature Reviews Endocrinology*, 11(9), 547–564. <https://doi.org/10.1038/nrendo.2015.112>
- Boublata, N. E. I., Habbachi, S., Saadane, F. Z., Bouzar, A., & Habbachi, W. (2021). Effects of ethanolic extract of the *Cleome arabica* on sexual behavior in Wistar rats. *Journal of Animal Behaviour and Biometeorology*, 9(4). <https://doi.org/10.31893/JABB.21035>
- Boudou, F. (2022). *Effet thérapeutique de Curcuma longa sur la fonction reproductrice des rats mâles Wistar exposés aux métaux lourds (cas de l'aluminium)*.
- Boudou, F., Bendahmane-Salmi, M., Benabderrahmane, M., Belakredar, A., Benalia, A., Beghdadli, B., & Kandouci, B. D. A. (2020). Effect of Curcuma longa Aqueous Extract on Male Fertility in Aluminum Exposed Wistar Rats. *Journal of Drug Delivery and Therapeutics*, 10(5), 11–17. <https://doi.org/10.22270/jddt.v10i5.4275>
- Bouzana, A., Chekroud, Z., Bechecker, I., Sakhraoui, N., Bouzenad, N., & Bensouici, C. (2023). Phytochemical analysis by LC MS/MS and in vitro antioxidant activity of the Algerian endemic plant *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet. *Global Nest Journal*, 25(7), 113–119. <https://doi.org/10.30955/gnj.005047>
- Brady, V., Whisenant, M., Wang, X., Ly, V. K., Zhu, G., Aguilar, D., & Wu, H. (2022). Characterization of Symptoms and Symptom Clusters for Type 2 Diabetes Using a Large Nationwide Electronic Health Record Database. *Diabetes Spectrum*, 35(2), 159–170. <https://doi.org/10.2337/DS21-0064>
- Brindisi, M., Bouzidi, C., Frattaruolo, L., Loizzo, M. R., Cappello, M. S., Dugay, A., Deguin, B., Lauria, G., Cappello, A. R., & Tundis, R. (2021). New insights into the antioxidant and anti-inflammatory effects of italian *salvia officinalis* leaf and flower extracts in lipopolysaccharide and tumor-mediated inflammation models. *Antioxidants*, 10(2), 1–20.

<https://doi.org/10.3390/antiox10020311>

- Brown, G. R., & Nemes, C. (2008). The exploratory behaviour of rats in the hole-board apparatus: Is head-dipping a valid measure of neophilia? *Behavioural Processes*, 78(3), 442–448. <https://doi.org/10.1016/j.beproc.2008.02.019>
- Brunert, D., & Rothermel, M. (2021). Extrinsic neuromodulation in the rodent olfactory bulb. *Cell and Tissue Research*, 383(1), 507–524. <https://doi.org/10.1007/s00441-020-03365-9>
- Buhur, A., Gürel, Ç., Kuşçu, G. C., Yiğittürk, G., Oltulu, F., Karabay Yavaşoğlu, N. Ü., Uysal, A., & Yavaşoğlu, A. (2023). Is losartan a promising agent for the treatment of type 1 diabetes-induced testicular germ cell apoptosis in rats? *Molecular Biology Reports*, 50(3), 2195–2205. <https://doi.org/10.1007/s11033-022-08172-9>
- Bursal, E., Yılmaz, M. A., İzol, E., Türkan, F., Atalar, M. N., Murahari, M., Aras, A., & Ahmad, M. (2021). Enzyme inhibitory function and phytochemical profile of *Inula discoidea* using in vitro and in silico methods. *Biophysical Chemistry*, 277(May), 106629. <https://doi.org/10.1016/j.bpc.2021.106629>
- Calabrò, R. S., Cacciola, A., Bruschetta, D., Milardi, D., Quattrini, F., Sciarrone, F., la Rosa, G., Bramanti, P., & Anastasi, G. (2019). Neuroanatomy and function of human sexual behavior: A neglected or unknown issue? *Brain and Behavior*, 9(12), 1–17. <https://doi.org/10.1002/brb3.1389>
- Caliskan, H., Akat, F., Omercioglu, G., Bastug, G., Ficicilar, H., & Bastug, M. (2020). Aerobic exercise has an anxiolytic effect on streptozotocin-induced diabetic rats. *Acta Neurobiologiae Experimentalis*, 80(3), 245–255. <https://doi.org/10.21307/ane-2020-022>
- Caliskan, H., Akat, F., Tatar, Y., Zaloglu, N., Dursun, A. D., Bastug, M., & Ficicilar, H. (2019). Effects of exercise training on anxiety in diabetic rats. *Behavioural Brain Research*, 376(July), 112084. <https://doi.org/10.1016/j.bbr.2019.112084>
- Carvalho, M. G., Silva, K. M., Aristizabal, V. H. V., Ortiz, P. E. O., Paranzini, C. S., Melchert, A., Amaro, J. L., & Souza, F. F. (2021). Effects of Obesity and Diabetes on Sperm Cell Proteomics in Rats. *Journal of Proteome Research*, 20(5), 2628–2642. <https://doi.org/10.1021/acs.jproteome.0c01044>
- Castellano, J. M., Navarro, V. M., Roa, J., Pineda, R., Sánchez-Garrido, M. A., García-Galiano, D., Vigo, E., Dieguez, C., Aguilar, E., Pinilla, L., & Tena-Sempere, M. (2009). Alterations in hypothalamic kiSS-1 system in experimental diabetes: Early changes and functional consequences. *Endocrinology*, 150(2), 784–794. <https://doi.org/10.1210/en.2008-0849>
- Chaudhari, Y. S., Matkar, S. S., Bhujbal, S. S., Walunj, V. A., Bhor, N. S., & Vyavhare, R. D. (2023). Diabetes Mellitus: A Review. *International Journal of Advanced Research in Science, Communication and Technology*, 3(1), 16–22. <https://doi.org/10.48175/ijarsct-8551>
- Cherian, P., Bergfeld, W. F., Belsito, D. V., Klaassen, C. D., Liebler, D. C., Marks, J. G., Peterson, L. A., Shank, R. C., Slaga, T. J., Snyder, P. W., Fiume, M., & Heldreth, B. (2021). Safety Assessment of Soy-Derived Ingredients as Used in Cosmetics. *International Journal of Toxicology*. <https://doi.org/10.1177/10915818241231249>
- Chianese, R., & Pierantoni, R. (2021). Mitochondrial reactive oxygen species (ROS) production alters sperm quality. *Antioxidants*, 10(1), 1–19. <https://doi.org/10.3390/antiox10010092>
- Chu, X., & Ågmo, A. (2016). Sociosexual interactions in rats: Are they relevant for

- understanding human sexual behavior? *International Journal of Psychological Research*, 9(2), 76–95. <https://doi.org/10.21500/20112084.2339>
- Chu, X., Snoeren, E., Södersten, P., & Ågmo, A. (2021). Sexual incentive motivation and male and female copulatory behavior in female rats given androgen from postnatal day 20. *Physiology and Behavior*, 237. <https://doi.org/10.1016/j.physbeh.2021.113460>
- Condorelli, R. A., Vignera, S. La, Mongioi, L. M., Alamo, A., & Calogero, A. E. (2018). Diabetes mellitus and infertility: Different pathophysiological effects in type 1 and type 2 on sperm function. *Frontiers in Endocrinology*, 9(MAY). <https://doi.org/10.3389/fendo.2018.00268>
- Crisler, R., Johnston, N. A., Sivula, C., & Budelsky, C. L. (2019). Functional anatomy and physiology. In *The Laboratory Rat*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-814338-4.00004-0>
- Dagna, S., Wojciech, M., & Ewa, K.-R. (2020). Streptozotocin - an antibiotic used to induce diabetes on experimental animals. *Journal of Education, Health and Sport*, 2020;10(9)(Dm), 906–909. <https://doi.org/10.12775/JEHS.2020.10.09.110>
- Davies, K. P. (2015). Development and therapeutic applications of nitric oxide-releasing materials. *Future Science OA*, 1(1). <https://doi.org/10.4155/fso.15.50>
- Davoodi, F., Taheri, S., Raisi, A., Rajabzadeh, A., Ahmadvand, H., Hablolvarid, M. H., & Zakian, A. (2020). Investigating the sperm parameters, oxidative stress and histopathological effects of salvia miltiorrhiza hydroalcoholic extract in the prevention of testicular ischemia reperfusion damage in rats. *Theriogenology*, 144, 98–106. <https://doi.org/10.1016/j.theriogenology.2020.01.002>
- Dean, R. C., & Lue, T. F. (2005). Physiology of penile erection and pathophysiology of erectile dysfunction. *Urologic Clinics of North America*, 32(4), 379–395. <https://doi.org/10.1016/j.ucl.2005.08.007>
- Derouich, M., Bouhlali, E. D. T., Hmidani, A., Bammou, M., Bourkhis, B., Sellam, K., & Alem, C. (2020). Assessment of total polyphenols, flavonoids and anti-inflammatory potential of three Apiaceae species grown in the Southeast of Morocco. *Scientific African*, 9, 0–15. <https://doi.org/10.1016/j.sciaf.2020.e00507>
- Dick, B., Koller, C., Herzog, B., Greenberg, J., & Hellstrom, W. J. G. (2020). The Role of Hormones in Male Sexual Function. *Current Sexual Health Reports*, 12(3), 101–112. <https://doi.org/10.1007/s11930-020-00271-7>
- Dušková, M., & Pospíšilová, H. (2011). The role of non-aromatizable testosterone metabolite in metabolic pathways. *Physiological Research*, 60(2), 253–261. <https://doi.org/10.33549/physiolres.932080>
- El awady, M. E., Mohamed, S. S., Abo Elsoud, M. M., Mahmoud, M. G., Anwar, M. M., Ahmed, M. M., Eltaher, A., Magdeldin, S., Attallah, A., Elhagry, A. E., & Abdelhamid, S. A. (2024). Insight into antioxidant and anti-inflammatory effects of marine bacterial natural exopolysaccharide (EPSSM) using carrageenan-induced paw edema in rats. *Scientific Reports*, 14(1), 1–23. <https://doi.org/10.1038/s41598-024-53502-5>
- El Helew, E. A., Hamed, W. S., Moustafa, A. M., & Sakkara, Z. A. (2024). Structural changes in testes of Streptozotocin induced diabetic rats and possible protective effect of royal jelly: light and electron microscopic study. *Ultrastructural Pathology*, 48(1), 1–15.

