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PHYTOCHEMICAL SCREENING, ACUTE TOXICITY, ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF THE AQUEOUS EXTRACT OF *Lavandula stoekas* (L.) FLOWERS IN MICE

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ABSTRACT

The objective of the study was to identify phytochemical constituents of *Lavandula stoekas* and to determine the anti-inflammatory and analgesic activities as well as the acute toxic effects of the aqueous extract of *Lavandula stoekas* in mice. The LD50, preliminary phytochemical screening, anti-inflammatory and analgesic potentials of the aqueous extract from the flowers of *Lavandula stoekas* L (AEFLS) were investigated in mice using carragenin (inducing paw oedema), formalin (inducing nociception), paw licking, acetic acid (inducing writhing) in mice. The LD50 was found to be > 5000 mg/Kg body weight. Phytochemical screening revealed the presence of coumarins, steroids, tannins, saponins, flavonoids, and terpenoids. Concerning the acetic acid writhing test, the percentage of inhibition was obtained at the dose of 400 and 600 mg/kg (60.27 and 56.54%), close to that of the aspirin drug (62.03%). For formalin-induced nociception, the tested extract at 600 mg/kg showed a higher percentage of inhibition compared to aspirin, in early (62.07 and 57.18%), and in late phase (77.37 and 74.01%), respectively. The extract at 200, 400 and 600 mg/kg body weight reduced significantly the formation of edema induced by carrageenan. At the end, our data showed that the AEFLS has a significant antalgic and anti-inflammatory activity which supports its use in traditional herbal medicine practice.

Keywords: *Lavandula stoekas*; aqueous extract; anti-inflammatory; analgesic; acute toxicity; phytochemical screening.

INTRODUCTION

Pain and inflammation are unpleasant and non-specific symptoms of many diseases. Prescription of common anti-inflammatory and analgesic drugs may be limited due to their side effects [1] such as: gastrointestinal irritation, ulceration, bleeding. Opioids used against the pain are accompanied by side effects such as addiction. Thus, the use of plants and plant extracts are considered to be a source of

new chemical substances with potential therapeutic effects and have shown essential roles as effective anti-inflammatory agents with less complication.

According to the World Health Organization (WHO), three-quarters of the world's people depend on traditional medicines for their cure [2]. The variety of climatic and geographic conditions in Algeria provides a rich source of vegetation, including many plant species [3]. Among these plants, we were interested in studying the species: *Lavandula stoechas* (Lamiaceae). Indeed, various parts of this plant have been widely used in traditional Algerian medicine because it is known to protect against migraines, depression, and epilepsy [4]. *Lavandula stoekas* extracts are also used for their positive effects on wounds, urinary tract infections, heart disease, and eczema [5]. Although these substances are used to treat a wide variety of ailments and symptoms, no adequate experimental study was performed regarding their effectiveness.

Therefore, the present study was carried out to identify phytochemical constituents, acute toxic effects and to assess the anti-inflammatory and analgesic potential of the aqueous extract of *Lavandula stoekas* flowers against induced inflammation and pain in mice.

MATERIALS AND METHODS

Plant Collection and Identification

The aerial parts of *Lavandula stoekas* L, were collected in May 2016 in the mountainous area of Chreaa belonging to Blida region (in the North of Algeria). Identification of the plant was confirmed by Higher National Agronomic School Botany Department (Algiers, Algeria). A voucher specimen was deposited at the Giffen Herbarium of Higher National Veterinary School in Algiers for future reference.

Aqueous Extract Preparation

In this study, 50 g of *Lavandula stoekas* powder (dried flowers) were mixed with 500 mL of distilled water and macerated on a magnetic agitator for 72 hours at room temperature. The mixture was filtered twice through cotton wool, then through the Whatman filter paper (Number 1). The filtrate was evaporated to dryness using under vacuum in a rotary evaporator at 40°C to obtain a powder. After that, it was lyophilized using the lyophilizer for 12 hours and the collected product was preserved in a refrigerator at 4°C for further use. Lyophilized flowers had then a dried form.

Experimental Animals

Swiss albino mice ± 20 g of either sex were obtained from the Pasteur Institute of Algiers (Algeria). The animals were maintained under standard environmental conditions (Temperature of 22 ± 3°C, relative humidity: 55-65% and 12 hours light/ dark cycle) and had free access to food and water ad libitum.

