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ANALGESIC ACTIVITY OF ETHANOLIC AND ALKALOIDIC EXTRACTS OF DELPHINIUM STAPHISAGRIA SEED

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KEYWORDS:
Analgesic Activity,
Diterpenoids alkaloids,
Delphinium staphisagria,
Ethanolic extract, Medicinal plants.

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ABSTRACT

We evaluate the analgesic activity of the ethanolic and Alkaloidic extracts obtained from Delphinium staphisagria seeds, using chemical and the Tail flick models which will induce acute pain in mice and rats. This study was carried out by using sex Swiss mice (20-30g) and Wistar male rats (180-200g). The ethanolic extract was prepared by using maceration at room temperature (25°C) over the period of 24 hours. The effect of ethanolic and alkaloidic extracts of Delphinium staphisagria seeds was investigated for analgesic activity using acetic acid-induced abdominal writhing (Koster test) and Tail immersion method (Tail flick test). The analgesic activity of ethanolic and alkaloidic extracts of Delphinium staphisagria seeds at the dose of (40 and 20 mg/kg, p.o.) respectively showed significant (p<0.001) reducing in abdominal writhing when compared with control and standard drug (Aspirin, 200 mg/kg, p.o.). However, ethanolic extract at the dose 40mg/kg; p.o., and Alkaloidic extract at the dose 20 mg/kg, p.o., showed significant (p<0.001) central analgesic action when compared with control and morphine (5 mg/kg, s.c.) as reference drug. We conclude that, the alkaloidic extract of Delphinium staphisagria is a notable, central, and peripheral analgesic activity, these data provide pharmacological basis for its therapeutic efficacy on pains.

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INTRODUCTION:
Morocco is fortunate to have such varied climate that almost any medicinal plant can grow. The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genera. Diterpenoids types have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties. Delphinium staphisagria owned Renonculaceae family is a species characterized by its richness in diterpenoids alkaloids. Analgesia is to reduce the perception of pain without altering the level of consciousness, vigilance or memory of the patient, a large number of products have been used as analgesic, most of these agents produced several side effects including dyspepsia and gastrointestinal complications ranging from unspecified symptoms (nausea, vomiting and diarrhoea) to severe complications (ulcer, bleeding and perforation) and represents major limitations in their clinical use.

Recently, many natural medicines derived from medicinal plants, were considered as the effective and safer for the treatment of various diseases including inflammation and pain. Aconitum and Delphinium plants have been medicinally used for centuries. The pharmacological effects of Aconitum plants are attributed to their characteristic diterpenoid alkaloids, a group of complex natural products displaying a lot of interesting chemistry and biological activities. The analgesic activities of C18- and C19-diterpenoid alkaloids have been extensively investigated since 1981, among which 3-acetylaconitine, lappaconitine and crassicauline A, have been reported to exhibit marked analgesic activities and have been developed to be analgesic drugs clinically used for the treatment of various pains in China. As compared with the known analgesics, such as morphine, methadone, etc., all these three alkaloids induced neither morphine-like tolerance nor physical dependence.

Delphinium plants are a large species within the family Renonculaceae, largely distributed throughout the northern hemisphere region, such as Asia, Europe, and North America, while a few occur in equatorial Africa. The chemical constituents of plants belonging to genus Delphinium have been extensively studies and are best known for their diverse C19 and C20 Diterpene alkaloids.

Over 40 alkaloids with differing structures and toxicity have been identified, the amount and types of alkaloids varies greatly between the different larkspur species, within different larkspur populations, and different phenological grown stages. Individual plants often contain 15 or more alkaloids.

In 1976, Pelletier isolated, by chromatography and crystallization, a methoxyl- containing bis-diterpene alkaloids which they designated as Staphisine C$_4$H$_{60}$N$_2$O$_2$ and they find that Jacobs
Staphisine is a mixture of Staphisine and a companion non methoxyl staphidine. In addition, they have isolated two new amine-containing bis-diterpene alkaloids named staphinine and Staphimine. The mother liquors which had been accumulated during the isolation of delphinine from the seeds of Delphinium staphisagria were found to contain a relatively large amorphous fraction of alkaloids. By a combination of gradient pH extractions and chromatographic technique Pelletier and al. isolated Delphidine. Three new diterpenoids alkaloids and eight known alkaloids were isolated from the aerial parts of Delphinium staphisagria gathered in Morocco. Diterpenoid alkaloids, found in plants of the genera Aconitum and Delphinium, are of the C18, C19 and C20 diterpenoides types have been the targets of considerable interest of medicinal chemists for a broad range of demonstrated pharmacological properties: arrhythmogenic (neurocardiotoxic), local anesthetic, antiarrhythmic, curariform, analgesic, hypotensive, anti-inflammatory, spasmylytic, neurotropic and psychotropic.