<https://doi.org/10.1080/01913123.2023.2277170>

- ElBanna, A. H., Osman, A. S., Hossny, A., ElBanna, H., & Abo El-Ela, F. I. (2023). Dose-dependent effects of taurine against testicular damage in a streptozotocin-induced type 1 diabetes mellitus rat model. *International Journal of Immunopathology and Pharmacology*, 37, 1–15. <https://doi.org/10.1177/03946320231172745>
- Emil, A. B., Hassan, N. H., Ibrahim, S., Hassanen, E. I., Eldin, Z. E., & Ali, S. E. (2024). Propolis extract nanoparticles alleviate diabetes-induced reproductive dysfunction in male rats: antidiabetic, antioxidant, and steroidogenesis modulatory role. *Scientific Reports*, 14(1), 1–22. <https://doi.org/10.1038/s41598-024-81949-z>
- Faddladdeen, K. A. jalil. (2022). The possible protective and therapeutic effects of ginger and cinnamon on the testis and coda epididymis of streptozotocin-induced-diabetic rats: Histological and biochemical studies. *Saudi Journal of Biological Sciences*, 29(12), 103452. <https://doi.org/10.1016/j.sjbs.2022.103452>
- Feng, T. Y., Lv, D. L., Zhang, X., Du, Y. Q., Yuan, Y. T., Chen, M. J., Xi, H. M., Li, Y., Han, N., & Hu, J. H. (2020). Rosmarinic acid improves boar sperm quality, antioxidant capacity and energy metabolism at 17°C via AMPK activation. *Reproduction in Domestic Animals*, 55(12), 1714–1724. <https://doi.org/10.1111/rda.13828>
- Feng, Y., Shi, T., Fu, Y., & Lv, B. (2022). Traditional chinese medicine to prevent and treat diabetic erectile dysfunction. *Frontiers in Pharmacology*, 13(September). <https://doi.org/10.3389/fphar.2022.956173>
- Festa, J., Hussain, A., Al-Hareth, Z., Bailey, S. J., Singh, H., & Da Boit, M. (2024). Phenolic Metabolites Protocatechuic Acid and Vanillic Acid Improve Nitric Oxide Bioavailability via the Akt-eNOS Pathway in Response to TNF- α Induced Oxidative Stress and Inflammation in Endothelial Cells. *Metabolites*, 14(11). <https://doi.org/10.3390/metabo14110613>
- Fidan, H. S., Kilinc, F. M., Yilmaz, M. A., Akdeniz, M., Yener, I., Firat, M., Onay, A., Kolak, U., & Ertas, A. (2021). Comparison of chemical and biological properties of in vivo and in vitro samples of *Salvia siirtica* Kahraman, Celep & Dogan extracts prepared with different solvents. *South African Journal of Botany*, 142, 421–429. <https://doi.org/10.1016/j.sajb.2021.07.029>
- Gaderpour, S., Ghiasi, R., Hamidian, G., Heydari, H., & Keyhanmanesh, R. (2020). Voluntary exercise improves spermatogenesis and testicular apoptosis in type 2 diabetic rats through alteration in oxidative stress and mir-34a/SIRT1/p53 pathway. *Iranian Journal of Basic Medical Sciences*, 24(1), 58–65. <https://doi.org/10.22038/ijbms.2020.49498.11314>
- Gaunay, G., Nagler, H. M., & Stember, D. S. (2013). Reproductive Sequelae of Diabetes in Male Patients. *Endocrinology and Metabolism Clinics of North America*, 42(4), 899–914. <https://doi.org/10.1016/j.ecl.2013.07.003>
- Gawlik, V., Schmidt, S., Scheepers, A., Wennemuth, G., Augustin, R., Aumüller, G., Moser, M., Al-Hasani, H., Kluge, R., Joost, H. G., & Schürmann, A. (2008). Targeted disruption of Slc2a8 (GLUT8) reduces motility and mitochondrial potential of spermatozoa. *Molecular Membrane Biology*, 25(3), 224–235. <https://doi.org/10.1080/09687680701855405>
- Gaytán-Tocavén, L., Aguilar-Moreno, A., Ortiz, J., Alcauter, S., Antonio-Cabrera, E., & Paredes, R. G. (2023). Identification of neural circuits controlling male sexual behavior

- and sexual motivation by manganese-enhanced magnetic resonance imaging. *Frontiers in Behavioral Neuroscience*, 17. <https://doi.org/10.3389/fnbeh.2023.1301406>
- Ghasemi, A., & Jeddi, S. (2023). Streptozotocin As a Tool for Induction of Rat Models of Diabetes: a Practical Guide. *EXCLI Journal*, 22, 274–294. <https://doi.org/10.17179/excli2022-5720>
- Ghasemi, H., Karimi, J., Goodarzi, M. T., Khodadadi, I., Tavailani, H., Moridi, H., & Kheiripour, N. (2016). Seminal plasma zinc and magnesium levels and their relation to spermatozoa parameters in semen of diabetic men. *International Journal of Diabetes in Developing Countries*, 36(1), 34–39. <https://doi.org/10.1007/s13410-015-0408-y>
- Ghorbani, A., & Esmailizadeh, M. (2017). Pharmacological properties of *Salvia officinalis* and its components. *Journal of Traditional and Complementary Medicine*, 7(4), 433–440. <https://doi.org/10.1016/j.jtcme.2016.12.014>
- Giribabu, N., Kumar, K. E. swa., Rekha, S. S. wapn., Muniandy, S., & Salleh, N. (2014). Chlorophytum borivilianum root extract maintains near normal blood glucose, insulin and lipid profile levels and prevents oxidative stress in the pancreas of streptozotocin-induced adult male diabetic rats. *International Journal of Medical Sciences*, 11(11), 1172–1184. <https://doi.org/10.7150/ijms.9056>
- Gómez, O., Romero, A., Terrado, J., & Mesonero, J. E. (2006). Differential expression of glucose transporter GLUT8 during mouse spermatogenesis. *Reproduction*, 131(1), 63–70. <https://doi.org/10.1530/rep.1.00750>
- González-Gallego, J., García-Mediavilla, M. V., Sánchez-Campos, S., & Tuñó, M. J. (2010). Fruit polyphenols, immunity and inflammation. *British Journal of Nutrition*, 104(SUPPL.3). <https://doi.org/10.1017/S0007114510003910>
- Grande, G., & Foresta, C. (2023). Male Reproduction: From Pathophysiology to Clinical Assessment. *Practical Clinical Andrology*, 161–172. https://doi.org/10.1007/978-3-031-11701-5_12
- Grohs, L., Cheng, L., Cönen, S., Haddad, B. G., Bülow, A., Toklucu, I., Ernst, L., Körner, J., Schmalzing, G., Lampert, A., Machtens, J. P., & Hausmann, R. (2023). Corrigendum: Diclofenac and other non-steroidal anti-inflammatory drugs (NSAIDs) are competitive antagonists of the human P2X3 receptor. *Frontiers in Pharmacology*, 14. <https://doi.org/10.3389/fphar.2023.1225522>
- Gualtieri, R., Kalthur, G., Barbato, V., Longobardi, S., Di Rella, F., Adiga, S. K., & Talevi, R. (2021). Sperm oxidative stress during in vitro manipulation and its effects on sperm function and embryo development. *Antioxidants*, 10(7), 1–24. <https://doi.org/10.3390/antiox10071025>
- Gülçin, İ., Tel, A. Z., Gören, A. C., Taslimi, P., & Alwasel, S. H. (2019). Sage (*Salvia pilifera*): determination of its polyphenol contents, anticholinergic, antidiabetic and antioxidant activities. *Journal of Food Measurement and Characterization*, 13(3), 2062–2074. <https://doi.org/10.1007/s11694-019-00127-2>
- Güven, L., Ertürk, A., Yılmaz, M. A., Alwasel, S., & Gülçin, İ. (2024). *Alchemilla pseudocartalinica* Juz: Phytochemical Screening by UPLC-MS/MS, Molecular Docking, Anti-oxidant, Anti-diabetic, Anti-glaucoma, and Anti-Alzheimer Effects. *Records of Natural Products*, 18(2), 251–272. <https://doi.org/10.25135/rnp.448.2312.2993>

- Hamza, N., Berke, B., Umar, A., Cheze, C., Gin, H., & Moore, N. (2019). A review of Algerian medicinal plants used in the treatment of diabetes. *Journal of Ethnopharmacology*, 238(July 2018), 111841. <https://doi.org/10.1016/j.jep.2019.111841>
- Hamzé, R., Delangre, E., Tolu, S., Moreau, M., Janel, N., Bailbé, D., & Movassat, J. (2022). Type 2 Diabetes Mellitus and Alzheimer's Disease: Shared Molecular Mechanisms and Potential Common Therapeutic Targets. *International Journal of Molecular Sciences*, 23(23). <https://doi.org/10.3390/ijms232315287>
- Hargaden, M., & Singer, L. (2012). Anatomy, Physiology, and Behavior. In *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* (pp. 575–602). Elsevier Inc. <https://doi.org/10.1016/B978-01-2-380920-9.00020-1>
- Harter, C. J. L., Kavanagh, G. S., & Smith, J. T. (2018). The role of kisspeptin neurons in reproduction and metabolism. *Journal of Endocrinology*, 238(3), R173–R183. <https://doi.org/10.1530/JOE-18-0108>
- Hasan, M. K., Akhter, S., Fatema, K., Hossain, M. R., Sultana, T., & Uzzaman, M. (2023). Selective modification of diclofenac to reduce the adverse effects; A computer-aided drug design approach. *Informatics in Medicine Unlocked*, 36(November 2022), 101159. <https://doi.org/10.1016/j.imu.2023.101159>
- Hedge, I. C. (1974). NOTES FROM THE ROYAL BOTANIC GARDEN EDINBURGH. In *A REVISION OF SALVIA IN AFRICA INCLUDING MADAGASCAR AND THE CANARY ISLANDS* (Vol. 33, Issue 1).
- Heijkoop, R., Huijgens, P. T., & Snoeren, E. M. (2017). Assessment of sexual behavior in rats: The potentials and pitfalls. *Behavioural Brain Research*. <https://doi.org/10.1016/j.bbr.2017.10.029>
- Himanshu, Dharmila, Sarkar, D., & Nutan. (2020). A review of behavioral tests to evaluate different types of anxiety and anti-anxiety effects. *Clinical Psychopharmacology and Neuroscience*, 18(3), 341–351. <https://doi.org/10.9758/CPN.2020.18.3.341>
- Hoglen, N. E. G., & Manoli, D. S. (2022). Cupid's quiver: Integrating sensory cues in rodent mating systems. *Frontiers in Neural Circuits*, 16(July), 1–16. <https://doi.org/10.3389/fncir.2022.944895>
- Hosseini-pour, M., Asgari, R., Kermani, J., Goodarzi, N., & Bakhtiari, M. (2022). The antioxidant effects of hydroalcoholic extract of Ashrasi date palm on sperm parameters and DNA fragmentation in diabetic rats. *Animal Models and Experimental Medicine*, 5(3), 281–287. <https://doi.org/10.1002/ame2.12222>
- Huang, A. C., Yeh, T. C., Wu, N. C., Yeh, C. Y., Lin, P. H., & Yeh, K. Y. (2022). Protective Effects of Epigallocatechin Gallate for Male Sexual Dysfunction in Streptozotocin-Induced Diabetic Rats. *International Journal of Molecular Sciences*, 23(17). <https://doi.org/10.3390/ijms23179759>
- Huang, R., Chen, J., Guo, B., Jiang, C., & Sun, W. (2024). Diabetes-induced male infertility: potential mechanisms and treatment options. *Molecular Medicine*, 30(1). <https://doi.org/10.1186/s10020-023-00771-x>
- Huijgens, P. T., Guarraci, F. A., Olivier, J. D. A., & Snoeren, E. M. S. (2021). Male rat sexual behavior: Insights from inter-copulatory intervals. *Behavioural Processes*, 190(June), 104458. <https://doi.org/10.1016/j.beproc.2021.104458>

