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Studing the effect of exercise on the expression of inducible nitric oxide synthase and heat shock protein 70 in the brains of mice with induced Parkinson`s disease

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Dedication

I would like to dedicate this thesis to my husband Abdulla who has supported me in all stages of my study, I also would like to dedicate this work for my kids, the flowers of my life, to father and mother vestl souls, to my brothers and sisters.

RESUME

La maladie de Parkinson est une maladie neurodégénérative commun. La carence en dopamine est considérée comme responsable du développement de la maladie de Parkinson. L'entraînement physique a été associé à des améliorations chez les patients atteints de la maladie de Parkinson.L'objectif de la présente étude est d'explorer l'effet de l'entraînement sur l'expression de HSP70 et iNOS dans le cerveau des souris atteintes de la maladie de Parkinson induite.Quarante souris albinos ont été sélectionnés et ils sont attribuées en quatre groupes: contrôle sédentaire (CS, n = 10), exercice contrôle (EC, N = 10), et un groupe pour induire la maladie de Parkinson (GMP, N = 10) et un groupe de 10 souris albinos maladie de Parkinson qui suivé l'exercice (EPD, N = 10). Le Protocole de MPTP a été utilisé pour induire la maladie de Parkinson par des injections de 10 doses de MPTP (25 mg / kg) et du probénécide (250 mg / kg) pendant 5 semaines. Après la formation de protocoles et apres l'exercice sur tapis roulant est terminé.

Les échantillons de tissus cérébraux ont été évalués par immunohistochimie pour examiner l'expression de HSP70 dans les quatre groupes d'animaux.Les résultats de la présente étude montrent que l'expression de HSP70 a été réduite dans le cerveau des souris atteintes de la maladie de Parkinson de manière significative (P <0,05) en comparaison avec des groupes témoins. Les résultats ont également montré que l'entraînement physique a augmenté l'expression de HSP70 dans EC de façon significative (P <0,05) par rapport au groupe de contrôle, et non significative (P> 0,05) dans EPD par rapport à GMD.Bien que l'augmentation de l'expression de HSP70 dans la maladie de Parkinson nest été pas significatif donc il a un rôle potentiel dans l'amélioration de l'état des souris atteintes de la maladie de Parkinson et il peut avoir un rôle thérapeutique potentiel.Les données de la présente étude ont également montré une expression significative de iNOS dans les cerveaux de rats avec la maladie de Parkinson induite par rapport au groupe témoin (p 0,000) et par rapport au groupe de contrôle, et cette expression était significativement diminuée dans le groupe exercé (P 0,000).

Mots clés : Métaux lourds, Plomb, Maladie de Parkinson, Souris, HSP70

ABSTRACT

Parkinson disease is a common neurodegenerative disease. Deficiency of dopamine is thought to be responsible for development of Parkinson Disease. Exercise training has been associated with improvements in patients with Parkinson Disease. The objective of the present study is to explore the effect of exercise training on the expression of HSP70 and iNOS in brains of mice with induced Parkinson Disease.

Forty albino mice were selected and assigned into four groups: Sedentary control (SC, N=10), exercised control (EC, N=10), Parkinson Disease (PD, N=10) and exercised Parkinson Disease (EPD, N=10). MPTP protocol was used to induce Parkinson Disease by injections of 10 doses of MPTP (25 mg/kg) and probenecid (250 mg/kg) over 5 weeks. After the protocols treadmill exercise training had been finished, samples from the brain tissues were assessed by immunohistochemistry to examine the expression of HSP70 in the four groups of animals.

The results of the present study showed that the expression of HSP70 was reduced in the brain of mice with Parkinson Disease significantly ($P \leq 0.05$) compared with control groups. The results also showed that exercise training increased the expression of HSP70 in EC significantly ($P \leq 0.05$) compared with control group, and insignificantly ($P>0.05$) in EPD compared with PD.

Although the increased expression of HSP70 in exercised Parkinson Disease was not significant, it has a potential role in improved the status of mice with Parkinson Disease and it may have a potential therapeutic role.

The data of the present study also showed significant expression of iNOS in brainsof rats with induced Parkinson Disease compared with control group (P 0.000) compared with control group, and this expression was significantly decreased in exercised group (P 0.000).

Keys Words: Heavy metals, lead, Parkinson disease, mice, HSP70

ملخص

مرض باركنسون هومن الأمراض العصبية الشائعة ذات التاكل العصبي. ويعتقد أن نقص الدوبامين من الأسباب الرئيسية التي تؤدي إلى تطور هذا المرض.وقد بينت الدراسات أن التمارين الرياضية تلعب دوراً هاماً في تحسين الوضع السريري لمرضى الباركنسون. وقد هدفت هذه الدراسة الى استكشاف تاثير التمارين الرياضية على تركيز المؤشرين الحيويين (HSP70وiNOS) في ادمغة الفئران المستحدث فيها مرض الباركنسون.وقد تم اختيار أربعين فارا وتوزيعها إلى أربع مجموعات: المجموعة الضابطة (SC، N = 10)، مجموعة التدريب (ECN = 10,)، مجموعة مرض باركنسون (PD، N = 10) ومجموعة مرض باركنسون المتدربة (EPDN = 10,). ولقد تم استخدام بروتوكول MPTP لاستحداث مرض باركنسون عن طريق حقن 10جرعات (25ملغ/كغم) والبروبينسيد (250 ملغ/كغ) لمدة 5 أسابيع. وبعد أن انتهت التدريبات الرياضية, تم أخذ عينات نسيجية من الدماغ وفحصها عن طريق الاصباغ المناعية الكيميائية لفحص تركيز المؤشرين الحيويين (HSP70وiNOS) في مجموعات الدراسة.

وأظهرت نتائج الدراسة أن تركيز HSP70 انخفض في أدمغة الفئران المصابة بمرض الباركنسون بشكل إحصائي هام (P<0.05) مقارنة مع مجموعة السيطرة. وأظهرت النتائج أيضاً أن التمرينات زادت تركيز HSP70 في مجموعة التدريب (P<0.05) مقارنة مع مجموعة السيطرة، (P <0.05) في مجموعة باركنسون المتدربة مقارنة مع مجموعة باركنسون.

كما أظهرت بيانات هذه الدراسة وجود تراكيز هامة من iNOS في أدمغة الفئران الصابة بمرض الباركنسون مقارنة مع مجموعة السيطرة (P0.000). وهذا التركيز قد انخفض بشكل ملحوظ في مجموعة التدريب (P 0.000).

الاستنتاجات: بينت الدراسة أن التمارين الرياضية تساعد في تحسين الوضع الصحي لمرضى الباركنسون وذلك من خلال زيادة تركيز المؤشر الحيوي HSP70أو خفض تركيز المؤشر الحيوي iNOS في أدمغة الفئران المصابة بمرض الباركنسون. وعلاوة على ذلك، فقد أشارت نتائج هذه الدراسة إلى امكانية استهداف هذه البروتينات كاهداف علاجية.**الكلمات الدالة:** المعادن الثقيلة، الرصاصة مرض الرعاش.

Recommendations

1- Exerise traninig is recomemended regulary for wide spectrum of people either healthy or diseased.
2- Patients with PD are recommended to perform exercise training to improve their health status.
3- It is recommended to use HSP70 and iNOS inhibitors by pharmaceutical companies as new therapeutic option fo PD.

Abbreviations

APV: Amino phosphonoValeric

AD: aldehyde dehydrogenase

ADHD: Attention defcit Hyperactivity disorder

AMD:acid mine drainage

ATSDR: Agency for Toxicsubstances and Disease Registry

BDNF: Brain- derived neurotrophic Factor

BG: Basal Ganglia

BLL: Blood Lead Level

CAMKII: Calcium\calmadulin Kinase II

CDC: Centers for Disease Control

COMT: catechol-O- methyltransferase

CREB:cAMP Response element binding protein

DA: Dopamine

eNOS: endothelial NOS

EPSCs: Excitatory postsynaptic Currents

GA: Geldanamycin

GABA: gamma-aminobutyric acid

GABA: glutamate amby-aminobutyric acid

HSP: heat shock protein

iNOS: inducible NOS

IPSCs: Inhibitory postsynaptic Currents

IQ:Intelligence quotient

LBs: LewyBodies

L-DOPA: L-dihydroxyphenylalanine

MAKP: mitogen activated Protein Kinase

MAO: monoamine oxidase

MPTP: N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine

NINDS: National Institute of Neurological Disorders and Stroke

NMDAR: N-mehyle D-aspartate Receptor

nNOS: Neuronal NOS

NO: Nitric oxide

NOS: Nitric oxide synthase

NR1: NMAD Receptor

Pb: Lead (Plumbum)

PD: Parkinson's disease

ROS: reactive oxygen species

SH: sulphydrylgroupe

SPM: Suspended particulate matters

TH: Tyrosine hydroxylase

UCH-L1: Ubiquitin carboxyl-terminal hydrolase L1

UPDRS:The Unified Parkinson's Disease Rating scale

VGCCs: Voltage – gated calcium channels

VPA: valproic acid

VTA: ventral tegmental area

αSN :α-synuclein

µg/dL: micrograms Per deciliter

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Chapter One

Heavy metals and neurotoxicity

INTRODUCTION

The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). “Heavy metals” is a general collective term, which applies to the group of metals and metalloids with atomic density grea-ter than 4 g/cm3, or 5 times or more, greater than water (Huton and Symon,1986; Battarbee et al., 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino et al., 1995, Hawkes, 1997). However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements. Environment is defined as the totality of circumstances surrounding an organism or group of organisms especi-ally, the combination of external physical conditions that affect and influence the growth, development and survival of organisms (Farlex, 2005).

It consists of the flora, fauna and the abiotic, and includes the aquatic, terrestrial and atmospheric habitats. The environment is considered in terms of the most tangible aspects like air, water and food, and the less tangible, though no less important, the communities we live in (Gore, 1997).

A pollutant is any substance in theenvironment,which causes objection-able effects, impairing the welfare of the environment, reducing the quality of life and may eventually cause death. Such a substance has to be present in the enviro-nment beyond a set or tolerance limit, which could be either a desirableor acceptable limit. Hence, environ-mental pollution is the presence of a pollutant in the envi-ronment; air, water and soil, which may be poisonous or toxic and will cause harm to living things in the polluted (Duru.ibe et al., 2007).

1. Heavy Metal Emission

Heavy metals can be emitted into the environment by both natural and anthropogenic causes. The major caus-es of emission are the anthropogenic sources specifically mining operations (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu, 1989). In some cases, even long aft-er mining activities have ceased, the emitted metals con-tinue to persist in the environment. Peplow (1999) repor-ted that hard rock mines operate from 5-15 years until the minerals are depleted, but metal contamination that occurs as aconsequence of hard rock mining persist for hundreds of years after the cessation of mining opera-tions. Apart from mining operations, mercury is introduc-ed into the environment through cosmetic products as well as manufacturing processes like making of sodium hydroxide. Heavy metals are emitted both in elemental and comp-ound (organic and inorganic) forms. Anthropogenic sour-esof emission are the various industrial point sources including former and present mining sites, foundries and smelters, combustion by-products and traffics (UNEP / GPA, 2004).

Cadmium is released as a by- product of zinc (and occasionally lead) refining; lead is emitted du-ring its mining and smelting activities, from automobile exhausts (by combustion of petroleum fuels treated with tetraethyl lead antiknock) and from old lead paints; mercury is emitted by the degassing of the earth's crust. Generally, metals are emitted during their mining and processing activities (Lenntech, 2004). Environmental pollution by heavy metals is very promi-nent in areas of mining and old mine sites and pollution reduces with increasing distance away from mining sites (Peplow, 1999).

These metals are leached out and in sloppy areas, are carried by acid water downstream or run-off to the sea. Through mining activities, water bodies are most emphatically polluted (Garbarino et al., 1995; INECAR, 2000). The potential for contamination is incre-ased when mining exposes metal-bearing ores rather than natural exposure of ore bodies through

erosion (Garbarino et al., 1995), and when mined ores are dumped on the earth surfaces in manual dressing proce-sses. Through rivers and streams, the metals are trans-ported as either dissolved species in water or as an integral part of suspended sediments, (dissolved species in water have the greatest potential of causing the most deleterious effects).

They may then be stored in river bed sediments or seep into the underground water thereby contaminating water from underground sources, particu-larly wells; and the extent of contamination will depend on the nearness of the well to the mining site. Wells located near mining sites have been reported to contain heavy metals at levels that exceed drinking water criteria (Garbarino et al., 1995; Peplow, 1999).

1.2. Chemistryof Heavy Metal Pollution

Mining activities and other geochemical processes often result in the generation of acid mine drainage (AMD), a phenomenon commonly associated withmining activities. It is generated when pyrite (FeS2) and other sulphide minerals in the aquifer and present and former mining sites are exposed to air and water in the presence of oxidizing bacteria, such as Thiobacillus ferrooxidans, and oxidised to produce metal ions, sulphate and acidity (Ogwuegbu and Muhanga, 2005).

$2FeS2 + 7O2 + 2H2O \quad 2FeSO4 + 2H2SO4$

$2FeSO4 + 2H2SO4 \quad Fe2(SO4)3 + SO2 + 2H2O$

$Fe2(SO4)3 + 2FeAsS + 9/2O2 + 3H2O \quad 2H3AsO4 + 4FeSO4 + S$

1.3.Human Exposure Through Food, Air and Water

Heavy metal pollution of surface and underground water sources results in considerable soil pollution and pollution increases when mined ores aredumped on the ground surface for manual dressing (Garbarino et al.,1995;INECAR, 2000). Surface dumping exposes the metals to air and rain thereby generating

much AMD. When agricultural soils are polluted, these metals are taken upby plants and consequently accumulate in their tissues (Trueby, 2003).Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues, and milk, if lactating (Habashi, 1992; Garbarino et al., 1995; Horsfall and Spiff, 1999; Peplow, 1999). Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders. In summary, all living organisms within a given ecosystem are variously contaminated along their cycles of food chain.

1.4. Human Exposure through Industrial Products

Industrial products that are used in homes, and which have been produced with heavy metals are sources of human exposure to such heavy metals. Mercury expos-ure is through disinfectants (like mercurochrome), anti-fungal agents, toiletries, creams and organo-metallics (McCluggage, 1991); cadmium exposure is through nickel/cadmium batteries and artist paints; lead exposure is through wine bottle wraps, mirror coatings, batteries, old paints and tiles and linolein amongst others. Infants are more susceptible to the endangering effects of expo-sure to heavy metals.

1.5. Occupational Exposure

Heavy metal exposure occurs significantly by occupa-tional exposure. Workers of the mining and production of cadmium, chromium, lead, mercury, gold and silver have been reported to be thus exposed; also inhabitants around industrial sites of heavy metal mining and proces-sing, are exposed through air by suspended particulate matters (SPM) (Heyer, 1985; USDOL, 2004; Ogwuegbu and Muhanga, 2005).

1.6. Biochemistry of Toxicity

The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted to their stable oxidation states (Zn2+) and combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds. The equations below show their reactions during bond formation with the sulphydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH3) (Ogwuegbu and Ijioma, 2003).

The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme.

1.7. Human Health and Heavy Metals Exposure

Metals, a major category of globally-distributed pollutants, are natural elements that have been extracted from the earth and harnessed for human industry and products for millenia. (An exception to metals being "natural" is plutonium, the material at the heart of nuclear weapons,created by man through the processing of uranium.) (Low et al., 2000). Metals are notable for their wide environmental dispersion from such activity; their tendency to accumulate in select tissues of the human body; and their overall potential to be toxic even at relatively minor levels of exposure. Some metals, such as copper and iron, are essential to life and play irreplaceable roles in, for example, the functioning of critical enzyme systems (Kapaj et al., 2006).

Other metals are xenobiotics, i.e., they have no useful role in human physiology (and most other living organisms) and, even worse, as in the case of lead and mercury, may be toxic even at trace levels of exposure. Even those metals that are essential, however, have the potential to turn harmful at very high levels of exposure, a reflection of a very basic tenet of toxicology--"the dose

makes the poison." One reflection of the importance of metals relative to other potential hazards is their ranking by the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), which lists all hazards present in toxic waste sites according to their prevalence and the severity of their toxicity. The first, second, third, and sixth hazards on the list are heavy metals: lead, mercury, arsenic, and cadmium, respectively. Exposure to metals can occur through a variety of routes. Metals may be inhaled as dust or fume (tiny particulate matter, such as the lead oxide particles produced by the combustion of leaded gasoline) (Peterson et al., 2006).

Some metals can be vaporized (e.g., mercury vapor in the manufacture of fluorescent lamps) and inhaled. Metals may also be ingested involuntarily through food and drink. The amount that is actually absorbed from the digestive tract can vary widely, depending on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it distributes in tissues and organs. Excretion typically occurs primarily through the kidneys and digestive tract, but metals tend to persist in some storage sites, like the liver, bones, and kidneys, for years or decades.The toxicity of metals most commonly involves the brain and the kidney, but othermanifestations occur, and some metals, such as arsenic, are clearly capable of causing cancer. An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache. This makes the diagnosis of metals toxicity in a clinical setting very difficult unless a clinician has the knowledge and training to suspect the diagnosis and is able to order the correct diagnostic test. Chronic exposure to metals at a high enough level to cause chronic toxicity effects (such as hypertension in individuals exposed to lead and renal toxicity inindividuals exposed to cadmium) can also occur in individuals who have no symptoms (ASTDR, 2005).

Much about metals toxicity, such as the genetic factors that may render some individuals especially vulnerable to metals toxicity, remains a subject of intense investigation. It is possible that low-level metals exposure contributes

much more towards the causation of chronic diseaseand impaired functioning than previously thought.This chapter focuses on exposure to the four “heavy” metals on the ATSDR listmentioned above —lead, mercury, arsenic, and cadmium—as they are arguably the mostimportant metal toxins from a global, as well as U.S. perspective. Some additional remarks arealso made regarding a few other metals of concern. (Exposure to arsenic and lead in drinking wateris covered by John Balbus in Chapter 3, Water Quality and Water Resources)(ASTDR, 2005).

2. Lead

Exposure For centuries, lead has been mined and used in industry and in household products. Modern industrialization, with the introduction of lead in mass-produced plumbing, solder used in food cans, paint, ceramic ware, and countless other products resulted in a marked rise in population exposures in the 20th century. The dominant source of worldwide dispersion of lead into the environment (and into people) for the past 50 years has clearly been the use of lead organic compounds as antiknock motor vehicle fuel additives. Since leaded gasoline was introduced in 1923, its combustion and resulting contamination of the atmosphere has increased background levels everywhere, including the ice cap covering Northern Greenland (Fig. 1), where there is no industry and few cars and people.

Although a worldwide phase-out of leaded gasoline is in progress ,it is still being used all over the world. The current annual worldwide production of lead is approximately 5.4 million tons and continues to rise. Sixty percent of lead is used for the manufacturing of batteries (automobile batteries, in particular), while the remainder is used in the production of pigments, glazes, solder,plastics, cable sheathing, ammunition, weights, gasoline additive, and a variety of other products.Such industries continue to pose a significant risk to workers, as well as surrounding communities.In response to these risks, many developed countries over the last 25 years have implemented regulatory action that has effectively decreased actual exposures to the generalpopulation. However, exposures remain

high or are increasing in many developing countriesthrough a rapid increase in vehicles combusting leaded gasoline and polluting industries (some ofwhich have been “exported” by corporations in developed countries seeking relief fromregulations). Moreover, some segments of the population in developed countries (such as theU.S.) remain at high risk of exposure because of the persistence of lead paint, lead plumbing, andlead-contaminated soil and dust, particularly in areas of old urban housing. A number of factors can modify the impact of lead exposures. For example, water with alower pH (such as drinking water stemming from the collection of untreated “acid rain”) willleach more lead out of plumbing connected by lead solder than more alkaline water. Lead fromsoil tends to concentrate in root vegetables (e.g., onion)and leafy green vegetables (e.g., spinach).Individuals will absorb more lead in their food if their diets are deficient in calcium, iron, or zinc.Other more unusual sources of lead exposure also continue to be sporadically found, such asimproperly glazed ceramics, lead crystal, imported candies, certain herbal folk remedies, andvinyl plastic toys.ToxicityLead has been the intense focus of environmental health research for many decades.

Studies inhumans were greatly assisted by the development of methods (such as graphite furnace atomicabsorption spectroscopy) for the accurate and reliable measurement of lead in blood (measured inunits of micrograms per deciliter [mg/dL]), a technique that is now widely available and used forsurveillance and monitoring, as well as research. The general body of literature on lead toxicity indicates that, depending on the dose, leadexposure in children and adults can cause a wide spectrum of health problems, ranging fromconvulsions, coma, renal failure, and death at the high end to subtle effects on metabolism andintelligence at the low end of exposures. Children (and developing fetuses) appear to beparticularly vulnerable to the neurotoxic effects of lead.

A plethora of well-designed prospectiveepidemiologic studies has convincingly demonstrated that low-level lead exposure in children lessthan five

years of age (with blood lead levels in the 5-25 mg/dL range) results in deficits inintellectual development as manifested by lost intelligence quotient points.As a result, in theU.S., the Centers for Disease Control (CD) lowered the allowable amount of lead in a child'sblood from 25 to 10 mg/dL and recommended universal blood lead screening of all children between the ages of six months and five years.However, a number of issues still remain unresolved with respect to lead toxicity in children. Among the most important is the risk posed to the fetus posed by mobilization of long-ved skeletal stores of lead in pregnant women.Recent research has clearly demonstrated thataternal bone lead stores are mobilized at an accelerated rate during pregnancy and lactation ande associated with decrements in birth weight, growth rate, and mental development. Sinceone lead stores persist for decades,it is possible that lead can remain a threat to fetal healthany years after environmental exposure had actually been curtailed. In contrast to children, adults are generally allowed by regulations to be exposed to highermounts of lead. In the U.S., for example, the Occupational Safety and Health Administrationquires that the blood lead levels of exposed workers be maintained below 40 mg/dL as a way ofreventing toxic effects to nerves, the brain, kidney, reproductive organs, and heart. This standardprobably outdated, however. First, the standard does not protect the fetuses of women who become pregnant while on the job (or even if they leave the job for several years because of the isue of bone lead mobilization, as discussed above).

Second, recent epidemiologic studies have linked blood lead levels in the range of 7-40 mg/dL with evidence of toxicity in adults, such as neurobehavioral decrementsand renal impairments.Third, recent studies using a newlydeveloped technique, K-x-ray fluorescence, to directly measure bone lead levels (as opposed toblood lead levels) have provided evidence demonstrating that cumulative lead exposure inindividuals with blood lead levels well below 40 mg/dL is a major risk factor for the developmentof hypertension,cardiac conduction delays,and cognitive impairments.Finally, even as research progresses to delineate the full toxicologic implications of leadexposure, investigations at the interface of genetics and environmental health are beginning

touncover subgroups of individuals who may be particularly susceptible to the toxicity of lead.

2.1.Neurotoxic efects of Pb2+: Results from Epidemiological Studies

The neurological efects of Pb2+ in exposed children have been a driving factor in reducing the level of Pb2+ in the environment (Gilbert and Weiss, 2006). In 1991 the United States Centers for Disease Control and Prevention (CDC) lowered the defnition of Pb2+ intoxication to 10 µg/dL BLL (the current regulatory level) motivated by the evidence from several studies that children with BLL of at least 10 µg/dL had impaired intellectual function (CDC, 1991). More recently, studies have shown that the dose-response of Pb2+ on IQ in children is non-linear, with lower exposures of Pb2+ resulting in a greater rate of IQ loss than at higher exposures (Canfield etal.,2003; Lanphear et al., 2005;Hu et al., 2006;Jusko et al., 2008). These data clearly demonstrate that the majority of the estimated IQ loss in Pb2+-exposed children occurs during the frst 10 µg/dL, and many studies have suggested a lack of a threshold for the efects of Pb2+ on IQ (Canfield etal.,2003; Lanphear et al., 2005; Jusko et al., 2008).

A large, internationally-pooled analysis of Pb2+-exposed children estimated that children with BLLs of 10 µg/dL experience a defcit of about 6.2 IQ points relative to children with estimated BLLs of 1 µg/dL (Lanphear et al., 2005).

This is comparable to the defcit of 7.4 IQ points observed in children with BLLs of 10 µg/dL in another large study (Jusko et al., 2008). On an individual level, a decrease in IQ of 6-7 points would be difcult to detect. However, the efect of a population decrease in IQ of this magnitude is quite signifcant. By shifing the normal distribution of IQ scores lower, the number of children with impaired intelligence would increase signifcantly while the number of exceptionally gifed children would decrease (Gilbert and Weiss, 2006). Several researchers have studied this efect from an economical standpoint

and suggest that the monetary cost of such an efect may total over 40 billion dollars for one age group alone. Over a 20-year period, one generation, this loss may amount to nearly 800 billion dollars (Landrigan et al.,2002; Gilbert and Weiss, 2006). In addition to the cognitive defcits associated with Pb2+ exposure, children with elevated BLLs experience behavioral defcits. School children with elevated BLLs are more likely to act out in class, display antisocial behavior, and have trouble paying attention (Bellinger et al.,1994; Needleman et al., 1996; Royet al., 2009).Cumulative childhood Pb2+ exposure was associated with a higher incidence in behavioral problems in 8-year-old children (Leviton et al.,1993; Bellinger et al.,1994). These behavioral efects appear to have a phenotype similar to attention-defcit hyperactivity disorder (ADHD).

Furthermore, recent studies have identifed that childhood Pb2+ exposure is positivity associated with ADHD diagnosis (Froehlich et al., 2009; Roy et al., 2009).The cognitive and behavioral defcits of Pb2+-exposed children persist even afer the cessation of Pb2+ exposure (White et al., 2007), and chelation therapy is unable to remediate the efect of Pb2+ on cognition (Chisolm et al., 1990; Rogan et al., 2001; Dietrich et al., 2004). Prenatal and/or childhood Pb2+ exposure was associated with anti-social and delinquent behavior as adolescents (Dietrich et al., 2001), an increased likelihood be an adjudicated delinquent (Needleman et al., 2002), or to be arrested as an adult (Wright et al., 2008).

Furthermore, childhood Pb2+ exposure may predict adult cognitive function (Mazumdaret al., 2011). Children who experience elevated Pb2+ levels are more likely to have decreased brain volume in adulthood in specifc brain regions (Cecil et al., 2008). These changes could account for altered behavior and cognition in adults exposed to Pb2+ as children. Tus, developmental Pb2+ exposure in humans results in long-lasting efects on

cognition and behavior even afer cessation of exposure. Possible Mechanism of Pb2+.

2.2. Neurotoxicity: Results from experimental animal studies

It is believed that Pb2+ targets the learning and memory processes of he brain by inhibiting the N-methyl-D-aspartate receptor (NMDAR), which is essential for hippocampus-mediated learning and memory (Morris et al., 1982, 1986). The NMDAR is essential for learning spatial navigation tasks n animal models (Morris et al., 1986),and animals which have been developmentally xposed to Pb2+ exhibit similar learning defcits as those with absent or mpaired NMDARs (Morris et al., 1982, 1986; Tsienet al., 1996). The NMDAR is composed of an obligatory NR1 subunit and one or more accessory subunits from the NR2 and NR3 families. In the ippocampus, NR2A and NR2B are the most abundant NR2 family members. Pb2+ is a potent, non-competitive antagonist of the NMDAR (Alkondon et al., 1990; Guilarte and Miceli, 1992; Guilarte, Miceli and Jett, 1994; Ruan et al., 1998). Evidence suggests that Pb2+ binds the Zn2+ regulatory site of the NMDAR in a voltage-independent manner (Guilarte et al., 1995; Yamada et al.,1995; Gavazzo et al., 2008).