In this study we report for the first time the analgesic activity of Delphinium staphisagria seeds extracts, the selection of plants for this study was based on ethnobotanical data and on their traditional use in the treatment of inflammations, this study may justifies its use in traditional medicine.

MATERIALS AND METHODS:

Plant materials: Seeds of Delphinium staphisagria were collected based on ethnopharmacological information, from villages around the region Chefchaoun, northern Morocco on September 2010, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (N° RAB 65077) was deposited in the herbarium of botany department of scientific institute of Rabat.

Preparation of extracts

Ethanolic extract

Seeds of Delphinium Staphisagria were extracted with 80% Ethanol by maceration at room temperature (25°C) over the period of 24 hours. 250 g of seeds material and one liter of 80% Ethanol were used in the extraction. Ethanol, containing the extract, was then filtered through Whatman paper and the solvent was vacuum distilled at 50°C in rotary evaporator. Final extract was a dark green semi-solid in percentage dry weight of 5.58%.

Alkaloidic extract

The ethanolic extract was treated with 0.5 M H2SO4 and filtered. The acid solution was extracted with CHCl3 to give a crude material (6.44g). Acid aqueous phase was neutralized to pH 7 and
extracted with CHCl$_3$ to give a crude materiel (5.36g). Neutral aqueous phase was basified with 20% NaOH to pH 12 and extracted with CHCl$_3$ to give a crude alkaloidic material (1.45g). Ethanolic and Alkaloid extracts were kept in deep freezer at –20°C until use$^{20}$.

**Animals**

Female Swiss mice (20-30g) (Offa-credo, France) were used in the acute toxicity from estimation of the LD$_{50}$; The Analgesic activity was done using male Wistar rats (180-200g) and Swiss mice. The animals were acquired from the animal centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty-Rabat. All animals had free access to food and water; they were housed under standard environmental conditions on a 12/12h light/dark cycle. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509.

**Acute toxicity**

LD$_{50}$ values were determined as described by OECD 423$^{22, 23, 24}$. The test consists on a stepwise procedure with the use of three female mice per step. The method permits estimation of an LD$_{50}$ with a confidence interval and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS)$^{25}$. After a single dose administration, mice were placed in individual clear plastic cages and all animals were observed for possible mortality cases (24h) and behavioural changes followed by daily weight monitoring for 14 days.

**Analgesic activity**

The evaluation of the analgesic activity of the ethanolic and Alkaloidic extracts obtained from *Delphinium staphisagria* seeds was carried out by using two different methods that used thermal stimuli (Tail Flick test), and chemical stimuli (Koster test)$^{26, 27}$.

**Acetic acid-induced writhing response in mice**

A chemical visceral pain model used in this test has been described by Koster. Swiss Offa-credo mice were selected one day prior to each test and were divided into groups of six mice each. The total number of cramps following intraperitoneal administration of acetic acid solution (3% with 300 mg /kg i.p) was recorded over a period of 20 min, starting 5 min after acetic acid injection, the mice were treated with ethanolic extract of seeds of Delphinium staphisagria (20 and 40 mg/kg, p.o); alkaloidic extract (10 and 20mg/kg, p.o) or standard drug (aspirin, 200 mg/kg, p.o), 30 min before administration of acetic acid, the number of cramps and stretching was recorded and permitted to express the percentage of protection using the following ratio (control mean-treated mean) x 100/control mean.
Tail flick test

The nociception was assessed by using a meter LE 7106 Tail-flick PANLAB (part of the Harvard Bioscience Family Spain), the tail flick test response was done by measuring the time taken to withdraw the tail from the heat source. Each rat was encased in a small aluminum chamber with the middle portion of the tail placed over the light Bern of the tail flick apparatus. A maximum cut-off time of 12 seconds was observed to minimize undue tissue damage as a result of over exposure of the tail to heat\textsuperscript{39, 40}. Morphine (5mg/kg s.c), was used as positive control and \textit{Delphinium staphisagria} ethanolic extract was administrated (20, 40 mg/kg; p.o.), and alkaloid extract (10, 20 mg/kg; p.o.) The reading was taken 15, 30, 60 and 120 min after administration of ethanolic extract (20, 40mg/kg; p.o.) and alkaloid extract (10, 20mg/kg; p.o.) of \textit{Delphinium staphisagria} seeds to different groups of six animals.