- Huijgens, P. T., Heijkoop, R., & Snoeren, E. M. S. (2021). Silencing and stimulating the medial amygdala impairs ejaculation but not sexual incentive motivation in male rats. *Behavioural Brain Research*, 405. <https://doi.org/10.1016/j.bbr.2021.113206>
- Hull, E. M., & Dominguez, J. M. (2015). Male Sexual Behavior. In *Knobil and Neill's Physiology of Reproduction: Two-Volume Set* (Fourth Ed, Vol. 2). Elsevier. <https://doi.org/10.1016/B978-0-12-397175-3.00049-1>
- IDF Diabetes Atlas. (2021). International Diabetes Federation. In *Diabetes Research and Clinical Practice* (Vol. 102, Issue 2). <https://doi.org/10.1016/j.diabres.2013.10.013>
- Iovino, M., Messana, T., Iovino, E., De Pergola, G., Guastamacchia, E., Giagulli, V. A., & Triggiani, V. (2019). Neuroendocrine Mechanisms Involved in Male Sexual and Emotional Behavior. *Endocrine, Metabolic & Immune Disorders - Drug Targets*, 19(4), 472–480. <https://doi.org/10.2174/1871530319666190131155310>
- Jafari, H., Mirzaiinajmabadi, K., Roudsari, R. L., & Rakhshkhorshid, M. (2021). The factors affecting male infertility: A systematic review. *International Journal of Reproductive BioMedicine*, 19(8), 681–688. <https://doi.org/10.18502/ijrm.v19i8.9615>
- Jain, G. C., & Jangir, R. N. (2014). Modulation of diabetes-mellitus-induced male reproductive dysfunctions in experimental animal models with medicinal plants. *Pharmacognosy Reviews*, 8(16), 113–121. <https://doi.org/10.4103/0973-7847.134245>
- Jash, S. K., Gorai, D., & Roy, R. (2016). SALVIA GENUS AND TRITERPENOID. *Jurnal Ilmu Pendidikan*, 7(2), 809–820. [https://doi.org/10.13040/IJPSR.0975-8232.7\(12\).4710-32](https://doi.org/10.13040/IJPSR.0975-8232.7(12).4710-32)
- Jažo, Z., Glumac, M., Paštar, V., Bektić, S., Radan, M., & Carev, I. (2023). Chemical Composition and Biological Activity of Salvia officinalis L. Essential Oil. *Plants*, 12(9), 1–16. <https://doi.org/10.3390/plants12091794>
- Jennings, K. J., & de Lecea, L. (2020). Neural and hormonal control of sexual behavior. In *Endocrinology* (Vol. 161, Issue 10, pp. 1–13). <https://doi.org/10.1210/endocr/bqaa150>
- Jiang, Q., Maresch, C. C., Petry, S. F., Paradowska-Dogan, A., Bhushan, S., Chang, Y., Wrenzycki, C., Schuppe, H. C., Houska, P., Hartmann, M. F., Wudy, S. A., Shi, L., & Linn, T. (2020). Elevated CCL2 causes Leydig cell malfunction in metabolic syndrome. *JCI Insight*, 5(21), 1–20. <https://doi.org/10.1172/jci.insight.134882>
- Kahloula, K. (2010). *Effets de l'exposition chronique au plomb pendant la période de la gestation et lactation sur les fonctions neurocomportementales chez les jeunes rats Wistar . Impact sur les récepteurs glutamatergiques.*
- Kanedi, M., Busman, H., Mandasari, R. A., & Pratami, G. D. (2019). Plant extracts of Suruhan (Peperomia pellucida L. Kunth) ameliorate infertility of male mice with alloxan-induced hyperglycemia. *International Journal of Biomedical Research*, 10(02), 10–13. <https://doi.org/10.7439/ijbr.v10i2.5039>
- Karabaşoğlu, C., & Erbas, O. (2021). Rat Sexual Behaviours. *Journal of Experimental and Basic Medical Sciences*, October. <https://doi.org/10.5606/jebms.2021.75650>
- Karagacili, H., Yilmaz, M. A., Ertürk, A., Kiziltas, H., Güven, L., Alwasel, S. H., & Gulcin, İ. (2023). Comprehensive Metabolite Profiling of Berdav Propolis Using LC-MS/MS: Determination of Antioxidant, Anticholinergic, Antiglaucoma, and Antidiabetic Effects. *Molecules*, 28(4), 1–20. <https://doi.org/10.3390/molecules28041739>

- Kashif, S., Razdan, R., & Illuri, R. (2019). Potential of phloroglucinol to improve erectile dysfunction associated with streptozotocin-induced diabetes in rats. *Journal of Integrative Medicine Journal*, 17(4), 282–287. <https://doi.org/10.1016/j.joim.2019.04.002>
- Keyhanmanesh, R., Hamidian, G., Alipour, M. R., Ranjbar, M., & Oghbaei, H. (2018). Protective effects of sodium nitrate against testicular apoptosis and spermatogenesis impairments in streptozotocin-induced diabetic male rats. *Life Sciences*, 211, 63–73. <https://doi.org/10.1016/j.lfs.2018.09.019>
- Khafaji, S. S. (2023). Antioxidant, anti-inflammatory, and anti-reprotoxic effects of kaempferol and vitamin E on lead acetate-induced testicular toxicity in male rats. *Open Veterinary Journal*, 13(12), 1683–1695. <https://doi.org/10.5455/OVJ.2023.v13.i12.17>
- Khenifi, M. L., Serseg, T., Migas, P., Krauze-baranowska, M., Özdemir, S., Bensouici, C., Alghonaim, M. I., Al-khafaji, K., Alsalamah, S. A., Boudjeniba, M., Yousfi, M., Boufahja, F., & Bendif, H. (2023). Flavonoids from Fenugreek Seeds. *Molecules*, 28, 7798.
- Khodadadi, H., Khaledi, S., Roshan, M. M., Eslampour, M., Moradi, S., & Mortazavi, P. (2024). Efficacy of *Salvia officinalis* Extract against Infertility in Oxidative Stress Conditions. *Journal of Medicinal Plants and By-Products*, 13(3), 629–636. <https://doi.org/10.22034/jmpb.2023.363418.1600>
- Knoblaugh Sue E., Lawrence True, Maria Tretiakova, R. R. H. (2018). MALE REPRODUCTIVE SYSTEM. In *Comparative Anatomy and Histology (Second edition): a mouse, rat, and human atlas*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-802900-8.00018-X>
- Kotian, S. R., Kumar, A., Mallik, S. B., Bhat, N. P., Souza, A. D., & Pandey, A. K. (2019). Effect of diabetes on the Male reproductive system—A histomorphological study. *Journal of Morphological Sciences*, 36(1), 17–23. <https://doi.org/10.1055/s-0039-1683405>
- Kowalczyk, A., Włodarczyk, M., Tuberoso, C. I. G., & Fecka, I. (2020). Analysis of polyphenolic composition and stability of magistral preparation based on *salviae officinalis folium*. *Acta Poloniae Pharmaceutica - Drug Research*, 77(1), 131–143. <https://doi.org/10.32383/appdr/112832>
- Kuedo, Z., Sangsuriyawong, A., Klaypradit, W., Tipmanee, V., & Chonpathompikunlert, P. (2016). Effects of astaxanthin from *Litopenaeus vannamei* on carrageenan-induced edema and pain behavior in mice. *Molecules*, 21(3), 1–14. <https://doi.org/10.3390/molecules21030382>
- Laoung-On, J., Saenphet, K., Jaikang, C., & Sudwan, P. (2021). Effect of *moringa oleifera* lam. Leaf tea on sexual behavior and reproductive function in male rats. *Plants*, 10(10), 1–17. <https://doi.org/10.3390/plants10102019>
- Le Moëne, O., & Ågmo, A. (2019). Modeling Human Sexual Motivation in Rodents: Some Caveats. *Frontiers in Behavioral Neuroscience*, 13(August), 1–25. <https://doi.org/10.3389/fnbeh.2019.00187>
- Lehtimäki, J., Ventura-Aquino, E., Chu, X., Paredes, R. G., & Ågmo, A. (2021). Sexual Incentive Motivation and Copulatory Behavior in Male Rats Treated With the Adrenergic α 2-Adrenoceptor Agonists Tasipimidine and Fadolmidine: Implications for Treatment of Premature Ejaculation. *Journal of Sexual Medicine*, 18(10), 1677–1689. <https://doi.org/10.1016/j.jsxm.2021.07.010>

- Lekmine, S., Benslama, O., Kadi, K., Martín-García, A. I., Yilmaz, M. A., Akkal, S., Boumegoura, A., Alhomida, A. S., Ola, M. S., & Ali, A. (2023). LC/MS-MS Analysis of Phenolic Compounds in Hyoscyamus albus L. Extract: In Vitro Antidiabetic Activity, In Silico Molecular Docking, and In Vivo Investigation against STZ-Induced Diabetic Mice. *Pharmaceuticals*, 16(7). <https://doi.org/10.3390/ph16071015>
- Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 51(2), 216–226. <https://doi.org/10.1007/s00125-007-0886-7>
- Lin, Y. S., Peng, W. H., Shih, M. F., & Cherng, J. Y. (2021). Anxiolytic effect of an extract of *Salvia miltiorrhiza* Bunge (Danshen) in mice. *Journal of Ethnopharmacology*, 264(July 2020), 113285. <https://doi.org/10.1016/j.jep.2020.113285>
- Lin, Y. W., Hsu, T. H., & Yen, P. H. (2013). Mouse sperm acquire a new structure o. The apical hook during epididymal maturation. *Asian Journal of Andrology*, 15(4), 523–528. <https://doi.org/10.1038/aja.2013.46>
- Llahana, S., Follin, C., Yedinak, C., Grossman, A., Davies, K., & Keil, M. F. (2019). Anatomy and Physiology of the Hypothalamic-Pituitary- Gonadal (HPG) Axis. *Advanced Practice in Endocrinology Nursing*, 1–1337. <https://doi.org/10.1007/978-3-319-99817-6>
- Ma, B., Zhang, J., Zhu, Z., Zhao, A., Zhou, Y., Ying, H., & Zhang, Q. (2019). Luteolin Ameliorates Testis Injury and Blood–Testis Barrier Disruption through the Nrf2 Signaling Pathway and by Upregulating Cx43. *Molecular Nutrition and Food Research*, 63(10), 1–17. <https://doi.org/10.1002/mnfr.201800843>
- Ma, Y., Yang, H. Z., Xu, L. M., Huang, Y. R., Dai, H. L., & Kang, X. N. (2015). Testosterone regulates the autophagic clearance of androgen binding protein in rat Sertoli cells. *Scientific Reports*, 5, 1–8. <https://doi.org/10.1038/srep08894>
- Machado, J. A., Araújo, D. B., & Lima-maximino, M. (2024). Flavonoids as anxiolytics in animal tests : Bibliometry and meta-analysis of the effects of flavonoids on anxiety-like behavior in animal tests. *BioRxiv*, 1–50.
- Mahanem, M. N., Puvaratnesh, S., Umarqayum, A., & A, S. N. (2024). Pro-fertility effect of Ficus carica fruit extract in streptozotocin-induced male rats. *Asian Pacific Journal of Reproduction*, 13(November 2023), 34–41. <https://doi.org/10.4103/apjr.apjr>
- Mahdjoub, M. M., Benzitoune, N., Maiz, Y., Aouadi, N. E. H., Bouhenna, M. M., & Kadri, N. (2023). HPLC-DAD Screening and Antioxidant Activity of Phenolic Compounds of *Salvia balansae* de Noé Leaves Extract. *Journal of Research in Pharmacy*, 27(3), 1076–1085. <https://doi.org/10.29228/jrp.400>
- Maleki, S. J., Crespo, J. F., & Cabanillas, B. (2019). Anti-inflammatory effects of flavonoids. *Food Chemistry*, 299(July). <https://doi.org/10.1016/j.foodchem.2019.125124>
- Mannucci, A., Argento, F. R., Fini, E., Coccia, M. E., Taddei, N., Becatti, M., & Fiorillo, C. (2022). The Impact of Oxidative Stress in Male Infertility. *Frontiers in Molecular Biosciences*, 8(January), 1–9. <https://doi.org/10.3389/fmolb.2021.799294>
- Mansouri, M. T., Hemmati, A. A., Naghizadeh, B., Mard, S. A., Rezaie, A., & Ghorbanzadeh, B. (2015). A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. *Indian Journal of Pharmacology*, 47(3), 292–298. <https://doi.org/10.4103/0253-7613.157127>
- Martin, L. J., & Touaibia, M. (2020). Improvement of testicular steroidogenesis using

