Abstract: *Lavandula stoechas* L. is a widely used medicinal plant. In Moroccan traditional medicine, it is employed for the treatment of different illness and as an anxiolytic, analgesic or anti-inflammatory remedy. The purpose of this investigation was to assess the antioxidant, antinociceptive and anti-inflammatory effects of the ethanolic extract of Moroccan *Lavandula stoechas* L. in animal models. Therefore, *in vitro* anti-oxidant activity was appraised via two techniques, the 2,2-Diphenyl-1-Picryl-Hydrayl (DPPH) radical scavenging method and reducing power approach. For the *in vivo* trials, the ethanolic extract of the herb was orally administered (0.5, 0.6 and 1 g/kg, p.o.). Furthermore, acetic acid induced-writhing, formalin induced-paw-licking, thermal hyperalgesia and tail-flick experiments were applied to examine the antalgic activity. The xylene induced ear-edema trial was achieved to estimate the anti-inflammatory activity. The results revealed an important anti-oxidant activity of our extract. Moreover, in the writhing-test, we observed that the concentrate inhibited abdominal contractions engendered by the acetic-acid in mice. In the hot-plate and the tail-flick tests, the extract caused a notable central analgesic reaction, the latency to the thermal stimuli intensified in a dose dependent way. Additionally, considering the formalin test, the pre-treatment with the extract reduced the licking-time in the first and late phase of the response. Lastly, the xylene induced ear-edema test demonstrated that the plant’s extract remarkably restrained the edema formation occasioned by the xylene, which was confirmed by histological verification. This study allowed us to conclude that *Lavandula stoechas* is an ultimate medicinal plant that possesses antioxidant and anti-inflammatory activities along with the central and peripheral anti nociceptive properties.

Keywords: *Lavandula stoechas* L., Antioxidant Activity, Anti-Nociceptive Activity, Anti-Inflammatory Effect
industries. The medicinal eminence of the plant is well described and the drugs composed from this plant are recorded in many Pharmacopoeia (Gören et al., 2002; Algieri et al., 2016). For instance, Silexan, an oral lavender oil capsule preparation, has significant effect in the remedy of ‘sub-syndromal’ anxiety condition (Kasper et al., 2010). Indeed, traditional medicine practitioners in Morocco recommend this plant as antispasmodic and because of its anti nociceptive, calmative, aseptic and anti-microbial potencies. Lavender also has positive consequences on lesions, urinal-infections, cardiac problems and atopic dermatitis (Gülçin et al., 2004). It has been applied in epilepsy, depression, headaches, diabetes and as calmative therapy (Gülen et al., 2000; Gülçin et al., 2004).

Pain is one of the most predominant ill that restricts the productivity and reduces the quality of life. Although there is a plentitude of efficient and extensively utilized analgesics, there is some concern in regard to their safeness and side effects such as drowsiness, dizziness, upset stomach making their use problematic (Zhao et al., 2003).

Drugs of natural origin are a prominent source for the medication of various ubiquitous ailments. The research and analysis of plants utilized as pain relievers and anti-inflammatory agents in popular ethno medicine is one among the most profitable and relevant approaches in the search for current chemical substances with potential therapeutic uses (Algieri et al., 2016).

Phytochemical investigations reported distinct secondary metabolites in Lavandula species (Shimizu et al., 1990; Gülçin et al., 2004) such as diterpenes (Politi et al., 2002), tri terpenes (Topcu et al., 2001), coumarins (Shimizu et al., 1990) and phenolic composites (Gabrieli and Kokkalou, 2003). Secondary metabolites are largely employed in food, pharmaceutical, chemical, cosmetics productions and agriculture (Ceylan et al., 2015).

In this study, anti-oxidant potentiality of Lavandula stoechas was assessed via DPPH technique and the reducing power assay. The total phenolic compounds, the flavonoids and tannins contents were also assessed. Additionally, we examined the anti-nociceptive property of the aerial parts’ total extract in hot-plate, tail immersion, writhing and formalin trials. The anti-inflammatory effect was assessed using the xylene producing ear-edema test. Moreover, the possible mechanism implicated in the anti-nociceptive and anti-inflammatory properties was explored as well.

