Phytochemical and Pharmaco-Toxicological Assessment of Hydro Ethanolic Extract of *Taverniera aegyptiaca* Boiss

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INTRODUCTION

Although there are number of drugs (e.g. diclofenac and ibuprofen) that have been used as anti-inflammatory and analgesic, most of these products caused several side effects including dyspepsia and gastrointestinal complications ranging from unspecified symptoms like nausea, vomiting and diarrhea to severe complications like ulcer, bleeding and perforation. Thus, there are continues search for potent and less toxic anti-inflammatory and analgesic drugs. Plant extracts have been the most attractive sources for a long time due to its safety and various bioactive contents. Many recent studies reported medicinal plants and their extracts, fractions and isolates with anti-inflammatory, analgesic and antiulcer properties. Research into medicinal plants having pain-relieving action and anti-inflammatory properties is a logical research strategy in the search for new analgesic and anti-inflammatory drugs. The Fabaceae (Luguminosae) family is a rich source of many important compounds. Research into soybean metabolites has mainly focused on flavonoids, because this particular class of compounds has been strongly associated with numerous health benefits. The Fabaceae family is the third largest family among the angiosperms after the Orchidaceae and Asteraceae, consisting of more than 700 genera and about 20000 species of trees, shrubs, vines, and herbs worldwide. It is the second largest family of medicinal plants, containing over 490 species used as traditional medicine. The genus *Taverniera* belongs to Fabaceae family and contains 15 species distributed in Egypt to India and northeast Africa. Only two species were recorded in Egypt: *T. aegyptiaca* Boiss and *T. lappacea* Forssk. *T. aegyptiaca* is a perennial shrubby plant grows naturally in Delta, Red Sea and Sinai, and common in the Red Sea. It is edible by animals such as goats, sheep and camels. To the best of our knowledge, the aerial part of the plant species involved in this study is being screened for the first time.

ABSTRACT

*Taverniera aegyptiaca* Boiss is a wild plant is grown at the Red Sea coast, Egypt. Traditional uses of this species drive us to evaluate the phytochemical and pharmaco-toxicological aspects of the hydro ethanolic extract of the aerial parts of *T. aegyptiaca*. Phytochemical screening revealed the presence of various bioactive secondary metabolites as flavonoids, terpenes, glycosides, saponins and sterols compounds which might be responsible for their medicinal attributes. Alkaloids and tannins were not detected. The safety of ethanolic extract of *T. aegyptiaca* is evidenced by the high LD₅₀ value of the extract (>5g/kg). In addition, there wasn’t significant modification in the general behavior of the animals and deaths after 72 hours post-administration. Oral administration of 500 mg kg⁻¹ hydro ethanolic extract of *T. aegyptiaca*, significantly inhibited the nociception to acetic acid induced writhes and increases in the latency to response of tail to thermal stimulation. Furthermore, pretreatment of rats with *T. aegyptiaca* extract reduced the ulcer index and produced protection in ethanol induced ulceration model. The extract induced all pharmacological effects in a dose dependent manner. These findings suggested that *T. aegyptiaca* can be used as a promising source of new antinociceptive anti-inflammatory and anti-ulcerogenic agent.

Keywords: *Taverniera aegyptiaca* Boiss, Phytochemical, antinociceptive, anti-inflammatory, anti-ulcerogenic
substitute for Yashtimadhu (G. glabra Linn.) in the management of Amlapitta disease (hyper acidity) in India\textsuperscript{19}. The goal of the current study focuses on preliminary phytochemical and pharmacological (analgesic, anti-inflammatory and antiulcer) screening of the hydro ethanolic extract of the aerial parts of \textit{T. aegyptiaca} growing wild in the Red Sea coasts, Egypt.

**MATERIAL AND METHODS**

**Plant material**

In this study, we used the aerial part of \textit{T. aegyptiaca} Boiss. This plant has a common local name; Dahasir and no synonyms are recorded for this plant. It grows wild in the Red Sea coast from the side of Gulf of Suez (Qusair – MarsaAlam road, approximately 47 km- Qusair), Egypt. The plant was collected in May 2013 and was identified by Dr. Attia, Desert Research Center, Egypt. A voucher specimen for the plant has been deposited at the Herbarium of Desert Research Center, Ministry of Agriculture, Egypt.

**Preparation of plant extract**

The aerial parts of \textit{T. aegyptiaca} Boiss plant were shade dried then grinded. The powder (500 g) from the plant was submitted to exhaustive maceration utilizing 70% ethanol in a water bath at 40°C (3 x 2L, each 48h). The hydro alcoholic solutions obtained were combined together then concentrated in a rotary evaporator at 40°C under reduced pressure till dryness, resulting in a crude hydro ethanolic extract was 34.5 g. The extract was stored at 4°C for the biological investigation.

