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Anti- inflammatory activity of *Laurus nobilis* leaves extract *in vivo*.

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ABSTRACT

The therapeutic potential of *Laurus nobilis,* a common plant grown in Algeria, has been validated by numerous studies. The present study aimed to highlight the anti- inflammatory activity of ethanolic extract *Laurus nobilis* extract (EELN) in vivo. The anti-inflammatory activity of EELN was tested in mice weighting (25 ± 5) g. Either PBS vehicle (control group), ethanolic extract (50mg/kg) or diclofenac (50 mg/kg), were administered (p.o) for 60 min before an oedema was induced in the mice paw by subcutaneous injection of carrageenan. The mouse-paw volume was measured from 1 h to 6 h after carrageenan injection. Plasma fibrinogen, serum albumin and C-reactive protein (CRP) levels were determined for each group. The EELN and the standard (diclofenac) reduced significantly ($p < 0.05$) paw oedema by 98.68% ± 11.24 % and 77.19 ± 0.87%, respectively compared to the control after the sixth hour after injection of carrageenan. Biomarkers of inflammation (serum albumin, C-reactive protein CRP and plasma fibrinogen) levels were significantly ($p < 0.05$) optimized in EELN group (assay) compared to the control. The obtained results revealed that the ethanolic extract of L. nobilis leaves has a significant in vivo anti-inflammatory activity.

1.Introduction

Laurus nobilis is a member of the Lauraceae family, which includes 32 genera and about 2000-2500 species. Laurus, Latin name, of Celtic origin meaning "evergreen", referring to the plant's evergreen foliage (Gedouari *et al.,* 2021). The leaves have been widely used and known as a seasoning and medicinal herb since ancient Greek and Roman times. Interestingly, this herb that has long been used in food as a condiment and in traditional medicine has, in fact, properties that may suggest new applications (El-Mijalli *et al.*, 2022).

L. nobilis has been shown to have antioxidant, antimicrobial activities, as well as anticonvulsant properties. The leaves have been used to treat rheumatic pains, arthritis, skin inflammation, and asthma (Berendika *et al.,* 2022).

Inflammation is defined a response of a tissue to a deleterious stimulus, like irritant agents, physical injury, and pathogens, which is described by increased vascular permeability, changes in blood flow, and leucocytes migration to the affected sites. Pain is a hostile sensory and emotional experience from tissue damage and acts as a signal to warn against further insults (Odira *et al.,* 2022). There are many anti-inflammatory and analgesic treatments for inflammation and pain however, they are unaffordable and inaccessible particularly in low income and remote settings, they are of low efficacy and have secondary effects with life-threatening consequences (Olela *et al.,* 2020). In this regard, we aim to investigate natural products, especially medicinal plants, as one of the most promising therapeutic agents for inflammatory diseases. The objective of this study was to explore the anti-inflammatory and analgesic effects of *L. nobilis* leaves extract on mice.

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2.Matériel et méthodes:

2-1. Plant leaves and extraction method

Leaves of *L. nobilis* were purchased from local market of Mostaganem, Algeria and preserved in airtight containers. A Soxhlet apparatus was used for extraction. 200 g of dried leaves were powdered then 2 L of ethanol was added. The extraction was lasted for 8-10 h until the dissolution of the soluble constituents in the solvent. After filtration, a rotary evaporator (Buchi, Switzerland; temp: 60°C; pressure: 175 mbar) was used to evaporate the solvent to yield semi solid mass. The obtained extract was collected and stored at 4°C until use (Yahla *et al.,* 2021).

2-2. Animal model

Male Swiss albino's mice weighting between 20 and 30 g, obtained from Pasteur institute of Algiers, Algeria were used in this study. Mice were fed a standard diet (Animal Food, Bouzereah, Algiers, Algeria) and tap water *ad libitum* for two weeks in an air-conditioned room at 22 ± 1°C and 55 ± 10% relative humidity with regular 12 h-dark-light cycle for stabilizing all metabolic conditions. This experiment was agreed by the Algerian Ethics Committee for Research on Animals of Abdelhamid Ibn Badis University of Mostaganem (ECRA/AIBUM).

2-3. Anti-inflammatory paw oedema test

The method of Winter *et al.* (1962) was realized to explore the anti-inflammatory effect of the EELN against carrageenan-induced paw oedema in mice. Mice were divided into 3 groups, a control group (G1) receiving intraperitoneally (ip) the phosphate buffer saline (PBS) solution, the treated group (G2) received 50 mg/Kg of the ethanolic extract of *L. nobilis* EELN whereas the experimental group (G3) received an ip anti-inflammatory drug (50 mg diclofenac/kg). Mice of each group were provided with tap water and food before the experiment. First, volumes of left hind paw to the tibio-tarsal articulation were measured by a plethysmograph.