Materials and Methods

Animals

The experimentations were accomplished using Swiss Albino male and female mice (28 and 35 g) and Sprague Dawley male rats (180 and 220 g) raised in the central animal care facilities of Cadi Ayyad University, Marrakech, Morocco. Animals were housed under supervised temperature (22±2°C), humidity (60%) and 12h-light-dark cycle with an ad libitum access to food and water. All efforts were performed to reduce the animal suffering and to diminish the number of animals utilized. All the experimental protocols were accomplished in conformity with the European Council Directive (EEC, 1986/609) and judiciously confirmed by the Council Committee of research laboratories of the Faculty of Sciences, Cadi Ayyad University of Marrakech.

Plant Materials

The Lavandula stoechas’ aerial parts were collected from Demnat region, Morocco. Pr. Ahmed Ouhammad (Professor Responsible of the Regional Herbarium “MARK” at the Samlalia Science Faculty, Cadi Ayyad University, Marrakech) had identified and classified the plant material and a voucher specimen (Mark - 8259) was stored at the faculty herbarium.

Preparation of Ethanolic Extract

The Lavandula stoechas’ aerial parts were dried and triturated in order to get a powder. The dried powdered aerial parts (120 g) of the plant were extracted with ethanol (500 mL, 80% v/v) in a Soxhlet equipment for 16 h at 64°C. In addition, to get the total extract (34 g), the rotary device was utilized to eliminate the solvent at 45°C.

Acute Toxicity

The toxicity examination was achieved out using male and female Swiss Albino mice. The animals were assigned at random to one control lot and seven treated lots, including 3 animals per sex. The treated groups got orally the plant extract at different doses: 100, 200, 500, 1000, 2000, 4000 and 5000 mg/kg. After the administration of the concentrate, the animals were observed for general behavioural changes or clinical signs and mortality. The observation was continuous for the first 4 h; then, for the next 24 h, they were observed every hour. Moreover, for the next 48 h the observation was one at 6 h intervals. The treated groups were kept under observation for one week for further changes.

Phytochemical Evaluation of the Ethanolic Extract of Lavandula Stoechas

Evaluation of Total Phenolic (TPC), Flavonoids (TFC) and Condensed Tannin Compounds (TTC)

The total phenolic composite of Lavandula stoechas (LS) ethanolic extract was appraised via the Folin-Ciocalteu technique (Singleton et al., 1999). One hundred micro liter (µl) of the extract was diluted with 3.7 mL of distilled water and 200 µL of Folin-Ciocalteu reagent was included. After 3 min, 20% sodium carbonate (1 milliliter of Na2CO3) was added. The solution was agitated and incubated in dark for 45 min at 25°C. Then, the absorbance
was interpreted at 725 nm a UV-Vis spectro photometer (VR-2000, Spain). The total phenolic concentration of LS ethanolic extract is described in terms of milligram equivalent Gallic acid per gram of the dry matter of extract (mg EGA/g). All specimens were realized three times.

The flavonoids compound of LS extract was estimated based on the aluminum tri-chloride technique (Zhihen et al., 1999). Aliquots (200 µL) of diluted extract were added to 5% NaNO2 solution (60 µL), followed by 10% AlCl3 (40 µL) and incubated for 6 min, followed by the addition of NaOH (400 µL, 1M). Then, distilled water (500 µL) was added. The absorbance of the solution was examined at 510 nm. The TFC in LS was mentioned as milligram catechin per gram of the extract (mg ECAT/g).

Tannins in LS extract were estimated as succeeding (Xu and Chang, 2007). Sample extract (300 µL) was added to a solution of vanillin (3 mM), 4% methanol and HCl (1 mL). The absorbance was determined at 500 nm. The results were revealed as catechin per gram of the extract.