- flavonoids and isoflavonoids for prevention of late-onset male hypogonadism. *Antioxidants*, 9(3), 1–17. <https://doi.org/10.3390/antiox9030237>
- Matsumoto, J., Urakawa, S., Hori, E., de Araujo, M. F. P., Sakuma, Y., Ono, T., & Nishijo, H. (2012). Neuronal responses in the nucleus accumbens shell during sexual behavior in male rats. *Journal of Neuroscience*, 32(5), 1672–1686. <https://doi.org/10.1523/JNEUROSCI.5140-11.2012>
- Maynard, R. L., & Downes, N. (2019). Dissection of the Adult Rat. *Anatomy and Histology of the Laboratory Rat in Toxicology and Biomedical Research*, 317–339. <https://doi.org/10.1016/b978-0-12-811837-5.00025-3>
- Mekki, S., Belhocine, M., Bouzouina, M., Chaouad, B., & Mostari, A. (2023). Therapeutic effects of *Salvia balansae* on metabolic disorders and testicular dysfunction mediated by a high-fat diet in Wistar rats. *Mediterranean Journal of Nutrition and Metabolism*, 16(1), 21–39. <https://doi.org/10.3233/MNM-220094>
- Melis, M. R., Sanna, F., & Argiolas, A. (2022). Dopamine, Erectile Function and Male Sexual Behavior from the Past to the Present: A Review. *Brain Sciences*, 12(7). <https://doi.org/10.3390/brainsci12070826>
- Menkveld, R., Holleboom, C. A. G., & Rhemrev, J. P. T. (2011). Measurement and significance of sperm morphology. *Asian Journal of Andrology*, 13(1), 59–68. <https://doi.org/10.1038/aja.2010.67>
- Minas, A., Camargo, M., Alves, M. G., & Bertolla, R. P. (2024). Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis; A narrative review. *Iranian Journal of Basic Medical Sciences*, 27(1), 3–11. <https://doi.org/10.22038/IJBMS.2023.69604.15173>
- Minaz, N., Razdan, R., Hammock, B. D., Mujwar, S., & Goswami, S. K. (2019). Impact of diabetes on male sexual function in streptozotocin-induced diabetic rats: Protective role of soluble epoxide hydrolase inhibitor. *Biomedicine and Pharmacotherapy*, 115(December 2018), 108897. <https://doi.org/10.1016/j.biopha.2019.108897>
- Mirza, F. J., Amber, S., Sumera, Hassan, D., Ahmed, T., & Zahid, S. (2021). Rosmarinic acid and ursolic acid alleviate deficits in cognition, synaptic regulation and adult hippocampal neurogenesis in an A β 1-42-induced mouse model of Alzheimer's disease. *Phytomedicine*, 83(January), 153490. <https://doi.org/10.1016/j.phymed.2021.153490>
- Mitchell, R. T., O'Hara, L., & Smith, L. B. (2017). Gonadotropin and steroid hormone control of spermatogonial differentiation. In *The Biology of Mammalian Spermatogonia*. https://doi.org/10.1007/978-1-4939-7505-1_7
- Mocan, A., Babotă, M., Pop, A., Fizeșan, I., Diuzheva, A., Locatelli, M., Carradori, S., Campestre, C., Menghini, L., Sîsea, C. R., Sokovic, M., Zengin, G., Păltinean, R., Bădăraș, S., Vodnar, D. C., & Crișan, G. (2020). Chemical constituents and biologic activities of sage species: A comparison between *salvia officinalis* L., *s. glutinosa* L. and *s. transsylvanica* (Schur ex Griseb. & Schenk) Schur. *Antioxidants*, 9(6). <https://doi.org/10.3390/antiox9060480>
- Mohamadi, Y., Jameie, S. B. E., Akbari, M., Staji, M., Moradi, F., Mokhtari, T., Khanehzad, M., & Hassanzadeh, G. (2015). Hyperglycemia decreased medial amygdala projections to medial Preoptic area in experimental model of diabetes mellitus. *Acta Medica Iranica*, 53(1), 1–7.

- Mohamed, N. A., Ahmed, O. M., Hozayen, W. G., & Ahmed, M. A. (2017). Ameliorative effects of bee pollen and date palm pollen on the glycemic state and male sexual dysfunctions in streptozotocin-Induced diabetic wistar rats. *Biomedicine & Pharmacotherapy*, 97, 9–18. <https://doi.org/10.1016/j.biopha.2017.10.117>
- Moreira, N., Sandini, T. M., Reis-Silva, T. M., Navas-Suárez, P., Auada, A. V. V., Lebrun, I., Flório, J. C., Bernardi, M. M., & Spinosa, H. S. (2017). Ivermectin reduces motor coordination, serum testosterone, and central neurotransmitter levels but does not affect sexual motivation in male rats. *Reproductive Toxicology*, 74, 195–203. <https://doi.org/10.1016/j.reprotox.2017.10.002>
- Moreira, N., Vicente, F. L., Sandini, T. M., Martinelli, E. C. L., Navas-Suárez, P. E., Reis-Silva, T. M., & Spinosa, H. S. (2020). Effects of ivermectin treatment during prepubertal and pubertal period on sexual parameters and sexual behavior in adulthood in rats. *Research in Veterinary Science*, 129(August 2019), 21–27. <https://doi.org/10.1016/j.rvsc.2019.12.013>
- Moreno-Castilla, C., Hernandez, M., Bergua, M., Alvarez, M. C., Arce, M. A., Rodriguez, K., Martinez-Alonso, M., Iglesias, M., Mateu, M., Santos, M. D., Pacheco, L. R., Blasco, Y., Martin, E., Balsells, N., Aranda, N., & Mauricio, D. (2013). Low-Carbohydrate diet for the treatment of gestational diabetes mellitus: A randomized controlled trial. *Diabetes Care*, 36(8), 2233–2238. <https://doi.org/10.2337/dc12-2714>
- Mostari, A., Benabdeli, K., & Vela, E. (2020). Le littoral de Mostaganem (Algérie), une “zone importante pour les plantes” (ZIP) autant négligée que menacée. *Flora Mediterranea*, 30(October), 207–233. <https://doi.org/10.7320/FLMEDIT30.207>
- Mostari, A., Limam, M., & Vela, E. (2016). Données préliminaires à l’évaluation des menaces selon les critères de la liste rouge UICN pour *Salvia balansae* De Noé, endémique d’Algérie. In F. Médail & G. Domina (Eds.), *XV Organization for the Phyto-Taxonomic Investigation of the Mediterranean Area Meeting* (Issue June, pp. 10–11). FLORA MEDITERRANEA 26. <https://doi.org/10.13140/RG.2.2.27359.61607>
- Müller, L., Gnoyke, S., Popken, A. M., & Böhm, V. (2010). Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Science and Technology*, 43(6), 992–999. <https://doi.org/10.1016/j.lwt.2010.02.004>
- Nadeem, M., Imran, M., Gondal, T. A., Imran, A., Shahbaz, M., Amir, R. M., & Sajid, M. W. (2019). Applied sciences therapeutic potential of rosmarinic acid: A comprehensive review. *Appl.Sci*, 9(3139), 23.
- Nagarajan, P., Gudde, R., & Srinivasan, R. (2021). Essentials of Laboratory Animal Science: Principles and Practices. In *Essentials of Laboratory Animal Science: Principles and Practices*. <https://doi.org/10.1007/978-981-16-0987-9>
- Nah, W. H., Koh, I. K., Ahn, H. S., Kim, M. J., Kang, H. G., Jun, J. H., & Gye, M. C. (2012). Effect of *Spirulina maxima* on spermatogenesis and steroidogenesis in streptozotocin-induced type i diabetic male rats. *Food Chemistry*, 134(1), 173–179. <https://doi.org/10.1016/j.foodchem.2012.02.085>
- Nasiri, K., Akbari, A., Nimrouzi, M., Ruyvaran, M., & Mohamadian, A. (2021). Safflower seed oil improves steroidogenesis and spermatogenesis in rats with type II diabetes mellitus by modulating the genes expression involved in steroidogenesis, inflammation and oxidative stress. *Journal of Ethnopharmacology*, 275(October 2020), 114139.