Since Zn2+ binds with high afnity at a regulatory site on the NR2A subunit (Fayyazuddin et al., 2000), but with lower afnity to the NR2B subunit (Rachline et al., 2005), this suggests preferential sensitivity of NR2A-NMDARs for Pb2+ (Guilarte et al., 1995; Gavazzo et al., 2008). In support of this hypothesis, electrophysiological studies on recombinant receptors demonstrate that Pb2+ more potently inhibits NR2A-NMDARs than NR2B-NMDARs (Yamada et al.,1995; Omelchenko et al., 1996), or the tri-heteromeric form, NR1/NR2A/NR2B-NMDAR (Omelchenko et al., 1996). In addition to acting as an NMDAR antagonist, Pb2+ exposure also disrupts normal NMDAR ontogeny. Chronic developmental Pb2+ exposure results in decreased NR2A content in the hippocampus (Guilarte and McGlothan, 1998;

Nihei MK, Guilarte, 1999; Nihei et al., 2000), and altered expression of NR1 splice variants (Guilarte etal., 2000; Zhang et al., 2002; Guilarte and, McGlothan, 2003). In contrast, NR2B mRNA levels either remained unchanged or slightly increased in rats developmentally exposed to Pb2+ (Guilarte and McGlothan, 1998; Nihei MK, Guilarte, 1999; Nihei et al., 2000; Zhang et al., 2002). Together, these data suggest that Pb2+ delays the normal developmental witch of increased NR2A incorporation in NMDARs with synapse maturation (Toscano et al., 2002; Toscano and Guilarte., 2005).

Similar trends have also been observed in culturedneuron systems(Xu and Rajanna, 2006; Neal et al., 2011) and suggest that Pb2+ exposure may cause lasting changes in NMDAR subunit composition and expression. In addition to hippocampal changes in NMDAR subunit expression and ontogeny, Pb2+ may alter the cellular distribution of NMDAR populations. We have shown that Pb2+ exposure during synaptic development in hippocampal cultures reduces the levels of synaptic NR2A-NMDARs with a concomitant increase in extrasynaptic NR2B-NMDARs (Neal et al., 2011). Tis is signifcant because the NR2 family members are linked to diferential MAPK signaling (Kim et al., 2005), pro-death or pro-life signaling (Soriano et al., 2008), and diferential induction of nuclear gene expression (Hardingham et al., 2002). In particular, NR2A-NMDAR activation is linked to cell survival pathways and cyclic AMP response element binding protein (CREB) activation while NR2B-NMDAR activation is linked to cell death pathways and CREB shutof (Hardingham et al., 2002). Thus, changes in synaptic localization of NMDARs by Pb2+ could alter downstream NMDAR-mediated signaling. Supporting this hypothesis, chronic developmental Pb2+ exposure results in altered MAPK signaling (Cordova et al., 2004), calcium/calmodulin kinase II (CaMKII) activity (Toscano et al., 2005), and altered CREB phosphorylation and binding afnity (Toscano et al., 2002; 2003).CREB is a transcription factor for many immediate early genes (IEGs),

which play an essential role in memory consolidation and are expressed as a result of NMDAR activity (Bourtchuladze et al., 1994). Altered IEG expression in animals exposed to Pb2+ has been observed (Kim et al.,2002) indicating that altered CREB activity due to Pb2+-mediated disruption of NMDAR signaling may result in impaired learning and memory processes.Pb2+ exposure can cause defcits in neurotransmission.

Rats chronically exposed to low levels of Pb2+ have reduced Ca2+-dependent glutamate and γ-aminobutyric acid (GABA) release in the hippocampus (Lasley and Gilbert, 1996; 2002; Xiao et al., 2006), which indicates presynaptic neuron dysfunction during Pb2+ exposure. In cultured hippocampal neurons (Braga, Pereira and Albuquerque, 1999)and in brain slices (Xiao et al., 2006), Pb2+ exposure impairs excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs).

EPSCs and IPSCsare dependent upon neurotransmitter release from the presynaptic neuron, thus, reductions in EPSCs and IPSCs indicate a defcit in neurotransmission in both the glutamatergic and GABAergic systems as a result of Pb2+ exposure.A recent study from our laboratory has shown that Pb2+ exposure in cultured hippocampal neurons during synaptic development resulted in altered presynaptic protein expression and defcits in vesicular neurotransmitter release (Neal et al., 2010). Pb2+ exposure reduced the expression of key presynaptic proteins involved in vesicular release, such as synaptophysin (Syn) and synaptobrevin (Syb). Reductions of vesicular release proteins were associated with both glutamatergic and GABAergic synapses, consistent with electrophysiological observations regarding EPSC and IPSC generation during Pb2+ exposure (Braga, Pereira and Albuquerque, 1999; Xiao et al., 2006).

Vesicular release in Pb2+-exposed neurons was signifcantly impaired relative to control conditions as determined by live-imaging studies using the synaptic vesicle dye FM 1-43 (Neal et al., 2010). Together, animal and

cell culture studies indicate a role for Pb2+ in presynaptic dysfunction which results in reduced neurotransmission (Neal and Guilarte, 2010). One molecular mechanism by which Pb2+ may disrupt neurotransmission is by inhibiting neuronal voltage-gated calcium (Ca2+) channels (VGCCs) (Penget al., 2002). Removal of extracellular Ca2+ from hippocampal slice cultures resulted in identical efects on IPSC frequency as Pb2+ exposure, suggesting that the Pb2+-induced inhibition of IPSC frequency occurred via reduction of Ca2+ infux through VGCCs (Xiao et al., 2006). Inhibition of presynaptic VGCCs may prevent the necessary rise in internal Ca2+ required for fast, Ca2+-dependent vesicular release, thus interfering with neurotransmission. However, the efects of Pb2+ we observed on presynaptic protein expression were dependent on NMDAR activity, based on comparison studies with thespecifcNMDAR antagonistaminophosphonovaleric acid (APV,which does not inhibit VGCCs) which resulted in similar efects as Pb2+ exposure (Neal et al., 2010).

Thus, while Pb2+ inhibits VGCCs, which may result in impaired neurotransmission, VGCC inhibition by Pb2+ is not exclusively responsible for the presynaptic efects of Pb2+ and long-term NMDAR inhibition plays an important role in these efects.An emerging theme in the mechanism of Pb2+ neurotoxicity is the disruption of intracellular Ca2+ dynamics. Inhibition of either VGCCs or NMDARs by Pb2+ would result in a signifcant reduction of Ca2+ entry into the cell. Tis is important because Ca2+ signaling is essential for synaptic development and plasticity (Konur and Ghosh, 2005; Waites and Garner, 2011) and perturbation of these processes can lead to neurological disease states (Mirnics et al., 2001; Waites and Garner, 2011). One key Ca2+-dependent pathway involved in synaptic development and neurotransmitter release is brain-derived neurotrophic factor (BDNF) signaling (Shieh et al., 1998; Shieh and Ghosh, 1999; Chen et al., 2003; Matsuda et al., 2009). BDNF is a trans-synaptic signaling molecule that is released from both axons and dendrites (Matsuda et al., 2009). We have recently shown

that BDNF levels are reduced in Pb2+-exposed cultures and that exogenous BDNF supplementation during Pb2+ exposure can fully mitigate the efects of Pb2+ on presynaptic function and protein expression (Neal et al., 2010). Furthermore, BDNF expression and release are dependent on Ca2+ signaling, and both NMDAR- and VGCC-dependent Ca2+ pathways have been implicated in BDNF neurotransmission (Jiang et al., 2005;Walz et al., 2006; Matsuda et al., 2009).

Interestingly, NMDAR-dependent release of BDNF may play a greater role in dendritic BDNF release rather than axonic BDNF (Matsuda et al., 2009). This would support our hypothesis that NMDAR-dependent release of BDNF is disrupted during Pb2+ exposure (Neal et al., 2010; Neal and Guilarte, 2010), since the majority of NMDARs are postsynaptically located (Wenthold et al., 2003).Regardless of whether Ca2+ disruption occurs via block of NMDAR or VGCC (or both), BDNF expression and release are impaired during Pb2+ exposure, which has efects on synaptic development (Neal et al., 2010) and may cause long-term impairment of hippocampal function in vivo. Interestingly in an animal study investigating the efects of environmental enrichment on Pb2+ exposure, animals exposed to Pb2+ but living in an enriched environment did not exhibit the defcits in spatial learning tasks usually observed in rats chronically exposed to Pb2+ (Guilarte et al., 2003). In fact, Pb2+-exposed rats living in an enriched environment performed equally as rats which were not exposed to Pb2+. Furthermore, the Pb2+-exposed rats living in enriched environments exhibited elevated mRNA levels of BDNF relative to Pb2+-exposed rats living in normal conditions.

This indicates that BDNF may be implicated in vivo in the efects of Pb2+ on learning and memory.To summarize, Pb2+ remains a neurotoxiciant of concern due to its ubiquitious environmental presence and the absence of “safe” levels of exposure. Pb2+ exposure can cause both behavioral and cognitive defcits in children at very low (<10 µg/dL blood lead) levels of exposure. Recent progress has been made in the understanding of the cellular mechanism of Pb2+ toxicity, but further work is needed to address intervention and/or remediation strategies.

Chapter two

Parkinson's Disease

Introduction

The purpose of the present study is to show how exercise benefits patients who have Parkinson's disease (PD). The present chapter introduces an overview of PD. It also covers introductory parts to both of stress proteins and iNOS and their roles in PD.

1.1 -AnOverviewofParkinson'sDisease

Parkinson's disease (PD) is known as a chronic-progressive and disabling neurological disorder and the second most common neurodegenerative disease after Alzheimer's disease (Tolosa, 2006).

From a pathologic point of view, PD can be defined by nigrostriatal loss of dopaminergic cells and Lewy bodies in the surviving cells on autopsy. Furthermore, PD may be manifested from a clinical point of view by a broad spectrum of motor and non-motor features. The four cardinal features of PD can be grouped under the acronym TRAP: tremor at rest, rigidity, akinesia (or bradykinesia) and postural instability. This syndrome is labeled "parkinsonism" and may also occur in other medical conditions than idiopathic PD, such as dementia with Lewy bodies, cerebrovascular disease, the so called parkinsonian plus syndromes or as side effect after administration of neuroleptic medication. The presence of akinesia and one of the other symptoms are considered sufficient for the clinical diagnosis of parkinsonism. Diagnostic criteria have been developed by the UK Parkinson's Disease Society Brain Bank and the National Institute of Neurological Disorders and Stroke (NINDS) (Tolosa, 2006). Other diagnostic criteria for clinical subgroups of the disease were suggested by Larsen et al (1994). According to a study conducted by Jankovic (2008), flexed posture and motor blocks (freezing) have been included among classic features of PD.

It has been shown that the diagnosis of PD is still based on the presence of a combination of cardinal motor features, associated and exclusionary symptoms, and response to levodopa (Rao, 2003).

The Unified Parkinson's Disease Rating scale (UPDRS) is regarded as the most established scale for assessing motor dysfunction, disability and impairment (Fahn, 1987).

1.2 -Epidemiology of PD

Studies indicated that the incidence and prevalence of PD increase with age, but the trend observed is that published numbers vary widely across studies and countries, which reflects differences in methodology and diagnostic criteria. Studies based on metaanalyses indicate that about 1.6% of persons 65 years of age or older are affected by the disease (Rijk, 1997). Other studies have indicated that the incidence studies give a rate of about 17 per 100 000 per year in the overall population and the highest incidence is generally between 70 and 79 years of age (Twelves, 2003).

According to a study published by Alves (2009), it has been found that the annual incidence rate to be 12.6 per 100 000 inhabitants, age-adjusted to the 1991European population structure.Other studies, as the study conducted by Taylor (2007), men are more likely to develop Parkinson's disease than women.

1.3 -Clinical Picture

Disease is presented usually as unilateral and insidious. The course of disease is relentlessly progressive, with gradually increasing motor symptoms, and development of a range of non-motor symptoms, increasing functional impairment and disability, such as autonomic dysfunction, pain, skin problems, sleep disturbances and neuropsychiatric symptoms (Chaudhuri , 2006). Patients with PD usually suffer from significant functional impairment, a poor health-related quality of life (HRQOL), and increased mortality compared with the general population (Poewe, 1998).

1.4 -Pathology of PD

Several brain regions and neurotransmitter systems have been identified to be involved in the pathogenesis of PD besides to the defining loss of nigrostriatal dopaminergic neurons. Braak (2004) proposed that there is a sequential rostral progression of the pathological involvement. Accordingly, in majority of cases, brain stem nuclei such as serotonergic raphe nuclei, the

adrenergic locus coeruleus, as well as dopaminergic nuclei such as ventral tegmental area are involved.

Other studies reported that the major cholinergic nuclei in the basal forebrain and limbic structures are also involved rather early in course, and in the final stages, neocortical involvement is common. Whereas the nigrostriatal pathology is the main cause of the motor symptoms, the widespread extra-striatal pathologies may contribute to the wide variety of non-motor symptoms in PD (Karagulle, 2008; Lerner, 2008; Frisina, 2009). It has been reported that the pathological hallmarks of PD are the loss of the nigrostriatal dopaminergic neurons and the presence of intraneuronal proteinacious cytoplasmic inclusions, termed “Lewy Bodies” (LBs). The cell bodies of nigrostriatal neurons are in the SNpc, and they project, primarily to the putamen. The loss of these neurons, which normally contain conspicuous amounts of neuro melanin (Marsden, 1983), produces the classic gross neuropathological finding of SNpc depigmentation (Figure 1).

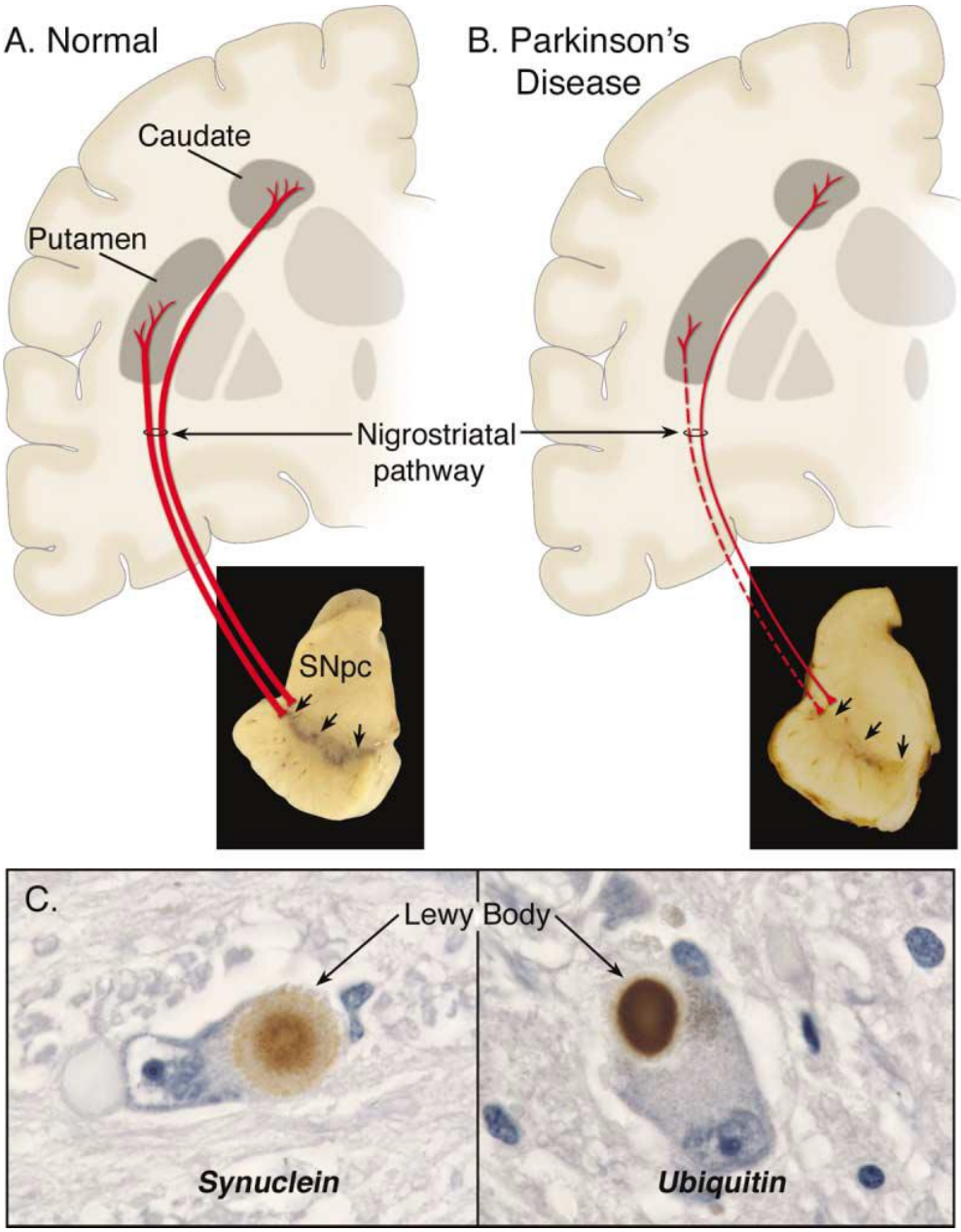


Figure1. Neuropathology of Parkinson's Disease (A) Schematic representation of the normal nigrostriatal pathway (in red). It is composed of dopaminergic neurons whose cell bodies are located in the substantia nigra pars compacta (SNpc; see arrows).

These neurons project (thick solid red lines) to the basal ganglia and synapse in the striatum (i.e., putamen and caudate nucleus). The photograph demonstrates the normal pigmentation of the SNpc, produced by neuromelanin within the dopaminergic neurons. (B) Schematic representation of the diseased nigrostriatal pathway (in red). In Parkinson's disease, the nigrostriatal pathway

degenerates. There is a marked loss of dopaminergic neurons that project to the putamen (dashed line) and a much more modest loss of those that project to the caudate (thin red solid line). The photograph demonstrates depigmentation (i.e., loss of dark-brown pigment neuromelanin; arrows) of the SNpc due to the marked loss of dopaminergic neurons. (C) Immunohistochemical labeling of intraneuronal inclusions, termed Lewy bodies, in a SNpc dopaminergic neuron. Immunostaining with an antibody against -synuclein reveals a Lewy body (black arrow) with an intensely immunoreactive central zone surrounded by a faintly immunoreactive peripheral zone (left photograph). Conversely, immunostaining with an antibody against ubiquitin yeilds more diffuse immunoreactivity within the Lewy body (right photograph). Source: Dauer, Serge Przedborski (2003)

1.5-Etiology of PD

Although the etiology of PD remains largely unknown, Etiology is largely unknown, but it is hypothesized that PD is caused by interplay of genetic and environmental causes. Recent findings regarding genetics of PD have enhanced the understanding of basic disease mechanisms, for example the exploring the central role of synuclein, the key element of the Lewy body. Mutations in the synuclein gene (SNCA) were found in autosomal dominant PD. Other findings revealed the identification of other mutations to contribute to familial cases of PD (Kurz, 2006).

1.6-Management of PD

The current treatment of PD is mainly medical and its aim is to alleviate thesymptoms. A cure is not available. Treatment of Parkinson's disease is complex because of the chronic-progressive course of the disease and the wide range of motor and non-motor symptoms demanding different strategies. Drugs include L-dopa, dopamine receptor agonists, anticholinergic drugs, and antiglutamatergic drugs (Rascol, 2002).

1.7-The relationship between physical activity and PD

Many studies have been carried out to investigate effectiveness of physical activity in collaboration with pharmacological treatment in PD patient. Although there is mounting evidence that physical activity can improve the patients'

quality of life, there is no general consensus on the type of physical activity program most useful for patients with PD (Pellecchiam 2004).

1.8-Nitric Oxide (NO)

Nitric oxide (NO) is gas in nature and it is produced by nitric oxide synthase (NOS) family of enzymes from L-arginine (Seet, 2010; Juurlink, 1999). It is known as a highly reactive signaling molecule having a few seconds of life time (Juurlink, 1999). Furthermore, it diffuses with ease (Juurlink, 1999). Nitric oxide is membrane permeable and can diffuse into dopaminergic neurons (Ara, 1998). Thus NO receptors are signaling transduction for intracellular communication. Once generated, the cell cannot control the local concentration of NO. Calabrese et al., shows that the activity of NO can be influenced by the degree of its synthesis and the activity is terminated with its reaction with its substrate (Seet, 2010). This is implicated in many physiological and pathological processes within the mammalian body (Moncada, 1997).

1.9- Heat Shock Proteins

Heat shock proteins (HSPs) have been studied extensively in literature and proved to provide an intrinsic mechanism to defend the cell against external diverse physiological stress that may initiate a cascade of events affecting cell structure and function. It has been assumed that, due to the high conservation of HSPs throughout the evolution, these proteins may have a vital role in protecting cells from injury. HSPs are composed of several classes of proteins according to their molecular weight, which include high-molecular-mass HSPs (≥100kD), HSP90 (81 to 99kD), HSP70 (65 to 80kD), HSP60 (55 to 64kD), HSP40 (35 to 54kD) and small HSPs (≤34kD) (Hart, 1996).

It has been indicated that different classes of HSPs to play a diversity role in governing proper protein assembly, folding, and translocation (Hart, 1996; Hightower, 1991). Furthermore, regulation of these HSPs synthesis has been found to create a unique defense system to maintain cellular protein homeostasis and to ensure cell survival (Hightower, 1991).

The understanding of HSPs' function is based on two main lines of evidence: (1) the clearance of waste proteins requires protein folding machinery called chaperones (Hart, 1996), and (2) HSPs chaperones bind to denatured proteins to promote their degradation (Hightower, 1991). Other evidence suggests that HSPs may actively participate in an array of cellular processes, including cytoprotection (Benn, 2004) and HSPs dysfunction may contribute to the pathogenesis of PD, a disease characterized by conformational changes in proteins that result in misfolding, aggregation and intracellular Lewy Body formation (Meriin, 2005).

1.10-Significance of the Study

The significance of the present study comes from introducing a model for PD and testing two important cellular signaling mechanisms so that the effect of exercise will be strongly explored to explain how exercise benefits patients with PD. At the same time, it is also expected that the possibility to identify therapeutic targets for PD will be highlighted. It is worth mentioning that the model adopted through this study will open the door for other studies to investigate the therapeutic potential for certain plants to be studies.

1.11-Study Hypothesis

The main hypothesis of the study states that exercise will help micewith PD through induction of changes on cellular level through increasing the expression of HSP70 and reducing the expression of iNOS in brains of micewith PD.

1.12-Study Objectives

1- To induce the PD model in mice.

2- To study associated changes of PD in brains of mice such as the expression of HSP70 and iNOS and to compare the expression of these proteins in control groups.

ChapterThree

Review Of Literature

Dopamine

2.Review Of Literature

A comprehensive literature review was conducted utilizing the following electronic databases: MEDLINE, CINAHL, EBSCOHOST, Google Scholars and Science direct. Key words were used when searching the databases were Parkinson's disease, stress proteins, iNOS, animal models for PD and cellular changes. All key words were linked with PD.

2.1-Dopamine Synthesis and Release

Dopamine (DA) is a catecholamine neurotransmitter, synthesized in the terminals of dopaminergic neurons (Stryer, 1995). The terminal end of these neurons is mainly in the striatal region of the basal ganglia (Guyton, 2006). Figure2shows Dopamine synthesis, storage, and release.L-dihydroxyphenylalanine (L-DOPA) and aromatic L-amino acid decarboxylase is produced by sequence of actions of tyrosine hydroxylase (TH) on tyrosine then L-DOPA converted into dopamine by DOPA decarboxylase. DA is transported into storage vesicles by a vesicular membrane transporter (T). Releasing of DA is stimulated by depolarization and entry of Ca^{+2} (Stryer, 1995).

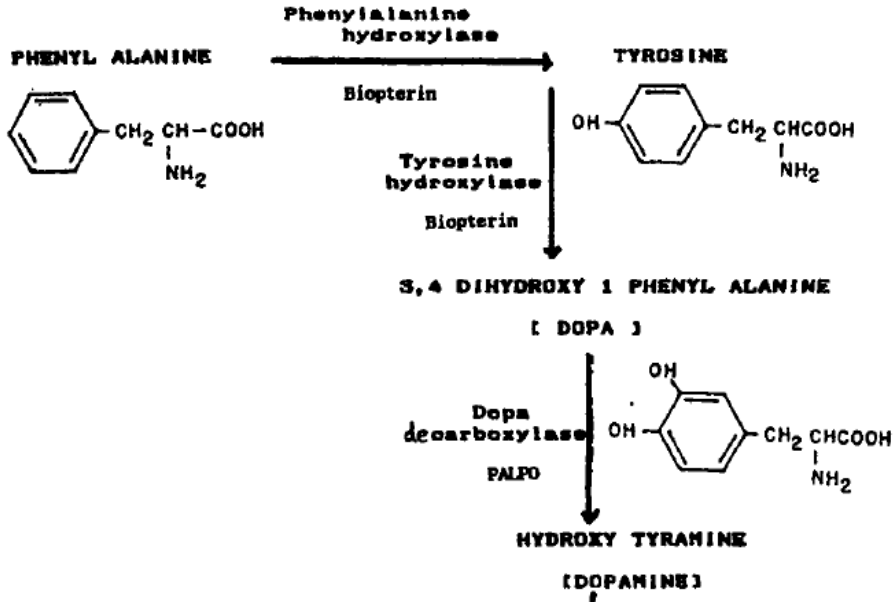


Figure2: Dopamine synthesis from amino acid tyrosine by the action of tyrosine

Hydroxylase

Dopamine activates five known types of dopamine receptors—D_1, D_2, D_3, D_4, and D_5. These five types are divided into two groups on the bases of their pharmacological and structural properties. The first group is D1: contain D_1 and D_5. The second group is D2: contain D_2, D_3, and D_4. Each of the five dopamine receptor proteins has a prominent anatomical distribution in the brain. The D_1 and D_2 proteins are ample in the striatum and are the most important receptor sites with respect to the causes and treatment of PD. The D_4 and D_5 proteins are largely extrastriatal, whereas D_3 expression is low in the caudate and putamen but more ample in the nucleus accumbens and olfactory tubercle. The action of DA is terminated in two ways either by reuptake into the nerve terminal, where DA may be restored or uptake into the postsynaptic cell, where DA is metabolized. Finally the metabolism of DA is a series of actions of many enzymes such as catechol-O- methyltransferase (COMT), monoamine oxidase (MAO), and aldehyde dehydrogenase (AD) (Stryer, 1995; Muller, 2007; Katzung, 2007; Moss, 2008). The D_1 and D_2 receptors are important in brain regions involved in PD. The DA has an inhibitory effect on brain regions (Pardridge, 2005).