In vitro Anti-oxidant property

DPPH (2,2-Diphenyl 1-Picrylhydrazyle) Free Radical Scavenging Activity

In order to evaluate the free radical scavenging property of LS ethanolic extract, we utilized the stable free radical 2,2-Diphenyl-1-Picryl Hydrazyl radical (DPPH) as described by Burits and Bucar (2000). Briefly, 50 µL of various extract concentrations were mixed with 2 milliliter of 60 mM methanol solution of DPPH. Furthermore, the samples’ absorbance was noted at 517 nm after 20 min of incubation in the dark at room temperature.

Blank solution was produced using the same amount of methanol and DPPH. As positive control, Quercetin and Butylated Hydroxytoluene (BHT) were utilized. To estimate the inhibition of the DPPH (%), the following equation was utilized (Badakhshan et al., 2012):

\[ I\% = \left( \frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100 \]

Where \( A_{blank} \) is the absorbance of the control and \( A_{sample} \) is the absorbance of the test compound. The sample concentration providing 50% Inhibition (IC50) was calculated from the graphic by plotting inhibition percentages against sample concentrations.

Reducing Power Assay

As mentioned by Oyaizu (1986), the reducing power of the LS ethanolic extract was assessed. 1 milliliter of different sample concentrations was added to the phosphate buffer (2.5 mL, 200 mM, Ph 6.6) and potassium ferricyanide (2.5 mL, 1%). The resulted solution was incubated for 20 min at 50°C; after incubation period, 2.5 mL of 10% Trichloroacetic Acid (TCA) was mixed with the solution and then centrifuged at 3000 rpm for ten minutes.

2.5 mL of the upper layer solution was added to 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride (FeCl3). The determination of coloration formed by the reduction of Fe3+ at 700 nm was utilized to examine the sample concentration providing 0.5 of absorbance (IC50). Ascorbic acid, BHT and quercetin were employed as positive controls.

Assessment of Anti-Nociceptive Activity

Hot Plate Test

The heated area of a hot-plate analgesia meter (Ugo Basil, Italy Socril DS-37®) was conserved at 55±0.2°C. Animals were located in an acrylic cylinder (20 cm in diameter) on the warmed area and the period (in seconds) after placing the animal on the hot plate and the manifestation of either the licking of the rear paw or the escape from the platform was monitored as response latency. A cut-off duration of twenty seconds was retained to prevent the paw surface injury (Shaheen et al., 2000).

Treated animals received orally the LS ethanolic extract, which was dissolved in distilled water (500, 600 and 1000 mg/kg). Control lot took distilled water orally at 0.1 mL/10 g. Morphine was administered at 10 mg/kg (intra-peritoneal).

Writhing-Test

The anti-nociceptive activity was investigated via the writhing test engendered, in mice, by acetic-acid at a concentration of 0.6% (0.1 mL/10 mg, (intra-peritoneal)). The various doses were given orally forty-five minutes earlier than the nociceptive agent. Five minutes after the acid injection, the number of writhes and stretching movements (contraction of the abdominal musculature and extension of hind limbs) was counted for a period of 30 min. Acetylsalicylic acid ASA (200 mg/kg body weight) was used as pharmacological reference (De Miranda et al., 2001; Aboufatima et al., 2021).

Anti-nociceptive activity was expressed as the reduction in the number of abdominal constrictions between the control animals and the mice pre-treated with compounds.

Inhibition percentage in the nociceptive response has been calculated; defined as follows:

\[ I = \left[ \frac{(NC - NT)}{NC} \right] \times 100 \]

Where:

\( NC \) : Number of contortions for the control group
\( NT \) : Number of contortions of a group under test

Formalin Test

The formalin test was carried out as previously described by De Miranda et al. (2001) with slight modification, twenty microliters of a 2% (20 µL) formalin solution were subcutaneously injected into the right posterior paw of the mice. Immediately after the injection, the animal was placed in a transparent enclosure.
Throughout 5 min prior to this procedure, each mouse is allowed to adapt the testing box and left freely moving and exploring (habituation). For 30 min after injection, the time (in seconds) that the animal spent licking the injected paw was recorded.