One hour after the administration of the PBS solution to the control group, 50 mg/kg of the EELN to the treated group and a dose of 50 mg/Kg of diclofenac to the experimental group, 100 µL of 0.5% carrageen solution was injected into the footpad of the hind paws of mice in all groups. The paw volume was measured at intervals of 1, 3, and 6 hours. The anti-inflammatory activity of EELN was compared with that of 50 mg/kg diclofenac.

The inhibition percentage of the inflammation was determined from the formula:

$$Inhibition\ \% = (D - D\,t)/\,D0 \times 100$$

where D is the diameter of injected paw,

D 0 is the average inflammation of the control group of mice at a given time 0;

and D t is the average of diameters of hind paw oedema of the drug treated (i.e. EELN or reference diclofenac) mice at the same time.

2-4. Biochemical analysis

At the end of the experimentation, mice were anesthised then sacrificed. Blood was collected and centrifuged, Plasma fibrinogen, serum albumin and C-reactive protein (CRP) levels were determined for each group.

2-5. Statistical analysis

Results are expressed as means ± SEM. The statistical analysis was accomplished using software statbox pro. (Version 6.40, Statbox pro). Differences between the means were assessed with one-way ANOVA. Treatment differences means were determined with Newman -Keuls test. Differences were considered significant at $p^{<} 0.05$.

3. Results and discussion

3-1. Anti-inflammatory effect

Inflammation consists in a natural response of the immune system, characterized by a mechanism that represents a chain of organized and dynamic responses including both vascular and cellular events with specific humoral secretion (Al-Mijalli *et al.,* 2022).

Results concerning effect of EELN on carrageenan induced oedema are illustrated on figure 1. After 1 h of pre-treatment with EELN, percentage increase in paw volume was significantly ($p < 0.05$) inhibited in treated mice compared to controls. This reduction was preserved for 3 h. The values were similar in the three groups at the fourth hour following the EELN treatment. According to diclofenac sodium anti-inflammatory activity, the diclofenac sodium group have shown an important reduction in percentage increase in paw volume induced by carrageenan.

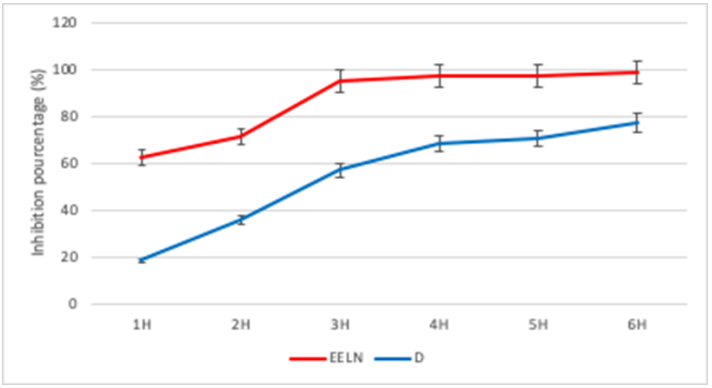

Fig. 1. Percentage inhibition of oedema by 50 mg/kg of EELN and 50mg/kg of diclofenac sodium at different interval times. EELN: 50mg/Kg EELN group, D: 50 mg/kg diclofenac group

The anti-inflammatory effect of EELN was highly significant ($p < 0.05$) through the three hours post injection. However, this effect was similar to that of the diclofenac at the fourth hour of experiment.

The present study has revealed that 50 mg/kg of ethanolic extract of *L. nobilis* leaves (EELN) induces a significant ($p < 0.05$) anti-inflammatory activity in mice.

Carrageenan-induced induced paw oedema is the typical experimental model of acute inflammation. Moreover, the experimental model reveals a high level of reproducibility. Its effect is biphasic. The initial phase is due to the secretion of histamine and serotonin (5 -HT) (0-2 h), while plateau phase is provided by a kinin-like substance (3 h) and a second accelerating phase of swelling is attributed to prostaglandins (PG) liberation (> 4 h) (Yahla *et al.,* 2021).

Consequently, the EELN induced significant reduction of oedema indicating an anti-inflammatory effect dose dependent. Among the

probable mechanisms involved in this effect, there will be the inhibition of formation of phlogistic mediators like kinins, prostaglandins, etc., modulation of mediators reaction with their respective receptors, and blockade of receptor action (Raval *et al.*, 2017).