**Animals**

Wistar albino rats (220-250 g) and Swiss male mice (20–22 g) were purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt. Animals were maintained in the Animal House of Pharmacology Department, Faculty of Veterinary Medicine, Cairo University under controlled hygienic conditions. The animals were maintained under controlled conditions of temperature (23 ± 20°C), humidity (50 ± 5%) and 12 hours light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. Animals were fed on locally manufactured pellets and water was provided \textit{ad libitum}. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Faculty of Veterinary Medicine, Cairo University.

**Acute Toxicity study**

Lorke (1983)\textsuperscript{16} method was used to determine the acute toxicity of the hydro ethanolic extract of \textit{T. aegyptiaca}. Three groups of 5 mice each were administered 100, 500 and 1000 mg/kg body weight (b.wt.) of the extract orally. The mice were observed for 24 h for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another four groups of 5 mice each were administered 2000, 3000, 4000 and 5000 mg/kg of both extracts orally. The animals were observed for 72 h and the signs of toxicity such as behavioral changes, locomotion, convulsion, and number of mortality in each group within the period were recorded. The LD\textsubscript{50} values were then calculated as the geometric mean of the highest non-lethal and the lowest lethal doses mathematically according to Kerber method (Pershin, 1971)\textsuperscript{17} using the following formula: \(LD_{50} = LD_{100} \Sigma (z \times d/m)\)

where \(z\) is a half of sum of animal quantity died from two next doses; \(d\) is the interval between two next doses and \(m\) is the number of animals/group.

**Evaluation of the antinociceptive effect**

**Acetic acid induced writhing test (Peripheral analgesic activity)**

The peripheral of the hydro ethanolic extract of \textit{T. aegyptiaca} was measured by the acetic acid induced writhing test as described earlier\textsuperscript{18}. Mice were fasted for 24 h with water given \textit{ad libitum}. At the beginning of the experiment, mice were treated orally with either 2% Tween-80, hydro ethanolic extract of \textit{T. aegyptiaca} (250 or 500 mg kg\textsuperscript{b.wt.}) or diclofenac sodium (50 mg kg\textsuperscript{b.wt.}). One hour later, animals were injected intraperitoneally with acetic acid (0.7%) at a dose of 0.1 mL/10g of kg\textsuperscript{b.wt.} was used to create typical stretching response. Animals were then placed in an observation box. The total number of writhes (abdominal constrictions) was counted under a double blind observation for 10 min, 10 min after the application of acetic acid.

**Radiant heat tail-flick method (Central analgesic activity)**

The central analgesic activity of the hydro ethanolic extract of \textit{T. aegyptiaca} was studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails as described by Janssen \textit{et al.} (1963)\textsuperscript{19}. Mice were fasted for 24 h with water given \textit{ad libitum} and were pretreated orally with either 2% Tween-80, hydro ethanolic extract of \textit{T. aegyptiaca} (250 or 500 mg kg\textsuperscript{b.wt.}), or diclofenac sodium (50 mg kg\textsuperscript{b.wt.}). After 30 min, 1–2 cm of the tail of mice was immersed in water bath kept constant at 55°C. The time taken by the mice to deflect their tails was recorded as the reaction time. The cut-off reaction time was fixed at 10 second to avoid any tissue damage.

**Evaluation of the Anti-inflammatory effect**

In this experiment, formaldehyde-induced rat hind paw edema was used as the animal model of acute inflammation (Saha \textit{et al.}, 2007)\textsuperscript{18}. Briefly, acute inflammation was produced by subplantar injection of 0.2 ml formaldehyde (1%,w/v) into the rat hind paw, in the right hind paw of the rats 1h after the oral administration of tested materials. The paw volume was measured by plethysmometer (Ugo Basile, Italy) at 1, 2, 3, and 4 h after the formaldehyde injection. The hydro ethanolic extract of \textit{T. aegyptiaca} was administered at 250 and 500 mg kg\textsuperscript{b.wt.} Diclofenac sodium (50 mg kg\textsuperscript{b.wt.}) was used as standard anti-inflammatory agent. The inhibition of inflammation was calculated using the formula, % inhibition = \(100 \times (V_{C} - V_t)/V_{C}\), Where ‘\(V_{C}\)’ represents edema volume in control and ‘\(V_t\)’ edema volume in group treated with test extracts.

**Evaluation of the anti-ulcerogenic effect**

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Induction of ulcer experimentally with ethanol in rats was employed to evaluate the antiulcer activity of the hydroethanolic extract of *T. aegyptiaca*. All the rats used were fasted for eighteen hours but were given water *ad libitum* till the start of the experiment.

**Ethanol-induced gastric ulceration**

Male adult albino rats were used for the experiment. They were randomized into six groups of five rats each. Food was withdrawn 24 h and water 2 h before the commencement of experiment. Group 1 (control) received equal volume of only saline instead of plant extracts, Groups 2, 3, were pretreated with the hydroethanolic extract of *T. aegyptiaca* orally at 250 and 500 mg kg\(^{-1}\) b.wt.; Group 4 received ranitidine (60 mg/kg b.wt. dissolved in distilled water). One hour later, all groups were administered with ethanol 50% in a dose of 10 ml/kg. One hour after ethanol administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored.

