Phytochemical Screening, Anti-inflammatory Activity and Acute Toxicity of Hydro-ethanolic, Flavonoid, Tannin and Mucilage Extracts of *Lavandula stoechas* L. from Morocco.

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ABSTRACT
The present work showed phytochemical screening, anti-inflammatory activities and sub-acute toxicity of hydro-ethanolic and polyphenols (flavonoid, tannin and mucilage) extracts from aerial part (branches, flowers and leaves) of *Lavandula stoechas* L. The anti-inflammatory activity was evaluated by Carrageenan-Induced Rat Paw Edema method. Sub-acute toxicity of the hydro-ethanolic extract and its fractions was evaluated *in vivo* after topical application of creams and some biochemical parameters were determined. Phytochemical screening of extract of *L. stoechas* revealed a presence of tannins, catechic tannins, flavonoids, sterols, coumarins, quinones, leucoanthocyans and mucilages compounds. The hydro-ethanolic extract of *L. stoechas* (5 and 10 %) inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. At dose of 10 %, *L. stoechas* produced a significant inhibition of inflammation at 74±7 % compared to 69±10.3 % for diclofenac at 1 %. Flavonoid and mucilage extracts showed significant effect in reduction of edema (85.1 ± 6.2 and 61.71± 7.3 % respectively). No significant variation was observed in the body and Relative Organ Weights (ROW) between the control and the treated group. Furthermore, no renal, hepatic and blood dysfunctions were noted in treated animals compared to control.

Key words: Sub-acute toxicity, anti-inflammatory effect, *Lavandula stoechas* L., flavonoids, tannins, mucilages.

INTRODUCTION
The inflammatory process involves a series of events that can be elicited by numerous stimuli, for example, infection agents, ischemia, and antigen–antibody interactions, chemical, thermal or mechanical injury. The inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms: an acute one characterized by local vasodilatation and increased capillary permeability, a sub-acute phase characterized by infiltration of leukocyte and phagocyte cells and a chronic proliferative phase, in which tissue degeneration and fibrosis occur. Inflammatory diseases are currently treated with steroidal and non steroidal anti-inflammatory drugs (NSAIDs). Despite their widespread use, NSAIDs associate with severe adverse effects; the most common is gastrointestinal bleeding\(^1\). For this reason, safer compounds with fewer side effects are needed. Many potential anti-inflammatory agents are evaluated in the pharmaceutical industry and animal models are used extensively in testing them. Winter et al.\(^2\). Polyphenols are abundant phytochemicals in berries, fruit and vegetables and constitute a large class of compounds which have attracted research attention due to their potential human health benefits\(^3\). Among the various sub-classes of polyphenols, the anthocyanins (water soluble pigments) and ellagitannins (hydrolyzable tannins) have been implicated with a wide range of biological properties including anti-inflammatory effects\(^4,5\). Polyphenols can exert their anti-inflammatory properties at multiple levels, through the modulation of mitogen-activated protein kinases (MAPK) signalling pathways\(^6,7\) and NF-kB and AP-1 transcription factors\(^8\), inhibition of the production of inflammatory cytokines and chemokines, suppressing the activity of cyclooxygenase (COX)\(^9\) and inducible nitric oxide synthase (iNOS) and thereby decreasing the production of reactive oxygen and nitrogen species (ROS/RNS). *L. stoechas* is widely distributed in the Mediterranean region. This species is one of the most explored lavenders in the world. Some studies considered the antibacterial\(^10,11\), antifungal\(^10,12\), and antioxidant\(^10,13,14\), properties of *L. stoechas*. The decoction of this plant is used traditionally in Morocco for the treatment of painful illnesses like inflammatory diseases, cystitis, nephritis and rheumatic arthritis\(^15\). Therefore, the current study designed

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The Ultrasound assisted extraction

MATERIALS AND METHODS

Plant collection

The aerial parts of L. stoechas are collected in the province of Taounate in Morocco, between April and June 2014. Then they are air-dried at 40 °C with forced ventilation for two days. The botanical identification and authenticated voucher specimens deposited in the Herbarium of The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

The Ultrasound-assisted extraction

Twenty grams of aerial part powder of L. stoechas are mixed with 150 mL of n-hexane at 35 kHz frequency during 45 min, and with a temperature lower than 25 °C. After the extraction, the mixture filtered under vacuum through Whatman paper, and the solvent was removed. Then the plant’s material re-extracted again with a mixture of ethanol /water (4:1 (v/v)) for 45 min under the same conditions. The final extract recuperated from the mixture (ethanol/water) after filtration by Watman paper and evaporation under vacuum at 40°C on a rotary evaporator.

Phytochemical screening

The hydro-ethanolic extract is screened for phytochemical constituents (coumarins, leucoanthocyans, flavonoids, mucilags, tannins, sterols and terpens, quinones and cardiac glycosids) using a simple qualitative methods as described in the study of 16, 17.