The obtained results are in line with those of Guedouari et al., (2021), who also reported that the ethanol extract of leaves (30.62%) exhibited a reduction in oedema higher than that of the reference Diclofenac sodium 50 mg (17.59%)

3-2. Biochemical analysis

Serum albumin, C-reactive protein (CRP) and plasma fibrinogen levels are represented in figures 2, 3 and 4, respectively. Data of figures 2 and 3 showed that separate administration of 50 mg/kg of EELN or diclofenac has decreased plasma levels of CRP by 42.8 and 36.3%, and those of fibrinogen by 54 and 18%, respectively, compared to controls.

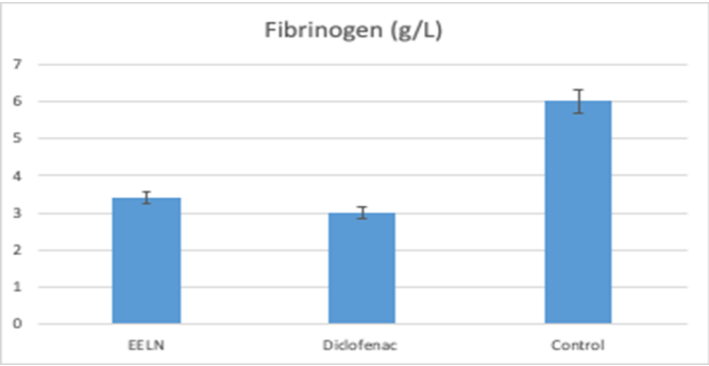

Fig. 2. Plasma fibrinogen levels (g/L) in mice groups.. The bars represent mean ± SD (n = 3).

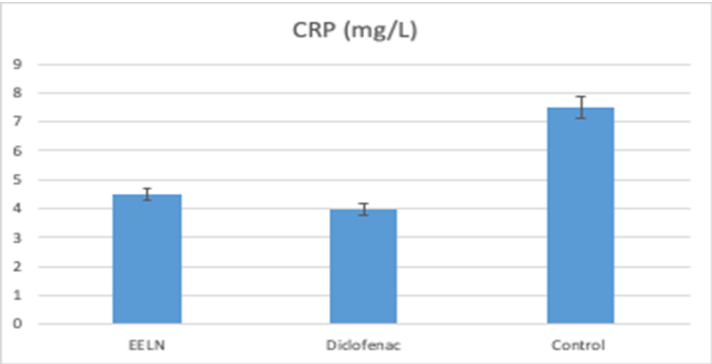

Fig. 3. C-reactive protein (CRP) levels (mg/L) in mice groups. The bars represent mean ± SD (n = 3).

Normal rates of serum albumin were observed in 50mg/Kg EELN (G1) and diclofenac (G2)-treated mice groups. However, a reduced serum albumin value was observed in control group as shown in figure 4.

The present study shows that EELN regulated plasma fibrinogen and C-reactive protein CRP levels. These results are in accordance with those described by literature highlighting the exerted anti- inflammatory effect. EELN reduced significantly the yeast-induced hyperpyrexia and inhibited carrageenan-induced pedal oedema. It also exhibited significant increase in fibrinogen level (Mohammed *et al.*, 2021).

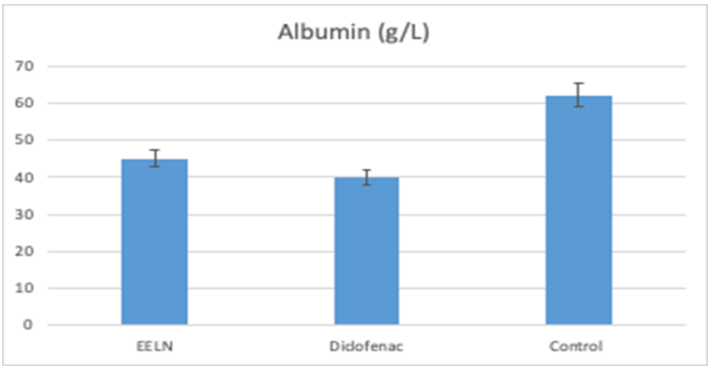

Fig. 4. Serum albumin levels (g/L) in mice groups. The bars represent mean ± SD (n = 3).

Moreover, we have explored the effect of EELN on serum albumin levels. Obtained results showed that the extract enhanced serum albumin level as well as diclofenac, whereas mice of the control group presented a reduction in albumin level. Tissue proteins denaturation is one of the well-documented inflammatory and arthritis causes. The protein denaturation bioassay was assessed in vitro for the anti-inflammatory property of methanol extract of EELN leaves. Similar results were reported by Casamassima *et al.* (2017).

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4. Conclusion

In conclusion, this study confirmed that the ethanolic extract of *L. nobilis* leaves exerts a significant anti-inflammatory effect against carrageenan induced paw inflammation in mice.

Déclaration conflit d'intérêt

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