There are two distinctive phases of intense licking performance that have been recognized. Early phase (the first five minutes) is primarily due to the C-fiber activation as a result of the peripheral stimulus caused by formalin (Tjølsen et al., 1992). A second burst of licking behavior occurs after 15 to 30 min and it is related to the inflammatory response elicited by formalin. This phase is termed inflammatory (Hunskaar and Hole, 1987; Milano et al., 2008).

The animals were pre-treated orally with the extract or injected with Acetylsalicylic Acid (ASA) (200 mg/kg b.w. i.p.) or morphine (10 mg/kg b.w. s.c.), before the formalin injection.

Tail-Immersion Nociceptive Test

Rats in groups of six each were placed in individual restraining cages and the nociceptive reaction time in seconds was noted when the tail was immersed in a fixed-temperature water bath maintained at 55±1°C. The nociceptive end-point was characterized by a violent tail jerk. 15 sec was the time limit imposed for all animals that did not respond to the test (Sewell and Spence, 1976; Aboufatima et al., 2021).

Anti-Inflammatory Activity

Xylene Induced Mouse Ear Edema

For inducing an acute inflammation, an application of 30µl of xylene was applied topically on the anterior and posterior surfaces on the right ear (Shang et al., 2011). Therefore, six groups of mice were tested: The control group (saline solution 0.9% i.p.), four treated groups with the LS extract (500, 600 and 1000 mg/kg p.o.) and a group of diclofenac (10 mg/kg i.p.). 30 min after the xylene usage, mice of the six groups of animals were given a euthanasia and 2 ear punches were collected and weighed. Comparing right and left ear weights, the edema’s degree was estimated (Kebbou et al., 2019).

For the histology evaluation, 6 samples of inflamed ears from both the control and treated groups were taken and fixed in formaldehyde (10%). Fixed ear tissues have been embedded in parafin and sliced into 3-4 µm sections. Slices were mounted on glass slides, stained with Hematoxylin and Eosin (HE) for pathological studies (Sudoh et al., 2004).

Statistical Analysis

The results were expressed as mean ± Standard Errors of the Mean (SEM). The comparison between the various groups was carried out with one-way Analysis (ANOVA) and the repeated measures ANOVA model monitored by Tukey’s post hoc test. A value of p<0.05 was assumed to be statistically significant.

Results

Acute Toxicity

In this study, the oral administration of *Lavandula stoechas*’ extract at the doses from 100 to 5000 mg/kg had no effect on animals’ behavioral responses and body weight. Furthermore, no allergic manifestation nor mortality during the period of 48 h after administration were noticed or even after seven days observation. Therefore, it can be indicated that ethanol extract has low toxicity profile and the LD50 is greater than 5 g/kg (b.w.).

Total Phenolic Compounds, Flavonoids and Tannins Content Determination

The quantitative analysis of the total phenols, flavonoids and condensed tannins showed that the ethanolic extract contains total phenols, flavonoids and condensed tannins levels of 83.90 mg EGA/g dry extract, 72.80 mg ECAT/g and 37.90 mg ECAT/g dry extract respectively (Table 1).

In vitro Antioxidant Activity

Antioxidant activity was evaluated using two complimentary in vitro antioxidant tests. Concentrations that led to 50% Inhibition (IC50) are presented in Table 2. The lower IC50 value reflects a higher protective action.

The results showed that the ethanolic extract of *Lavandula stoechas* revealed an important antioxidant activity, the lowest IC50 was obtained with reducing power assay (IC50 = 40.10±0.01) followed by DPPH (IC50 = 54.46±0.07). Comparing with Quercetin, BHT and Ascorbic acid, the IC50 values of the plant’s extract were less effective than those of synthetic antioxidant agent.