The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Laboratory Research Council of Higher National Veterinary School, Algiers Algeria. All the experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Phytochemical Screening

The phytochemical screening was performed to assess the qualitative chemical

composition of the aqueous extract from *Lavandula stoekas* flowers such as: alkaloids, glycosides, steroids, tannins, saponins, coumarins, flavonoids, and terpenoids, using commonly employed precipitation and coloration reactions to identify the major secondary metabolites, with the standard procedures described by Harborne [6] and Trease and Evans [7]. These tests were useful to estimate pharmacological activities that might be due to the presence of secondary metabolites in the flowers of this plant.

Acute Toxicity Study

Acute toxicity test was performed as per OECD guideline 423 for testing of chemicals (2001). Five healthy young adult albino nulliparous, non-pregnant female mice weighing about 20-30 g were administered a single oral dose of 5000 mg/kg of AEFLS in distilled water, while the control group received water vehicle. Animals were observed individually for first 30 min, then for the first 24 h, with special attention given during the first 4 h, and daily thereafter. This observation was made for a total of 14 days to note toxicity signs like changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and in order to record any mortalities.

Analgesic Activity

Acetic acid-induced writhing test in mice

The method described by Koster [8] was used. Thirty mice were randomly distributed into five groups of six mice each.

Group I (control) were administered distilled water (10 ml/kg).

Group II (positive control) received standard drug aspirin (at oral dose of 150 mg/kg). Remaining groups were treated orally as follows:

Groups III: They received AEFLS at a dose of 200 mg/kg.

Groups IV: They received AEFLS at a dose of 400 mg/kg.

Groups V: They received AEFLS at a dose of 600 mg/kg.

All treatments were administered orally. Then, 60 minutes after administration of standard drug and test samples, each mouse was injected with 0.6% acetic acid at the dose of 10 mL/kg body weight intraperitoneally. The number of writhes (a syndrome characterized by a wave of contraction of abdominal musculature followed by extension of hind-limb) was counted for 30 minutes.

The percentage of inhibition data were calculated and reported according to the following formula:

$$\% \text{ Inhibition } = \frac{\text{Mean number of writhes(Control)} - \text{Mean number of writhes(Test)}}{\text{Mean number of writhes(Control)}} \times 100$$

Formalin Induced Pain in Mice

The anti-nociceptive activity of the drugs was determined using the formalin test, according to the procedure described by Viana et al. [9]. Thirty mice were randomly distributed into five groups of six mice. Twenty microliters of 5% formalin was injected into the dorsal surface of the right hind paw of mice 30 min after administration of different doses of AEFLS (200, 400 and 600 mg/kg body weight intraperitoneally), aspirin (150 mg/kg), and distilled water (10 ml/kg, orally) to the control groups of animals. The time (in seconds) spent on licking and biting of the injected paw was

taken as an indicator of pain response. Responses were measured for 5 minutes (first phase) and 15-30 minutes (second phase) after formalin injection, representing the neurogenic and inflammatory pain response, respectively. Analgesic effect was expressed as a reduction in the time spent in licking or biting of the injected paw.

Anti-inflammatory Study

Carrageenan-induced paw edema

The method previously described by Winter et al. [10] was used. Thirty mice were randomly divided into five groups of six mice each. They were orally administered distilled water (1 ml/kg), AEFLS (200, 400 and 600 mg/kg) and diclofenac (10 mg/kg). After 60 min, edema was induced with the injection of 0.1 ml carrageenan. Carrageenan was prepared as 1% solution in 0.9% NaCl. The perimeter of paw was measured by using vernier callipers. Measurements were taken at 0–4 h after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation T, Thickness of paw in control group; T0, Thickness of paw edema in the test compound treated group.

$$\% \text{ inhibition of edema} = (T - T0 / T) \times 100$$

Statistical Analysis

Statistical analysis was performed using STATISTICA (Version 10, Stat Soft France, 2003). All the values were expressed as mean ± SD. The data were assessed using one-way analysis of variance (ANOVA). Statistical significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

As it is illustrated in Table 1, the screening allowed us to characterize the different families of existing chemical compounds in the AEFLS. Data revealed the presence of phytochemicals considered as active chemical constituents (coumarins, steroids, tannins, saponins, flavonoids, and terpenoids). However, alkaloids were not detected.