Statistical analysis

The results were reported as mean±S.E.M., and analyzed by one-way ANOVA followed by student’s t-test used for statistical evaluation. A value of p< 0.05 was considered significant.

RESULTS

Acute toxicity

During the 14 days, the evolution of the weight was established. Abdominal contraction was observed by 20-25min after the oral administration of \textit{Delphinium staphisagria} seeds ethanolic and alkaloidic extracts. All the animals treated with this extract presented feebleness, hypothermia and insufficiency respiratory. The LD\textsubscript{50} value was ranged from 200 to 300mg/kg. These results showed that, the ethanolic extract was evaluated as less toxic extract for seeds of \textit{Delphinium staphisagria} (LD\textsubscript{50}=300 mg/kg). However, the alkaloid extract to be the most toxic extract with LD50 equate to 200 mg/kg. Under the system of global harmonization of chemicals (GHS), these products are classified category 3 and 4, respectively, due to the LD\textsubscript{50} was lower than 2000mg/kg (Table 1).

Table. 1 LD\textsubscript{50} (mg/kg) and extraction yields (%) of \textit{Delphinium staphisagria} seeds ethanolic and alkaloidic extracts

<table>
<thead>
<tr>
<th>Phytochemical analysis</th>
<th>Ethanolic extract</th>
<th>Acid extract</th>
<th>Alkaloidic extract</th>
<th>Alkaloidic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delphinine</td>
<td>Delphinine</td>
<td>19-oxodihydroatisine, Isoazitine Azitine</td>
<td>14-acetylneoline; Chasmanine 14-acetylchasmanine Neoline</td>
</tr>
<tr>
<td>LD\textsubscript{50} (mg/kg; p.o.)</td>
<td>300</td>
<td>300</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Extraction yields (%)</td>
<td>5.58</td>
<td>2.57</td>
<td>2.14</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Mice of each group (n=3) were received single dose and they were examined for 14 days to determine LD\textsubscript{50} and any behavioral changes. p.o.: mean oral route.
Acetic acid-induced writhing in mice
The inhibition percentages of writhing for extracts are shown in Table 2. The reference drug Aspirin inhibited 51.81% of the number of writhing elicited by acetic acid. The ethanolic extract (40 mg/kg; p.o.) of Delphinium staphisagria seeds restrained the writhing reflex induced by acetic acid with an inhibition percentage of 50.17%. The alkaloidal extract (20mg/kg; p.o.) possess the highest analgesic properties with an inhibition percentage of 69.3%. The lowest activity was observed for ethanolic extract (20mg/kg; p.o.) (Table 2).

Table 2 The effect of Delphinium staphisagria ethanolic extract (EE) and Alkaloidic extracts (AE) on acetic acid induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment response</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Number of writhing (per 20 min)</th>
<th>Inhibition of writhing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>50.50 ± 2.81</td>
<td></td>
</tr>
<tr>
<td>Aspirine</td>
<td>200mg/kg</td>
<td>24.33 ± 2.87</td>
<td>51.82%</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>20mg/kg</td>
<td>31.50 ± 3.08</td>
<td>37.62%</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>40mg/kg</td>
<td>25.16 ± 3.06</td>
<td>50.17%</td>
</tr>
<tr>
<td>Alkaloidic extract</td>
<td>10mg/kg</td>
<td>21.66 ± 2.42</td>
<td>57.10%</td>
</tr>
<tr>
<td>Alkaloidic extract</td>
<td>20mg/kg</td>
<td>15.50 ± 1.51</td>
<td>69.3%</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M.; n= 6 per group; P< 0.001 versus the control group; n: mean number of animals; p.o.: mean oral, EE: ethanolic extract, AE: Alkaloidic extract.

Effect of ethanolic and alkaloidic extracts on Tail Flick test
As shown in figure 1, it can be clearly observed that morphine produced a significant increase (p<0.001) in the response time in the Tail flick experiment. The ethanolic (40 mg/kg, p.o.) and the alkaloidic extract (20 mg/kg, p.o.) significant (p<0.001) increased the reaction time respectively (6.46 ± 0.17); (7.50 sec ±0.22 sec) at 45 min in the Tail flick experiment which comparable with morphine (5 mg/kg, s.c.), the reaction time was (7.59 ±0.10sec) (p<0.001) (Fig.1).