2.2-Basal Ganglia (BG)

Basal ganglia consist of subcortical nuclei that play important functions including motor (skilled limb movements) (Meredith, 2009), cognitive (habit learning) and memorial behaviors, limbic role (Morris, 2010). Basal ganglia include the following nuclei and divisions: striatum (caudate and putamen), globus pallidus (GP) which is divided into (externa (GPe) and interna (GPi)), substantia nigra (SN) with its two parts (pars compacter (SNpc) and reticularis (SNpr)), and subthalamic nucleus (STN). These nuclei are connected to each other via complex projections and functions (Tugwell, 2008; Parent, 2005). Figure 3 shows these parts.

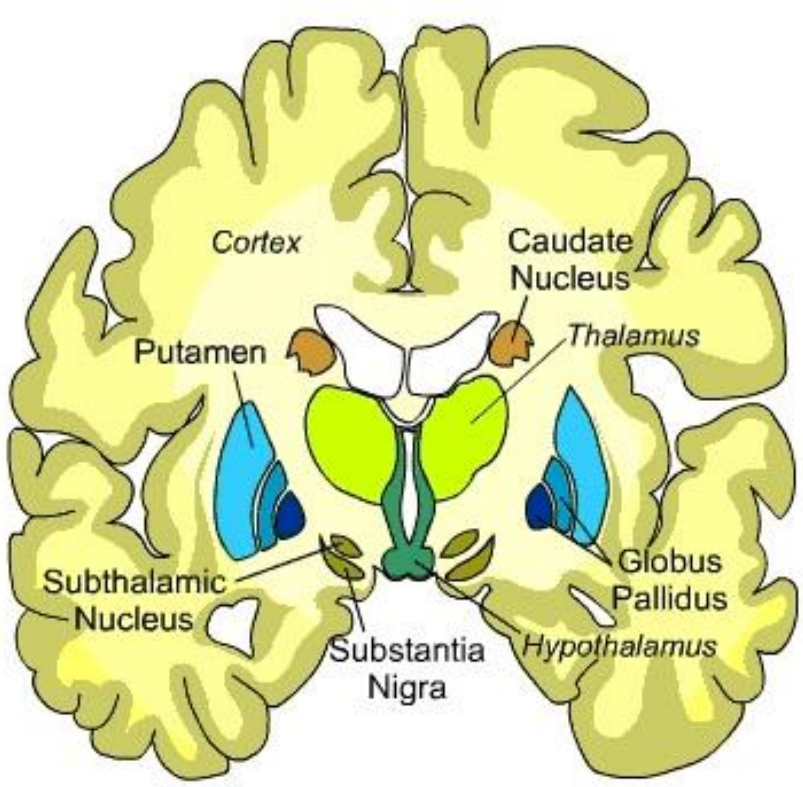


Figure 3: Basal ganglia (Striatum (Caudate and putamen), globus pallidus (GP) (externa (GPe) and interna (GPi)), substantia nigra (SN), and subthalamic nucleus (STN)).

2.2.1-Circuit Connections of Basal Ganglia

Many authors mentioned that basal ganglia have two pathways direct and indirect (Go and No-Go) pathways (Morris, 2010). When the cortex activates the striatum via glutamate, cells of striatum project inhibitory neurons via production of GABA (*gamma*-aminobutyric acid) to SNpr-Gpi complex. SNpr and Gpi are continuously having an inhibiting effect on the thalamus (by GABA). Then the thalami constantly stimulate the cortex via glutamate.

The cortex will then send stimulation massage via lateral corticospinal tract (motor pathway) to the muscles, resulting in a hyper-kinetic behavior. This is the direct (Go) pathway (Yelnik, 2002; Nicola, 2007; Morris, 2007).

The indirect (No-Go) pathway starts as the direct pathway starts from the striatum. Once the cortex stimulates the striatum, a group of neurons from striatum sends inhibitory axons to cells of Gpe. Gpe is constantly inhibiting the STN. STN is set to always stimulate the SNpr-Gpi complex, which main task is

to inhibit the thalamus. The end result is decreased stimulation of the cortex by the thalamus, less stimulation of the muscles through less and favoring hypo-kinetic lateral corticospinal tracts stimulation (Yelnik, 2002; Nicola, 2007; Morris, 2007). Theses pathways are shown in figure 4

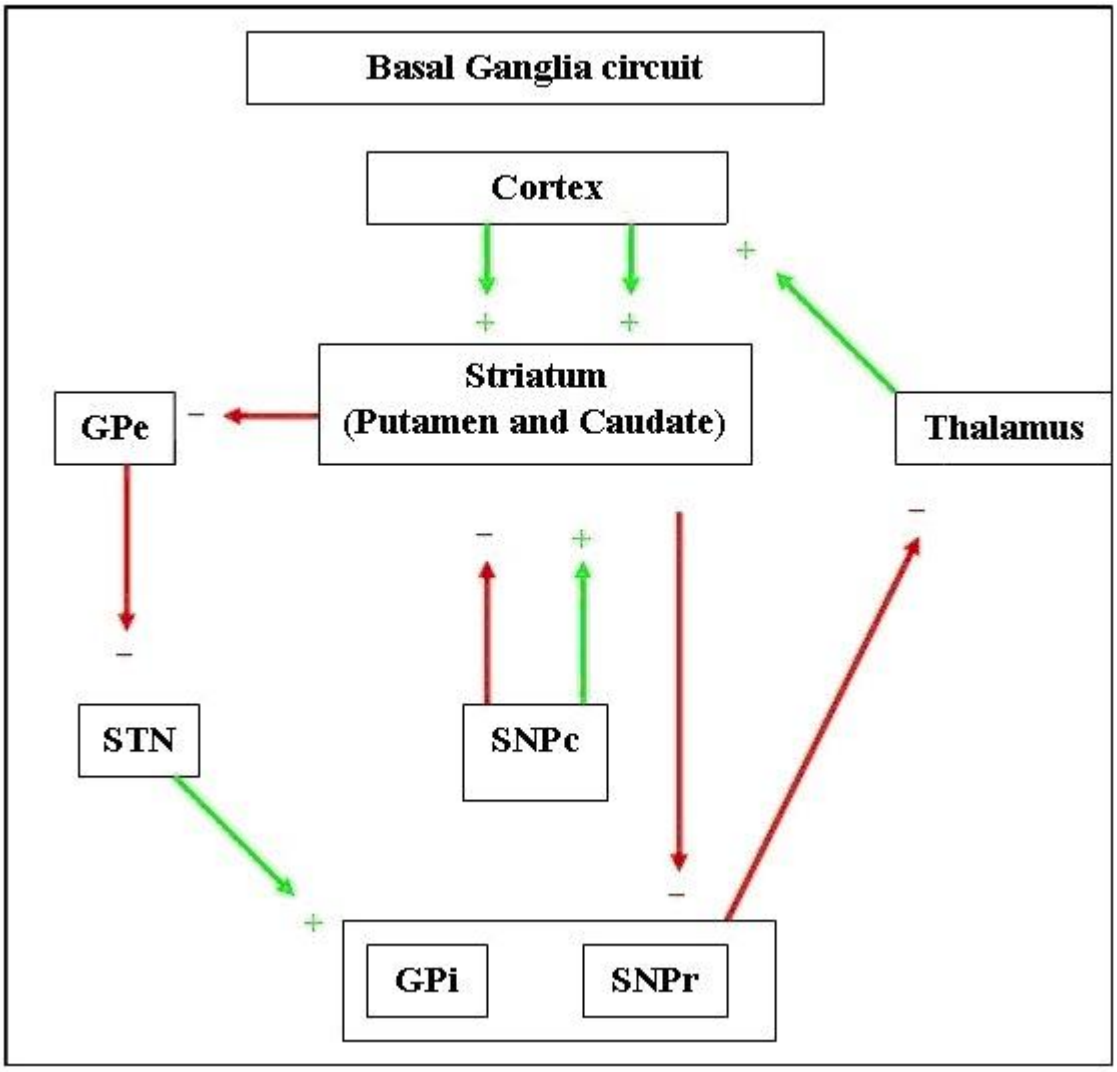


Figure 4: Direct pathway (*Cortex***to***striatum***to***Gpi and SNPr***to***Thalamus***to***Cortex*), Indirect pathway (*cortex***to***striatum***to***GPe***to***STN***to***GPi and SNPr***to***Thalamus***to***Cortex*).

Dopamine played important role between these pathways. It had an excitatory effect on the direct pathway and an inhibitory effect on the indirect pathway, thus decreasing the inhibitory effect of the system and making possible the execution of movement and behavior. So it is an important substance to solve the paradox

between direct (Go) pathway and indirect (No-Go) pathway so there is no inherent contradiction in the human body in terms of voluntary motion. In the Parkinsonian state, the absence of dopamine results in disinhibition of the output nuclei (GPi and SNr) and an increased inhibition of the thalamocortical projection, which leads to reduction or absence of movement (bradykinesia or akinesia) (Yelnik, 2002; Nicola, 2007; Morris, 2007). Another study shows that the degeneration of dopamine (DA) system in basal ganglia elicits a secondary, extranigral cascade of the degeneration of other transmitter system such as noradrenaline, serotonergic, and cholinergic (Weingarten, 1998).

2.3-Cardinal Symptoms of Parkinson's disease

Parkinson's disease has different impact in each patient. It may progress quickly in some cases, in others it does not. Furthermore, some cases experience severs motor disruption and become severely disable, but other cases experience minor motor disruption. PD has four cardinal symptoms Tremor, Bradykinesia, Rigidity, Postural instability.

2.3.1-Tremor

Involuntary rhythmic shaking occurs at rest tends to decrease or stop when the affected part is active. Some patients experience tremor of the jaw or foot (Tugwell, 2008). Furthermore, it is the most visible symptom and often begins in one extremity and worsens with stress, fatigue and cold weather. Tremor is usually unilateral that may affect one arm or leg (Morris, 2000). Moreover, the tremor affecting the thumb and first finger produces what is called pill rolling effect (Starkstein, 2002; Tugwell, 2008). Tremor is the first symptom to appear in 3/4 of patients with PD (Tugwell, 2008).

2.3.2-Bradykinesia

is another important symptom. The patients report slowing down of motion and difficulty in performing living activities such as dressing and walking and it is the most annoying symptoms. Furthermore, patients report difficulty in initiation of movements (Guttman, 2003).

2.3.3 Rigidity

is defined as a constant resistance to passive movement. This is become obvious when the examiner for example tends to extend flexed elbow on PD patients. This type of resistance is called cogwheel rigidity (Jerky movements) (Guttman, 2003).

2.3.4 Postural instability

is manifested as flexed posture (stooped posture) especially flexion of knees and hands. This problem is exacerbated with time and become troublesome feature. Reduced arm swing, and a shuffling gait may be very early features of the disease and inability to maintain balance (impairment of balance) (Guttman, 2003).

2.4-Stages of Parkinson's disease

Hoehn and Yahr PD stages (Jain, 2005).

Stage I: Unilateral embroilment only, usually with minimal or no functional disorder.

Stage II: Bilateral or midline embroilment, without affecting the Balance.

Stage III:Impairment of righting reactions. Instability of patient turns, this clear by pushing patient equilibrium state with feet together and eye closed. Functionally, the patients are to some extent restricted in their activities but may have the potential of some work, depending on the type of labour. Patients are physically capable to lead independent life, and their disability is mild to moderate.

Stage IV: Fully development PD, become severely disabling; the patient is still has the ability to walk and stand unaided but is noticeably incapacitated.

Stage V: Booking on the bed or wheelchair, transferring only with the assistance.

2.5-Causes of Parkinson's disease

The aforementioned symptoms are caused mainly by the marked loss of dopaminergic neurons in the substantia nigra pars compacta and hence depletion of the key neurotransmitter, dopamine (DA) (Chung, 2010; Nagatu, 2006; Galvin, 2006). In addition, smooth control of voluntary movement is attributed to the high concentration of DA in the basal ganglia of normal individuals

(Teismann, 2004). Although the apparent cause of the Parkinson is the marked depletion of dopaminergic neuron in SNpc, the underlying cause of this depletion in PD is still unknown. However, there is a belief that a combination of genetic and environmental factors might be involved in the development of the disease (Haavik, 1998; Oliveri, 2000; Au, 2005)

2.5.1-Genetic Causes of PD

In recent years, many studies have shown that there are several specific genetic mutations that cause PD (Tugwell, 2008, Au, 2005). The first gene that identified to be responsible for inherited PD is alpha-synuclein that was the starting point for studies determine a general role of this protein in neurodegeneration (Gasser, 2001; Steece, 2002; Chan, 2008; Yu, 2004). This mutation was identified in several Greek kindreds and most of them originate from a very small geographical area in Southern Greece (Steece, 2002). Despite the lack of knowledge about the functions of this protein, its importance in PD came from the discovery that it is the main component of Lewy bodies (abnormal aggregates of insoluble protein that develop inside nerve cells in PD) (Meredith, 2009; Spillantini, 2000).

Moreover, the detection of mutation in the alpha-synuclein gene led to find the important role of this protein in the formation of Lewy body and find that alpha-synuclein is the major component of Lewy bodies in PD (Lang, 1998), but evidence indicates that Lewy bodies are not essential for the pathogenesis and diagnosis of Parkinson's disease (Lang, 1998). At present, the evidences from many studies came convincingly to prove that genetic factors play an important role in the aetiology of at least a subset of patients with PD (Haavik, 1998; Steece, 2002). This evidence comes from postmortem studies of patients with parkin (Parkin is a protein which in humans is encoded by the PARK2 gene) mutations (Riess, 2006).

Recently, studies identified two types of mutation in alpha-synuclein gene located in chromosome 4. One mutation was reported with high percentage in a large Italian family and three smaller Greek families. The other one was reported in a family of German origin (Guttman, 2003; Steece, 2002; Tretter, 2004). For

instance, mutation in ubiquitin carboxyterminal hydrolase L1 gene (UCH-L1) has been identified in affected individuals in one family of German ancestry (Gasser, 2001). Recently, genetic markers on chromosome 4q21-q23 were found to be linked to PD phenotype in large Italian kindred with autosomal dominant PD (Haavik, 1998). Some studies have found that over expression of α-synuclein inhibit the tyrosine hydroxylase activity (TH), (TH: a rate-limiting enzyme for the synthesis of dopamine neurotransmitter) and dopamine synthesis, but little is known about the connection between the abnormality of α-synuclein and the expression of TH (Pardridge, 2005; Dickson, 2008; Ara, 1998; Yu, 2004; Jakowec, 2004).

Furthermore, loss of enzymatic activity and inactivation of dopamine synthesis have been seen after TH nitration by administration of MPTP (Pardridge, 2005; Dickson, 2008; Ara, 1998; Yu, 2004; Jakowec, 2004). In addition, there is evidence that α-synuclein can interact with the DA transporter (DAT) to regulate the amount of DA (Wenning, 2000). DAT is responsible for dopamine uptake at the synapse (Galvin, 2006; Jakowec, 2004; Jaber, 1997; Nutt, 2004). More recent works have revealed that the synthesis, storage and degradation of DA have been markedly changed in response to the lack of DAT in mice. This strongly suggests that the transporter is an important factor in regulating the balance of DA in the basal ganglia (Jaber, 1997) (figure 5, 6).

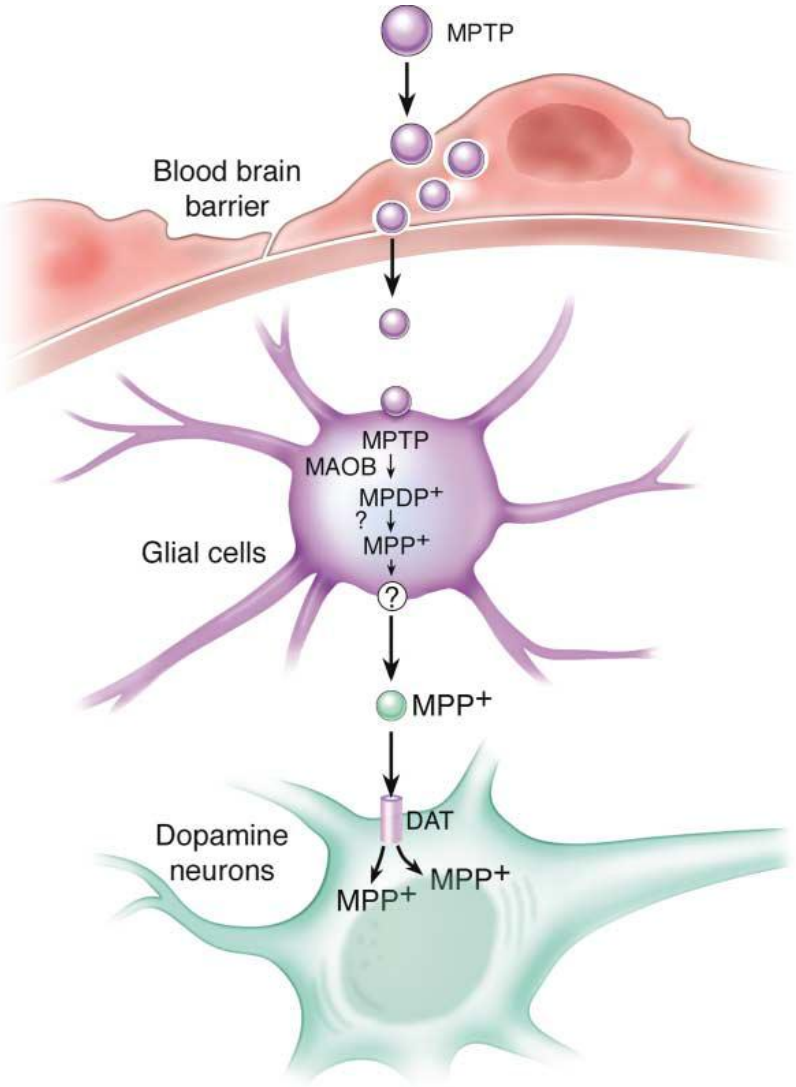


Figure 5: Schematic Representation of MPTP Metabolism**.** After systemic administration, MPTP crosses the blood-brain barrier. Once in the brain, MPTP is converted to MPDP ——————————— by MAO-B within nondopaminergic cells, such as glial cells and serotonergic neurons (not shown), and then to MPP ——————————— by an unknown mechanism (?). Thereafter, MPP_ is released, again by an unknown mechanism (?), into the extracellular space. MPP ——————————— is concentrated into dopa minergic neurons via the dopamine transporter (DAT).

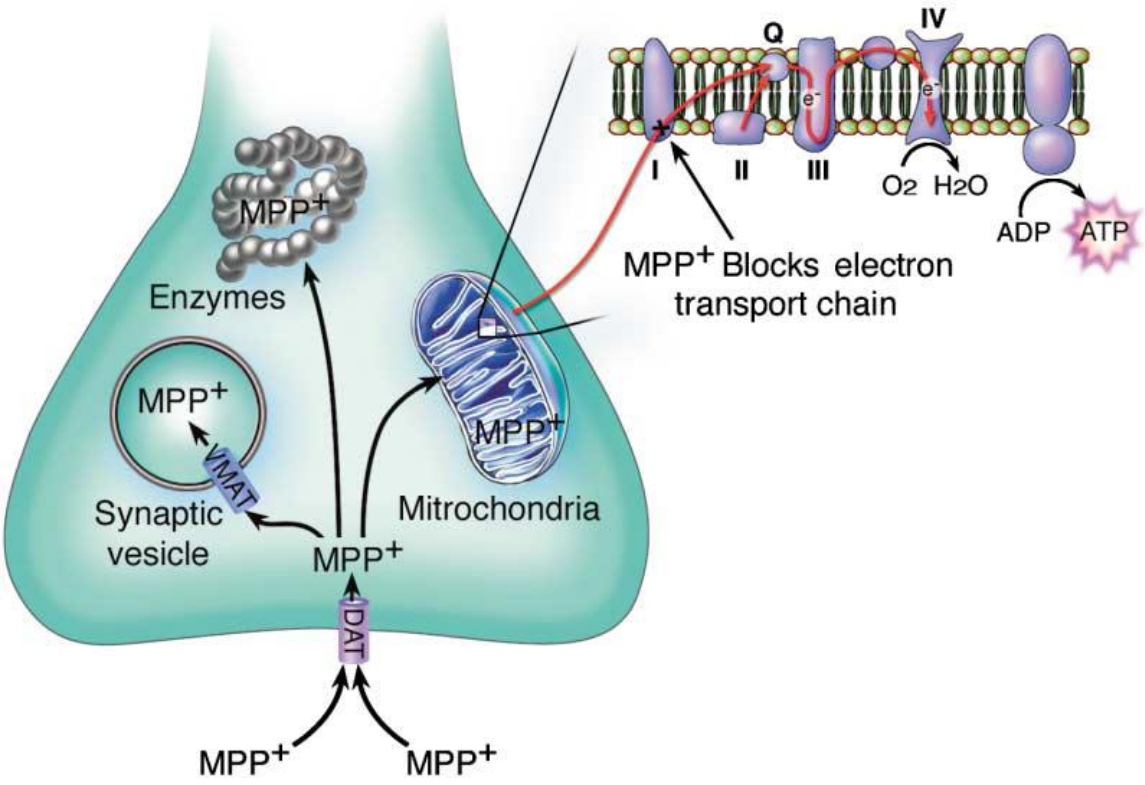


Figure 6: Schematic Representation of MPP+ Intracellular Pathways Inside dopaminergic neurons, MPP+ can follow one of three routes: (1) concentration into mitochondria through an active process (toxic); (2) interaction with cytosolic enzymes (toxic); (3) sequestration into synaptic vesicles via the vesicular monoamine transporters (VMAT; protective). Within the mitochondria, MPP+ blocks complex I (X), which interrupts the transfer of electrons from complex I to ubiquinone (Q). This perturbation enhances the production of reactive oxygen species (not shown) and decreases the synthesis of ATP.

2.5.2-Mitochondrial dysfunction

Many studies believed that the main factors in the pathogenesis of PD are the partial deficiencies of mitochondrial complex I (NADH-ubiquinone reductase complex, one of the five enzyme complexes of the inner mitochondrial membrane involved in oxidative phosphorylation) (Nagatu, 2006; Galvin, 2006; Naoi, 202; Shih, 2006). In PD brain, impairment in mitochondrial function is caused by decreasing the protein activity and protein content of mitochondrial complex I.Furthermore, complex I inhibition results is an enhancement of

production of reactive oxygen species (ROS) (Calabrese, 2000; Marella, 2009), which in turn will inhibit complex I (Gasser, 2001). Partial inhibition of complex I and/or an inherently higher ROS production in dopaminergic neurons lead over time to excessive oxidative stress and ATP deficit (Marella, 2009). This will be repeated continuously without interruption and enter in vicious cycle that finally would lead in cell death in the nigro-striatal pathway. It was shown that inhibition of complex I results from 1-methyl-4-pyridinium (MPP^+) derived from N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) (Tretter, 2004). Furthermore, it has been showed that impairment of mitochondrial function may result in increased ROS production, proteasome inhibition, and cell death, which was confirmed by the fact that the complex I inhibitor rotenone induced oxidized protein aggregation and DA cell death (Naoi, 2002). Furthermore, Calabrese et al., showed that there isdecrease in complex I activity in the substantia nigra of postmortem samples obtained from patients with Parkinson's disease (Seet, 2010).

2.5.3-Environmental factors

Oxidative stress is the imbalance between exposure to free radicals or other reactive species and antioxidant defenses. The involvement of oxidative stress in the pathogenesis of neurodegenerative disease such as PD and Alzheimer disease was postulated (Tarohda, 2005).

As an etiological factor or as contributory factor, oxidative stress has been suggested to be involved in the development of Parkinson's disease (Ben-Shachar, 1991) and plays an important role in mediating death in these neurons (Schierle, 1999). In addition to that, oxidative damage markers systemically have been elevated in PD, which may give the evidence about its relation to the onset and progression of PD (Tarohda, 2005). Other study provides a substantial body of evidence indicating that a state of oxidative stress exists in the SN of parkinsonian brains. This has been attributed to selective increase in levels of ferritin and iron in zona compacta of SN (Hou, 1999). There is experimental

evidence from studies on humans and animals in support of the hypothesis that oxidative stress contributes to the pathogenesis of PD (Ara, 1998).

Moreover, people with unusual exposure to herbicides and pesticides are more likely to develop Parkinson's disease than are people who don't have this exposure (Tugwell, 2008). Viral infections, inflammation, and traumatic events can cause Parkinsonian like symptoms (Tugwell, 2008).

2.6-Nitric Oxide (NO)

Nitric oxide (NO) is gas in nature and it is produced by nitric oxide synthase (NOS) family of enzymes from L-arginine (Seet, 2010; Juurlink, 1999).It is known as a highly reactive signaling molecule having a few second of life time (Juurlink, 1999). Furthermore, it diffuses with ease (Juurlink, 1999). Nitric oxide is membrane permeable and can diffuse into dopaminergic neurons (Ara, 1998). Thus NO receptors are signaling transduction for intracellular communication. Once generated, the cell cannot control the local concentration of NO. Calabrese et al., shows that its activity of NO can be influenced by the degree of its synthesis and the activity is terminated with its reaction with its substrate (Juurlink, 1999). This is implicated in many physiological and pathological processes within the mammalian body (Moncada, 1997).

2.6.2-Nitric Oxide Synthase (NOS)

There are three nitric oxide synthase (NOS) isoform, each one produced by different gene. The three forms of NOS are: Neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). They are produced by NOS1 gene, NOS2 gene, and NOS3 gene, respectively (Seet, 2010, HallS, 1994).The distribution of isoform in the body is not confined to a particular place wherefore we can find more than one isoforms in the same place. The obvious link between the three isoenzymes with the endothelium, neurons and inducibility is oversimplified. For example, eNOS has been found in the vascular endothelial cells and in platelets, as well as in certain neuronal populations in the brain. In addition, chronic exercise and pregnancy can induce the constitutive eNOS (Moncada, 1995), whereas, nNOS is located in the epithelium of the bronchi,

trachea, and in skeletal muscle.On the other hands iNOS seems to be present fundamentally in some tissues, including human bronchial epithelium, rat kidney and some fetal tissue (Moncada, 1995). Some differences have been identified between iNOS obtained from different tissues within the same species (Moncada, 1995).

Furthermore, several physiologic studies have proved potential role of NOS isoforms in the organization of neuronal cell biology and neurotransmission, control of total body sodium content and body fluid homeostasis, neuroendocrine biology, sexual function and myocyte /myoblast biology (Knowles, 1994).

Neuronal constitutive NO synthase is Ca^{+2} and calmodulin dependant and is distributed widely in the brain (Silvermam, 2009; Christopherson, 1997). The spinal cord and peripheral nervous system (Jakowec, 2004), neurons, and glial cell are mainly express nNOS (Hancock, 2008). The wide distribution of nNOS reflects its involvement in many biological functions (Moncada, 1995). Although eNOS is Ca^{+2} and calmodulin dependant, it is expressed in cerebral an endothelial cell that critically regulates cerebral blood flow. Activation of eNOS and nNOS can be achieved via increasing the intracellular calcium and formation of calcium/calmodulin complexes. In addition, nNOS has a dominant cytosolic localization, whereas, the eNOS is obligated to the plasma membrane (Seet, 2010).