Extraction of flavonoids

The extraction of flavonoids was performed following the method of Lee et al., with slight modifications, dried leaves of the L. stoechas were milled into powder and then extracted with mixture equal of water and ethanol (100 ml/100ml, V/V) for 60 min at 60 °C and filtered through filter paper with a porosity of 0.45 microns. The aqueous layer was then extracted with 200 ml of n-butanol for analysis and acidified with HCL-10% at pH 3. The butanol layer was evaporated by rotary evaporator at 40°C. The dry residue has extracted three times with 200 ml mixture of distilled water and ethyl acetate for analysis (100 ml: 100 ml) for one for 60 min at room temperature. The organic layer was basified with NaHCO3 to pH 9, and evaporated to dryness at 40 °C with a rotary evaporator.

Extraction of tannins

The crushed plants are steeped in a mixture of acetone / distilled water (35/15, v / v) for three days at room temperature. The solution was evaporated by rotary evaporator at 40°C. The aqueous phase is washed with 15 ml of dichloromethane to remove pigments and lipids. After separation of the organic phase, the aqueous phase was extracted twice with 15 ml of ethyl acetate. The two phase mixture is evaporated to dryness at 40 °C with a rotary evaporator.

Extraction of mucilages

The powdered material of L. stoechas has soaked in water for 5-6 h and boiled for 30 minutes and kept aside for 1 h for complete release of the mucilage into the water. The materiel squeezed from an eight fold muslin cloth bag to remove the mares from the solution. Acetone added to the filtrate to precipitate the mucilage in a quantity of three times the volume of the total filtrate. The mucilage was separated, dried in an oven at a temperature < 50 °C. The yielding was defined as follows: (crude extract weight/plant material weight) x 100.

Anti-inflammatory activity of the Hydro-Ethanolic Extract (HEE) and its fractions

Carrageenan-Induced Rat Paw Edema

Acute inflammation in the rats was produced according to the method described by Winter et al. Therefore; seven groups of five rats were used for this study.

Group 1 served as the control group receiving normal saline;

Group 2 were given topical application of Diclofenac gel (1%);

Groups 3 and 4 were given topical application of cream formulated in our laboratory by mixing the neutral cream with the HEE of L. stoechas at doses of 5% and 10%, b.w.

Groups 5, 6 and 7, were given topical application of creams formulated in our laboratory by mixing the neutral cream with the flavonoid, tannin and mucilage extracts at dose 4%, 3.6% and 1.5% respectively by basing on fractions yields.

The cream was applied 90 min before the induction of inflammation. The percentage of inflammation inhibition was calculated by the method previously described. The efficiency of creams was evaluated in comparison with Diclofenac at 1 %. The percentage inhibition was calculated thus:

\[
\% \text{ inhibition} = \left( \frac{(S_t−S_c)control−(S_t−S_c)treated}{(S_t−S_c)control} \right) \times 100
\]

Where: \( S_t \) = the mean paw size for each group after the carrageenan treatment.

And \( S_c \) = the mean paw size obtained for each group before the carrageenan treatment.

Sub-acute toxicity

Ten male rats are divided into 5 groups, each group containing 2 animals. All the groups were topical application of creams with Hydro-Ethanolic, Flavonoid, Tannin and Mucilage extracts of the recipe in a dose of

Table 1: Phytochemical screening of hydro-ethanolic extract of Lavandula Stoechas L.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Hydro-Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Catechic tannin</td>
<td>+</td>
</tr>
<tr>
<td>Gallic tannin</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Sterols and terpenes</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Leucoanthocyans</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosids</td>
<td>+</td>
</tr>
<tr>
<td>Mucilages</td>
<td>+</td>
</tr>
</tbody>
</table>

Presence of compounds is: (+) = present; (-) = absent chemical
RESULTS

Phytochemical screening and yielding extraction
As it is illustrated in Table 1, phytochemical screening of extract of *L. stoechas* revealed a presence of tannins, catechic tannins, flavonoids, sterols, coumarins, leucoanthocyanins and mucilages compounds. However, gallic tannins and quinones were not detected. These results are in accordance with other studies related to the *Lavandula* family. The species of this family produce flavonoids, tannins\(^2\) and coumarins\(^3\). The yields of bioactive compounds are shown in Table 2. Flavonoids are more abundant with a content of 4.6±0.8 % followed by Tannins and Mucilages that represent 3.6±0.7 % and 1.8±0.3 % of the leaf dry weight respectively.

Anti-inflammatory activity

Anti-inflammatory effect of hydro-ethanolic extract
Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 h. *L. stoechas* extract produced a maximum inhibition of inflammation at a dose of 5% (44±8.7%). We noted a maximum of inhibition of inflammation at 5h after treatment with *L. stoechas* extract at a dose of 10 % (74±7 %) compared to Diclofenac which produced an inhibition of 69.5 ± 10.3% (Figure 1). This dose of hydro-ethanolic extract from *L. stoechas* was therefore selected for the subsequent studies.