<https://doi.org/10.1016/j.jep.2021.114139>

- Nna, V. U., Bakar, A. B. A., Ahmad, A., & Mohamed, M. (2020). Diabetes-induced testicular oxidative stress, inflammation, and caspase-dependent apoptosis: the protective role of metformin. *Archives of Physiology and Biochemistry*, 126(5), 377–388. <https://doi.org/10.1080/13813455.2018.1543329>
- Nna, V. U., Bakar, A. B. A., & Mohamed, M. (2017). Diabetes mellitus-induced male reproductive impairment: The role of natural products: A review. *Journal of Applied Pharmaceutical Science*, 7(9), 233–242. <https://doi.org/10.7324/JAPS.2017.70932>
- Noor, S., Mohammad, T., Rub, M. A., Raza, A., Azum, N., Yadav, D. K., Hassan, M. I., & Asiri, A. M. (2022). Biomedical features and therapeutic potential of rosmarinic acid. *Archives of Pharmacal Research*, 45(4), 205–228. <https://doi.org/10.1007/s12272-022-01378-2>
- OECD. (2022). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. In *OECD Guidelines for the Testing of Chemicals, Section 4*. OECD Publishing, Paris. <https://doi.org/https://doi.org/10.1787/9789264071049-en>
- Oghbaei, H., Fattahi, A., Hamidian, G. R., Sadigh-Eteghad, S., Ziaee, M., & Mahmoudi, J. (2021). A closer look at the role of insulin for the regulation of male reproductive function. *General and Comparative Endocrinology*, 300(October 2020), 113643. <https://doi.org/10.1016/j.ygcen.2020.113643>
- Ogunyinka, B. I., Oyinloye, B. E., Osunsanmi, F. O., Opoku, A. R., & Kappo, A. P. (2017). Protective effects of parkia biglobosa protein isolate on streptozotocin-induced hepatic damage and oxidative stress in diabetic male rats. *Molecules*, 22(10), 78–86. <https://doi.org/10.3390/molecules22101654>
- Olvera-Roldán, E. O., Cristóbal-Luna, J. M., García-Martínez, Y., Mojica-Villegas, M. A., Pérez-Pastén-Borja, R., Gutiérrez-Salmeán, G., Pérez-Gutiérrez, S., García-Rodríguez, R. V., Madrigal-Santillán, E., Morales-González, J. A., & Chamorro-Cevallos, G. (2023). Effects of Spirulina maxima on a Model of Sexual Dysfunction in Streptozotocin-Induced Diabetic Male Rats. *Plants*, 12(4), 1–13. <https://doi.org/10.3390/plants12040722>
- Omar, S. M., Zahran, N. N., Alhotan, R. A., Hussein, E. O., Galik, B., & Saleh, A. A. (2024). Evaluation of Salvia hispanica as a Therapeutic Agent against Sodium Arsenic-Induced Testicular Toxicity in a Male Rats Model. *Life*, 14(1), 109. <https://doi.org/10.3390/life14010109>
- Omolaoye, T. S., & Du Plessis, S. S. (2018). Diabetes mellitus and male infertility. *Asian Pacific Journal of Reproduction*, 7(1), 6–14. <https://doi.org/10.4103/2305-0500.220978>
- Omolaoye, T. S., & Du Plessis, S. S. (2021). The effect of streptozotocin induced diabetes on sperm function: a closer look at AGEs, RAGEs, MAPKs and activation of the apoptotic pathway. *Toxicological Research*, 37(1), 35–46. <https://doi.org/10.1007/s43188-020-00040-7>
- Omolaoye, T. S., Skosana, B. T., & du Plessis, S. S. (2018). Diabetes mellitus- induction: Effect of different streptozotocin doses on male reproductive parameters. *Acta Histochemica*, 120(2), 103–109. <https://doi.org/10.1016/j.acthis.2017.12.005>
- Onder, A., Izgi, M. N., Cinar, A. S., Zengin, G., & Yilmaz, M. A. (2022). The characterization of phenolic compounds via LC-ESI-MS/MS, antioxidant, enzyme inhibitory activities of

- Salvia absconditiflora, Salvia sclarea, and Salvia palaestina: A comparative analysis. *South African Journal of Botany*, 150(January 2023), 313–322. <https://doi.org/10.1016/j.sajb.2022.07.030>
- Orzelska-Górka, J., Dos Santos Szewczyk, K., Gawrońska-Grzywacz, M., Herbet, M., Lesniak, A., Bielenica, A., Bujalska-Zadrożny, M., & Biała, G. (2023). Procognitive, Anxiolytic, and Antidepressant-like Properties of Hyperoside and Protocatechuic Acid Corresponding with the Increase in Serum Serotonin Level after Prolonged Treatment in Mice. *Pharmaceuticals*, 16(12). <https://doi.org/10.3390/ph16121691>
- Ott, A., Stolk, R. P., Van Harskamp, F., Pols, H. A. P., Hofman, A., & Breteler, M. M. B. (1999). Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology*, 53(9), 1937–1942. <https://doi.org/10.1212/wnl.53.9.1937>
- Otto, G. M., Franklin, C. L., & Clifford, C. B. (2015). Chapter 4 - Biology and Diseases of Rats. In *Laboratory Animal Medicine: Third Edition*. <https://doi.org/10.1016/B978-0-12-409527-4.00004-3>
- Palanichamy, C., Pavadai, P., Panneerselvam, T., Arunachalam, S., Ammunje, D. N., Kannan, S., Chandrasekaran, J., Sundar, K., Maszczyk, P., & Kunjiappan, S. (2022). *Aphrodisiac Performance of Bioactive Compounds from*.
- Pizarro Meléndez, G. P., Valero-Jara, V., Acevedo-Hernández, P., & Thomas-Valdés, S. (2024). Impact of polyphenols on stress and anxiety: a systematic review of molecular mechanisms and clinical evidence. *Critical Reviews in Food Science and Nutrition*, 64(8), 2340–2357. <https://doi.org/10.1080/10408398.2022.2122925>
- Pradidarcheep, Christian Wallner, Noshir F. Dabhoiwala, and W. H. L. (2019). Anatomy and physiology of the lower urinary tract. In *Japanese Journal of Clinical Urology* (Vol. 73, Issue 3). <https://doi.org/10.1007/978-3-642-16499-6>
- Raisi, A., Davoodi, F., & Mohammadi, R. (2022). Protective Effects of Rosmarinic Acid on Testicular Torsion Detorsion in an Animal Model. *Iranian Journal of Veterinary Surgery*, 17(2), 80–86. <https://doi.org/10.30500/ivsa.2022.327175.1293>
- Ramaswamy, S., & Weinbauer, G. F. (2014). Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. *Spermatogenesis*, 4(2), e996025. <https://doi.org/10.1080/21565562.2014.996025>
- Rebolledo-Solleiro, D., Araiza, L. F. O., Broccoli, L., Hansson, A. C., Rocha-Arrieta, L. L., Aguilar-Roblero, R., Crespo-Ramírez, M., Fuxe, K., & Pérez de la Mora, M. (2016). Dopamine D1 receptor activity is involved in the increased anxiety levels observed in STZ-induced diabetes in rats. *Behavioural Brain Research*, 313, 293–301. <https://doi.org/10.1016/j.bbr.2016.06.060>
- Reddy, K. P., Narayana Rao, M., Murthy, J. S. R., & Reddy, P. S. (2016). Lead aggravates the diabetic-induced reproductive toxicity in male Wistar rats. In *Toxicology Research* (Vol. 5, Issue 5). <https://doi.org/10.1039/c6tx00099a>
- Reis, G., Martins, S., Antunes, A., Carlos, M., Lemos, & Gomes, M. M. (2023). A Variant In The HNF4A Gene Causing MODY-1 In A Teenage Girl: Optimal Glycaemic Control After Transition To Sulfonylurea. *Journal of Clinical Studies & Medical Case Reports*, 1–5. <https://doi.org/10.24966/csmc-8801/1000154>
- Réus, G. Z., Carlessi, A. S., Silva, R. H., Ceretta, L. B., & Quevedo, J. (2019). Relationship of

- oxidative stress as a link between diabetes mellitus and major depressive disorder. *Oxidative Medicine and Cellular Longevity*, 2019(Mdd). <https://doi.org/10.1155/2019/8637970>
- Robaire, B., & Hinton, B. T. (2015). The Epididymis. In *Knobil and Neill's Physiology of Reproduction: Two-Volume Set* (Fourth Ed., Vol. 1). Elsevier. <https://doi.org/10.1016/B978-0-12-397175-3.00017-X>
- Saeedan, A. S., Soliman, G. A., Abdel-Rahman, R. F., Abd-Elsalam, R. M., Ogaly, H. A., Foudah, A. I., & Abdel-Kader, M. S. (2021). Artemisia judaica L. diminishes diabetes-induced reproductive dysfunction in male rats via activation of Nrf2/HO-1-mediated antioxidant responses. *Saudi Journal of Biological Sciences*, 28(3), 1713–1722. <https://doi.org/10.1016/J.SJBS.2020.12.013>
- Saikia, Q., Adhikari, K., Begum, T., Dutta, S., Hazarika, A., & Kalita, J. C. (2024). Erectile dysfunction: basics and its management using plant products. *Egyptian Journal of Basic and Applied Sciences*, 11(1), 25–41. <https://doi.org/10.1080/2314808X.2023.2300560>
- Sakamoto, H. (2010). The neurobiology of psychogenic erectile dysfunction in the spinal cord. *Journal of Andrology*, 31(6), 519–526. <https://doi.org/10.2164/jandrol.110.010041>
- Salaritabar, A., Darvishi, B., HadjiakhonDi, F., Manayi, A., Sureda, A., Nabavi, S. F., Fitzpatrick, L. R., Nabavi, S. M., & Bishayee, A. (2017). Therapeutic potential of flavonoids in inflammatory bowel Disease: A comprehensive review. *World Journal of Gastroenterology*, 23(28), 5097–5114. <https://doi.org/10.3748/wjg.v23.i28.5097>
- Saldanha, L. L., Bosqueiro, A. L. D., Bosqueiro, J. R., Camaforte, H. N. A. D. P., Vareda, P. M. P., Vilegas, W., Queiroz, E. F., Marcourt, L., & Wolfender, J.-L. (2021). Use of cyanoglucosides and pharmaceutical formulations thereof in the treatment of diabetes. *United States Patent*, 2, 2–6.
- Sandhiutami, N. M. D., Desmiaty, Y., Fahleni, & Fakhran, M. H. (2024). Antinociceptive and anti-inflammatory effect of Passiflora edulis leaves extract in acetic acid-induced pain and carrageenan-induced paw oedema in vivo. *Pharmacy Education*, 24(2), 78–85. <https://doi.org/10.46542/pe.2024.242.7885>
- Sanna, F., Porcu, P., & Fattore, L. (2020). Editorial: Sexual Behavior as a Model for the Study of Motivational Drive and Related Behaviors. In *Frontiers in Behavioral Neuroscience* (Vol. 14). <https://doi.org/10.3389/fnbeh.2020.00121>
- Saumya, S., & Basha, M. (2017). Fluoride Exposure Aggravates the Testicular Damage and Sperm Quality in Diabetic Mice: Protective Role of Ginseng and Banaba. *Biological Trace Element Research*, 177(2), 331–344. <https://doi.org/10.1007/s12011-016-0893-y>
- Sengupta, P., Dutta, S., Karkada, I. R., & Chinni, S. V. (2022). Endocrinopathies and male infertility. *Life*, 12(1), 1–23. <https://doi.org/10.3390/life12010010>
- Septiani, D., Angelina, M., & Kusmana, D. (2021). Aphrodisiac Activity of Java Ginseng (Talinum paniculatum Gaertn.) Leaves Ethanolic Extract on Libido Wistar Male Rats (Rattus norvegicus). *Hermina Health Sciences Journal*, 1(1), 27–33.
- Shea, J. L., Wong, P. Y., & Chen, Y. (2014). Free testosterone. Clinical utility and important analytical aspects of measurement. In *Advances in Clinical Chemistry* (1st ed., Vol. 63, Issue May 2014). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-800094-6.00002-9>
- Shen, J., Cheriyan, S., & Ko, E. (2017). Ejaculatory dysfunction: Retrograde ejaculation. The