Neuronal NOS and eNOS are similar in their dependant on Ca^{+2} but macrophage enzyme (iNOS) is different from these two enzymes in its independent on Ca^{+2}. In addition, activation of macrophage by a cytokine triggers iNOS to synthesize NO (Seet, 2010; Christopherson, 1997). Although iNOS induces low amounts of NO, a high level of NO are generated under pathogenic conditions to combat environmental insults in a wide range of cells upon induction (Hancock, 2008). Moreover, in responses to a variety of injuries, brain glial cells and invading macrophages can induce iNOS (Hancock, 2008).

Even though the levels of iNOS in the CNS are generally relatively low. The expression of iNOS increased in astrocytes and microglia in response to viral

infection and trauma. In addition, the activity of nNOS or eNOS is lower than the iNOS. Moreover, the induction of iNOS produces larger quantities of NO than nNOS or eNOS, this is usually linked with cellular pathology (Seet, 2010). In general, neurons containthelargest levels of NO throughout the body (Hancock, 2008).

2.6.3-Function of NO

Under normal concentrations nitric oxide (NO) performs vital and important physiological functions in the nervous and other systems. Small quantities of nitric oxide can regulate cerebral blood flow and local brain metabolism, gene expression, memory function, neuroendocrine secretion, neurotransmitter release, and play a key role in formation of the structure of organism, differentiation and growth of tissues and organs during development (morphogenesis) and synaptic plasticity (Levecque, 2003).

Furthermore, NO is a key element in signaling transduction pathways managing and controlling smooth muscle tone, platelet aggregation, host response to infection and a wide range of other physiological and pathophysiological processes (Seet, 2010; Levecque, 2003). Although cells are protected from toxic effects of NO, under pathological and physiological conditions, large amount of NO produces toxic environment (Seet, 2010; Levecque, 2003), tissue damage and oxidative stress, which result in a wide range of diseases, including rheumatoid arthritis, Alzheimer's disease, and may be responsible for neurodegeneration including Parkinson’s disease, amyotrophic lateral sclerosis, and Huntington’s disease (Levecque, 2003; Przedborski, 1996).

2.6.4-Nitric Oxide Synthase and Parkinson Disease

Karen et al., showed histopathological evidence that NO and glutamate in toxic dose may mediate certain neurodegenerative diseases (Przedborski, 1996). Postmortem studies, clinical findings, and evidence from experimental models revealed the role of NO in the degeneration of dopaminergic neurons in PD. Studies performed in the MPTP model of PD suggest that peroxynitrite, a reactive species formed by the nearly diffusion-limited reaction of nitric oxide

with superoxide, may be a mediator of nigrostriatal damage in PD (Ara, 1998). Over limit NO could contribute to the formation of free radicals that could be involved in the death of dopaminergic neurons, resulting in development of PD symptoms (Tuncel, 2009). Excess NO synthesis is likely to be involved in the progressive neuronal loss that distinguishes PD (Hancock, 2008). However, there is significant reduction in NO level in PD patients than in controls. The explanations for these low levels may be a faulty NO-dependent adaptation mechanism or the depletion of NO storage during the course of PD (Tuncel, 2009).

Moreover, as cytokines enhance the induction of NOS in brain; many studies suggest the role of glial derived NO in the pathogenesis of these diseases. Some studies showed that there was excessive formation of NO of glial origin in which NADPH diaphorase (a cytochemical marker of NOS activity) positive glial cells have been identified in the substantia nigra of postmortem brains obtained from individuals with Parkinson's disease (Seet, 2010).

Several animals and human studies showed that of the three isoforms, only the nNOS and iNOS are relevant with regard to their potential impact in neurodegeneration and glial response in PD (Kroncke, 1998). Several experiments showed that animals can be protected against MPTP by nNOS inhibitors (Seet, 2010; Kroncke, 1998). This gives evidence that the nNOS is responsible for MPTP neurotoxicity (Al-Jarrah, 2010). Furthermore, mutation in nNOS gene makes the mice more resistance to MPTP than wild-type mice. Similar experiments with iNOS show that this enzyme also plays a role in the dopaminergic neurons sensitivity (Kroncke, 1998). In human, overproduction of NO was detected in the substantia nigra of PD brain. It was correlated with high concentration of nNOS and iNOS (Al-Jarraha, 2010). Moreover, NOS inhibition protects against MPTP-induced loss of nigral neurons.Inhibition of NOS activity can stop MPTP-induced damage of dopaminergic nerve terminals in the striatum and the loss of dopaminergic cell bodies in the substantia nigra pars compacta (Dishman, 2006).

2.7-Parkinson Disease and Exercise

Exercises on a regular basis and motor skill training promote executive functions of cognition and some types of learning in the spinal cord (Yousefi, 2009). In central nervous system exercise have effects in prevention and treatment of obesity, depression, cancer, the decline in cognition associated with aging, and neurological disorders such as Parkinson's disease, ischemic stroke, Alzheimer's dementia, head and spinal cord injury (Yousefi, 2009). Another study found that exercise increases the level of blood vessel intensity and might consequently enhances and improves nerve cell survival through increased developing new blood vessels (angiogenesis) and has direct effect on the neurons (Pothakos, 2009). Furthermore, chronic physical activities have defensive effect against ischemic neuronal damage in the hippocampal formation and neurotoxic damage in the neostriatum. Moreover, these types of exercise have a role in maintenance of calcium balance, ATP production, and free radical management (Yousefi, 2009).

In contrast, little is known about the mechanisms behind the effect of physical activity and exercise in the form and function of the central nervous and peripheral nervous systems (Yousefi, 2009).Pothakos et al., and Al-Jarrah et al., have examined the positive impact of physical therapy (exercise) on functional outcomes of patients with PD like, posture, gait, balance and enhancing the activities of daily living and muscle strength better (Ahmad, 2009). A study was conducted in 1961 showed that physically active people have a lower threat of being diagnosed with PD (Pothakos, 2009). Clinical studies showed that exercise urges to improve motor performance and ambulation in PD patients (Petzinger, 2007).Moreover, in persons with idiopathic PD the high intensity resistance and balance training can help to get better balance and muscle strength (Herman, 2007). In addition, clinical and laboratory reports indicate that exercise may have neuroprotective effect and bring back dopaminergic and motor functions (Al-Jarrah, 2007). Intensive treadmill exercise leads to increased latency to fall (improved balance) (Hirsch, 2003; Schilling, 2010).

Also it leads to the refinement of motor performance in MPTP-lesioned mice Schilling et al. (2010) showed that compensatory changes in the MPTP-lesioned mice resulting in increased availability of synaptic dopamine through increase release, reduced uptake, and decreased decay. These changes can be obtained from physical activity or exercise. This in turn may play a more important role in preserving normal synaptic connections than the restoration of absolute dopamine levels (Schilling, 2010). Furthermore, other study findings pointed out that preserving endurance exercise in the early stage of neurodegenerative disorders may protect against or slowing the progression of neurodegeneration (Petzinger, 2007).

Running on treadmill sharply increases dopamine release and turnover and chronically up-regulates D2 receptors in the striatum of rats (Ahmad, 2010). In another study, high intensity treadmill exercise in the acute MPTP-treated mice results in behavioral recovery; however, the striatal expression of DAT is down-regulated and the expression of TH has not changed (Al-Jarrah, 2007). Although the link between DAT and TH is precisely not clear, it is predicted that the increased synaptic bioavailability of dopamine, through the downregulation of DAT, may lead to increased activation of the dopamine autoreceptor, leading to the downregulation of TH (Schilling, 2010).

Furthermore, increasing in the level of citrate synthase activity in the skeletal muscles and greater in the left ventricle of the heart were detected in the exercised Parkinsonian mice (Al-Jarrah, 2007). It is unclear whether the improvement of PD symptoms after exercise/balance training is simply owing to physical rehabilitation or to complex system involving neuronal adaptation and recovery (Al-Jarrah, 2007). Other study showed that walking on treadmill even at same walking speed on the ground improves gait and walk in PD patients with reduced gait variability (Petroske, 2001).

Effective endurance exercise training reverses the Parkinson's like behavioral deficits related to balance, regular movement and gait performance (Petzinger, 2007). Moreover, in chronic Parkinson's animals that subjected to exercise, there are higher numbers of ventral tegmental area (VTA) dopaminergic

neurons than the sedentary Parkinson's animals (Petzinger, 2007). In addition, exercise have upregulation and downregulation effect, dopamine receptor D_2, which is up regulated and its activation is important in eliciting motor behavior. Both dopamine transporter and tyrosine hydroxylase after 28 days of intense exercise are down regulated (Schilling, 2010).

Resistance training has proved a positive effect for persons with PD. Schilling et al., showed there are many differences in exercise programming and testing procedures, resistance training interventions have been effective in treating the symptoms of PD; increases in strength, gait, velocity, and functional mobility have been observed (Rennick, 1972).

2.8-HSPs and PD Pathophysiology

Many neurodegenerative disorders, including PD, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and other polyglutamine expansion disorders, are associated with degeneration and death of specific neuronal populations due to accumulation of certain abnormal polypeptides or proteins (Meriin, 2005). Numerous studies implicate that at least two components of cellular proteins are associated with PD: the ubiquitin proteasomal system (UPS) and the HSPs (Berke, 2003; Grunblatt, 2004).

Transcriptional analysis of multiple brain regions in PD indicates the impairment of multiple electron transport chain complexes and the dysfunction of UPS in PD, along with a robust induction of several forms of HSPs (Zhang, 2005). Inclusion bodies called Lewy bodies with aberrant misfolding and aggregative proteins are common pathological hallmark in PD, indicating that abnormality of protein homeostasis may contribute to the pathogenesis of the disease (McLean, 2002). Hsp70 and Torsin A, a homology to yeast Hsp104 and mutations of the gene causing dystonia, are colocalized with α-synuclein (αSN) containing Lewy bodies. Further, Dedmon (2005) found that Hsp70 could inhibit αSN fibril formation through preferential binding to prefibrillar species to change the characteristics of toxic αSN aggregates. This work therefore elucidates a specific role of Hsp70 in the pathogenesis of PD and supports a general concept

that chaperone action is a crucial aspect in protecting against the otherwise damaging consequences of protein misfolding. With ageing, the level of HSPs is decreased insufficiently to keep the cellular proteins homeostasis, which may give rise to certain diseases (Meriin, 2005; Berke; 2003).

2.9-PD-Related Gene Mutations and Possible Association with HSPs

During the last decade of discovery of several PD-associated mutant genes a remarkable progress has been made to help our understanding of the biology of PD. So far there are at least 6 genes and several loci that have been identified responsible to PD (Le, 2004; Moore, 2005). It is hypothesized that UPS dysfunction resulted from these defected genes may cause protein misfolding and aggregation, and eventually lead to nigral cell degeneration (McNaught, 2004). Polymorphisms in the 5' promoter regions of Hsp70 gene have been found significantly associated with PD (Wu, 2004).

2.10-Alpha-synuclein (αSN)

αSN, which plays a critical role in regulatingsynaptic vesicle size with particular relevance todopamine storage, was found to be the maincomponent in the Lewy body. Stress can increase theαSN protein aggregation and inclusion bodyformation (Macario, 2005); misfolding αSN can changeproteasome composition, impairproteasome-mediated protein degradation, alterprotein synthesis, and reduce the ability of cells towithstand stationary phase ageing (Chen, 2005). Threemutations of *αSN*, which show toxic gain-of-function,have been found in association with familial PD (Le, 2004; Moore, 2005). Inducible expression of mutant *αSN* in PC12 celllines can result in greater sensitivity to proteasomalimpairment, leading to mitochondrial abnormalitiesand neuronal cell death (Tanaka, 2001). αSN at nanomolarconcentration is able to increase Hsp70 protein level inPC12 cells, which can reduce αSN aggregation andtoxicity (Kluchen, 2004). In addition, the αSN protein has atendency to self-aggregate and the protein level ofαSN is increased in SNc with ageing (Cuervo, 2004).

2.11-Parkin

Parkinis a member of E3 ligase in the UPS (Shimura, 2000). Parkinmutations are thought to result in the improper targeting of its substrates for proteasomal degradation leading to potentially neurotoxic accumulation (Kim, 2003). Thus, great emphasis has been placed on the identification of substrates of parkin and their possible role in dopaminergic neuron loss in PD (Moore, 2005). Kalia *et al* showed that the bcl-2-associated athanogene 5 (BAG5) can enhance dopaminergic neuron death in a vivo model of PD through inhibiting the E3 ligase activity and the chaperone activity of Hsp70 (Liu, 2002).

2.12-Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)

UCH-L1, a highly abundant and neuronal specific protein that belongs to a family of deubiquitinating enzymes, is responsible for hydrolyzing polymeric ubiquitin chains to free ubiquitin monomers (Le, 2004; Moore, 2005). UCH-L1 might additionally act as a dimerization-dependent ubiquitin protein ligase (Liu, 2002) and maintain ubiquitin homeostasis by promoting the stability of ubiquitin monomers in vivo (Osaka, 2003). When *UCH-L1* mutates, ubiquitin recycling is reduced, which may lead to aggregation of aberrant proteins. It is found that UCH-L1 aggresomes colocalize with Hsp70, chaperone BiP, and other ubiquitinated proteins (Ardley, 2004), suggesting that UCH-L1 may interact with HSPs in an attempt to participate in protein degradation.*DJ-1* DJ-1 is a novel oncogene and mutations in thisgene can cause familial PD. It is reported that DJ-1mutations may result in oxidative stress andmitochondrial injury, which may lead to proteinaggregation and neuronal cell death (Le, 2004; Moore, 2005). Li et al (2005) reported that DJ-1 and its mutants are associatedwith Hsp70, CHIP and mtHsp70/Grp75, amitochondria-resident Hsp70; and DJ-1 and itsmutants are colocalized with Hsp70 and CHIP in cells.Furthermore, H2O2 treatment in cells enhances DJ-1interaction with mtHsp70 in mitochondria (Li, 2005). Thesefindings suggest that translocation of DJ-1 tomitochondria after oxidative stress is carried out bychaperones.

2.13-Protective Role of HSPs in PD

It has been reported that Hsp70 is associated with αSN, dopamine transporter (DAT), parkin, proteasome subunits, ubiquitin and UCH-L1 (Cuervo, 2004). Hsp70 is believed not only to protect cells from rotenone-mediated cytotoxicity but also to decrease soluble αSN aggregation (Zhou, 2004). Furthermore, Hsp70 can work as a putative anti-apoptotic factor to protect against neuronal cell death in PD (Benn, 2004; Meriin, 2005). These results highlight the possibility of using Hsp70 as a potential therapy for PD. Recent studies of function and inducer of Hsp90 also indicate its potential therapy for PD (Uryu, 2006; Waza, 2006).

2.14- Hsp90

Hsp90 is the main component of the cytosolic molecular chaperone complex that has been implicated in the negative regulation of the heat shock factor 1 (HSF1). HSF1 is responsible for the transcriptional activation of the heat shock genes including Hsp40, Hsp70, and Hsp90 (Bharadwaj, 1999), suggesting a regulatory role in Hsp90 synthesis at the transcriptional level. Hsp90 forms a multichaperone complex with Hsp70 and Hsp40 to regulate several regulatory proteins, such as steroid hormone receptors (Sabbah, 1996) and transcription factors (Zhang, 2006), and to modulate the protein translocation from peroxisomal to organelle (Crookes, 1998).

The interplay between these chaperones is of crucial importance for cell function and survival. Recently, Uryu *et al*. demonstrated that Hsp90 was predominantly increased in PD brains, which was in correlation with the elevated level of insoluble αSN. These alterations of Hsp90 in PD brain were recapitulated by neuropathological findings in αSN mutant transgenic mouse model of PD (Uryu, 2006). Furthermore, exposure of cells to proteasome inhibitors resulted in increased levels of Hsp90 (Uryu, 2006).Microglia, which plays a principal role of inflammation in brain (Mor, 1999), express high levels of Hsp90 following excitotoxic lesion in the mouse hippocampus (Jeon, 2004). The protective function of Hsp90 can be very important since inflammation evoked by microglia may increase the risk of PD. Recently, we have

demonstrated that (-)-Epigallocatechin gallate EGCG, a major monomer of green tea polyphenols, is a potent inhibitor of microglial activation (Li, 2004).

EGCG could directly bind to Hsp90 and stabilize the complex of Hsp90 (Palermo, 2005).Thus EGCG could be used to alleviate microglia-mediated dopaminergic neuronal injury in PD.

2.15- Hsp70

Auluck *et al*. (2002) reported that application of Hsp70 can prevent dopaminergic neuronal loss in αSN transgenic Drosophila and interference with endogenous chaperone activity can accelerate αSN toxicity. Furthermore, Lewy bodies in human postmortem tissues were usually immunostained positive for molecular chaperones, suggesting that chaperones may play a role in PD progression (Auluck, 2002). It has been reported that Hsp70 can enhance parkin binding and ubiquitinating of expanded polyglutamine protein *in vitro*, suggesting that Hsp70 may help recruit misfolded proteins as substrates for parkin E3 ubiquitin ligase activity (Tsai, 2003). This finding provides a direct evidence to show the Hsp70 can promote the activity of E3 ligase to degrade aberrant αSN.

It is postulated that Hsp70 itself or cooperating with other factors can protect the neurons from cytotoxicity caused by aberrant proteins. The crosstalk between the Hsp70 and UPS may provide a clue for the intrinsic mechanism of protein aggregation and degradation. Moreover, Hsp70 exerts anti-apoptotic activity by blocking the function of several key proapoptotic factors (Benn, 2004). Several studies have demonstrated that Hsp70 may play a role in neuroprotection against rotenone-mediated apoptosis in human dopaminergic cell line SH-SY5Y *in vitro* and against MPTP-induced nigral injury *in vivo* by inhibiting the proapoptotic factors as well as activating the survival pathway (Pan, 2005; Shen, 2005).

The ability of chaperones such as Hsp70 to protect against neurodegeneration provoked by disease-related proteins (including synuclein-

mediated dopaminergic neuron loss) is consistent with the view that soluble misfolded proteins are neurotoxic (Muchowski, 2002; Auluck et al., 2002) (figure 7).

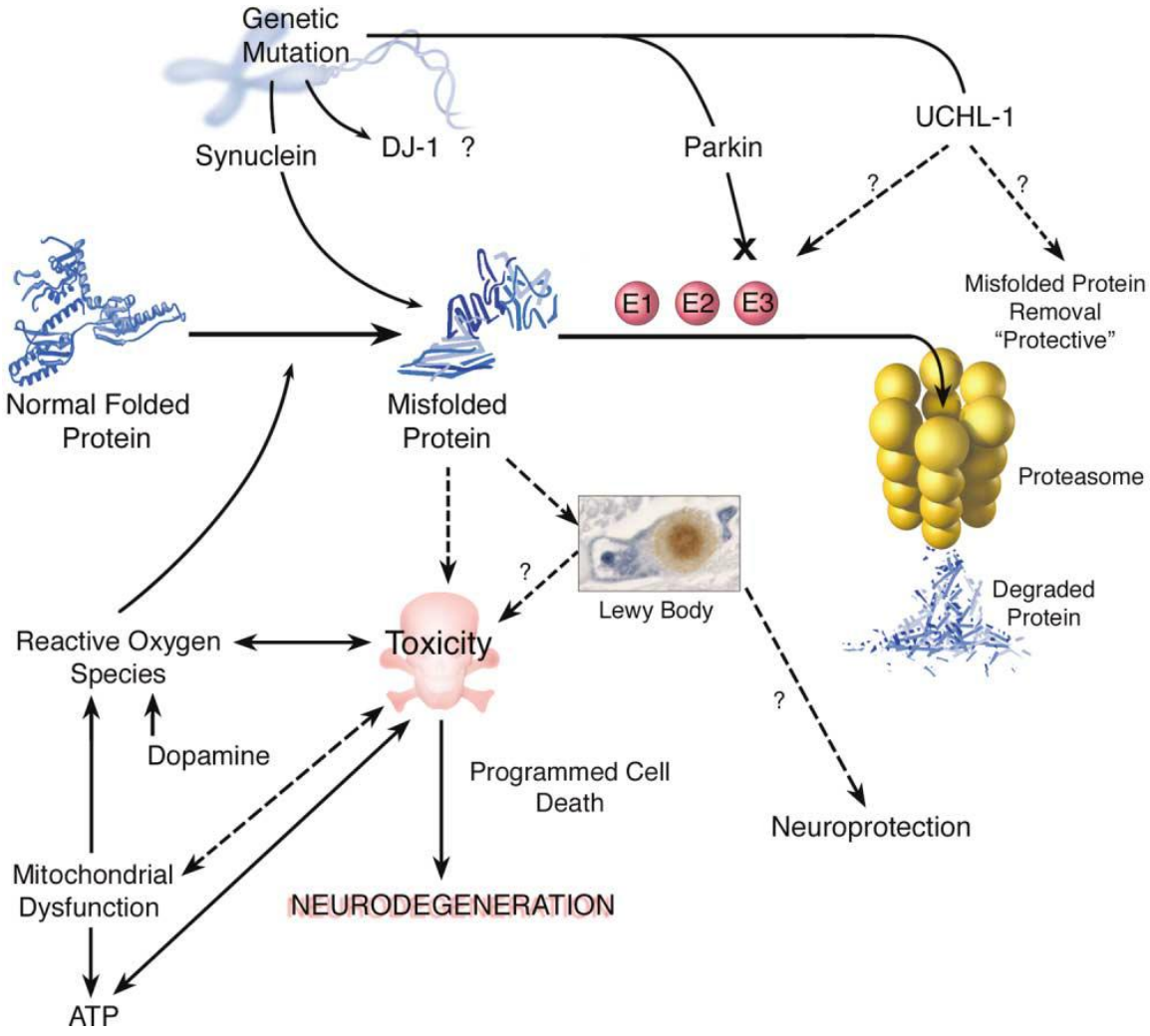


Figure7. Mechanisms of Neurodegeneration

A growing body of evidence, detailed in this review, suggests that the accumulation of misfolded proteins is likely to be a key event in PD neurodegeneration. Pathogenic mutations may directly induce abnormal protein conformations (as believed to be the case with synuclein) or damage the ability of the cellular machinery to detect and degrade misfolded proteins (Parkin, UCH-L1); the role of DJ-1 remains to be identified. Oxidative damage, linked to

mitochondrial dysfunction and abnormal dopamine metabolism, may also promote misfolded protein conformations. It remains unclear whether misfolded proteins directly cause toxicity or damage cells via the formation of protein aggregates (Lewy body). Controversy exists regarding whether Lewy bodies promote toxicity or protect a cell from harmful effects of misfolded proteins by sequestering them in an insoluble compartment away from cellular elements. Oxidative stress, energy crisis (i.e., ATP depletion) and the activation of the programmed cell death machinery are also believed to be factors that trigger the death of dopaminergic neurons in Parkinson's disease.

2.16- Small HSPs

Chaperone *Hsp25/27*(Hsp25 in mice and Hsp27 in humans), is an inhibitor of actin polymerization (Miron, 1991), which has been demonstrated to play a major role in actin filament dynamics in diverse cell types (Benn, 2004). In human endothelial cells, inhibition of p38-MAPK activation can abolish Hsp27phosphorylation, actin polymerization, and cell migration (Huot, 1997). p38-MAPK may act as an upstream activator of stress-inducible Hsp25/27 phosphorylation. It has been demonstrated that Hsp27 could bind to the microtubule associated protein tau and lead to decreased level of hyperphosphorylated tau and therefore enhance cell survival in AD (Shimura, 2004).

Another important function of Hsp27 is its protective effects on mitochondria pathway leading to inhibition of apoptosis (Concannon, 2003). It has been found that Hsp27 can block the tBID entering the mitochondria and reduce SMAC and Cytochrome C releasing from mitochondria so as to block the apoptotic process (Benn, 2004).

αB-crystallin Chaperone (Hsp22)*:* Increased expression and abnormal aggregation of small HSPs αB-crystallin has been detected in Lewy bodies and reactive astrocytes in various neurodegenerative diseases (Yun, 2002).. Rekas *et al* (2004*)* demonstrated that αB-crystallin was a potent inhibitor of αSN

fibrillization *in vitro*. αB-crystallin may redirect αSN from a fibril-formation pathway towards an amorphous aggregation pathway, thus reducing the amount of physiologically stable amyloid deposits in favor of easily degradable amorphous aggregates (Rekas, et al, 2004).

It has been reported that treatment with proteasomal inhibitors MG-132 or lactacystin in cultured rat brain oligodendrocytes can cause apoptotic cell death and induction of heat shock proteins in a time- and concentration-dependent manner (Goldbaum, 2004). Specifically in this study, αB-crystallin was up-regulated, and ubiquitinated proteins were accumulated. Meanwhile, the tau was dephosphorylated, which enhanced its microtubule-binding capacity (Goldbaum, 2004). These findings imply that αB-crystallin may work together with other HSPs, ubiquitin and microtubule associated proteins (MAPs) to cope with stressed conditions.

2.17- Potential Target for the Treatment of PD

Dong *et al*. (2005) reported that Hsp70 gene transferred to dopaminergic neurons by a recombinant adeno-associated virus significantly protected the mouse against MPTP-induced nigral dopaminergic neuron loss and striatal dopamine levels decline (Dong, 2005). Hsp70 attenuated MPTP induced apoptosis in the SNpc, and increased amphetamine-induced rotation (Dong, 2005). Collectively, these results demonstrate that increasing chaperone activity may be beneficial for the treatment of PD.

HSPs may exert protective function through two major pathways besides their own chaperon activity: reducing mitochondrial dysfunction and oxidative stress, and preventing UPS impairment.

2.18- Anti-apoptotic effects of HSPs in PD

Mitochondrial dysfunction is probably the leading cause of increased oxidative stress and apoptosis in PD. Dopaminergic neurons are more vulnerable to oxidative stress than other neurons because of the special substrate dopamine (Jenner, 2003). In general, apoptotic process can be divided into the three phases: induction (or triggering), transduction of signal, and execution. Theoretically, HSPs may modulate any of these apoptotic phases to rescue the cells (Beere, 2001). In addition, it has been reported that stable expression of wild-type αB-crystallin protects cancer cells from caspase-3 activation *in vitro*, indicating that small HSPs αB-crystallin is a novel inhibitor of the activation of apoptosis (Kamradt, 2005). Other gene products linked to monogenic forms of PD also appear to be implicated in mitochondrial dysfunction. Parkin can interact with leucine-rich repeat kinase 2 (Lrrk2) which is part of the mitochondrial outer membrane (Smith, 2005; West, 2005). Thus, Parkin may have an unexpected role in the regulation of normal mitochondrial function in an indirect way (Palacino, 2004; Winklhofer, 2003).

2.19- HSPs may promote the UPS in protein degradation

The UPS plays a pivotal role in the degradation of short-lived regulatory proteins which are components of cell cycle regulation, cell surface receptors, ion channels modulation, and antigen presentation (Schwartz, 1999). It is believed that once the disposal system fails to work, the substances, such as regulatory molecules p53, NFκB, and Bax that promote apoptosis, may accumulate to a high level that is harmful to the cell (Hernandez, 2004). A hypothesis for the etiology of PD is that subsets of neurons are vulnerable to a failure in proteasome-mediated protein turnover (Schwartz, 1999).