Effect of flavonoid, tannin and mucilage extracts on paw edema formation

The various fractions of *L. stoechas* (Flavonoid 4%, Tannin 3.6%, Mucilage 1.5 %) showed significant effect in decreasing edema (Table 3). The inhibition percentages were measured at 5h after inflammation induction 85.1 %, 38.29 %, and 61.71 %, respectively. Fraction I (flavonoids at 4 %) inhibits significantly carrageenan-induced edema mainly at 4 h (78%) and 5 h (85.1 %); fraction III (Mucilage at 1.5%) shows a significant inhibition at 4 h (56%) and 5 h (61.71%). No significant anti-inflammatory effect was observed (30%) in treated group with 3.6 % of fraction IV (tannins) as compared to control and ethanolic extract.

Sub-acute Toxicity

During the experiment, the rats did not show any observable signs of toxicity or morbidity. Furthermore, no mortality was recorded. There was no significant difference in the ROW of liver, kidney and spleen of the treated groups of rats when compared to the control group (Table 4).

Biochemical parameters

Table 5 shows the results of some biochemical parameters assessed in serum of rats treated with HEE, flavonoids, tannins and mucilages extracts. We noted any significant difference in hepatic functions indicated by AST and ALT.
values of treated rats at all extracts at different doses of 0%, 4%, 3.6% and 1.5% compared to the control. Furthermore, the values of all parameters related to renal and blood functions (urea, creatinine, glucose, triglycerides, cholesterol and total proteins) are not changed when we compared between control and treated animals (Table 5).

**DISCUSSION**

Flavonoids are a large group of naturally occurring compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. A variety of in vitro and in vivo experiments have shown that selected flavonoids possess antiallergic, antiinflammatory, antiviral and antigenitigant activities. Previous studies demonstrated also the role of flavonoids in anti-inflammation processes. The early phase (2 h after carrageenan injection) is attributed to the release of histamine and serotonin followed by a later phase of edema due to production of bradykinin and prostaglandins. This later phase has been attributed to the release of histamine and serotonin around 1 h, is attributed to the release of histamine and serotonin.

The body weight and fatalities model, the ethanol extract and two fractions showed anti-inflammatory activity. The results clearly highlighted the significant anti-inflammatory effect of hydro-ethanolic extract of *L. stoechas*, as it inhibited edema formation in a dose dependent manner and 10% showed maximum inhibition. Fraction I (flavonoids) inhibits significantly carrageenan-induced edema from 4 to 5 h. However, fraction III (mucilage) shows a significant inhibition only at 4 h. Flavonoids and mucilage fraction’s could be considered as the main chemical compounds responsible of the anti-inflammatory activity of the hydro-ethanolic extract from aerial part of *L. stoechas*. We could assume that the anti-inflammatory activity observed is due to a synergistic action of flavonoid and mucilage components contained in hydro-ethanolic extract. Several authors report that flavonoids and mucilage inhibits cyclooxygenase activity. The development of edema in the paw of the rat after the injection of carrageenan has been described as a biphasic event: the early phase, observed around 1 h, is attributed to the release of histamine and serotonin and the late phase is due to the release of prostaglandins. In this study, the extracts did not show any significant anti-inflammatory effect in the early phase but showed an important effect at the later phase after 4-5 h without any adverse toxic effects observed in vivo. Thus, the results suggest that extracts acts at the later phase involving arachidonic acid metabolites probably by the inhibition of cyclooxygenases. Furthermore, the safety of medicinal plants is important as safety of herbal medicine use has recently been questioned due to reports of illnesses and fatalities. The body weight monitoring showed that *L. stoechas* did not induce any significant changes in all animals. The animal body weight is also an important factor to evaluate the toxicity of substances. The reduction in body weight and internal organ weight can be a simple and sensitive index of toxicity after exposure to a toxic substance.
The present work, HEE of *L. stoechas* and its fractions did not induce any significant changes to the relative weight of the organs of rats (liver, kidneys, and spleen) and any modifications of the biochemical parameters too when compared to the control group. ALT is a more specific marker of liver cell damage, because it occurs more frequently in the liver while AST is also found in heart, skeletal muscle, kidneys, brain, pancreas and blood cells. In the liver, ALT is confined to cytoplasm, while AST is found in both mitochondria (80%) and cytoplasm (20%). In our study, we noted that level of ALT and AST were not affected, suggesting that HEE of *L. stoechas* and its fractions are not hepatotoxic.

**CONCLUSION**

The results obtained in the present study indicated that the hydro-ethanolic extract of *L. stoechas L.* possesses a good anti-inflammatory activity more than Diclofenac treatment. Flavonoids and Mucilages extracts of *L. stoechas* may be the potential therapeutic agent involved in inflammatory diseases, justifying the use of this plant in the traditional medicine in Morocco. It will be useful to further investigations to identify the structures flavonoids and mucilage’s responsible for this anti-inflammatory activity. These results suggested that the aerial part of *L. stoechas* can be used for treatment of inflammatory diseases without exhibiting any side toxic effect.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


39. Filippin F B, Reis K, Cemin L, Duzzioni M, Hermes E M, Souza L C. Novo intervalo de referência para alanina aminotransferase usando o sistema