- diagnosis and treatment of male infertility. In N. Aziz & A. Agarwal (Eds.), *International Journal of Fertility and Women's Medicine* (Vol. 46, Issue 3).
- Shi, G. J., Li, Z. M., Zheng, J., Chen, J., Han, X. X., Wu, J., Li, G. Y., Chang, Q., Li, Y. X., & Yu, J. Q. (2017). Diabetes associated with male reproductive system damages: Onset of presentation, pathophysiological mechanisms and drug intervention. *Biomedicine and Pharmacotherapy*, 90, 562–574. <https://doi.org/10.1016/j.biopha.2017.03.074>
- Shulman, L. M., & Spritzer, M. D. (2014). Changes in the sexual behavior and testosterone levels of male rats in response to daily interactions with estrus females. *Physiology and Behavior*, 133, 8–13. <https://doi.org/10.1016/j.physbeh.2014.05.001>
- Sliwowska, J. H., Woods, N. E., Alzahrani, A. R., Paspali, E., Tate, R. J., & Ferro, V. A. (2024). Kisspeptin a potential therapeutic target in treatment of both metabolic and reproductive dysfunction. *Journal of Diabetes*, 16(4). <https://doi.org/10.1111/1753-0407.13541>
- Snoeren, E. M. S., Veening, J. G., Olivier, B., & Oosting, R. S. (2014). Serotonin 1A receptors and sexual behavior in male rats: A review. *Pharmacology Biochemistry and Behavior*, 121, 102–114. <https://doi.org/10.1016/j.pbb.2013.11.007>
- Sok Yen, F., Shu Qin, C., Tan Shi Xuan, S., Jia Ying, P., Yi Le, H., Darmarajan, T., Gunasekaran, B., & Salvamani, S. (2021). Hypoglycemic Effects of Plant Flavonoids: A Review. *Evidence-Based Complementary and Alternative Medicine*, 2021, 12. <https://doi.org/10.1155/2021/2057333>
- Soliman, G. A., Saeedan, A. S., Abdel-Rahman, R. F., Ogaly, H. A., Abd-Elsalam, R. M., & Abdel-Kader, M. S. (2019). Olive leaves extract attenuates type II diabetes mellitus-induced testicular damage in rats: Molecular and biochemical study. *Saudi Pharmaceutical Journal*, 27(3), 326–340. <https://doi.org/10.1016/J.JSPS.2018.11.015>
- Son, Y. L., Ubuka, T., & Tsutsui, K. (2022). Regulation of stress response on the hypothalamic-pituitary-gonadal axis via gonadotropin-inhibitory hormone. *Frontiers in Neuroendocrinology*, 64(September 2021), 100953. <https://doi.org/10.1016/j.yfrne.2021.100953>
- Sotiropoulou, N. S., Megremi, S. F., & Tarantilis, P. (2020). Evaluation of Antioxidant Activity, Toxicity, and Phenolic Profile of Aqueous Extracts of Chamomile (*Matricaria chamomilla* L.) and Sage (*Salvia officinalis* L.) Prepared at Different Temperatures. *Applied Sciences*, 10(7). <https://doi.org/10.3390/app10072270>
- Spinella, M. (2007). The role of prefrontal systems in sexual behavior. *International Journal of Neuroscience*, 117(3), 369–385. <https://doi.org/10.1080/00207450600588980>
- Srinivasulu, K., & Changamma, C. (2017). A study on the effect of *Ocimum sanctum* (Linn.) leaf extract and ursolic acid on spermatogenesis in male rats. *Indian Journal of Pharmaceutical Sciences*, 79(1), 158–163. <https://doi.org/10.4172/pharmaceutical-sciences.1000213>
- Srivastava, N., & Pande, M. (2017). Protocols in semen biology (comparing assays). *Protocols in Semen Biology (Comparing Assays)*, 1–288. <https://doi.org/10.1007/978-981-10-5200-2>
- Sukhov, I. B., Derkach, K. V., & Shpakov, A. O. (2016). Comparative study of functional activity of the D2-dopaminergic system in the hypothalamus of rats with different models of diabetes mellitus. *Journal of Evolutionary Biochemistry and Physiology*, 52(3), 267–

269. <https://doi.org/10.1134/S0022093016030108>

- Sun, W., & Shahrajabian, M. H. (2023). Therapeutic Potential of Phenolic Compounds in Medicinal Plants—Natural Health Products for Human Health. *Molecules*, 28(4).
- Swerdloff, R. S., Dudley, R. E., Page, S. T., Wang, C., & Salameh, W. A. (2017). Dihydrotestosterone: Biochemistry, physiology, and clinical implications of elevated blood levels. *Endocrine Reviews*, 38(3), 220–254. <https://doi.org/10.1210/er.2016-1067>
- Tekin, B., ELMACIOĞLU, F., & ÜLKER, M. T. (2023). Alzheimer Disease-Type 3 Diabetes: Traditional Review. *Turkiye Klinikleri Journal of Health Sciences*, 8(1), 153–160. <https://doi.org/10.5336/healthsci.2022-94043>
- Uysal, I., Koçer, O., Mohammed, F. S., Lekesiz, Ö., Doğan, M., Şabik, A. E., Sevindik, E., Gerçeker, F. Ö., & Sevindik, M. (2023). Pharmacological and Nutritional Properties: Genus *Salvia*. *Advances in Pharmacology and Pharmacy*, 11(2), 140–155. <https://doi.org/10.13189/app.2023.110206>
- Vallianou, N. G., Stratigou, T., Geladari, E., Tessier, C. M., Mantzoros, C. S., & Dalamaga, M. (2021). Diabetes type 1: Can it be treated as an autoimmune disorder? *Reviews in Endocrine and Metabolic Disorders*, 22(4), 859–876. <https://doi.org/10.1007/s11154-021-09642-4>
- Vazquez, E., Navarro, M., Salazar, Y., Crespo, G., Bruges, G., Osorio, C., Tortorici, V., Vanegas, H., & López, M. (2015). Systemic changes following carrageenan-induced paw inflammation in rats. *Inflammation Research*, 64(5), 333–342. <https://doi.org/10.1007/s00011-015-0814-0>
- Vilmosh, N., Delev, D., Kostadinov, I., Zlatanova, H., Kotetarova, M., Kandilarov, I., & Kostadinova, I. (2022). Anxiolytic Effect of *Satureja montana* Dry Extract and its Active Compounds Rosmarinic Acid and Carvacrol in Acute Stress Experimental Model. *Journal of Integrative Neuroscience*, 21(5). <https://doi.org/10.31083/j.jin2105124>
- Wang, H., Zhao, T., Liu, Z., Danzengquzhen, Cisangzhuoma, Ma, J., Li, X., Huang, X., & Li, B. (2023). The neuromodulatory effects of flavonoids and gut Microbiota through the gut-brain axis. *Frontiers in Cellular and Infection Microbiology*, 13(June), 1–11. <https://doi.org/10.3389/fcimb.2023.1197646>
- Wang, Y. (2003). Male Reproductive Toxicology. *Current Protocols in Toxicology*, 16(16.6), 1–5. <https://doi.org/10.1002/0471140856.tx1606s14>. PMID: 20963758.
- Wankeu-Nya, M., Watcho, P., Deeh Defo, P. B., Ngadjui, E., Ngudefack, T. B., Kamtchouing, P., & Kamanyi, A. (2019). Aqueous and ethanol extracts of *Dracaena arborea* (Wild) Link (Dracaenaceae) alleviate reproductive complications of diabetes mellitus in rats. *Andrologia*, 51(10), 1–11. <https://doi.org/10.1111/and.13381>
- Wankeu-Nya, M., Watcho, P., Ngudefack, T. B., Carro-Juarez, M., Tapondjou, L., & Kamanyi, A. (2014). Effects of *Dracaena arborea* (Dracaenaceae) on sexual dysfunction in 4 weeks hyperglycemic male rats. *Asian Pacific Journal of Tropical Medicine*, 7(8), 609–619. [https://doi.org/10.1016/S1995-7645\(14\)60103-6](https://doi.org/10.1016/S1995-7645(14)60103-6)
- Whitney, K. M., & Suttie, A. W. (2018). Testis and Epididymis. In *Boorman's Pathology of the Rat*. Elsevier Inc. <https://doi.org/10.1016/b978-0-12-391448-4.00028-9>
- Widyarini, S., Sugiyono, Akrom, A. M., & Paryuni, A. D. (2023). Carrageenan-Induced Acute Inflammation on Back-Skin of Mice: Histopathological Features, Number of

- Inflammatory Cells, and Expression of COX-2, COX-1, and IL-6. *World's Veterinary Journal*, 13(4), 520–530. <https://doi.org/10.54203/SCIL.2023.WVJ55>
- World Health Organization. (2023). *Infertility prevalence estimates* (T. A. Ghebreyesus (ed.)).
- Xu, R., Wang, F., Zhang, Z., Zhang, Y., Tang, Y., Bi, J., Shi, C., Wang, D., Yang, H., Wang, Z., & Tang, Z. (2023). Diabetes-Induced Autophagy Dysregulation Engenders Testicular Impairment via Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, 2023. <https://doi.org/10.1155/2023/4365895>
- Yaris, E., Balur Adsız, L., Yener, I., Tuncay, E., Yilmaz, M. A., Akdeniz, M., Kaplaner, E., Firat, M., Ertas, A., & Kolak, U. (2021). Isolation of secondary metabolites of two endemic species: *Salvia rosifolia* Sm. and *Salvia cerino-pruinosa* Rech. f. var. *elazigensis* (Lamiaceae). *Journal of Food Measurement and Characterization*, 15(6), 4929–4938. <https://doi.org/10.1007/s11694-021-01065-8>
- Yaya, S. T., Louise, A. B. M., Sirabana, C., Koffi, S., Claude, M. J., & Flavien, raoré. (2023). Evaluation of the Effects of Aqueous Extract of *Cnestis ferruginea* on Sexual Behaviour in Male Rats. *Scholars Academic Journal of Biosciences*, 11(1), 16–22. <https://doi.org/10.36347/sajb.2023.v11i01.003>
- Yeniçeri, E., Altay, A., Koksall, E., Altın, S., Taslimi, P., Yılmaz, M. A., Cakir, O., Tarhan, A., & Kandemir, A. (2024). Phytochemical profile by LC-MS/MS analysis and evaluation of antioxidant, antidiabetic, anti-Alzheimer, and anticancer activity of *Onobrychis argyrea* leaf extracts. *European Journal of Integrative Medicine*, 66(January). <https://doi.org/10.1016/j.eujim.2024.102337>
- Yilmaz, G., Eruygur, N., Bona, G. E., Bona, M., Akdeniz, M., Yilmaz, M. A., & Ertas, A. (2023). Phytochemical analysis, antioxidant, and enzyme inhibition activity of five *Salvia* taxa from Turkey. *South African Journal of Botany*, 152(December 2022), 212–221. <https://doi.org/10.1016/j.sajb.2022.11.027>
- Yilmaz, M. A. (2020). Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC–MS/MS method validation. *Industrial Crops and Products*, 149(December 2019), 112347. <https://doi.org/10.1016/j.indcrop.2020.112347>
- Yilmaz, M. A., & Cakir, O. (2024). *Salvia* (Sage) Species, A Game-Changer Bioactive Plant: Focus on Antioxidant Properties. In *Natural Antioxidants and in vitro Antioxidant Assays* (pp. 71–98). <https://www.researchgate.net/publication/382625156>
- Yilmaz, M. A., Taslimi, P., Kılıç, Ö., Gülçin, İ., Dey, A., & Bursal, E. (2023). Unravelling the phenolic compound reserves, antioxidant and enzyme inhibitory activities of an endemic plant species, *Achillea pseudoaleppica*. *Journal of Biomolecular Structure and Dynamics*, 41(2), 445–456. <https://doi.org/10.1080/07391102.2021.2007792>
- Yolanda López Ramírez, Lucio, R. A., Ríos, E. A., López, J. L. T., Valderrama, L. R., & Lorenzana, M. G. (2019). Morphophysiology of the epididymis and spermatozoa of the Wistar rat after sexual satiety. In E. A. Ríos & G. F. Mascorro (Eds.), *A view from Mexico Spermatozoa* (p. 286).
- Yoon, J. H., Hwang, J. H., Son, S. U., Choi, J., You, S. W., Park, H., Cha, S. Y., & Maeng, S. (2023). How Can Insulin Resistance Cause Alzheimer's Disease? *International Journal of Molecular Sciences*, 24(4). <https://doi.org/10.3390/ijms24043506>