Table 1. Phytochemical constituents in the aqueous extract of the *Lavandula stoechas* flowers

Phytochemical constituents	Relative presence
Coumarins	+
Steroids	+
Tannins	+
Saponins	+
Flavonoids	+
Terpenoids	+
Alkaloids	-

+: presence of specific phytoconstituents;
−: absence of specific phytoconstituents

Many studies have confirmed that medicinal plants can contain a diverse range of bioactive molecules responsible for a collection of pharmacological properties [11]. For example, the presence of saponins, triterpenes, tannins, flavonoids, cardiac glycosides, and steroids which might be responsible for the obvious anti-inflammatory activities of the extracts of the plant.

Previous phytochemical works on the leaf and flowers of *Lavandula stoekas* revealed the presence of flavonoids, terpenoids, and steroids [12].

The presence of the chemical families detected for this plant in our study was confirmed previously by Baptisa et al. [13] for polyphenols and flavonoids, by Zoubi et al. [14] for tannins and sterols, and by Teixeira et al. [15] for polyphenols and terpenes.

Acute Toxicity

In this study, no animal death was registered and no sign of toxicity was observed in the behavior of mice during the 14-days of observation period following the administration of 5000 mg/kg of the AEFLS. Therefore, the approximate acute lethal dose (LD50) of this extract in female mice was estimated to be higher than 5000 mg/kg. Regulatory agencies generally agreed that once a compound reached a dose of 5 g/kg by the oral route (Category 5), it can be considered “Generally regarded as safe” (OCDE. 2000).

Effects of AEFLS on Acetic-Acid-Induced Writhing Response

All the doses of the extract reduced significantly the acetic acid induced writhing in mice. A dose-dependent increasing the inhibition of writhes was noted with AEFLS 200, 400, 600 mg/kg with the rates of 34.29%, 56.54 and 60.27%, respectively. The AEFLS at 600 mg/kg showed 60.27% of writhes inhibition, close to that of the standard aspirin (62.03%). The effect of aspirin was significantly better than AEFLS up to 200 mg/kg, but the effect of both up to 400 and 600 mg/kg mg/kg were close ($P > 0.05$) (Table 2).

The AEFLS showed a potent analgesic effect in the acetic acid induced writhing model. This may be due to the inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. In addition, it can be attributed to the inhibitory action on prostaglandin synthesis [16,17]. Moreover, components of AEFLS such as saponins, triterpenes, tannins, and flavonoids might be responsible for this action [18,19,20].

Effects of AEFLS on Formalin-induced Nociception

As shown in Table 3, AEFLS had significant ($P < 0.05$) anti-nociceptive activity in the formalin test. Treatment with AEFLS caused obvious decrease of the late phase pain responses induced by formalin, in a dose-dependent manner (200, 400, and 600 mg/kg) both the early (neurogenic, 0– 5 min) and late (inflammatory, 15–30 min) phases of the formalin-induced nociception method. At 600mg/kg, AEFLS produced similar reductions in both phases when compared with those results obtained for aspirin group (Table 3). However, all the doses inhibited the second phase significantly ($P > 0.05$) similar to aspirin 150 mg/kg.

Table 2. Acetic-acid-induced writhing response

Group	Dose (mg/kg)	Writhing times	Inhibition (%)
Control	-	32.45	
Aspirin	150	12.32±3.25*	62.03
AEFLS	600	12.89±2.40*	60.27
AEFLS	400	14.10±2.21*	56.54
AEFLS	200	21.32±3.05*	34.29

*The values were presented as mean ± standard deviation. *: the significance levels in comparison with the negative control (P< 0.05)*

Table 3. Effects of AEFLS in formalin induced edema in mice

Treatment group (n=6)	Doses (mg/kg)	Early phase (0-5 min)	% Inhibition	Late phase (15-30 min)	% Inhibition
Control	-	64.39±1.48		76.46±1.34	
Aspirin	150	27.57±1.56*	57.18	19.87±1.65*	74.01
AEFLS	600	24.42±2.03*	62.07	17.3±2.4*	77.37
AEFLS	400	36.3±1.06*	43.62	22.1±1.72*	71.09
AEFLS	200	42.12±2.43*	34.58	24.29±1.05*	68.23

*Value were presented as the mean±SD, *P<0.05, when compared to control*

The injection of formalin into plantar aponeurosis is biphasic, an early neurogenic component, followed by late tissue-mediated response [21]. The early response corresponded to the direct effect of formalin on pain fibers particularly, C fibers causing release of bradykinin and tachykinin [22]. On the other hand, the late phase was due to the inflammatory reaction with the release of some compounds such as serotonin, histamine, bradykinin, and prostaglandins [23].