Accumulation of ubiquitin conjugates has been reported in the pathologic lesions of many chronic neurodegenerative diseases, such as the neurofibrillary tangles in AD and brainstem Lewy bodies in PD (Winklhofer, 2003; Schwartz, 1999). Inhibition of proteasome activity will sensitize dopaminergic neurons to protein alterations and oxidative stress (Mytilineou, 2004). Hsp90, together with Hsc70, Hsp40 and 20S proteasome subunit are the effective components to

capture firefly luciferase during thermal inactiveness and to prevent it from undergoing an irreversible off-pathway (Minami, 2000). The 20S proteasome has been found in tight association with the molecular chaperone Hsp90 (Whittier, 2004). Composed within 26S proteasome subunit, they form a complex involved in a multitude of intracellular processes (Schwartz, 1999). In addition, Kim *et al*(1999)has demonstrated that the inhibition of proteasome can increase the expression of Hsp27 and Hsp70, implying that HSPs may act as compensation of UPS or work together to regulate the intracellular protein level. Robertson *et al.* supported the hypothesis by demonstrating that Hsp70 antisense oligomers enhanced proteasome inhibitor-induced apoptosis (Robertson, 1999).

All evidences above implicate that HSPs and UPS are participants in keeping proteins folding correctly. They provide an effective protein quality control system that is essential for cellular functions and survival in many tissues. Dysfunction of these systems leads to protein aggregation and inclusion body formation in dopaminergic neurons.

2.20- HSPs inducers and their potential application in PD

It is proposed that up-regulation of protective factors may benefit our cells, but overload of some proteins may be a burden for cells or even cause cancer. So we need to find better way to keep cells in delicate balance with maximal protective effects and minimal side effects. Cyclopentenone prostaglandin A1 (PGA1), an inducer of HSPs, has been shown to inhibit SH-SY5Y neuron apoptosis. PGA1 can protect against rotenone-induced neuronal degeneration by promoting the expression of HSPs as well as attenuating nuclear translocation of NF-kappaB and caspase-3 activation (Wang, 2002). Geldanamycin (GA) binds to an ATP site on HSP90 and blocks its interaction with HSF1 to promote HSF1 activation (Zou, 1998).

GA also sensitizes the stress response within normal physiological parameters to enhance chaperone activation and offerprotection against αSN neurotoxicity (Auluck, 2005). Furthermore, GA uncouples neuronal toxicity from

Lewy body and Lewy neurite formation so that dopaminergic neurons are protected from the effects of αSN expression despite the continued presence of or even increased inclusion pathology (Auluck, 2005). Significantly, GA does not alter the basal level of HSP70, suggesting that GA acts only to elevate chaperone levels in stressed cells and does not alter chaperone activity in neighboring, healthier cells (Auluck, 2005). Because αSN expression leads to a local elevation of inducible HSP70 in dopaminergic neurons (Auluck, 2002), these neurons should be preferentially targeted by GA treatment (Auluck, 2005). Its new derivative 17-Allylamino-17-demethoxygeldanamycin 17-AAG shares its important biological activities with less toxicity (Waza, 2006), which gives us a much bright perspective to use GA to induce specific HSPs expression and to attenuate the side effect.

There is feasibility to use Hsp70 as a pretreatment therapy because there are many nontoxic or low toxic Hsp70 inducers available, such as paeoniflorin (Yan, 2004), bimoclomol (co-inducer to increase the activity) (Nanasi, 2001; Hargitai, 2003), radicicol, and valproic acid (VPA) (Pan, 2005). These Hsp70 inducers can up-regulate Hsp70 effectively for reconfirmation of the cellular homeostasis. Thus, it is hope that modulates the stress response by inducers can be a promising target for treatment of PD.

Materials And Methods

III- Materials And Methods

3.1 Chronic model of PD

Forty male albino mice, 10-12 week old, weighting between 25-27g at the beginning of the study, were housed in a single cage. This cage was preserved at constant temperature and humidity on a 12-h/12-h light/dark cycle, with food pellets and water available ad libitum. These animals were given random numbers from 1-100, and each animal was labeled with a random number. We do the randomization to be sure that any animal has the same chance to be in any group of the study.

Further randomization was carried out through assigning animals into four groups: sedentary control (SC, N=10), exercise control (EC, N=10), sedentary Parkinson's disease (SPD, N=10), exercise Parkinson's disease (EPD, N=10).

All animal treatments, including anesthesia, were carried out strictly according to the guideline of the animal research committee at Jordan University of Science and Technology. Twenty mice (SPD and EPD) were used to obtainchronic mouse model of PD. These mice received 10 doses of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinex hydrochloride) by injection of 25mg/kg in saline sc, co-administrated with probenecid (250mg/kg in dimethyl sulfoxide, DMSO,i.p.). The formula for MPTP was considered as follows: 25 mg of MPTP for 1000g of mice weight, accordingly for one mouse, we need to use the following formula: weight of mouse (g)/1000g (25 mg MPTP). The resulting amount of MPTP was dissolved in normal saline. The weight of each mouse was taken and injected with the calculated MPTP.Probenecid was prepared for each mouse in the following formula: weight of mouse (g)/1000g (250 mg probenecid). The resulting amount of probenecid was dissolved in 0.5 ml dimethyl sulfoxide and injected intraperitoneally into the mouse.

The administration of the 10 doses took 5 weeks with interval of 3.5 days between consecutive doses. Probenecid inhibits the renal excretion of some drugs; thereby increase their plasma concentration and prolonging of their effects (Rennick, 1972; Lau, 1990). Petroske et al., 2001 discovered that probenecid

potentiates neurotoxicity by reducing the clearance of MPTP and its metabolites from kidney and brain. It was shown that probenecid inhibits the clearance of compounds from choroids plexus and parenchymal cells into plasma (Rennick, 1972). Probenecid will increase the accumulation of the MPTP toxic metabolites in the brain and the depletion of striatal DA would be amplified (Rennick, 1972; Lau, 1990). MPTP hydrochloride and probenecid were purchased from sigma-Aldrich (St. Louis, MO).

3.2.Endurance exercise protocol

In the experimental setting, there was no specific treadmill for animals, but there was one for Human. We modified the human treadmill through making a plastic box that permitted the mice to run through a series of lanes (16 lanes).

A modified treadmill with 16 lanes was utilized for exercise training (figure 8). Plastic box was placed on the human treadmill and divided into two compartments each has 8 separate lanes, so animals can see each other running on the treadmill. Following the chronic MPTP/ probenecid treatment, the exercised groups of mice were introduced to the treadmill slowly over the course of a week. We let the animals running daily to be able to reach the protocol limits.

When the speed of running reached 18m/min, the four weeks of exercise protocol began. Although the training protocol composed of running for 40 min/day for 5 days/week at a speed of 18 m/min with 0 inclinations. All mice even the sedentary groups were transported daily to training room to expose all animal to the same environment. In order to monitor the ability of the average daily running scores were mice run on daily basis categorized for the two exercise groups as following: mice that ran but with little assistance for 40 min take score 4, mice ran well, but need some encouragements by using brush take score 3, score 2 need continuous incitement to maintain the speed of running, finally the mice that did not complete the full 40 min protocol take score 1.

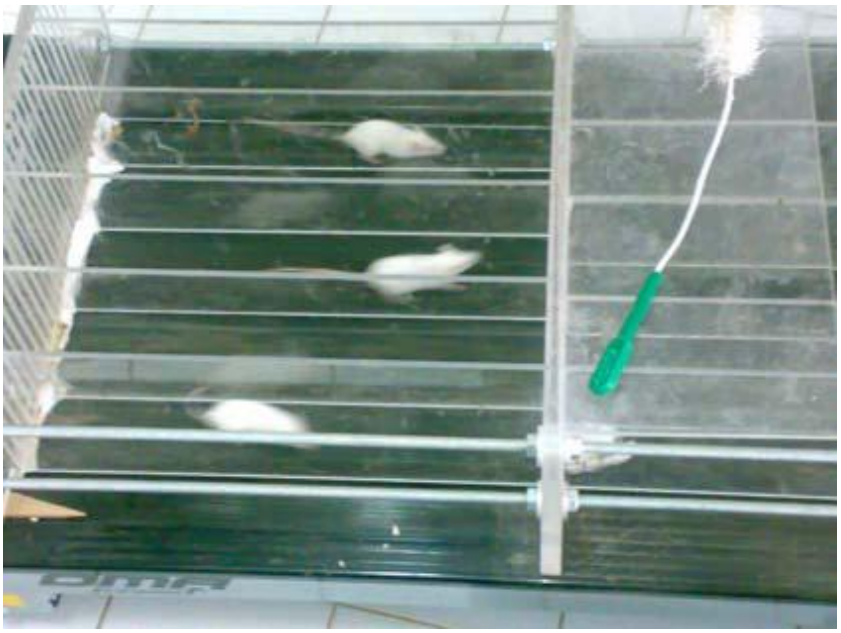


Figure 8: The modified human treadmill to train the rats according to our rehabilitation protocol.

At the end of the experiment, all animals were sacrified under anesthetic conditions as follows:

1- A container with cover was lined with a layer of cotton.

2- 20 ml of concentrated ether were poured over the cotton to saturate cotton with ether.

3- Each mouse was placed for 5 minutes in the container with ether-soaked cotton for anesthesis.

4- The mouse was then taken from the container and placed in a dissecting tray and its brain was removed.

5- The mouse brain was separated into two halves and put in a container with 0.4% formaldehyde for fixation purposes.

6- 0.4% formaldehyde was prepared as follows: 100 ml of 40% formaldehyde were added to 900 ml of distilled water.

3.3. Identification of striatal tissue

The neurons of striatum are divided into two compartments, the patch which is identified by patches of dense opiate receptor binding, and is enriched in enkephalin and substance p. The other compartment, the matrix, has a high acetyl cholinesterase activity.

To reach the striatum, we started sectioning the brain tissue at thickness of 5 micrometer and examined microscopically till white spots appeared which indicated the right tissue to take for immunostaining.

3.4. Brain section immunohistochemistry using nNOS and iNOS antibodies

Immunohistochemistry stain requires thefollowing steps:

A: Deparaffinization

In order to deparaffinize striatum sections, sections were incubated for 15 min in the oven at temperature of 65 C, then the sections were processed in the following sequence:

- Xylene for 5 minutes.
- Xylene for 5minutes.
- 100% ethanol for 5 minutes.
- 90% ethanol for 5 minutes.
- 80% ethanol for 5 minutes.
- 70% ethanol for 5 minutes.
- Distilled water for 5 minutes.

B: Antigen retrieval treatment

This step was performed to overcome the fixative effect in the following sequence:

1- Reveal solution (citrate base buffer 10X) was purchased from Biocare medical. It was freshly diluted as 1X to be ready for using.

5 ml of 10X reveal solution was diluted in 45 mldistilled water and mixed and became ready for use.

2- Sections were placed in a couplain jar containing 35ml reveal solution (1X) as previously prepared, the jar was covered by its cap and placed in a decloaker champer(Biocare medical, CB 910M) and incubated for 5 minutes at 121C.

3- When the cycle ended, the jar was taken from the chamber, and left to reach room temperature.

4- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2)for 5 minutes.

5- Sections were incubated with 1% H_2O_2in methanol for 30 minutes.

6- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2) for 5 minutes.

7- The area around the section was blotted dry and a circle was made around them by PAP pen (chemicon International).

8- Sections were incubated with 1% bovine serum albumin to prevent bachground staining for 20 minutes.

9- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2) for 5 minutes.

10- The primary antibodies of mouse anti-nNOS, mouse anti-iNOS, and anti-HSP70 (Santa Cruz biotechnology, SC-651, SC-648, SC-24), were diluted in a ratio 1:100 (primary antibody: diluent). Using micropipette, 20 µl of concentrated antibody were added to 980 µl of phosphate buffer saline (PBS, pH 7.2±0.2) in a vial and mixed using vortex (2500 RPM) for 30 seconds.

11- Then the primary antibody was applied until it covers the section, and then sections were incubated for an hour (at room temperature).

12- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2) for 5 minutes.

13- A biotinylated secondary antibody (ready to use from DAKO K0675) was applied by covering the sections (1-2 drops) for 30 minutes.

14- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2) for 5 minutes.

15- Streptavidin conjugated to horse raddish peroxidase enzyme (ready to use from DAKO K0675) was applied by covering the sections (1-2 drops) for 30 minutes.

16- Sections were washed with phosphate buffer saline (PBS, pH 7.2±0.2) for 5 minutes.

17- 1ml of liquid DAB (3,3' - diaminobenzidine) was placed in a vial, and 20 µl of 1% H_2O_2were added and mixed using vortex.

18- About 100 µl of DAB solution were added to each section for up to 7 minutes or till brown color developed.

19- Sections were washed with tab water for 5minutes.

20- Sections were counter stained by immersion the slides in Mayer's hematoxylin for 1minute, then the sections were washed from hematoxylin by tap water.

21- After that, sections were dehydrated through the following steps:

- 70% ethanol for 2 minutes.
- 80% ethanol for 2 minutes.
- 90% ethanol for 2 minutes.
- 100% ethanol for 2 minutes.

22- Sections were cleared by xylene for 2 minutes.

23- 1-2 drops of mounting medium, DPX (Dextrune plasterizar xylene) were applied to slides and a cover slip was placed on top of each. Regarding to negative control slides, negative slides was prepared, as other the slides prepared but without adding the primary antibody.

3.5. Analysis of tissues images

Slides were examined using compound Microscope. Ten random areas from each section were examined and photographed for all animals. Each photograph was analyzed using adobe photoshop software. The signals for brown color of immunoreactivity for nNOS and iNOS were set. The number of pixels for brown color was counted and compared to total number of pixels in the field. The average count for each treatment was calculated. The t test was used to

determine any statistical difference between all groups of mice. (The total pixels were 124848).

RESULTS

IV.Results

4.1. nNOS (N1) results

Analysis of the area showed no significant increase in the area occupied by nNOS in sedentary Parkinson disease mice compared to sedentary control mice (figure 9; P>0.421),this is clear in figure 10. Exercise training of parkinsonian animals caused a significant decrease of nNOS compared to sedentary Parkinson disease mice (figure 11; P<0.046), this is clear in figure 12. In addition, exercise training of parkinsonian mice caused a significant decrease of nNOS compared to exercise control (figure 13; P<0.012), this is clear in figure 14. In fact, there was no statically significant decrease in the nNOS in exercise control compared to sedentary control (figure 15; P>0.091), this is clear in figure 16.

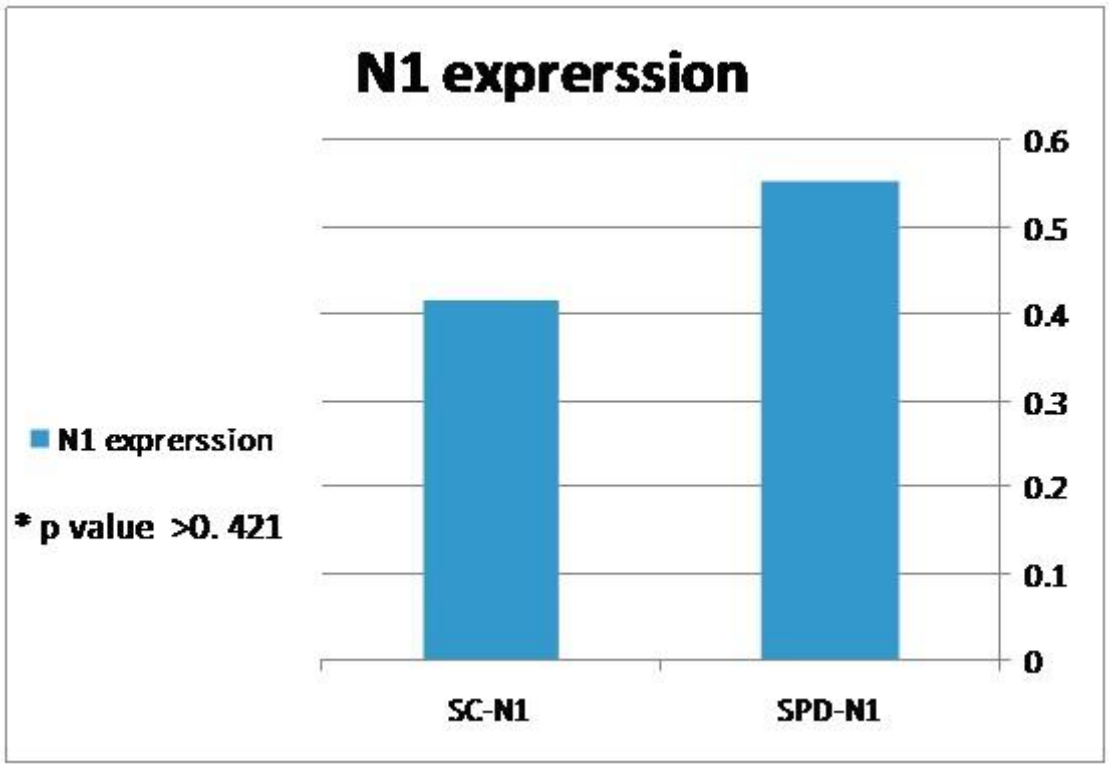
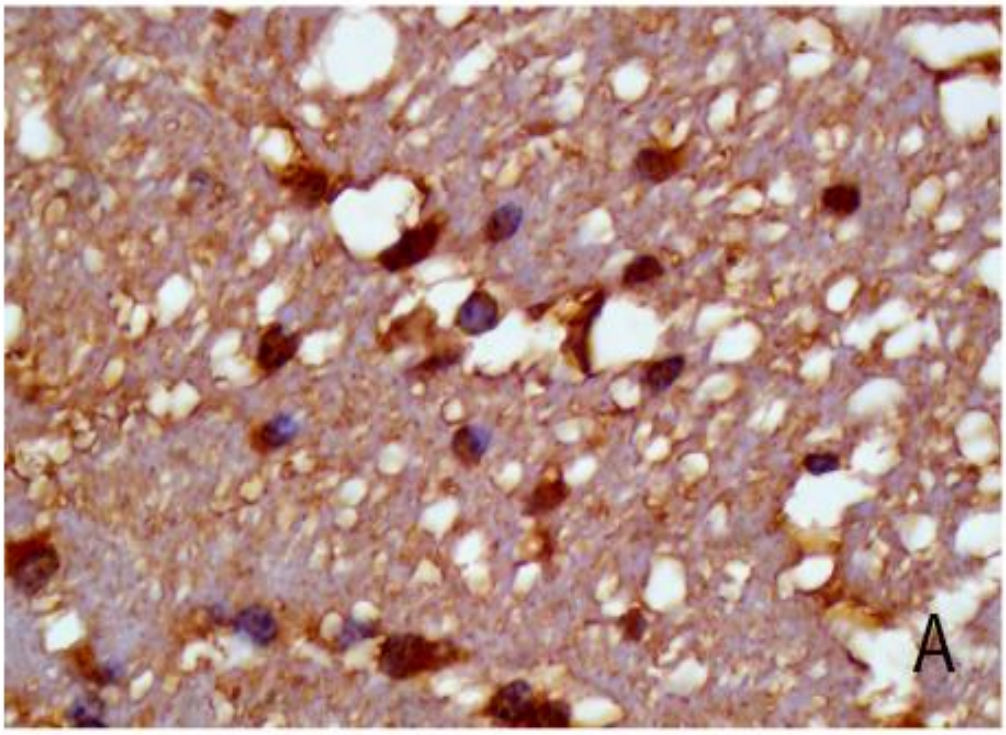


Figure9: A histogram represents the average expression of nNOS in the striatum of sedentary Parkinson's disease (**SPD**) and sedentary control (**SC**). There was no significant decrease of nNOS (P>0.421) between the two groups.



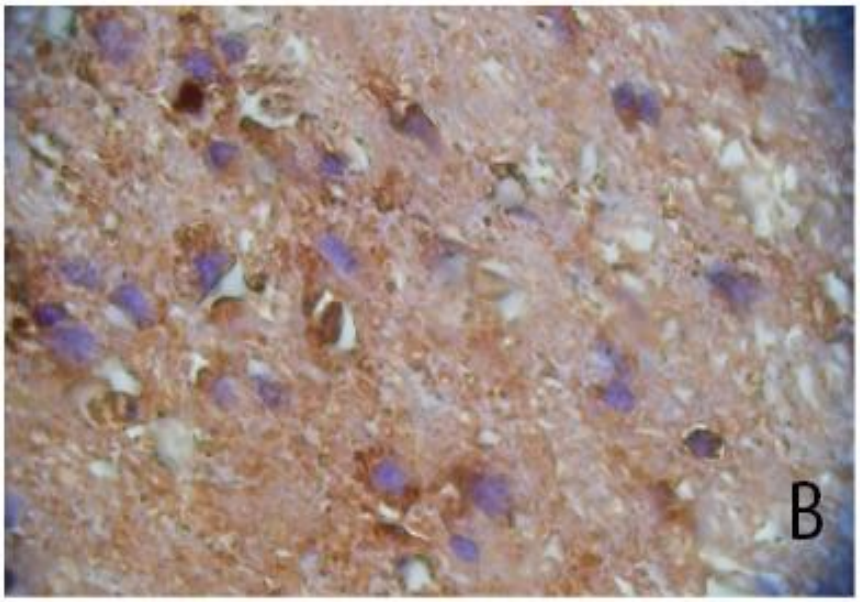


Figure 10: Photograph of the striatum of the mouse brain.

A: After MPTP treatment,B:without MPTP treatment. Intense immunoreactivity for nNOS is obvious in sedentary Parkinson's disease (A) and less intense in sedentary control (B).

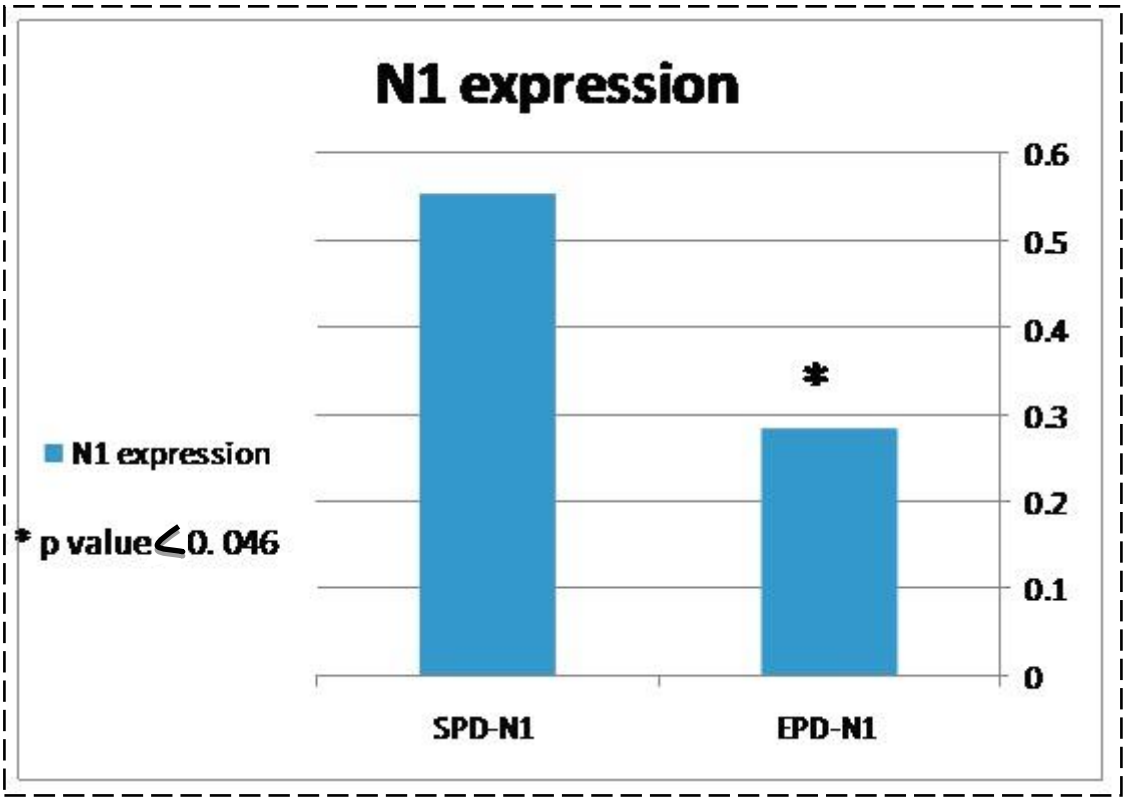


Figure11: A histogram represents the average expression of nNOS in the striatum of sedentary Parkinson's disease (**SPD**) and exercised Parkinson's disease (**EPD**). There was a significantdecrease of nNOS (P< 0.046) between SPD and EPD.

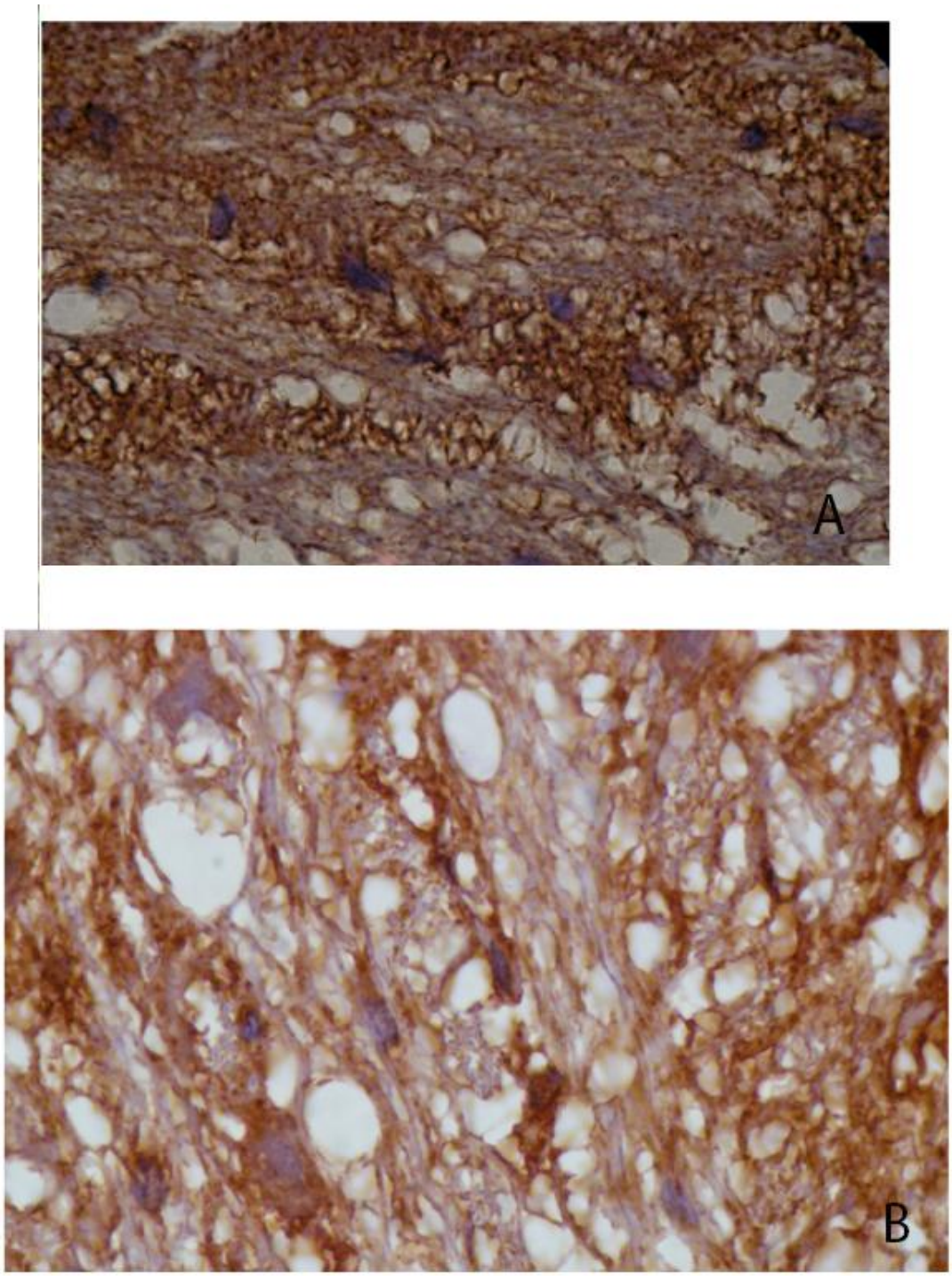


Figure12: Photograph of the striatum of the mouse brain after treatment with MPTP. Intense immunoreactivity for nNOS is obvious exercised Parkinson's disease (A) and less intense in sedentary Parkinson's disease (B).