Fig.1 Central Analgesic activity of ethanolic and alkaloidic extracts of Delphinium staphisagria by Tail Flick test. Data are expressed as mean ± S.E.M., (n = 6) P<0.001, statistically significant relative to control at 45 min.
DISCUSSION

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answer to their health problems in the plant environment\(^1\), \(^2\), \(^4\). Recently, the search for novel pharmacotherapy from medicinal plants for inflammation diseases has progressed significantly owing to their less side effects and better tolerability. Aromatherapy is currently used worldwide in the management of chronic pain\(^3\), \(^4\). We analyzed the effect of different doses of ethanolic extract and alkaloidic from Delphinium staphisagria seeds for their acute toxicity and analgesic activities. The LD\(_{50}\) value was ranged from 200 to 300mg/kg. These results showed that, the ethanolic extract was evaluated as less toxic extract for seeds of Delphinium staphisagria (LD\(_{50}\)=300 mg/kg). However, the alkaloid extract to be the most toxic extract with LD50 equate to 200 mg/kg. Preliminary phytochemical analysis of the ethanolic extract revealed the presence of flavonoides, alkaloids and dianthramides glycosides\(^20\), \(^28\), \(^29\). The alkaloidic is the most toxic, so seeds toxicity is in relation with the presence of alkaloids\(^26\).

The analgesic activities were evaluated using two laboratory models. The Tail Flick test was selected to investigate the central analgesic activity. Acetic acid-induced writhing response was selected to evaluate the peripheral analgesic effects.

In the acetic acid induced writhing test, all tested doses reduced significantly the number of writhing, as ethanolic as alkaloidic extracts demonstrated a significant analgesic effects, with an inhibition percentage of 37.62 %, 50.17 % at the doses 20 mg/kg and 40 mg/kg, respectively, for ethanolic extract, and with an inhibition percentage of 57.10%, 69.3% at the doses 10mg/kg and 20mg/kg respectively, for alkaloidic extract, as compared to the control group. Related studies have demonstrated that acetic acid indirectly induces the release of endogenous mediators of pain (such as prostaglandin, kinin, histamine….etc), that stimulate the nociceptive neurons, which are sensitive to non-steroidal anti-inflammatory drugs and opioïds\(^31\). The results obtained in this current study suggest that, the extracts for Delphinium staphisagria seeds possess peripheral analgesic properties; this particular activity is probably linked to their anti-inflammatory effects\(^30\).

In the Tail Flick test, the central analgesic effects were observed (Fig. 1), indicating that the ethanolic extracts of Delphinium staphisagria at the dose 20 mg/kg, don’t exhibit significant analgesic activity when compared with control and morphine treated animal. By increasing the doses to (40 mg/kg, 20mg/kg; p.o.) the ethanolic and alkaloidic extracts of Delphinium staphisagria produces significant (\(p<0.001\)) central analgesic action, because this extract increase the reflex time of removal the tail of rats by inhibition the pain respectively (6.46 sec±0.17) (7.50 sec ±0.18) at 45 min, these results were compared with morphine, (7.65 sec ±0.26) at the same time.

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These observations suggest that, the ethanolic and alkaloidic extracts of *Delphinium staphisagria* have a significant inhibitory activity in inflammation pain, and this activity may be related with the suppression of synthesis and/or release of endogenous pro-inflammatory substances. Alkaloids are commonly found to have analgesic activities\(^{30-33}\) are detected in the ethanolic seeds extract are known to have analgesic activity. The result obtained in this work demonstrated a high activity at low alkaloids extracts doses (10 and 20 mg/kg). However, alkaloids detected in the seeds extracts can be solely responsible for the analgesic activity. With those analgesic property alkaloids of *D. staphisagria* seeds can be considered an effective agent to treat diseases. As analgesics, this could be beneficial in searching for the potential analgesics that is equal or more active, but with lower toxicity, than currently clinical used C18- and C19-alkaloid- type analgesics\(^{30, 32}\). These chemical compositions were also identified in others plants extracts such as, *Stylosanthes fruticosa*\(^{33}\), *Pistacia integerrim*\(^{38, 39}\), *Hedyotis puberula*\(^{35-37}\)and *Argania spinosa*\(^{38}\), that have been provided to possess analgesic activity. Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the analgesic effect.

**CONCLUSION**

We conclude that, the alkaloidic extract of *Delphinium staphisagria* is a notable, central, and peripheral analgesic activity, these data provide pharmacological basis for its therapeutic efficacy on pains.

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**REFERENCES**