Assessment of Pharmacological Activities

Analgesic Tests

Writhing Test

The administration of the total extract at different doses caused a significant reduction in the number of writhing episodes induced by acetic acid compared to the control (p<0.001). The results for the second dose (600 mg/kg) and the third one (1000 mg/kg) were significant compared to the first dose (500 mg/kg) (Fig. 1). Moreover, it was stronger than that produced by ASA. On the other hand, no difference in the latency times was observed when the positive control mice were compared with those pretreated with the LS extract. The percentage of writhes inhibition was calculated as 59, 61% for ASA, 30, 36% for LS extract 500 mg/kg, 65, 46% for LS extract 600 mg/kg and 73, 54 % for LS extract 1000 mg/kg.
**Formalin Test**

The results of formalin test have been represented in Fig. 2A. The ethanolic extract produced a significant (p<0.001) analgesia in the first and the second phases. Indeed, at doses of 500, 600 and 1000 mg/kg paw licking time in the early phase was reduced by 45.84, 75.37 and 80 %, respectively. In the late phase, pretreatment with different doses of the ethanol extract has significant (p<0.001) effect against the duration of licking activity compared to the negative control group.

Morphine (10 mg/kg b.w.) and the ASA (200 mg/kg b.w.) injections produced marked inhibition (p<0.001) of both the neurogenic pain phase and the inflammatory pain phase of the formalin test (Fig. 2A).

Using the drug naxoxone reduced significantly both the analgesic effect of the morphine injection and the administration of the extract (EE.D3) (Fig. 2B).

**Hot-Plate Test**

In the hot-plate test, there were significant differences in the anti-nociceptive effect of plant’s extract at different doses. Administration of morphine at the dose of 10 mg/kg demonstrated a significant effect compared with the control group (p<0.001) (Fig. 3A).

Furthermore, these results also demonstrated that pretreatment with naloxone (a non-selective opioid receptor antagonist) significantly reversed the antinociceptive effect of morphine (10 mg/kg, i.p.) and ethanolic extract in the hot plate test (EE. D 3 = 1000 mg/kg) (Fig. 3B).

**Tail-Immersion Nociceptive Test**

The results of tail immersion induced nociception method revealed that the ethanolic extract had substantially (p<0.001) augmented the reaction time in the thermal stimuli. The ethanolic extract of the plant showed an anti-nociceptive activity against the tail immersion induced nociception. The 1000 mg/kg of ethanolic extract manifested a significantly (p<0.001) increased reaction time in the thermal pain stimuli in 60 min and 120 min after the extract administration (Fig. 4). Treatment with morphine also showed significant (p<0.001) nociception inhibition activity within 30 min.

**Effect of Ethanolic Extract of Lavandula Stoechas on Anti-Inflammatory Activity**

**Xylene Induced Mouse Ear Edema**

The anti-inflammatory effect of various treatments of *Lavandula stoechas* ethanolic extract in xylene-induced ear edema is depicted in Fig. 5. The ethanolic extract significantly inhibited edema formation induced by the xylene at the doses of 500, 600 and 1000 mg/kg (p<0.001), compared to the vehicle control group. Moreover, ethanolic extract dose-dependently decreased xylene-induced edema by 39.66, 47.66 and 58.83% at doses of 500, 600 and 1000 mg/kg, respectively. The anti-inflammatory effect of ethanolic extract at the highest dose tested (1000 mg/kg) was comparable to the effect of diclofenac (10 mg/kg).

As indicated in Fig. 6, histologic assessment of tissue in the ear indicated that application of xylene produced a marked increase in epidermal thickness, edema and infiltration of Polymorph Nuclear Leukocytes (PMN). Oral administration of *Lavandula stoechas* extract (500, 600 and 1000 mg/kg) and diclofenac (10 mg/kg) significantly reduced the indicated changes.