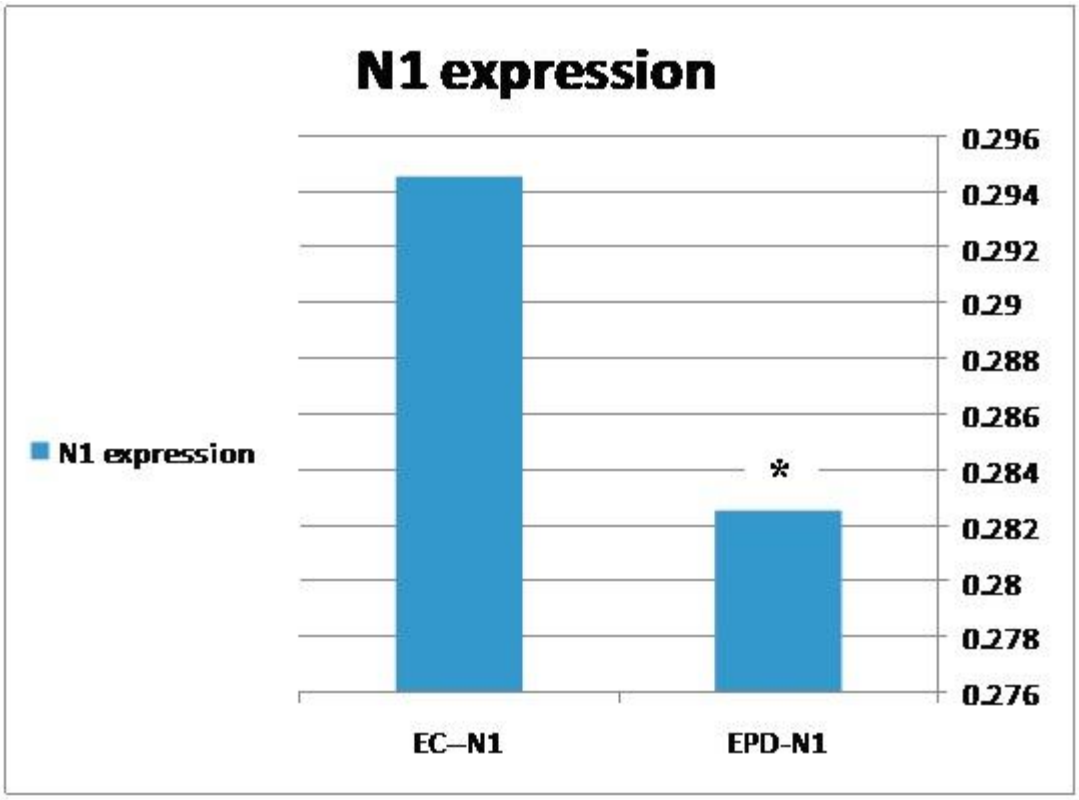


Figure 13: A histogram represents the average expression of nNOS in the striatum of exercise control (**EC**) and exercised Parkinson's disease (**EPD**). There was a significant decrease of nNOS ($P<0.012$) between the two groups.

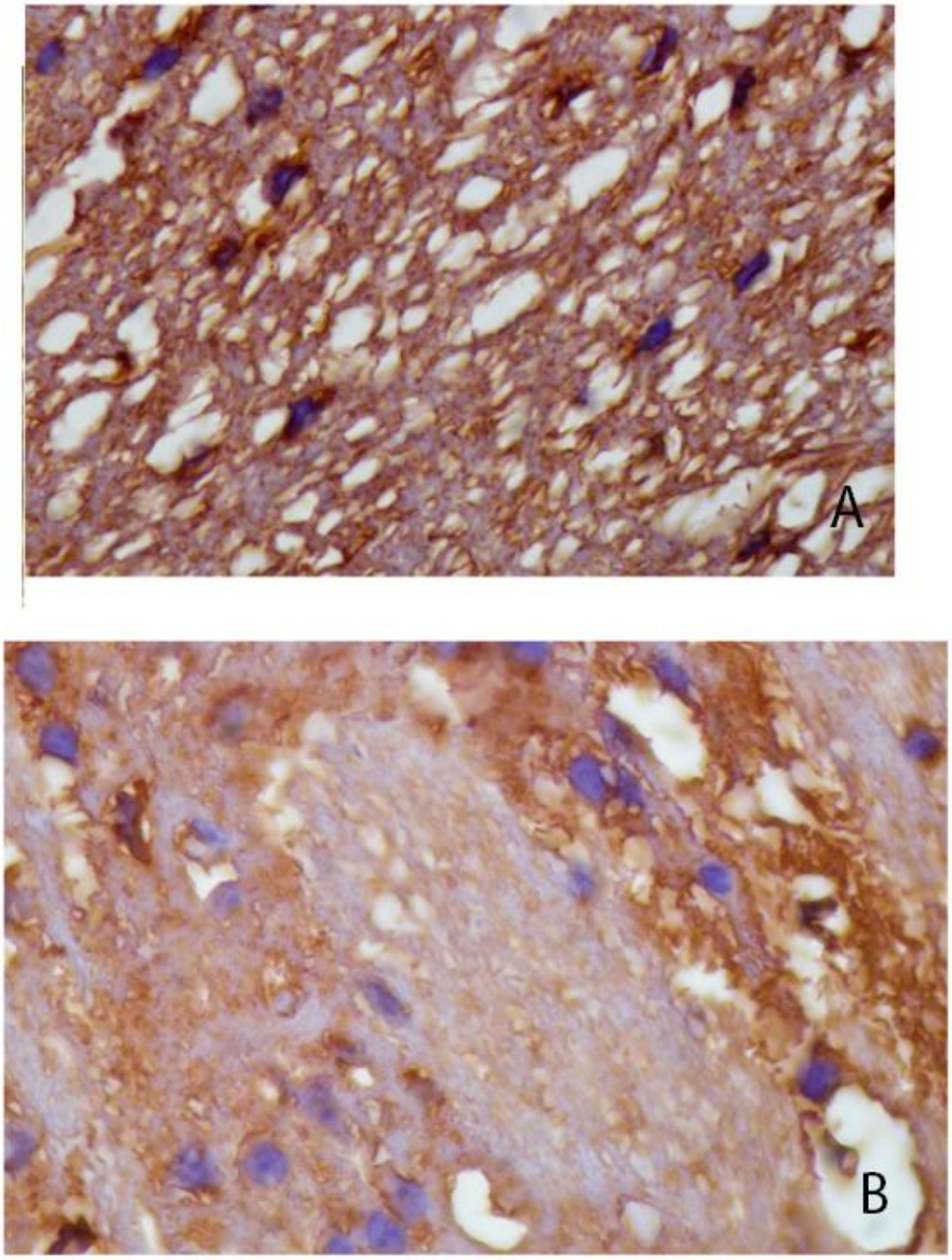


Figure 14: Photograph of the striatum of the mouse brain. A: after MPTP treatment, B: without MPTP treatment. Intense immunoreactivity for nNOS is obvious exercised control (A) and less intense in exercised Parkinson's disease (B).

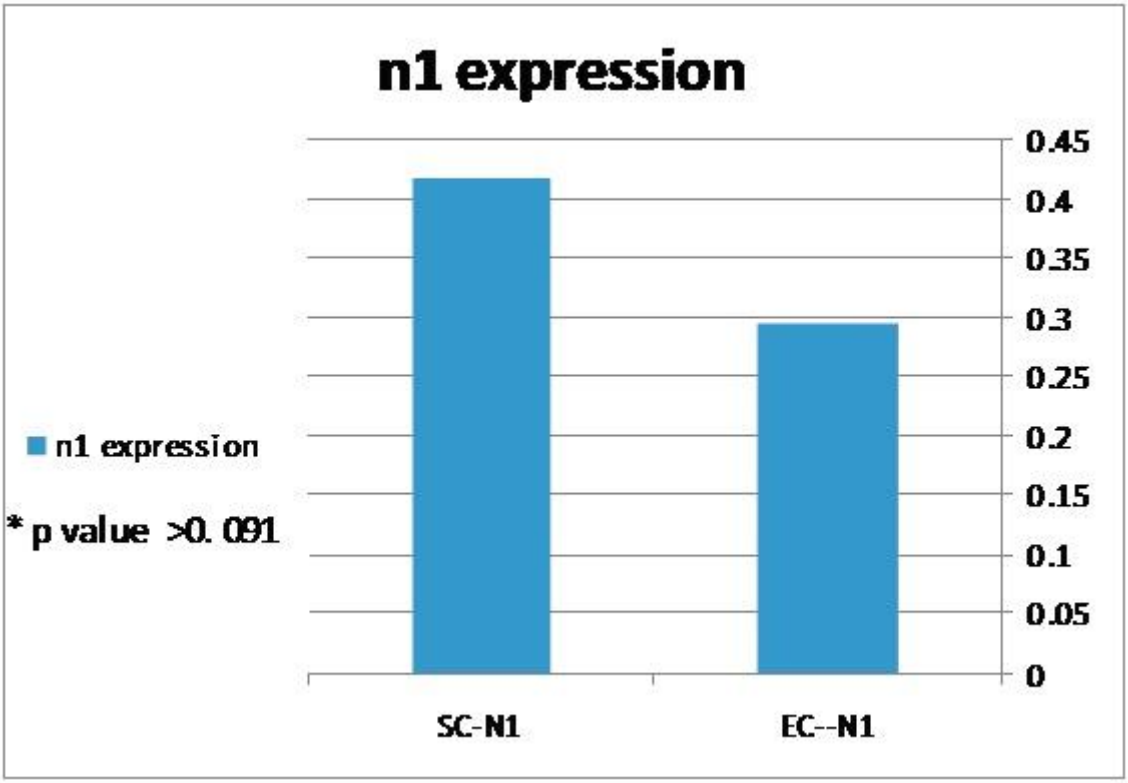


Figure 15: A histogram represents the average expression of nNOS in the striatum of sedentary control (**SC**) and exercised control (**EC**). There was no significant decrease of nNOS (P>0.091) between the two groups.

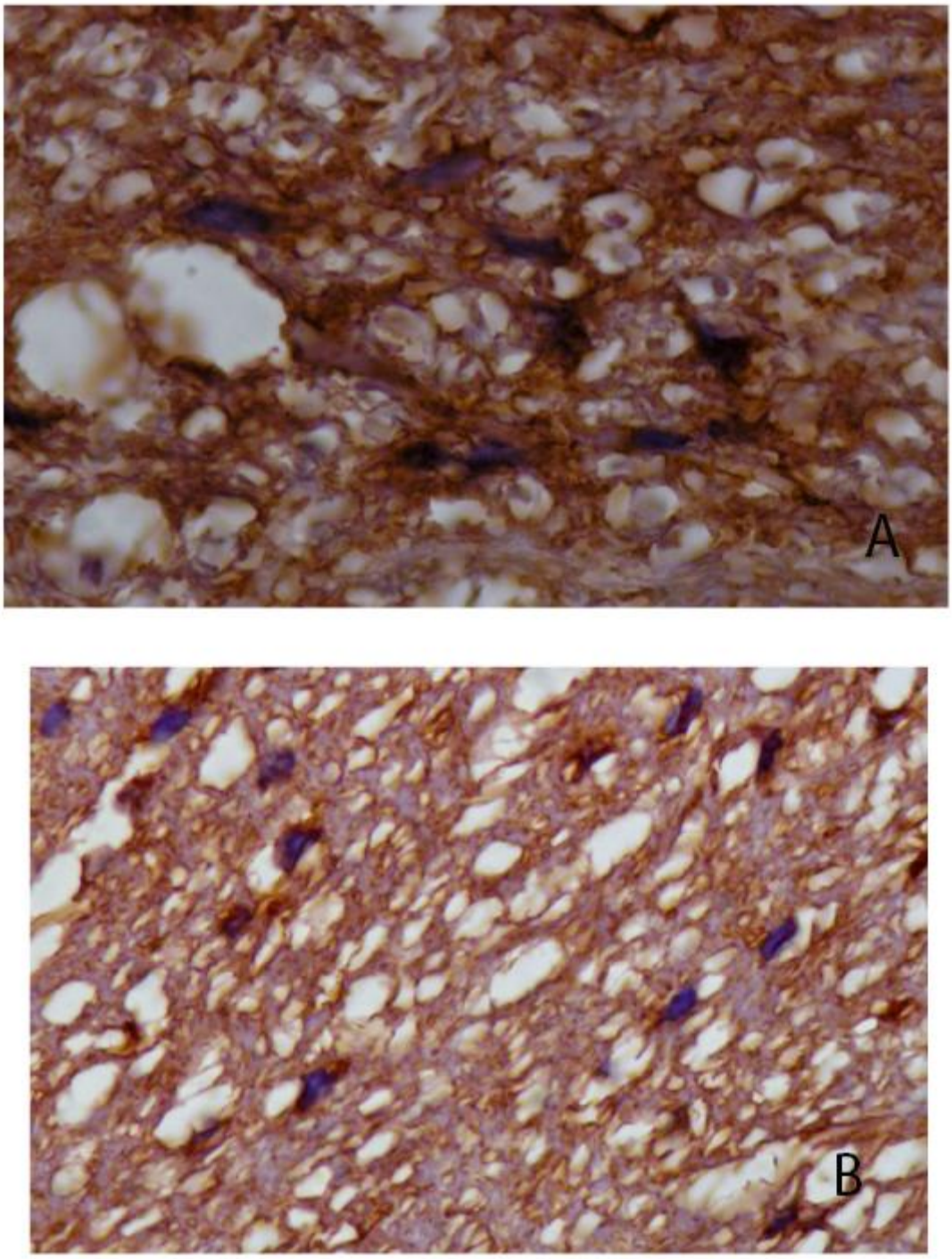


Figure16: Photograph of the striatum of the mouse brain without MPTP treatment. Intense immunoreactivity for nNOS is obvious sedentary control (A) and less intense in exercise control (B).

4.2- iNOS (N2) results

Analysis of the area showed a significant increase in area occupied by iNOS in sedentary Parkinson disease mice compared to sedentary control (figure 17; P<0.026), this is clear in figure 18. Furthermore, exercise training showed a significant increase in area occupied by iNOS in exercised Parkinson disease mice compared to exercised control mice (figure 19; P<0.036), this is clear in figure 20. In fact, there was no statistically difference in iNOS area in exercised Parkinson disease mice compared with sedentary control (figure 21; P> 0.46), this is clear in figure 22, also there was no statically difference in iNOS area in exercised Parkinson disease mice compared with sedentary Parkinson disease mice (figure 23; P>0.130), this is clear in figure 24.

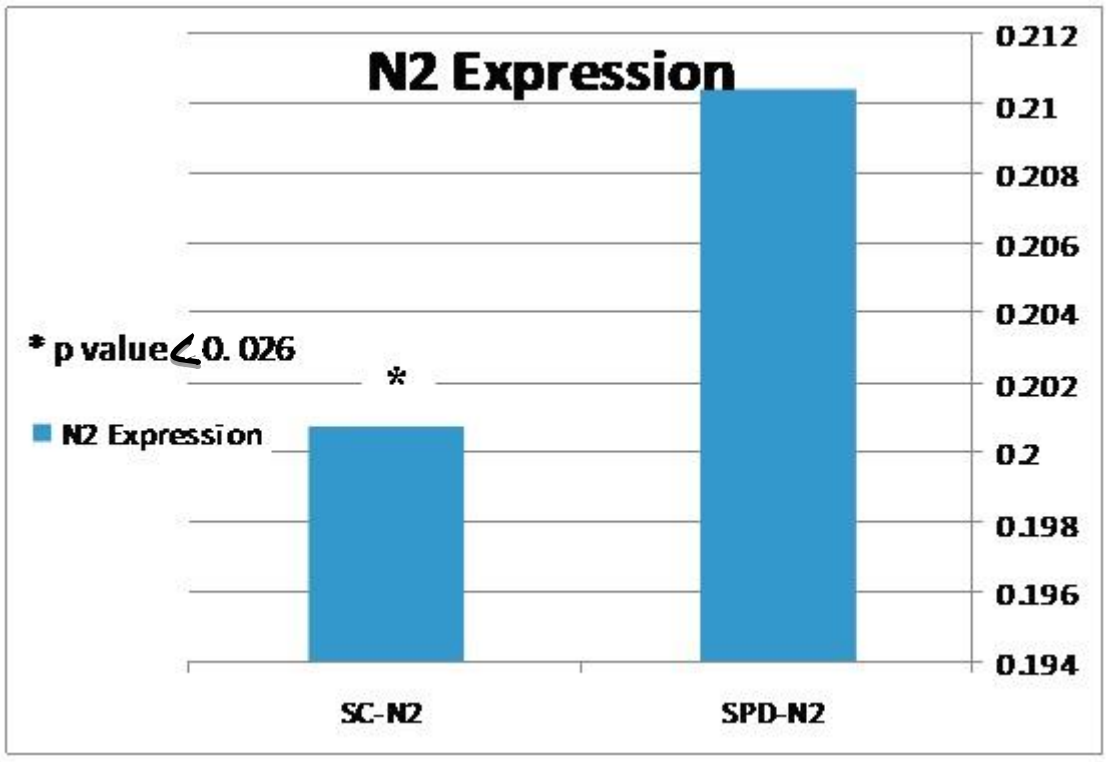


Figure 17: A histogram represents the average expression of iNOS in the striatum of sedentary control (**SC**) and sedentary Parkinson (**SPD**). There was a significant increase of iNOS (P<0.026) between the two groups.

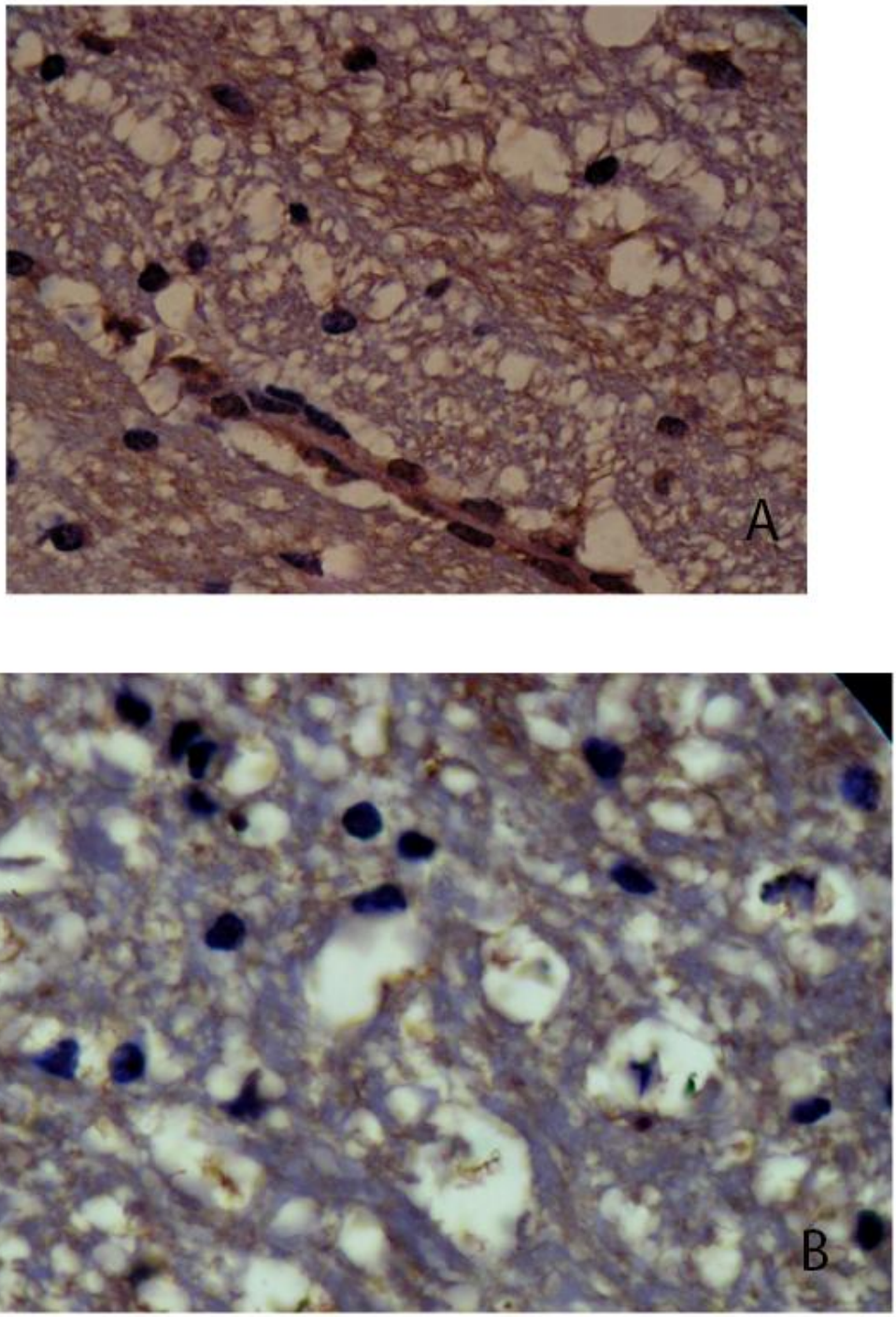


Figure 18: Photograph of the striatum of the mouse brain. A: after MPTP treatment, B: without MPTP treatment. Intense immunoreactivity for iNOS is obvious in sedentary Parkinson's disease (SPD) and less intense in sedentary control (SC).

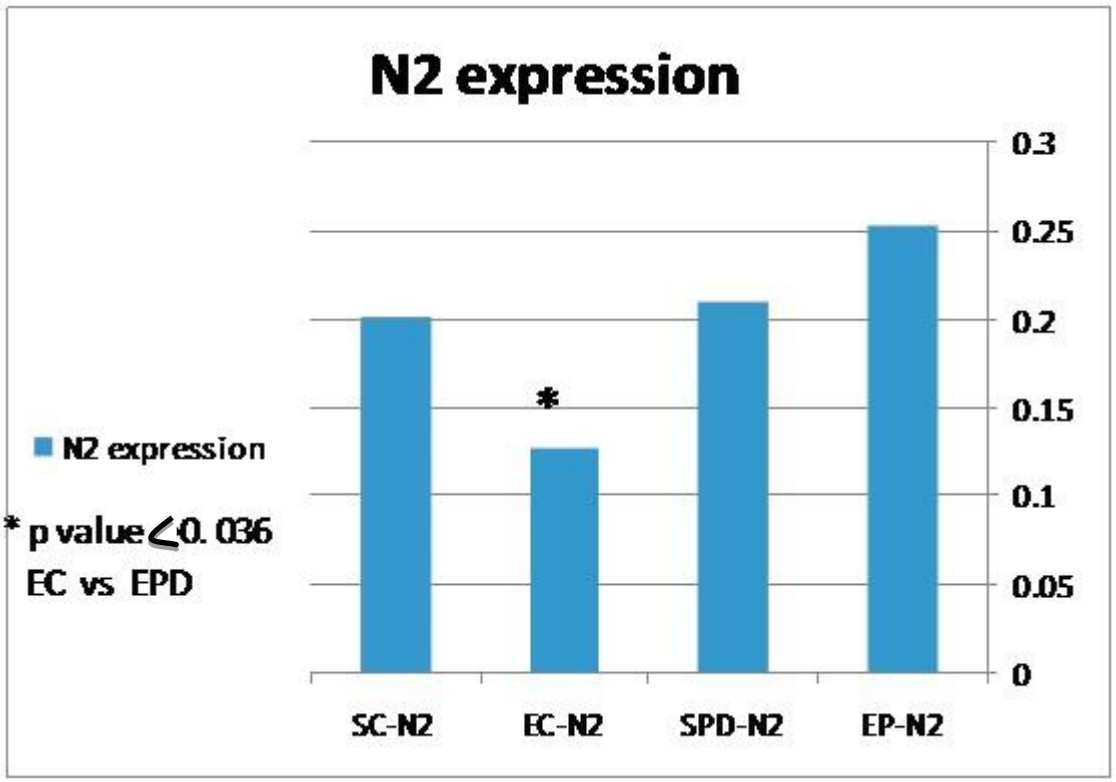


Figure 19: A histogram represents the average expression of iNOS in the striatum of sedentary control (**SC**), exercised control (**EC**), sedentary Parkinson (**SPD**) and exercise Parkinson (**EP**). There was a significant increase of iNOS ($P<0.036$) between exercised Parkinson disease and exercised control.