Centrally acting drugs (opioids) inhibit both phases, while peripherally acting drugs (non-steroidal anti-inflammatory drugs [NSAIDs]) inhibit only the second phase. The analgesic action of the extract may be due to its ability to inhibit cyclooxygenase, as in the case of NSAIDs. It is well known that NSAIDs were largely ineffective or causing weak inhibition against both models [24]. In addition, NSAIDs can attenuate the second phase of formalin-induced licking in dose-dependent manner [25]. In the formalin test, AEFLS reduced significantly the late phase of formalin-induced nociception. These results suggested that the extract resembled mild analgesics such as paracetamol and aspirin [26] and induced specific modulation of tonic pain compared with the phasic pain.

Effects of AEFLS on Carrageenan-induced Paw Edema

The anti-inflammatory activity of AEFLS using carrageenan-induced paw edema was summarized in (Table 4). Treatment with the extract (200, 400, and 600mg/kg) and the standard anti-inflammatory drug diclofenac (10 mg/kg) had shown significant suppression of edema at all-time points as compared to controls ($p<0.05$). Thus, the anti-inflammatory activity of the AEFLS at the dose levels of 200 mg/kg, 400 mg/kg, and 600 mg/kg was similar to that of diclofenac (reference drug). Also, the activity was found to be dose-dependent.

These results were in agreement with those reported previously [27,28]. *Lavandula stoechas* has been long used in Mediterranean traditional medicine for the treatment of inflammatory-related disorders. Data obtained in the present study supported this hypothesis. The extracts had shown to contain several polyphenols and flavonoids which could explain the effects observed since they exhibit antioxidant, immunomodulatory and anti-inflammatory properties [29,30]. The anti-inflammatory effect may be due to the inhibition of prostaglandin biosynthesis, which is similar to that produced by non-steroidal anti-inflammatory drugs such as diclofenac. However, exact mechanism of inhibition of prostaglandin synthesis could be a potential future perspective.

Table 4. Anti-inflammatory effect of AEFLS on carrageenan-induced paw edema

Groups	Dose (mg/kg)	Edema Size Means (mm) ± SD (% inhibition)				% inhibition
		1st h	2nd h	3rd h	4th h	
Control		4.94± 0.16	4.86± 0.2	4.95±.0.14	4.84±0.23	-
Carrageenan						
Carrageenan + diclofenac	10	3.08± 0.12* 37.64	2.01±0.21* 58.64	1.96± 0.14* 60.40	1.72± 0.12* 64.42	55.27
Carrageenan + AEFLS	600	3.14± 0.21* 36.43	2.12± .01* 56.37	1.93± 0.01* 61.01	1.62± 0.14* 65.90	54.92
Carrageenan + AEFLS	400	3.17± 0.13* 35.82	2.69± 0.13* 44.65	1.97± 0.02* 60.20	1.92±0.15* 60.33	50.25
Carrageenan + AEFLS	200	3.20± 0.15* 35.22	2.94± 0.22* 39.50	2.21± 0.01* 55.35	2.07± 0.04* 57.23	46.82

**P < 0.05 - significant compared to carragenan treated group*

CONCLUSION

This work demonstrated that the aqueous extract of *Lavandula stoekas* flowers exhibited anti-inflammatory and analgesic activities. Our present study also reported the presence of saponins, triterpenes, tannins; flavonoids which were responsible for the activities of this plant in traditional medicine. Acute toxicity test showed that the plant generally regarded as safe. It may be concluded that the current study supported the traditional use of the *Lavandula stoekas* flowers by the medical practitioners for several conditions such as stomachache, pain and inflammation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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