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**Fig. 1:** Effect of *Lavandula stoechas* ethanolic extract on the acetic acid-induced writhing behavior in mice. Ethanolic extract (D1 = 500 mg/kg, D2 = 600 mg/Kg and D3 = 1000 mg/kg, p.o.) and acetylsalicylic acid (ASA = 200 mg/kg, i.p.) were administered. The results are illustrated as mean ± SEM, ***p<0.001 vs. control, +++p<0.001 Vs. EE. D 1
Fig. 2: The antinociceptive effect of *Lavandula stoechas* ethanolic extract, Acetyl Salicylic Acid (ASA), Morphine (MOR) (A) and Naloxone (NALX) reversible effect (B) on formalin-induced pain in mice. The values are represented as mean ± SEM. The results are reported as mean ± SEM, ***p<0.001 Vs. Control, +++p<0.001 Vs. EE. D3
Fig. 3: The antinociceptive effect of *Lavandula stoechas* ethanolic extract, Morphine (MOR) (A) and reversal effect of naloxone (NALX) (B) on the hot plate test. Values are represented as the mean ± SEM. The results are reported as mean ± SEM, ***p<0.001 Vs. MOR; +++p<0.001 Vs. EED3

Fig. 4: Antinociceptive effect of ethanolic extract of *Lavandula stoechas*, Morphine (MOR) on the tail immersion test. Values are represented as the mean ± SEM. Results are presented as mean ± SEM, ***p<0.001 Vs. Control; +++p<0.001, ++p<0.01 Vs. EE.

Fig. 5: Effect of ethanolic extract of *Lavandula stoechas* on the inflammation response in the xylene test. Diclofenac (DICL = 10 mg/kg i.p.), ethanolic extract were administered 45 min before xylene application (EE. D 1 = 500 mg/kg, EE. D 2= 600 mg/kg and EE. D 3 = 1000 mg/kg p.o.). The findings are reported as mean ± SEM, ***p<0.001 Vs. control
Fig. 6: Tissue sections indicating the swelling of the ear caused by xylene treatment. (A) The normal ear; (B) model control group induced by xylene; (C) the treatment with diclofenac (10 mg/kg); (D) an ethanolic extract treatment (500 mg/kg); (E) the treatment with ethanolic extract (600 mg/kg); and (F) treatment with Ethanolic Extract (1000 mg/kg). 1: Dermis thickness; 2: Epidermal thickness. (HE ×100). Scale - bar = 15 µm

Table 1: Total phenolics, flavonoids and tannins content of ethanol extract of the Lavandula stoechas (Mean ± SEM). mg equivalent gallic acid per g of extract (mg EGA/g); mg equivalent catechin per g of the extract (mg ECAT/g); the Dry Weight (DW).

<table>
<thead>
<tr>
<th>Ethanol extract</th>
<th>Total phenolic contents (mg EGA/g DW)</th>
<th>Flavonoid contents (mg ECAT/g DW)</th>
<th>Tannin contents (mg ECAT/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>83.90±1.72</td>
<td>72.80±3.91</td>
<td>37.90±9.56</td>
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</tbody>
</table>

Table 2: IC50 (µg/ml) values of Lavandula stoechas ethanolic extract compared to synthetic antioxidants (Ascorbic acid, Quercetin and BHT). Results are presented as mean ± SEM

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>DPPH</th>
<th>Reducing power</th>
</tr>
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<tbody>
<tr>
<td>BHT</td>
<td>2.49±0.02</td>
<td>1.51±0.01</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.72±0.03</td>
<td>3.81±0.09</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.14±0.02</td>
<td>4.33±0.01</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>54.46±0.07</td>
<td>40.10±0.01</td>
</tr>
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Discussion

Nowadays, there are several pharmaceutical protocols to reduce pain and control inflammation. While most of these medications have been shown to be effective, their use may be accompanied by some alarming side effects (Lisa et al., 2020), hence limiting their long-term use. Therefore, the rising interest on alternative medicine could be justified. Within this frame of reference, our study focused on the anti-nociceptive, anti-inflammatory and antioxidant effects of the total ethanolic extract of Lavandula stoechas’ aerial parts.