- Zheng, H., Hu, Y., Zhou, J., Zhou, B., & Qi, S. (2024). Protective Effect of Black Rice Cyanidin-3-Glucoside on Testicular Damage in STZ-Induced Type 1 Diabetic Rats. *Foods*, 13(5). <https://doi.org/10.3390/foods13050727>
- Zhumaliyeva, G., Zhussupova, A., Zhusupova, G. E., Błońska-Sikora, E., Cerreto, A., Omirbekova, N., Zhunusbayeva, Z., Gemejiyeva, N., Ramazanova, M., Wrzosek, M., & Ross, S. A. (2023). Natural Compounds of Salvia L. Genus and Molecular Mechanism of Their Biological Activity. *Biomedicines*, 11(12). <https://doi.org/10.3390/biomedicines11123151>
- Zygmunt-Siembida, E., Wróblewski, H., Zimna, A., Wróblewska, K., Kozdra, M., Piasek, L., & Osińska, A. (2023). Gestational diabetes mellitus - pathogenesis, diagnosis, treatment and prognosis. *Quality in Sport*, 11(1), 11–15. <https://doi.org/10.12775/qs.2023.11.01.001>

Annexes

Annex 01:

Table S01. Analytical method validation parameters that belong to the LC-MS/MS method

No	Analytes	RT ^a	M.I. (m/z) ^b	F.I. (m/z) ^c	Ion. mode	Equation	r^2 ^d	RSD% ^e		Linearity Range (mg/L)	LOD/LOQ (µg/L) ^f	Recovery (%)		U ^g	Gr. No ⁱ
								Interday	Intraday			Interday	Intraday		
1	Quinic acid	3.0	190.8	93.0	Neg	$y = -0.0129989 + 2.97989 \times$	0.996	0.69	0.51	0.1-5	25.7/33.3	1.0011	1.0083	0.0372	1
2	Fumaric acid	3.9	115.2	40.9	Neg	$y = -0.0817862 + 1.03467 \times$	0.995	1.05	1.02	1-50	135.7/167.9	0.9963	1.0016	0.0091	1
3	Aconitic acid	4.0	172.8	129.0	Neg	$y = -0.7014530 + 32.9994 \times$	0.971	2.07	0.93	0.1-5	16.4/31.4	0.9968	1.0068	0.0247	1
4	Gallic acid	4.4	168.8	79.0	Neg	$y = 0.0547697 + 20.8152 \times$	0.999	1.60	0.81	0.1-5	13.2/17.0	1.0010	0.9947	0.0112	1
5	Epigallocatechin	6.7	304.8	219.0	Neg	$y = -0.00494986 + 0.0483704 \times$	0.998	1.22	0.73	1-50	237.5/265.9	0.9969	1.0040	0.0184	3
6	Protocatechuic acid	6.8	152.8	108.0	Neg	$y = 0.211373 + 12.8622 \times$	0.957	1.43	0.76	0.1-5	21.9/38.6	0.9972	1.0055	0.0350	1
7	Catechin	7.4	288.8	203.1	Neg	$y = -0.00370053 + 0.431369 \times$	0.999	2.14	1.08	0.2-10	55.0/78.0	1.0024	1.0045	0.0221	3
8	Gentisic acid	8.3	152.8	109.0	Neg	$y = -0.0238983 + 12.1494 \times$	0.997	1.81	1.22	0.1-5	18.5/28.2	0.9963	1.0077	0.0167	1
9	Chlorogenic acid	8.4	353.0	85.0	Neg	$y = 0.289983 + 36.3926 \times$	0.995	2.15	1.52	0.1-5	13.1/17.6	1.0000	1.0023	0.0213	1
10	Protocatechuic aldehyde	8.5	137.2	92.0	Neg	$y = 0.257085 + 25.4657 \times$	0.996	2.08	0.57	0.1-5	15.4/22.2	1.0002	0.9988	0.0396	1
11	Tannic acid	9.2	182.8	78.0	Neg	$y = 0.0126307 + 26.9263 \times$	0.999	2.40	1.16	0.05-2.5	15.3/22.7	0.9970	0.9950	0.0190	1
12	Epigallocatechin gallate	9.4	457.0	305.1	Neg	$y = -0.0380744 + 1.61233 \times$	0.999	1.30	0.63	0.2-10	61.0/86.0	0.9981	1.0079	0.0147	3
13	1,5-dicaffeoylquinic acid	9.8	515.0	191.0	Neg	$y = -0.0164044 + 16.6535 \times$	0.999	2.42	1.48	0.1-5	5.8/9.4	0.9983	0.9997	0.0306	1
14	4-OH Benzoic acid	10.5	137.2	65.0	Neg	$y = -0.0240747 + 5.06492 \times$	0.999	1.24	0.97	0.2-10	68.4/88.1	1.0032	1.0068	0.0237	1
15	Epicatechin	11.6	289.0	203.0	Neg	$y = -0.0172078 + 0.0833424 \times$	0.996	1.47	0.62	1-50	139.6/161.6	1.0013	1.0012	0.0221	3
16	Vanillic acid	11.8	166.8	108.0	Neg	$y = -0.0480183 + 0.779564 \times$	0.999	1.92	0.76	1-50	141.9/164.9	1.0022	0.9998	0.0145	1
17	Caffeic acid	12.1	179.0	134.0	Neg	$y = 0.120319 + 95.4610 \times$	0.999	1.11	1.25	0.05-2.5	7.7/9.5	1.0015	1.0042	0.0152	1
18	Syringic acid	12.6	196.8	166.9	Neg	$y = -0.0458599 + 0.663948 \times$	0.998	1.18	1.09	1-50	82.3/104.5	1.0006	1.0072	0.0129	1
19	Vanillin	13.9	153.1	125.0	Poz	$y = 0.00185898 + 20.7382 \times$	0.996	1.10	0.85	0.1-5	24.5/30.4	1.0009	0.9967	0.0122	1
20	Syringic aldehyde	14.6	181.0	151.1	Neg	$y = -0.0128684 + 7.90153 \times$	0.999	2.51	0.77	0.4-20	19.7/28.0	1.0001	0.9964	0.0215	1
21	Daidzin	15.2	417.1	199.0	Poz	$y = 9.45747 + 152.338 \times$	0.996	2.25	1.32	0.05-2.5	7.0/9.5	0.9955	1.0017	0.0202	2
22	Epicatechin gallate	15.5	441.0	289.0	Neg	$y = -0.0142216 + 1.06768 \times$	0.997	1.63	1.28	0.1-5	19.5/28.5	0.9984	0.9946	0.0229	3
23	Piceid	17.2	391.0	135/106.9	Poz	$y = 0.00772525 + 25.4181 \times$	0.999	1.94	1.16	0.05-2.5	13.8/17.8	1.0042	0.9979	0.0199	1
24	p-Coumaric acid	17.8	163.0	93.0	Neg	$y = 0.0249034 + 18.5180 \times$	0.999	1.92	1.43	0.1-5	25.9/34.9	1.0049	1.0001	0.0194	1
25	Ferulic acid-D3-IS ^h	18.8	196.2	152.1	Neg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.0170	1
26	Ferulic acid	18.8	192.8	149.0	Neg	$y = -0.0735254 + 1.34476 \times$	0.999	1.44	0.53	1-50	11.8/15.6	0.9951	0.9976	0.0181	1
27	Sinapic acid	18.9	222.8	193.0	Neg	$y = -0.0929932 + 0.836324 \times$	0.999	1.45	0.52	0.2-10	65.2/82.3	1.0031	1.0037	0.0317	1
28	Coumarin	20.9	146.9	103.1	Poz	$y = 0.0633397 + 136.508 \times$	0.999	2.11	1.54	0.05-2.5	214.2/247.3	0.9950	0.9958	0.0383	1

^aRT.: Retention time, ^bMI (m/z): Molecular ions of the standard analytes (m/z ratio), ^cFI (m/z): Fragment ions ^d r^2 : Coefficient of determination, ^eRSD: Relative standard deviation, ^fLOD/LOQ (µg/L): Limit of detection/quantification, ^gU (%): percent relative uncertainty at 95% confidence level ($k = 2$), ^hIS: Internal standard, ⁱGr. No: Represents grouping of internal standards, these numbers indicate which IS stands for which phenolic compound.