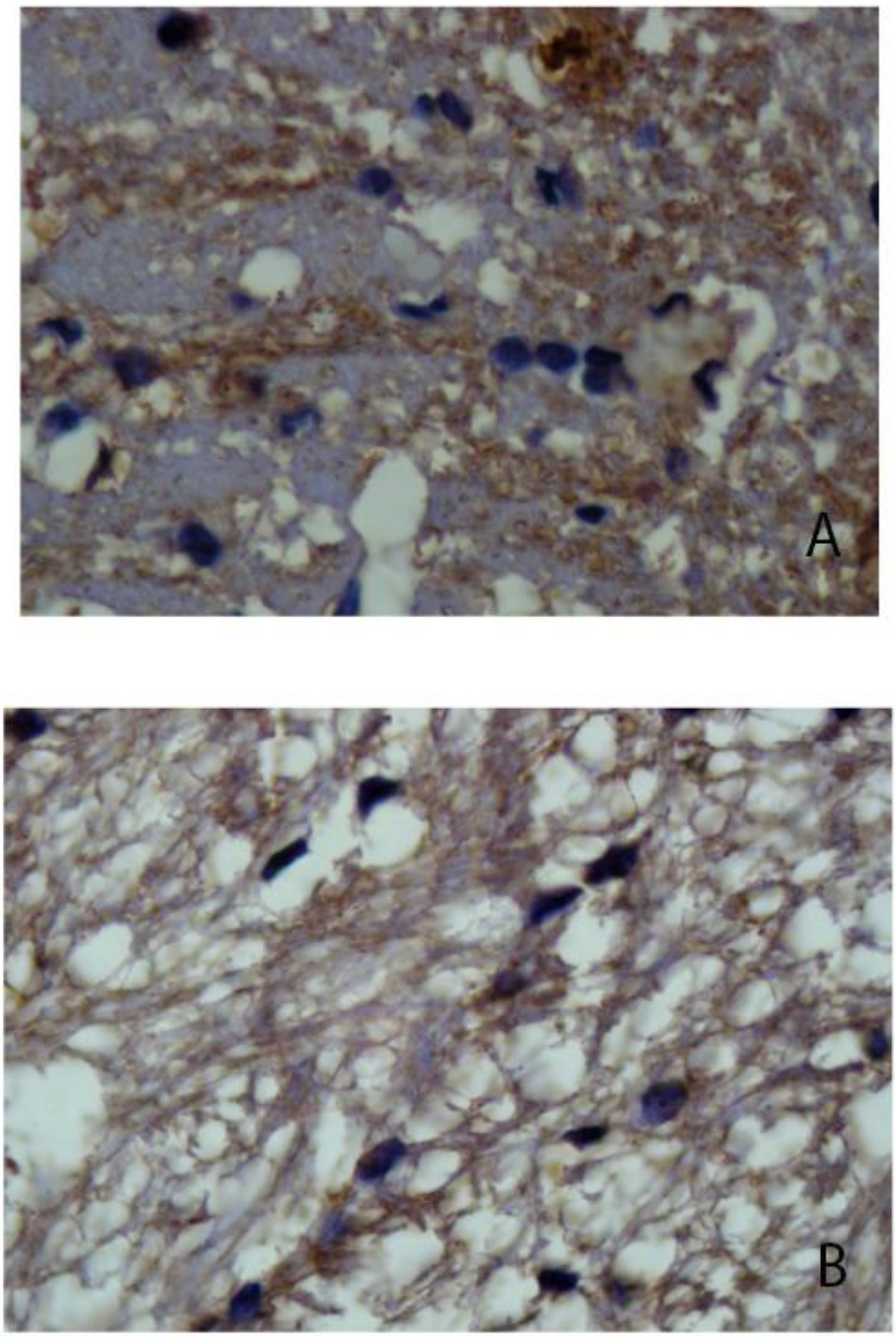


Figure 20: Photograph of the striatum of the mouse brain. A: after MPTP treatment (EPD), B: without MPTP treatment (EC). Intense immunoreactivity for iNOS is obvious exercised Parkinson's disease (A) and less intense in exercised control (B).

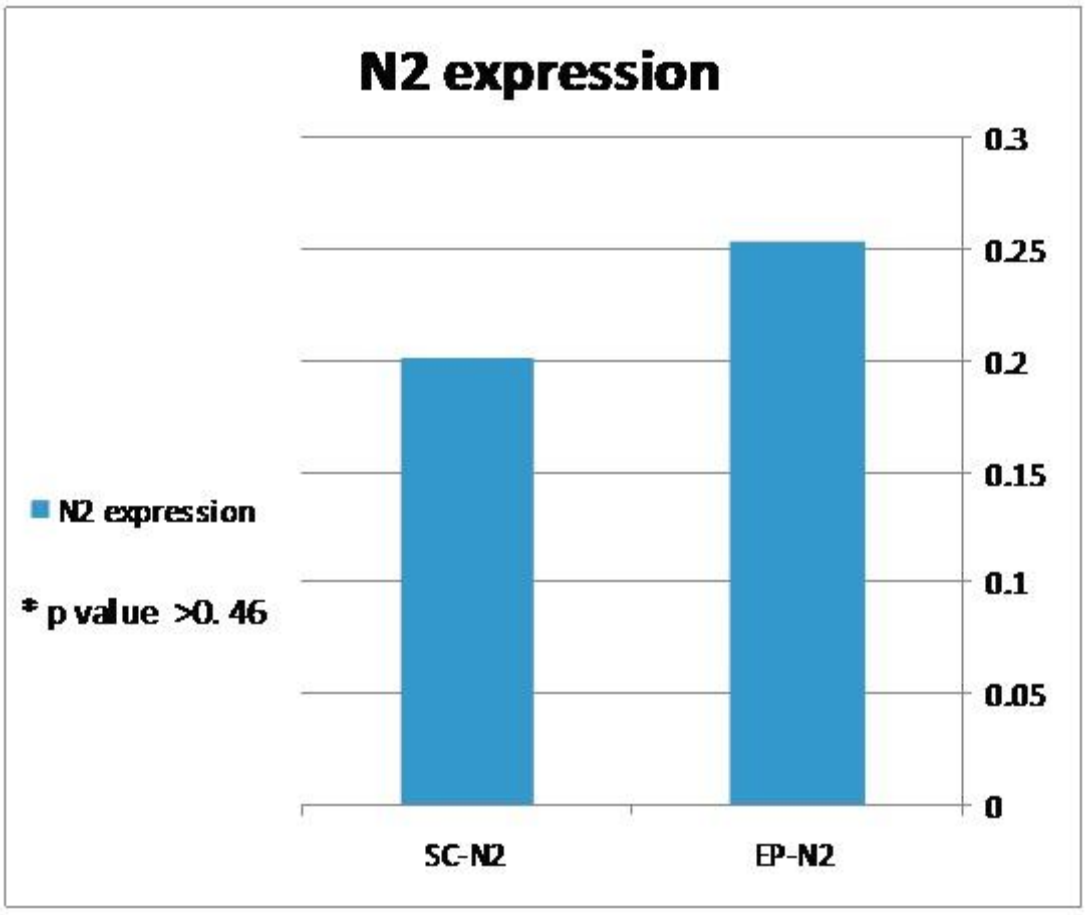


Figure 21: A histogram represents the average expression of iNOS in the striatum ofsedentary control (**SC**) and exercised Parkinson (**EP**). There was no significant increase of iNOS (P> 0.46) between the two groups.

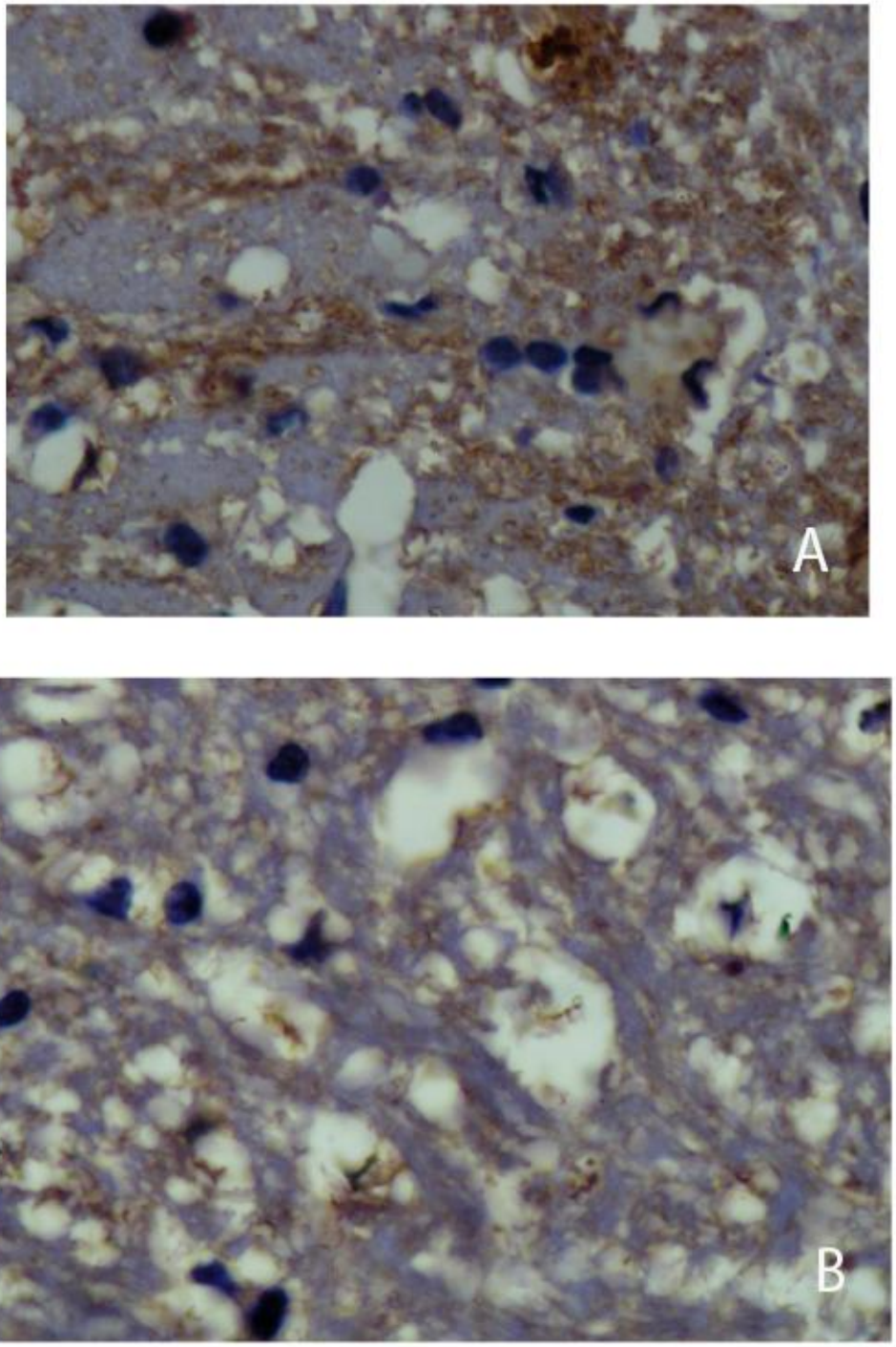


Figure 22: Photograph of the striatum of the mouse brain. A: after MPTP treatment (**EPD**), B: without MPTP treatment (**SC**). Intense immunoreactivity for iNOS is obvious exercised Parkinson's disease (A) and less intense in sedentary control (B).

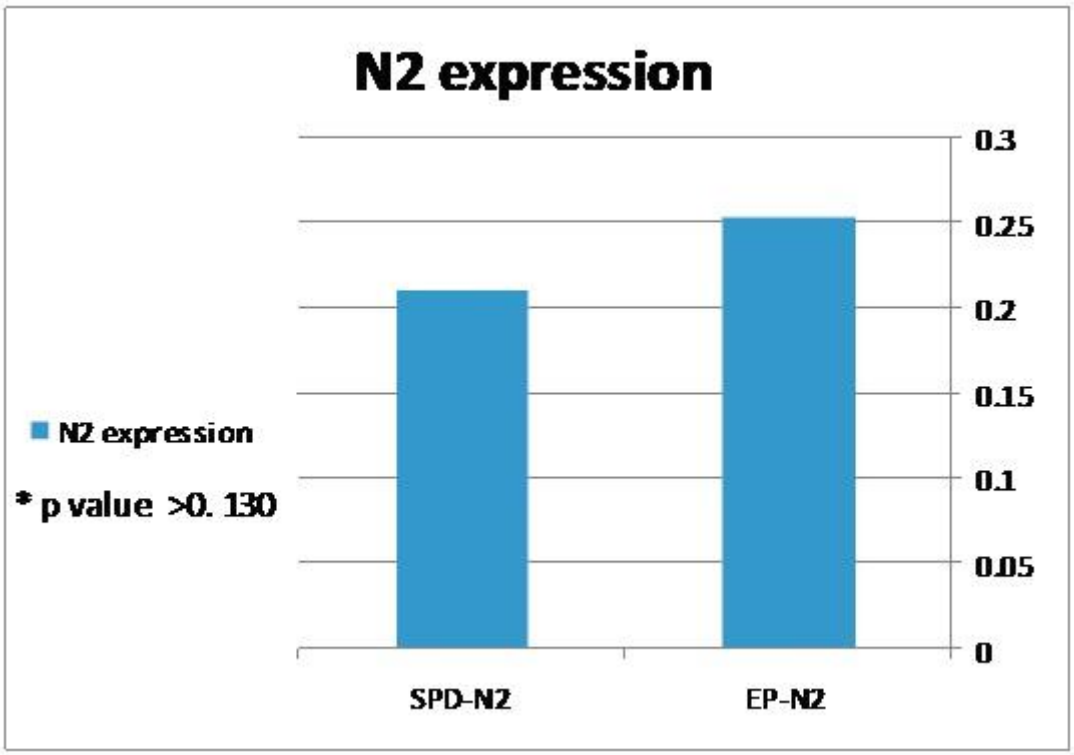


Figure 23: A histogram represents the average expression of iNOS in the striatum of sedentary Parkinson (**SPD**) and exercised Parkinson (**EP**). There was no significant increase of iNOS (P> 0.130) between the two groups.

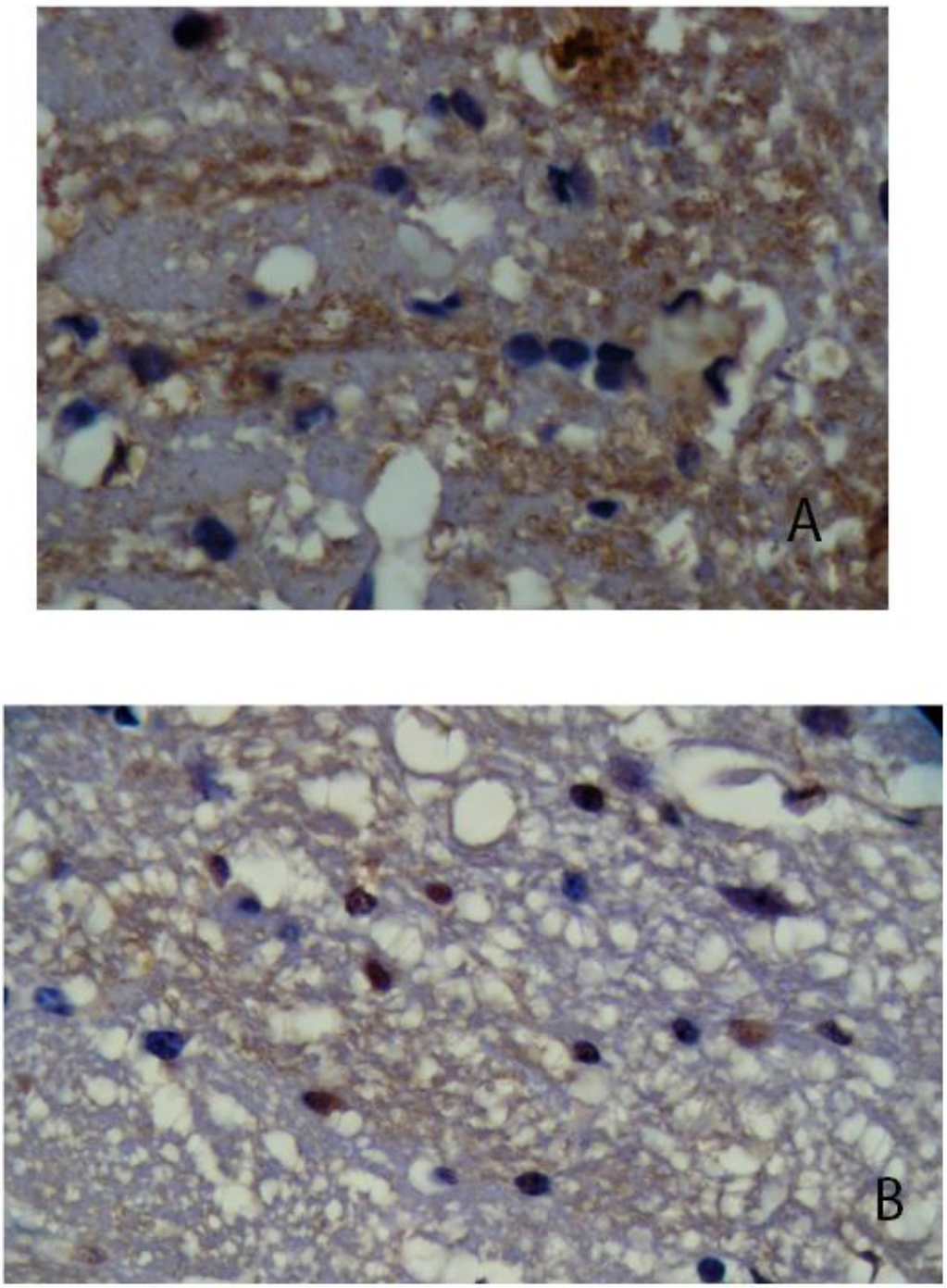


Figure 24: Photograph of the striatum of the mouse brain after treatment with MPTP. Intense immunoreactivity for iNOS is obvious exercised Parkinson's disease (A) and less intense in sedentary Parkinson's disease (B).

4.3 The Results for Heat Shock Protein (HSP70)

The expression of HSP70 was compared among four groups in the present study. The average expression rate of HSP70 was 0.045% in SC group. This rate was decreased in PD group to 0.0029. This decreased rate was statistically significant as shown by T test (p value $\leq$0.05). Exercise was able to increase to the expression rate of HSP70 to 0.0122%. But, this was not statistically significant (p value >0.05). Exercise was also able to increase significantly the expression rate of HSP70 in EC group compared with SC group to 0.106% (p value $\leq$0.05) (figure 25).

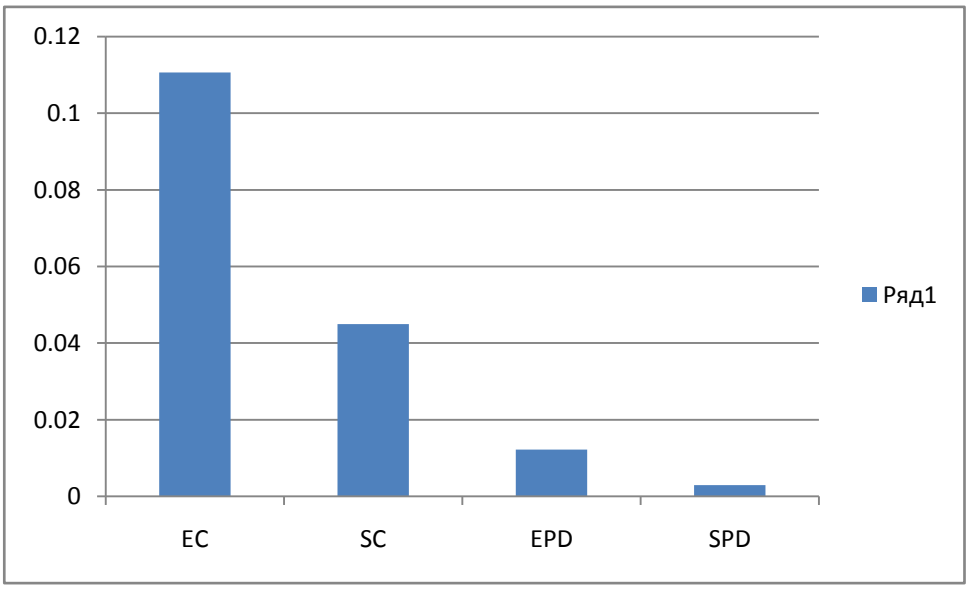


Figure 25: A histogram represents the average expression of HSP70 in the striatum of sedentary control and sedentary Parkinson.

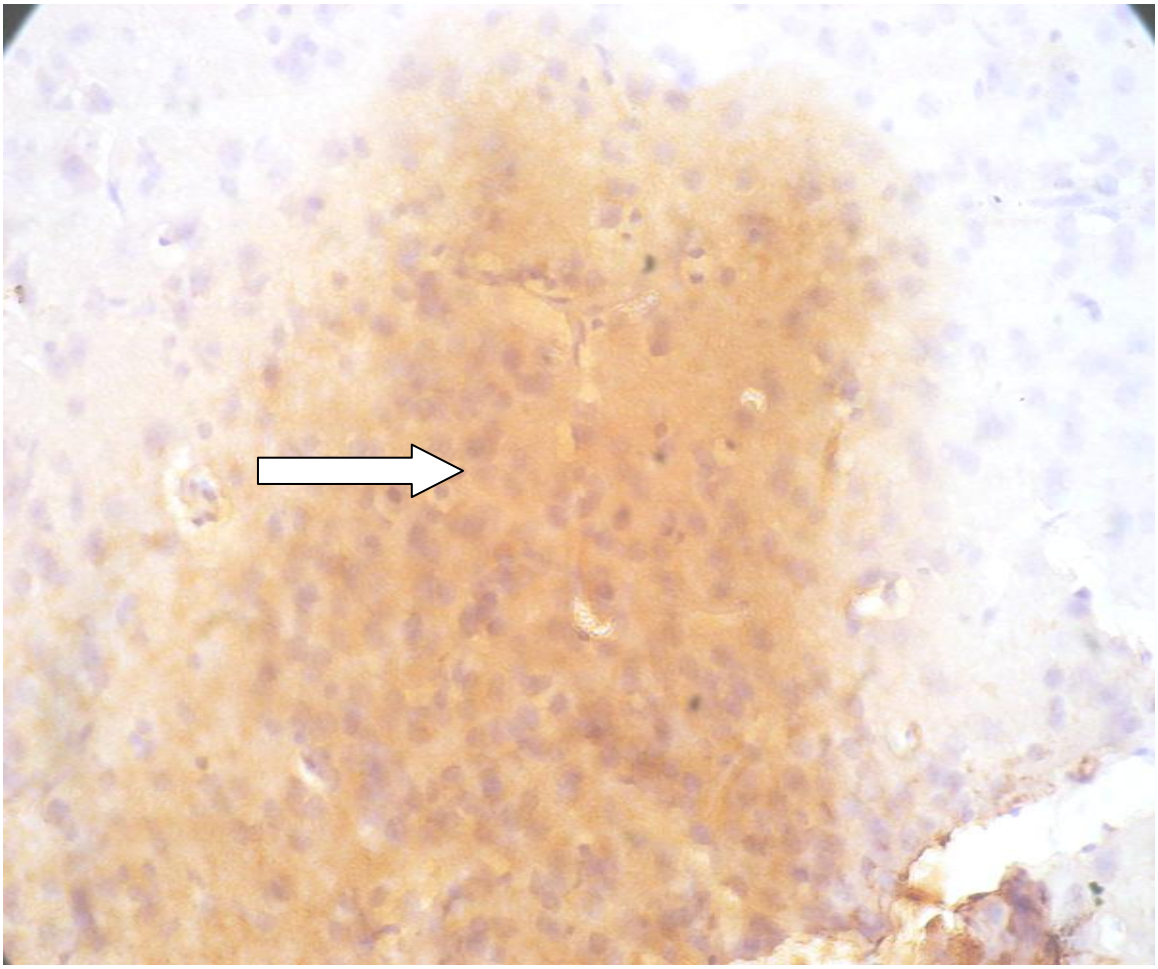


Figure 26: The expression of HSP70 in exercise control(EC) group. Cytoplasmic reactivity in brain tissue (arrow).

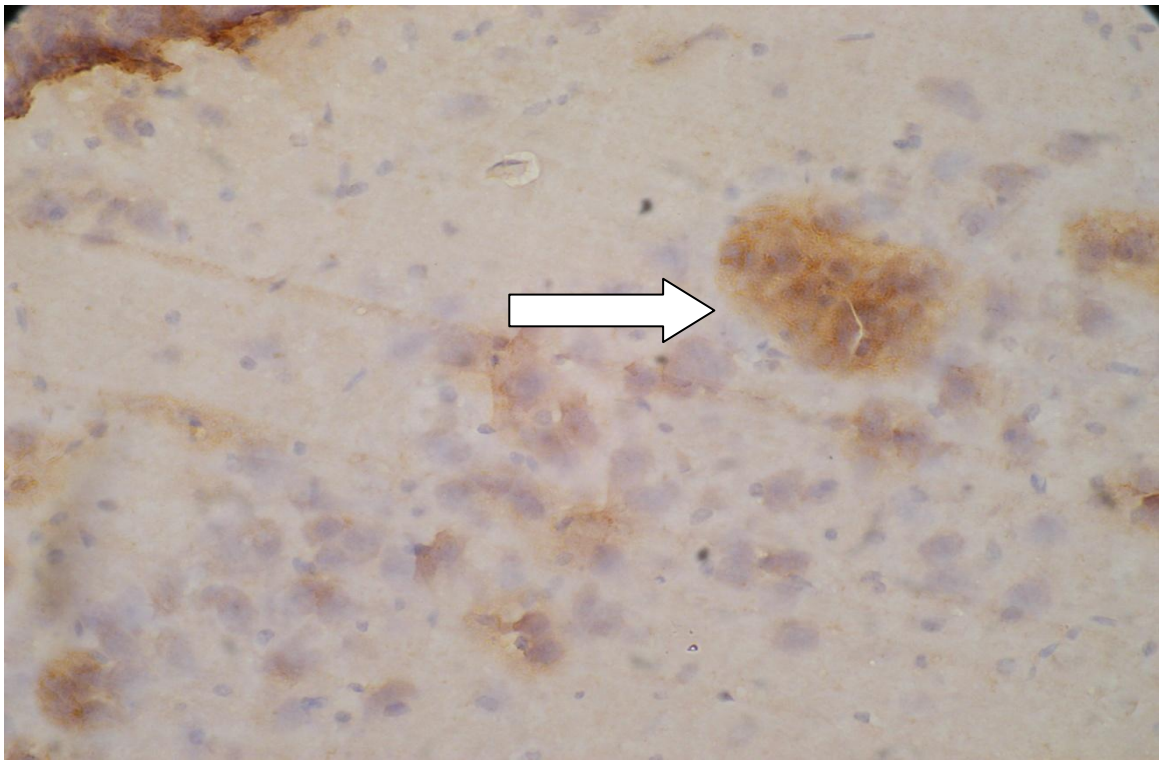


Figure 27: The expression of HSP70 in sedentary Parkinson disease(SPD)group 40X. note cytoplasmic reaction in brain tissue (arrows).

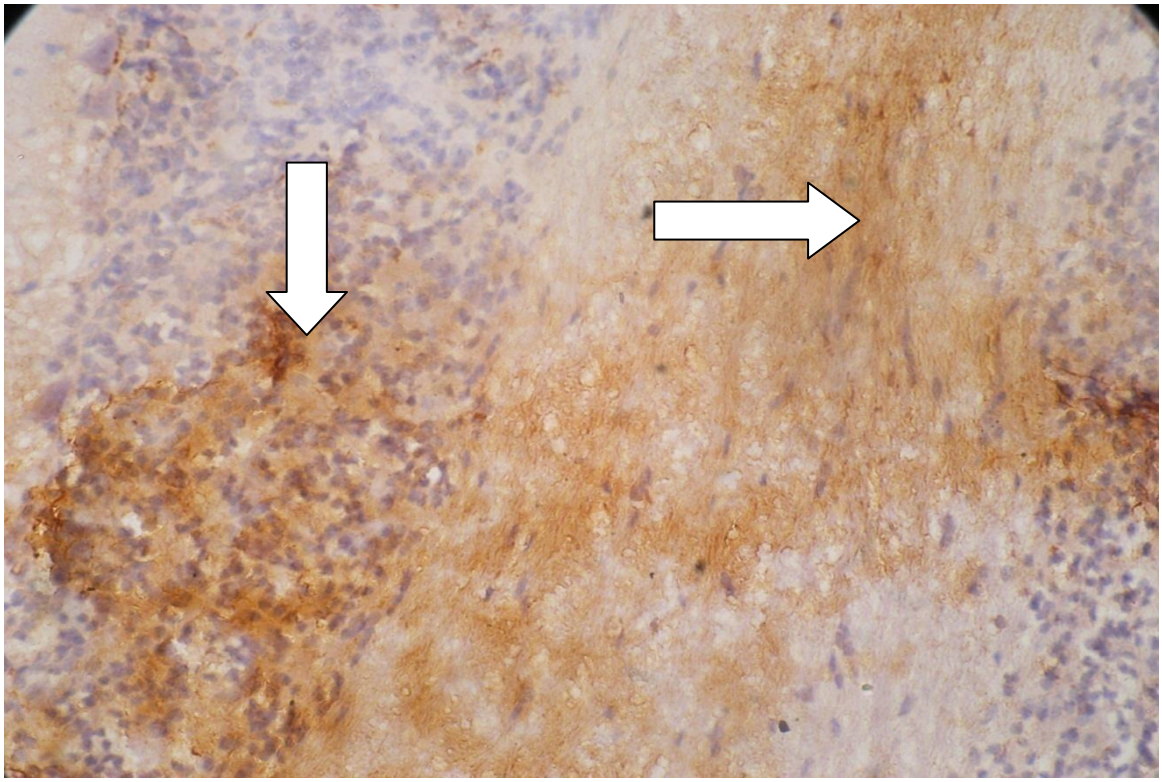


Figure 28: The expression of HSP70 in exercise Parkinson disease(EPD) group, 40 x. Note cytoplasmic and nuclear reactions in brain tissue (arrows).