To begin with, the anti-nociceptive activity has been performed in a wide range of acute pain models in rodents, such as, the formalin, acetic acid-induced writhing, hot-plate and tail-immersion nociceptive tests. These are classical models of nociception used in the screening of potential anti-nociceptive drugs or plant extracts. They were chosen to study both peripherally and centrally mediated effects. In fact, acetic acid-induced abdominal writhes helped to elucidate peripheral activity, whereas both the tail flick and hot plate approaches investigated the central pain mechanism; however, the formalin test investigated the central and peripheral pain pathways (Paulino et al., 2003). For instance, the results represented above lead us to confirm that the Lavandula stoechas ethanolic extract exerted a significant effect against pain in the four current anti-nociceptive tests in mice and rats. Moreover, significant elevation of pain threshold demonstrated by the extract at different doses tested in these models indicates relevant peripheral and central mediated activities.

Indeed, the acetic acid test is widely used for the evaluation of peripheral anti-nociceptive activity. Also known as the abdominal constriction response, it is highly sensitive and capable of detecting the anti-nociceptive effects of compounds (Bentley et al., 1981; Collier et al., 1968; Ouyomi et al., 1992). In this pharmacological test, because the intraperitoneal injection of acetic acid immediately activates somatic and visceral nociceptors innervating the peritoneum, inducing inflammation in the sub diaphragmatic visceral organs as well as in the subcutaneous muscle walls, the observed anti-nociceptive effect of acetic acid conditioning could be more than just
visceral (Kurihara et al., 2003). Indeed, when administered orally, the ethanolic extract of L. stoechas caused a strong analgesic effect decreasing the number of acetic acid-induced abdominal twinges compared to the control group. This finding suggests that the extract of plant may be well absorbed from the gastrointestinal tract and induces peripheral analgesic activity. This is supported by the finding that the twitch-lowering drug demonstrates analgesia via inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition, as reported by Loganayaki et al. (2012) and Alam et al. (2009).

The tail-immersion and hot plate nociceptive tests are pain tests in which central analgesics apply their anti-nociceptive activities via supra-spinal and spinal receptors (Sewell and Spence, 1976; Abbot and Melzack, 1982). Moreover, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in these tests in this study, the oral pretreatment with the Lavandula stoechas total extract showed a significant activity as well. Furthermore, the application of the opioid antagonist (naloxone) relatively reversed the analgesic effect of morphine and extract in the hot plate test. It could then be proposed that the ethanolic extract includes compounds that can act on the central nervous system and are therefore related to the opioid system.

Formalin test is a frequently used and well established model to study pain mechanisms and to evaluate the analgesic action of various endogenous compounds (Dubuisson and Dennis, 1977; Abbott et al., 1999; Olyuomi et al., 1992; Murray et al., 1988; Zhao et al., 2003). The intra-plantar formalin injection generates a reproducible syndrome of nociceptive behavior, which appears in two distinct phases. The first phase starts at the time of the injection and lasts about 10 min. However, the second phase starts at 10 min after the injection and lasts for about 50 min. Indeed, it is considered that the first phase results from a direct activation of nociceptive afferent fibers. Yet, the second phase is thought to be mediated by activation of central sensitized neurons due to peripheral inflammation and continued activity of primary afferents (Hunskaar and Hol, 1987; Zhao et al., 2003; Puig and Sorkin, 1996; Dubner and Ren, 1999; Milano et al., 2008). The application of formalin to a hind paw was shown by Dickenson and Sullivan (1987) to stimulate primary afferent C-fibers in a biphasic manner and to follow a time course similar to that observed in behavioral studies (Dickenson and Sullivan, 1987).