Table S01. Analytical method validation parameters that belong to the LC-MS/MS method (Continued)

No	Analytes	RT ^a	M.I. (m/z) ^b	F.I. (m/z) ^c	Ion. mode	Equation	r^{2d}	RSD% ^e		Linearity Range (mg/L)	LOD/LOQ (µg/L) ^f	Recovery (%)		U ^g	Gr. No
								Interday	Intraday			Interday	Intraday		
29	Salicylic acid	21.8	137.2	65.0	Neg	$y=0.239287+153.659 \times$	0.999	1.48	1.18	0.05-2.5	6.0/8.3	0.9950	0.9998	0.0158	1
30	Cynaroside	23.7	447.0	284.0	Neg	$y=0.280246+6.13360 \times$	0.997	1.56	1.12	0.05-2.5	12.1/16.0	1.0072	1.0002	0.0366	2
31	Miquelianin	24.1	477.0	150.9	Neg	$y=-0.00991585+5.50334 \times$	0.999	1.31	0.95	0.1-5	10.6/14.7	0.9934	0.9965	0.0220	2
32	Rutin-D3-IS ^h	25.5	612.2	304.1	Neg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	2
33	Rutin	25.6	608.9	301.0	Neg	$y=-0.0771907+2.89868 \times$	0.999	1.38	1.09	0.1-5	15.7/22.7	0.9977	1.0033	0.0247	2
34	isoquercitrin	25.6	463.0	271.0	Neg	$y=-0.111120+4.10546 \times$	0.998	2.13	0.78	0.1-5	8.7/13.5	1.0057	0.9963	0.0220	2
35	Hesperidin	25.8	611.2	449.0	Poz	$y=0.139055+13.2785 \times$	0.999	1.84	1.35	0.1-5	19.0/26.0	0.9967	1.0043	0.0335	2
36	<i>o</i> -Coumaric acid	26.1	162.8	93.0	Neg	$y=0.00837193+11.2147 \times$	0.999	2.11	1.46	0.1-5	31.8/40.4	1.0044	0.9986	0.0147	1
37	Genistin	26.3	431.0	239.0	Neg	$y=1.65808+7.57459 \times$	0.991	2.01	1.28	0.1-5	14.9/21.7	1.0062	1.0047	0.0083	2
38	Rosmarinic acid	26.6	359.0	197.0	Neg	$y=-0.0117238+8.04377 \times$	0.999	1.24	0.86	0.1-5	16.2/21.2	1.0056	1.0002	0.0130	1
39	Ellagic acid	27.6	301.0	284.0	Neg	$y=0.00877034+0.663741 \times$	0.999	1.57	1.23	0.4-20	56.9/71.0	1.0005	1.0048	0.0364	1
40	Cosmosiin	28.2	431.0	269.0	Neg	$y=-0.708662+8.62498 \times$	0.998	1.65	1.30	0.1-5	6.3/9.2	0.9940	0.9973	0.0083	2
41	Quercitrin	29.8	447.0	301.0	Neg	$y=-0.00153274+3.20368 \times$	0.999	2.24	1.16	0.1-5	4.8/6.4	0.9960	0.9978	0.0268	2
42	Astragalin	30.4	447.0	255.0	Neg	$y=0.00825333+3.51189 \times$	0.999	2.08	1.72	0.1-5	6.6/8.2	0.9968	0.9957	0.0114	2
43	Nicotiflorin	30.6	592.9	255.0/284.0	Neg	$y=0.00499333+2.62351 \times$	0.999	1.48	1.23	0.05-2.5	11.9/16.7	0.9954	1.0044	0.0108	2
44	Fisetin	30.6	285.0	163.0	Neg	$y=0.0365705+8.09472 \times$	0.999	1.75	1.19	0.1-5	10.1/12.7	0.9980	1.0042	0.0231	3
45	Daidzein	34.0	253.0	223.0	Neg	$y=-0.0329252+6.23004 \times$	0.999	2.18	1.73	0.1-5	9.8/11.6	0.9926	0.9963	0.0370	3
46	Quercetin-D3-IS ^h	35.6	304.0	275.9	Neg	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	3
47	Quercetin	35.7	301.0	272.9	Neg	$y=+0.00597342+3.39417 \times$	0.999	1.89	1.38	0.1-5	15.5/19.0	0.9967	0.9971	0.0175	3
48	Naringenin	35.9	270.9	119.0	Neg	$y=-0.00393403+14.6424 \times$	0.999	2.34	1.69	0.1-5	2.6/3.9	1.0062	1.0020	0.0392	3
49	Hesperetin	36.7	301.0	136.0/286.0	Neg	$y=+0.0442350+6.07160 \times$	0.999	2.47	2.13	0.1-5	7.1/9.1	0.9998	0.9963	0.0321	3
50	Luteolin	36.7	284.8	151.0/175.0	Neg	$y=-0.0541723+30.7422 \times$	0.999	1.67	1.28	0.05-2.5	2.6/4.1	0.9952	1.0029	0.0313	3
51	Genistein	36.9	269.0	135.0	Neg	$y=-0.00507501+12.1933 \times$	0.999	1.48	1.19	0.05-2.5	3.7/5.3	1.0069	1.0012	0.0337	3
52	Kaempferol	37.9	285.0	239.0	Neg	$y=-0.00459557+3.13754 \times$	0.999	1.49	1.26	0.05-2.5	10.2/15.4	0.9992	0.9990	0.0212	3
53	Apigenin	38.2	268.8	151.0/149.0	Neg	$y=0.119018+34.8730 \times$	0.998	1.17	0.96	0.05-2.5	1.3/2.0	0.9985	1.0003	0.0178	3
54	Amentoflavone	39.7	537.0	417.0	Neg	$y=0.727280+33.3658 \times$	0.992	1.35	1.12	0.05-2.5	2.8/5.1	0.9991	1.0044	0.0340	3
55	Chrysin	40.5	252.8	145.0/119.0	Neg	$y=-0.0777300+18.8873 \times$	0.999	1.46	1.21	0.05-2.5	1.5/2.8	0.9922	1.0050	0.0323	3
56	Acacetin	40.7	283.0	239.0	Neg	$y=-0.559818+163.062 \times$	0.997	1.67	1.28	0.02-1	1.5/2.5	0.9949	1.0011	0.0363	3

^aR.T.: Retention time, ^bMI (m/z): Molecular ions of the standard analytes (m/z ratio), ^cFI (m/z): Fragment ions ^d r^2 : Coefficient of determination, ^eRSD: Relative standard deviation, ^fLOD/LOQ (µg/L): Limit of detection/quantification, ^gU (%): percent relative uncertainty at 95% confidence level ($k = 2$), ^hIS: Internal standard, ⁱGr. No: Represents grouping of internal standards, these numbers indicate which IS stands for which phenolic compound.

Annex 02: Experimental evidence



Harvesting



Cleaning & drying



Grinding



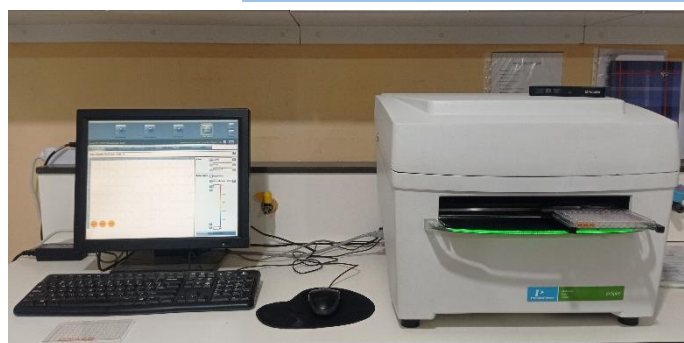
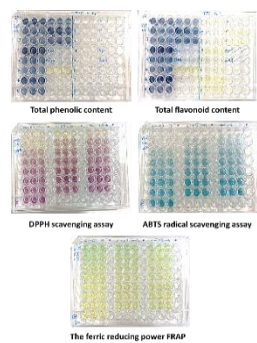
Decoction



Vacuum filtration



Freeze-drying



Determination of polyphenol, flavonoid contents and antioxidant activity of the extract



Sample preparation and quantification of phytochemicals by UHPLC-ESI-MS/MS method



Diabetes induction preparation



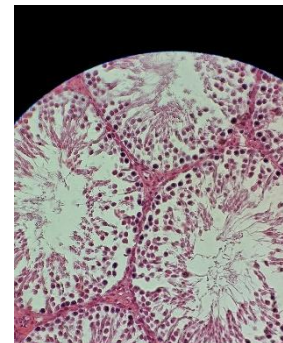
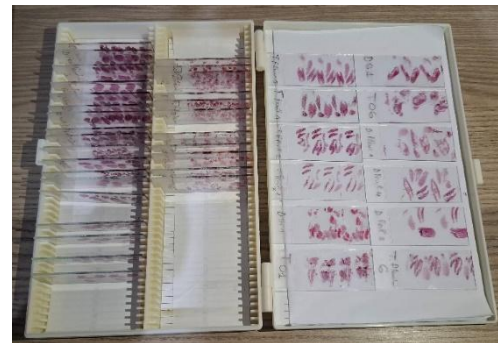
Force-feeding



Intraplantar injection



Leg thickness



Organ sampling and histopathological analysis



Sperm quality assessment

Scientific Publications and Communications:

Publications:

- **Wadjila S. Souidi**, Mansouria Belhocine, Mohamed Bouzouina, Mustafa A. Yilmaz, Ines Feknous, Oguz Cakir, Abbas Tarhan, Tayeb Sitayeb, and Abdelkader E. Dahou (2025). Phytochemical Profiling by UHPLC–ESI–MS/MS, In Vitro Antioxidant, In Vivo Antidiabetic, and Pro-Sexual Effects of *Salvia balansae* Noë Ex Coss. Flower Extract in Diabetic Male Rats. Chemistry & Biodiversity 0:e202402552 <https://doi.org/10.1002/cbdv.202402552>
- **Sendous W. Souidi**, Mansouria Belhocine, Ines Feknous, Chawki Bensouici, Tayeb Sitayeb, and Abdelkader A. Dahou (2025). Antidiabetic, antioxidant, and therapeutic effects of *Salvia balansae* leaf and stem extracts on sexual function in diabetic male rats. Archives of Biological Sciences ;77(1):27-36 <https://doi.org/10.2298/ABS241218003S>
- Ines Feknous, Djamel Ait Saada, Cerine Yasmine Boulahlib, Laura Alessandroni, **Sendous Wadjila Souidi**, Ouiza Ait Chabane, and Mohammed Gagaoua (2023). Poultry meat quality preservation by plant extracts: an overview. Meat Technology. 64(3), 80-101. <https://doi.org/10.18485/meattech.2023.64.3.2>

National communications:

- The fourth National Seminar of Biology and Health held at Djillali Liabes University of Sidi Bel Abbes on October 21, 2023: Effectiveness of sage aqueous leaf extract on reproductive function in streptozotocin induced diabetic male rat. **Sendous Wadjila SOUIDI**, Mansouria BELHOCINE, Mouhamed BOUZOUINA, Aicha MERABTI and Dallal KOURAT.
- The fourth National Seminar of Biology and Health held at Djillali Liabes University of Sidi Bel Abbes on October 21, 2023: Sexual behavior and sperm parameters in streptozotocin-induced diabetic rats. **Sendous Wadjila SOUIDI**, Mansouria Belhocine, Mouhamed Bouzouina, Hajer Ziadi and Nour El Houda Khenifer.
- The first National Pharmacognosy Day: Phytotherapy & Aromatherapy, held at Faculty of Medicine – Annaba on 8 November 2025: Exploring the Therapeutic Potential of *Salvia balansae* Flower Extract: UHPLC Profiling, Antioxidant, Antidiabetic, and Pro-Sexual Effects in Diabetic Male Rats. **Wadjila Sendous SOUIDI**, Mansouria BELHOCINE, Ines FEKNOUS, Hajer ZIADI, SiTayeb Tayeb.

Intenational communications:

- The first international seminar on biology, physiology and pathophysiology (ISBPP'23) Held at Hassiba Benbouali University of Chlef, from 18-19 October 2023 : Effect of sage aqueous extract on sexual behavior in Streptozotocin-induced diabetic male rat. **SOUIDI Wadjila Sendous**, BELHOCINE Mansouria, BOUZOUINA Mouhamed, KHENIFER Nour El Houda, BOUAZZA Sarah.
- The second international day of food technology, nutrition and human health held at National Higher School of Agronomy of Algiers on 20 Novembre 2024: Antidiabetic and anti-inflammatory effects of a sage species endemic to Mostaganem in Wistar rats. **SOUIDI Wadjila Sendous**, FEKNOUS Ines, BELHOCINE Mansouria, BOUZOUINA Mouhamed & TAYEB Si Tayeb.