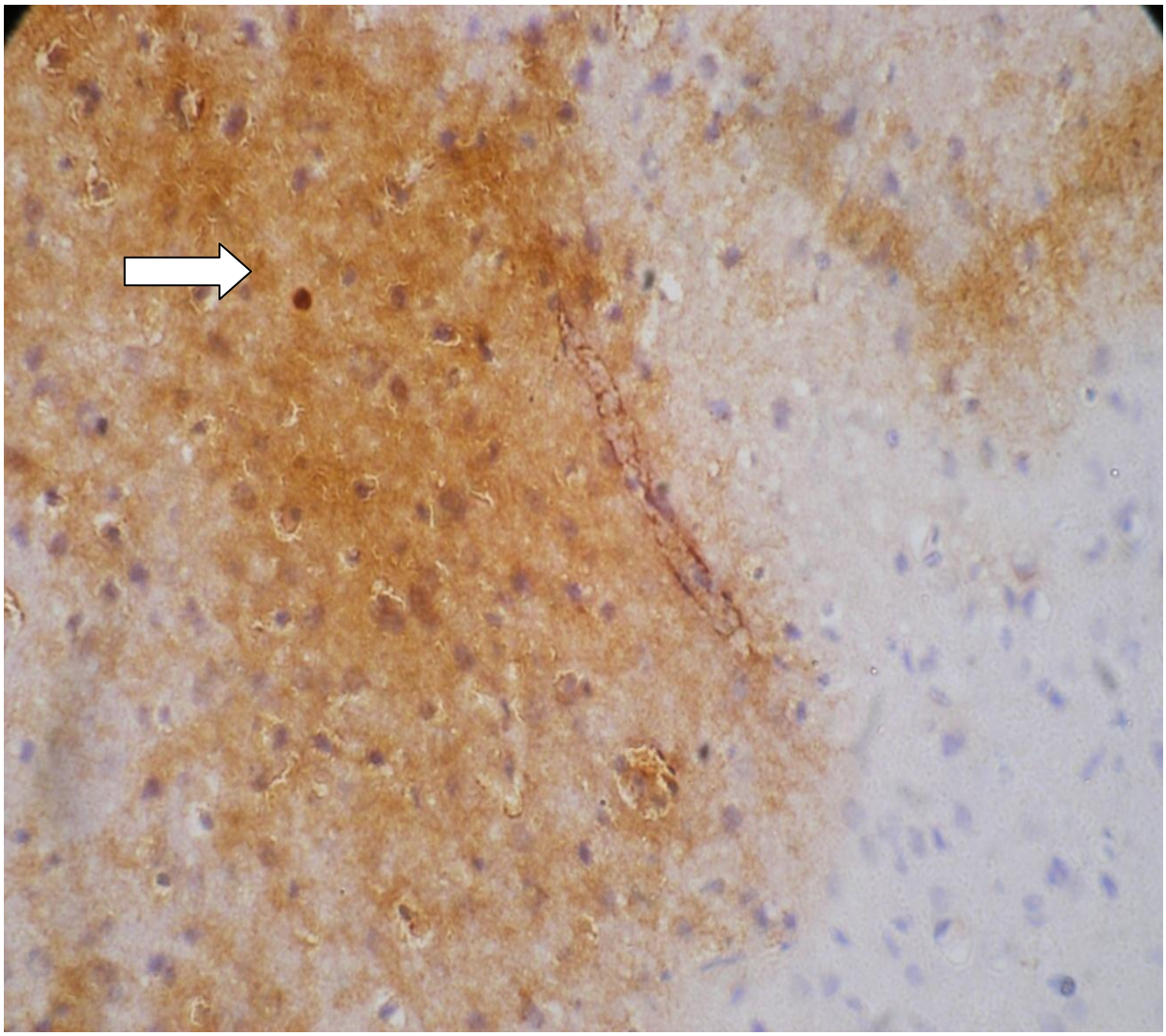


Figure 29: The expression of HSP70 in sedentary control(SC) group 40 X. ote cytoplasmic and nuclear reactions in brain tissue (arrows).

Discussion of Results

V- Discussion of results

The importance of physical therapy in patients with PD lies in reducing the severity of neurological degeneration, improving motor performance and quality of life (Taylor, 2007). No doubt that medication along with proper exercise therapies is more likely to improve the imbalance, immobility, and reduce accidents and the dangers of falling in early stages of PD unless there is hard evidence that there was a significant limitation to exercise (Kroncke, 1998).

Furthermore, the potential impact of exercise in PD as neuroprotective and neurorestorative has been formulated as theories, and the results have been highly debated. However, the convincing evidence showed the importance of exercise in PD was limited to clinical experiments (Kroncke, 1998). Previous data suggested that there was a possible positive connection between three factors that include chronic moderate exercise, increased nitrergic system and cognitive behavior in rats. Pietrelli et al., 2011 and Christian et al., 1999 showed that there was a significantupregulation of the nitrergic system in trained animals. In addition, an increased NOS activity in neurons of the cerebral cortex, hippocampus and striatum was found in trained animals (Al-Jarrah, 2010; Dishman, 2006). Recently, human studies showed that nitric oxide release from aorta, active and inactive muscles, and coronary arteries enhanced after a period of endurance exercise ranging from days to weeks (Yousefi, 2009).

Postmortem studies, clinical findings, and evidence from experimental models revealed the role of NO in the degeneration of dopaminergic neurons in PD (Jankovic, 2008). Eve et al., (1998) observed that there was a significant increase in NOS mRNA expression in the dorsal two-thirds of the STN of Parkinson's disease. Furthermore, Przedborski et al. (1996) showed by using histopathological technique that NO and glutamate in toxic dose may mediate certain neurodegenerative diseases.

In our study we found that both iNOS (N2) and nNOS (N1) were increased in the striatum of parkinsonian mice compared to controls. These data may suggest a role of both iNOS (N2) and nNOS (N1) in the development and

progression of PD. Our findings are in agreement with other several animal and human studies that showed potential impact of the nNOS and iNOS in neurodegeneration and glial response in PD (Tarohda, 2005). In this regard, many scientists worked hard to study the relationship between iNOS and nNOS in pathogenesis of PD. They found that there was a positive correlation between the concentration of nNOS and iNOS and the progression of PD (Ben, 1991; Twelves, 2003). On the other hand, mutation in nNOS gene makes the mice more resistance to MPTP than wild-type mice (Ben, 1991; Twelves, 2003). Similar experiments with iNOS showed that this enzyme also played a role in the dopaminergic neurons sensitivity (Ben, 1991; Twelves, 2003).

Running on treadmill increases dopamine release and turnover and chronically upregulates D2 receptors in the striatum of rats (Moncada, 1997). In another study, high intensity treadmill exercise in the acute MPTP-treated mice results in behavioral recovery; however, the striatal expression of DAT is down-regulated and the expression of TH has not been changed (Taylor, 2007). In contrast, little is known about the effect of physical activity and exercise in the form and function of the central nervous and peripheral nervous systems (Hou, 1999). In healthy humans, physical fitness and acute exercise together regulate nitric oxide formation. Jungersten et al., 1997 indicated that there was a positive linkage between physical fitness and basal NO formation. They studied nitrate level, the major stable end product of nitric oxide (NO) metabolism, in the plasma from groups of healthy subjects with different working capacities. They found that the athletic subjects have higher resting plasma level than the non-athletic controls.

In our study, we found that iNOS was increased in both controlled Parkinson's and exercised Parkinson's but the increase was more profound in exercised group. This suggests that exercise may have positive effect on the expression of iNOS in the striatum of Parkinson's mice. It was found that iNOS was increased in human leukocytes (Petzinger, 2007), heart of rats (Herman, 2007), and rat aortic endothelial cells (Al-Jarrah, 2007). This increase may be

due to increase endogenous NO- production and reflects an inflammatory response to exercise. These findings were supported by Niess et al., 2000, who studied the expression of iNOS in human leukocytes after 30 minutes of exhaustive running exercise in nonathletic subjects. They found that untrained subjects were capable to induce iNOS expression in response to exhaustive treadmill running (Petzinger, 2007). The increase of iNOS may due to the release of cytokines and other immunity mediators, which induce iNOS. Moreover, Husain and Hazelrigg (2002) found that the exercise training and chronic NOS inhibitor (nitro-L-arginine methyl ester, LNAME) administration lead to a significant induction of iNOS after exercise in heart of rats. This may be due to increase blood flow during exercise which leads to the upregulation of iNOS, or may be due to positive effect of exercise on catecholamines, and hence upregulation of iNOS expression. In addition, Yang et al. (2002) studied the effect of chronic exercise on rat aortic endothelial cells. They found that there was upregulation in iNOS mRNA expression. In the contrary, Liu et al. (2012) demonstrated that swimming exercise may lead to the decrease iNOS expression in hippocampus and prefrontal cortex.

In 2003, Gielen et al., found a significant reduction of iNOS in skeletal muscle of chronic heart failure patients after exercise training. On the other hand, Yang et al. (2003) studied iNOS expression in muscle samples during chronic exercise training. They found that there was no detection of iNOS expression in these samples. Regarding to nNOS, we found that nNOS was decreased in exercised Parkinson's. This suggests that exercise may have negative effect on the expression of nNOS in the striatum of Parkinson's mice. Since the development and progression of Parkinson disease is associated with high levels of nNOS (Ben, 1991), we expect that training could improve or at least hinder the progression of Parkinson patients. This is supported by the work of Hancock et al., 2008 who noticed that mutation in nNOS gene makes the mice more resistance to MPTP than wild-type mice. In the contrary, training lead to increased levels of nNOS in isolated skeletal muscle, islets of the pancreas and in the skeletal muscle of type 2 diabetes mellitus (Rennick, 1972; Meriin, 2005;

Berke, 2003; Grunblatt, 2004). On the other hand, there was no change in the nNOS amount in trained muscles compared to untrained ones (Rennick, 1972). This contrasts response of training may be due to tissue difference. Different tissues may show different immunological response. Frandsen et al., 2000 used human skeletal muscle to examine nNOS level, activity and distribution after endurance training. He found that there was no change in the nNOS amount in trained muscles compared to untrained one. On the other hand, during contraction, NO was produced at a higher rate compared to control using models of isolated skeletal muscle. Vassilak-opoulos et al., 2003 found that there was fourfold rise in gastrocnemius NOS activity and enhancement of nNOS expression to 240%.Ueda et al., 2003 examined the effect of exercise on islets of both control and trained mice.

The results showed that nNOS expression was enhanced and NO release was increased (Grunblatt, 2004). Furthermore, in skeletal muscles of type 2 diabetes mellitus, short term exercise training has an impact on the level of nNOS protein. There was 4-fold increase in nNOS protein expression in soleus muscle after 8 weeks of treadmill running (Meriin, 2005). In human, 6 weeks of exercise have no effect on nNOS expression on skeletal muscle (Berke, 2003). Furthermore, treadmill running enhanced the nNOS expression in hippocampal region in food deprived rats. This induction of nNOS expression may overcome the nNOS suppression caused by food deprivation (Zhang, 2005). In addition, exercise enhanced nNOS expression in Para-ventricular nucleus in food deprived animals (McLean, 2002). and in heart failure rats. Kim. S et al 2003 revealed that nNOS mRNA expression was significantly increased which result in increased NO synthesis in paraventricular nucleus of heart failure rats after exercise. The striatum, hippocampus and the paraventricular nuclei show the histological features of the nervous tissue.

We expect that tissues with almost identical features show the same immunological response. But it was not the case, the striatum of PD mice showed decrease levels of nNOS while the hippocampus and the paraventricular nucleus

showed increased levels of nNOS expression in rat after training (McLean, 2002). This contrast response cannot be attributed to technical or environmental factors because of the uniformity in handling all specimens. This difference may be attributed to difference in specific tissue response to exercise which lead to decreased expression nNOS in the striatum of PD mice after training and overexpression of nNOS in the hippocampus and the paraventricular nucleus of trained rat.

The findings of the present study showed significant decrease in the level of HSP70 in PD group significantly (p value <0.05). Our findings confirm the hypothesis thatchaperone action is a crucial aspect in protecting against the otherwise damaging consequences of protein misfoldingDedmon (2005).Our findings can also explain the other reported considerations in which there is declining level of heat shock protein with ageing, the level of HSPs is decreased insufficiently to keep the cellular proteins homeostasis, which may give rise to certain diseases such as PD (Meriin, 2005; Berke; 2003).

Other studies showed that Hsp70 is believed not only to protect cells from rotenone-mediated cytotoxicity but also to decrease soluble αSN aggregation (Zhou, 2004). Furthermore, Hsp70 can work as a putative anti-apoptotic factor to protect against neuronal cell death in PD (Benn, 2004; Meriin, 2005). These results highlight the possibility of using Hsp70 as a potential therapy for PD. Recent studies of function and inducer of Hsp90 also indicate its potential therapy for PD (Uryu, 2006; Waza, 2006).

The findings of our study also showed that exercise increased the level of HSP70 in EPD group insignificantly (p value>0.05). We believed that in part exercise can benefit patients with PD and this is attributed to increase HSP70 levels.

The results of the present study have also revealed significant expression of HSP70 attributed to exercise in EC group compared with SC group (p value <0.05). These findings explain how exercise can benefit normal people from

oxidative damage and how exercise training becomes crucial with age to compensate for deficiency of HSP with ageing (Meriin, 2005; Berke; 2003).

Taken together, the possibility of using HSP70 for targeting PD has been supported by findings of the present study.

Conclusion

VI- Conclusions

The effect of exercise on nNOS and iNOS expression was debatable. Many studies have demonstrated that exercise lead to increase amount of nNOS and iNOS while other studies showed decreased levels of nNOS and iNOS. These results did not give a solid evidence of the positive correlation between exercise and nNOS and iNOS expression. Our study is the first study that tried to elucidate the effect of exercise on the Parkinson disease in animal model. We showed a discrepancy in the levels of nNOS and iNOS in the striatum in control and Parkinsonian animal both at rest and with exercise. No doubt that exercise has a positive effect of behavioral changes in Parkinson patients, but whether these changes are due to the fluctuation of nNOS and iNOS needs more work to be done before we jump to this conclusion.

Regarding the expression of HSP70, it is obvious in view of our findings that the expression HSP70 has decreased in PD group and exercise benefits in increasing the expression of HSP70 even insignificantly in EPD group.

The findings of our data are consistent with other reported findings in which HSP could work as a therapeutic target for PD.

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Publication

THE EXPRESSION OF INOS IN MOUSEEXPERIMENTAL MODEL POINTS TOINFLAMMATORY CONDITIONS ASSOCIATEDWITH PARKINSON'S DISEASE

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Abstract

Parkinson's Disease (PD) is one of the most commonneurodegenerative diseases. Several molecular mechanisms are involved.The objective of conducting this study was to evaluate the expression ofiNOS in mouse experimental model of PD. PD was induced throughinjecting mice with 10 doses of MPTP (25 mg/kg) and probenecid (250mg/kg). Mice in control group were injected by saline (25 mg/kg). Immunohistochemical stains for iNOS in brain sections were carried outusing indirect immunoperoxidase techniques. Study findings showed thatthere was a significant difference in the expression level iNOS in studygroups ($P<0.001$), and experimental PD group had more expressed iNOSlevels compared with control group. Taken together, the present studyconfirmed the impact of induction of iNOS in the etiology of PD.

Keywords:

Introduction

Parkinson's disease (PD) is a chronic-progressive and disablingneurological disorder (Tolosa, 2006). From a pathologic point of view, Dcan be defined by nigrostriatal loss of dopaminergic cells and Lewybodies inthe surviving cells on autopsy. Furthermore, PD may bemanifested from aclinical point of view by a broad spectrum of motorand non-motor features.The four cardinal features of PD can be grouped under the acronymTRAP:tremor at rest, rigidity, akinesia (or bradykinesia) and postural instability.

This syndrome is labeled “parkinsonism” and may also occur in othermedical conditions than idiopathic PD, such as dementia with Lewybodies,cerebrovascular disease, the so called parkinsonian plussyndromes or as sideeffect after administration of neurolepticmedication. The presence ofakinesia and one of the other symptoms are considered sufficient for theclinical diagnosis of parkinsonism. Diagnostic criteria have been developedby the UK Parkinson’s Disease Society Brain Bank and the National Instituteof Neurological Disorders and Stroke (NINDS) (Tolosa, 2006). Otherdiagnostic criteria for clinical subgroups of the disease were suggested byLarsen et al (1994). According to a study conducted by Jankovic (2008),flexed posture and motor blocks (freezing) have been included among classicfeatures of PD. It has been shown that the diagnosis of PD is still based onthe presence of a combination of cardinal motor features, associated andexclusionary symptoms, and response to levodopa (Rao, 2003).

Nitric oxide (NO) is gas in nature and it is produced by nitric oxidesynthase (NOS) family of enzymes from L-arginine (Seet, 2010; Juurlink,1999). It is known as a highly reactive signaling molecule having a fewseconds of life time (Juurlink, 1999). Furthermore, it diffuses with ease(Juurlink, 1999). Nitric oxide is membrane permeable and can diffuse intodopaminergic neurons (Ara, 1998). Thus NO receptors are signalingtransduction for intracellular communication. Once generated, the cell cannotcontrol the local concentration of NO. Calabrese et al., shows that theactivity of NO can be influenced by the degree of its synthesis and the

activity is terminated with its reaction with its substrate (Seet, 2010). This isimplicated in many physiological and pathological processes within the mammalian body (Moncada, 1997).

It has been shown that a histopathological evidence that NO andglutamate in toxic dose may mediate certain neurodegenerative diseases(Przedborski, 1996). Postmortem studies, clinical findings, and evidencefrom experimental models revealed the role of NO in the degeneration ofdopaminergic neurons in PD. Studies performed in the MPTP model of PDsuggest that peroxynitrite, a reactive species formed by the nearly diffusionlimitedreaction of nitric oxide with superoxide, may be a mediator ofnigrostriatal damage in PD (Ara, 1998). Over limit NO could contribute tothe formation of free radicals that could be involved in the death ofdopaminergic neurons, resulting in development of PD symptoms (Tuncel,2009). Excess NO synthesis is likely to be involved in the progressiveneuronal loss that distinguishes PD (Hancock, 2008). However, there issignificant reduction in NO level in PD patients than in controls. Theexplanations for these low levels may be a faulty NO-dependent adaptationmechanism or the depletion of NO storage during the course of PD (Tuncel,2009).

Moreover, as cytokines enhance the induction of NOS in brain; manystudies suggest the role of glial derived NO in the pathogenesis of thesediseases. Some studies showed that there was excessive formation of NOofglial origin in which NADPH diaphorase (a cytochemical marker of NOSactivity) positive glial cells have been identified in the substantianigra ofpostmortem brains obtained from individuals with Parkinson’s disease (Seet,2010).

Several animals and human studies showed that of the three isoforms,only the nNOS and iNOS are relevant with regard to their potential impact inneurodegeneration and glial response in PD (Kroncke, 1998). Severalexperiments showed that animals can be protected against MPTP

by nNOSinhibitors (Seet, 2010; Kroncke, 1998). This gives evidence that the nNOS isresponsible for MPTP neurotoxicity (Al-Jarrah, 2010). Furthermore,mutation in nNOS gene makes the mice more resistance to MPTP than wildtypemice. Similar experiments with iNOS show that this enzyme also playsa role in the dopaminergic neurons sensitivity (Kroncke, 1998). In human,overproduction of NO was detected in the substantia nigra of PD brain. Itwas correlated with high concentration of nNOS and iNOS (Al-Jarraha,2010). Moreover, NOS inhibition protects against MPTP-induced loss ofnigral neurons. Inhibition of NOS activity can stop MPTP-induced damageof dopaminergic nerve terminals in the striatum and the loss of dopaminergiccell bodies in the substantia nigra pars compacta (Dishman, 2006).

Study objectives

The main objective of the current study is to study the expression ofiNOS in mice experimental model of PD using immunohistochemicaltechniques.

Methodology

Twenty Albino mice were selected randomly and assigned into 2groups: Control group (N=10), PD group (N=10).

The animals were housed in individual cages under identicalconditions (22 ± 1 °C, free access to standard chow and water, 12 hoursdark/light cycle). PD was induced by injecting mice with 10 doses of MPTP(25 mg/kg) and probenecid (250 mg/kg) (chemicals were obtained fromSigma Chemical Co. (St. Louis, MO, USA)). Mice in control group wereinjected by saline (25 mg/kg).

iNOS immunostaining of brain tissue

The mice were sacrificed, and their brains were removed and fixed in10% formalin, embedded in paraffin, and sliced into 3 micometer thicksections. Then, the 3 μm thick sections were processed

viaimmunohistochemistry using an antibody to iNOS (Santa Cruzbiotechnology). So, the 3 micron thick paraffin-embedded sections mountedon glass slides were deparaffinized in xylene for 2 minutes twice, andsubsequently rehydrated through serially descending dilutions of alcohol(starting with 100%, and ended with 70%) followed by water (2 minutes foreach step). After that, sections were processed for antigen retrieval in thereveal solution (RV 1000M, Biocare Medical, Concord, CA) under pressurein the Decloaking chamber (Biocare medical) for 2 minutes. Tissue sectionswere then cooled down to room temperature, and incubated with 3%hydrogen peroxidase in methanol for 5 minutes. After washing the sectionsin phosphate buffered saline (PBS), they were incubated with iNOS antibody(Santa Cruz Biotechnology), with the dilution recommended by the vendor,at room temperature for one hour. Next, the sections were washed in PBSand treated with secondary antibodies and Streptavidin usingImmunoCruz™ goat ABC Staining System (sc-2023). Diaminobenzidine(DAB) was applied for 2 minutes or longer, until the desired intensity wasdeveloped, and then the slides were washed with tap water to stop thereaction. Negative control sections were processed without the primaryantibody. All sections were then counterstained with hematoxylin andviewed under the light microscope. Ten slides of brain tissues from eachanimal group were evaluated for iNOS expression byimmunohistochemistry.

Data analysis

The sections were photographed with digital camera. Photoshopsoftware was used. The slides from each group were analyzed by countingthe total pixels area occupied by positive staining. iNOS expression wasanalyzed, in the different brain tissues, and statistically compared among the2 different groups using paired student t-test.

Differences in iNOS expressionwere considered statistically significant at P value < 0.05.

Results

The expression of iNOS in study groups

The study findings showed that the expression level of iNOS incontrol group was 0.125 and this expression level was further increased inParkinson's disease group, 0.21 (Figure 1). This variation in the expressionlevel in study groups was statistically significant (P< 0.001) .

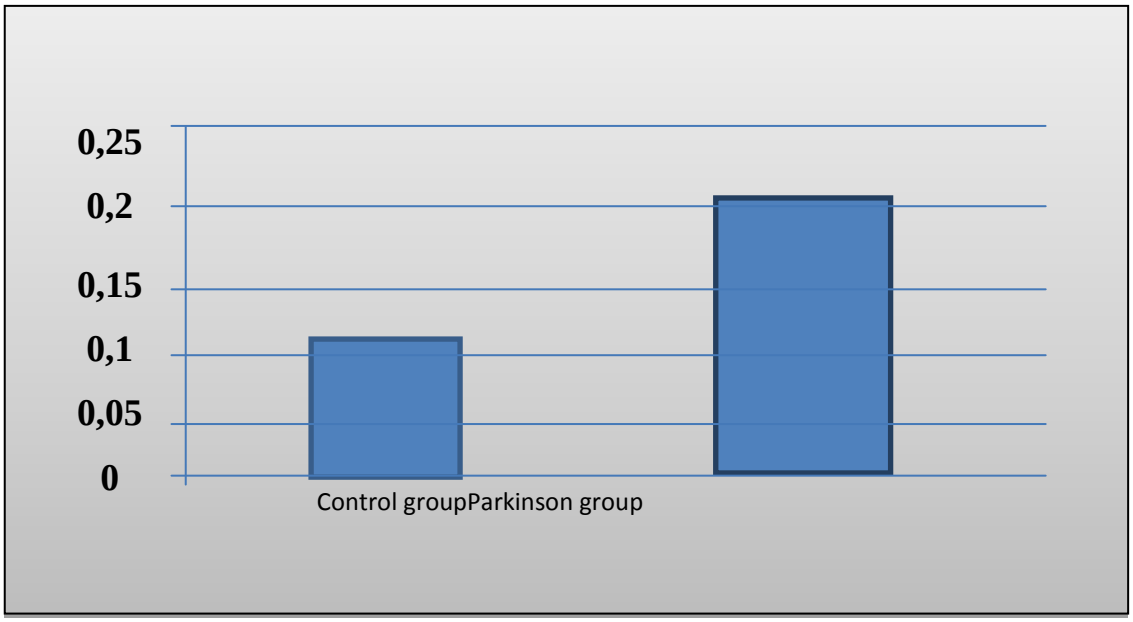

Figure 1: comparison of expression level of iNOS among study groups

Discussion

The present study was conducted to evaluate the expression level ofiNOS in PD compared with control group.The results of the present study clearly identified a significantinvolvement of iNOS in the etiology of PD. This finding agrees with otherstudies in which it was found that iNOS has impacts in neurodegenerationand glial response in PD and plays a role in the dopaminergic neuronssensitivity (Kroncke, 1998).

The increase of iNOS is possibly due to the release of cytokines andother immunity mediators, which induce iNOS. Moreover, Husain

andHazelrigg (2002) found that the exercise training and chronic NOSinhibitor(nitro-L-arginine methyl ester, LNAME) administration lead to a significantinduction of iNOS after exercise in heart of rats. This may be due to increaseblood flow during exercise which leads to the upregulation of iNOS, or maybe due to positive effect of exercise on catecholamines, and henceupregulation of iNOS expression.In human, overproduction of NO was detected in the substantia nigraof PD brain. It was correlated with high concentration of nNOS and iNOS(Al-Jarraha, 2010). Moreover, NOS inhibition protects against MPTPinducedloss of nigral neurons. Inhibition of NOS activity can stop MPTPinduceddamage of dopaminergic nerve terminals in the striatum and the lossof dopaminergic cell bodies in the substantia nigra pars compacta (Dishman,2006).

Conclusion

The present study confirmed the impact of induction of iNOS in theetiology of PD.

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