In this way, it has been shown that formalin intra-plantarly injected in animal’s hind paw increased significantly in the early phase the release of aspartate, taurine, glutamate, citrulline, glycine, serine, glutamine, PGE2 and asparagine; while in the second phase, it augmented the liberation of the citrulline, PGE2, glutamate and aspartate. In similar studies conducted on thymoquinone (Abdel-fattah et al., 2000), supraspinal opioid systems, particularly the M1 and K opioid receptor subtypes, have been shown to be involved in the early phase. Whereas the antinociception induced by thymoquinone in related late phase is due to the inhibition of inflammatory mediators (Houghton et al., 1995).

The Lavandula stoechas ethanolic extract showed nociceptive activity by inhibiting both phases of the formalin response. Indeed, the manifestation of the late phase is due to inflammation causing a release of serotonin, prostaglandins, histamine and bradykinin, which at least to some extent, can lead to sensitization of central nociceptive neurons (Verma et al., 2005). It then implies that the anti-nociceptive activity of the extract possibly resulted from its central and peripheral action, which might also suggest an anti-inflammatory action.

Since prostaglandins are important mediators of pain and lavender extract, due to the rich composition in phenolic flavonoids and flavonols compounds (Ezzoubi et al., 2014) can inhibit the production of thromboxane and prostaglandins (Picq et al., 1991), mediators involved in the maintenance and initiation of the pain and inflammation cascade. Therefore, Lavandula stoechas ethanolic extract may induce its analgesic and anti-inflammatory effects in this way.

To screen for the effects of anti-inflammatory drugs, xylene-induced acute inflammation in the ear of mice was used (Kou et al., 2005). Indeed, our study examined the impacts of Lavandula stoechas on acute inflammation induced by the xylene model. The application of xylene caused a significant increase in the weight of the mouse ear due to the inflammatory reaction. These ear weight increases were used as pertinent markers of anti-inflammatory effects (Ojewole, 2005). For example, pretreatment with the plant extract inhibited the increase in ear weight in a dose-dependent manner. Furthermore, the histopathological manifestations such as inflammatory cell infiltration, vasodilatation and edematous skin changes were decreased on a dose-dependent basis. This inhibition could be considered as a direct evidence that the extract of Lavandula stoechas has positive effects on decreasing the acute inflammatory reaction in comparison with those of diclofenac.

The anti-inflammatory action of this extract could be attributed to its chemical composition. Indeed, several major constituents of Lavandula sp. can explain their positive effects, including polyphenols (Somani et al., 2015). In fact, flavonoids are polyphenols that show antioxidant properties that can contribute to the anti-inflammatory effects exerted by this extract in this experimental model of inflammation (Comalada et al., 2005; 2006).
Conclusion

Significant analgesic and anti-inflammatory properties of the total extract of the aerial parts of the plant were found in these models, which suggests a reasonable basis for the popular and traditional uses of this plant for certain painful and inflammatory conditions.

Many mechanisms are involved in the anti-inflammatory and analgesic actions of the drugs and should be investigated for these fractions in the future. In addition, further biological and phytochemical assays are proposed to determine the chemical active constituents involved in these activities.

Acknowledgment

We are grateful to Prof. A. Ouhammou for his help in identifying plant material and we thank Mr. A. Regragui for the provision of animals.

Data Availability

All of the data used to substantiate the findings are included in the article.

Author’s Contributions

Hind Ferehan, Mehdi Ait Laaradia, Sara Ouafquir and Rachida Aboutafatima: Conception and design of study, Acquisition of data, Analysis and/or interpretation of data, Drafting the manuscript, Revising the manuscript critically for important intellectual content, Final approval of the version submitted and any revised version.

Soad Moubtakir: Conception and design of study, Acquisition of data, Analysis and/or interpretation of data, Final approval of the version submitted and any revised version.

Zahra Sokar: Conception and design of study, Acquisition of data, Analysis and/or interpretation of data, Drafting the manuscript, Final approval of the version submitted and any revised version.

Ethics

This article is an original work done by the authors. The corresponding author asserts that the other authors have read and approved the manuscript and no ethical issues could be involved.

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