Lesions in the glandular part of stomach were measured under illuminated magnifying microscope (10x). Long lesions were counted and measured along greater length. Petechial lesions were counted with the aid of 1-mm squares grid. Each five petechial lesions were taken as 1 mm ulcer. The sum of total length of long ulcers and petechial lesions in each group of rats were divided by its number to calculate the ulcer index (mm). The curative ratio was determined by the following formula: Curative ratio = (control ulcer index-test ulcer index) / (control ulcer index) X 100

**Preliminary phytochemical screening**

The extract was screened for the presence of unsaturated sterols & triterpenes, carbohydrates & glycosides, flavonoids, saponins, tannins and alkaloids with thin layer chromatography (TLC) as described by Stahl (1969). Analytical precoated Silica Gel 60 F254 plates from Merck were used. Elution and development was carried out with different solvent systems: ethyl acetate-methanol-water (100:13.5:10, v/v/v), ethyl acetate-formic acid-glacial acetic acid-water (64:32:12:8, v/v/v/v), chloroform-glacial acetic acid-methanol-water (100:11:11:27, v/v/v/v), chloroform-methanol-water (64:50:10, v/v/v), benzene-ethyl acetate (86:14, v/v) and toluene-ethyl acetate (93:7, v/v). After development in the solvents the plates were dried and sprayed with Dragendorff’s, AlCl\(_3\), and antimony trichloride for the discovery of alkaloids, flavonoids, and unsaturated sterols & triterpenes. Detection of saponins, tannins and carbohydrate & glycosides is carried out using anisaldehyde–sulphuric acid, ferric chloride and naphthoresorcinol reagents, respectively by (Wagner *et al.*, 1983)\(^2\). Detection was carried out by visualization in visible light and under UV light (\(λ\): 366 nm).

**Statistical analysis**

Results are expressed as mean ± standard error (S.E.) of six observations. Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA). P-values of 0.05 and 0.001 were significant.

**RESULTS AND DISCUSSION**

The safety of the extract is evidenced by the high LD\(_{50}\) value of the extract (>5g/kg). In addition, there were no significant modification in the general behavior of the animals nor were there death after 72 hours at the highest administered dose (5g/kg) of the hydroethanolic extract of *T. aegyptiaca*. Studies carried out to access the safety of this plant extract using mice revealed a high margin of safety LD\(_{50}\) > 5 g/kg. The results of preliminary phytochemical screening of the aerial parts of *T. aegyptiaca* is shown in (Table 1) and revealed that the plant is rich with flavonoids and to lesser extent terpenes, glycosides, saponins and sterols compounds. In this respect, new triterpenoidal saponins of oleane type and 7 isoflavones and a flavanol together with the known oleanolic acid 3-beta-O-beta-glucoside were isolated and identified from *T. aegyptiaca*\(^6\). The identification of the isolated compounds was done on the basis of chemical and spectral evidences. Flavonoids possessed antioxidant activity because of their redox properties which let them act as reducing agents and quencher of singlet oxygen. Some studies have already claimed that flavonoids also possessed anti-inflammatory action\(^7\). Therefore, both anti-inflammatory and antioxidant effect could be supposed either as the protective action against any oxidative stress or inhibition of enzymes (e.g., cyclooxygenase) of prostaglandin pathway of inflammatory process\(^8\). Oral administration of 500 mg kg\(^{-1}\) hydroethanolic extract of *T. aegyptiaca*, significantly (\(P < 0.001\)) inhibited the nociception to acetic acid-induced writhes. The protection percent was 42.10 of the control mice. This value was compared to 58.70% protection for the standard diclofenac sodium (50 mg kg\(^{-1}\)). The smaller dose (250 mg kg\(^{-1}\)) of *T. aegyptiaca* induced 19.43% protection (Table 2). Oral administration of 500 mg kg\(^{-1}\) hydroethanolic extract of *T. aegyptiaca* significantly (\(P < 0.05\)) increases in the latency to response of tail to thermal stimulation. The protection percent was 46.79 of the control mice. This value was compared to 59.02% protection for the standard diclofenac sodium (50 mg kg\(^{-1}\)). The smaller dose (250 mg kg\(^{-1}\)) of *T. aegyptiaca* induced 20% protection (Table 3). It is clear that the anti-nociceptive of *T. aegyptiaca* was potent. In the present study, two animal models for investigation of the

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th><em>Taverniera aegyptiaca</em> Bioass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsaturated sterols and/or</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates and/or</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
</tr>
</tbody>
</table>

\((++): \text{rich } (+): \text{present } (-): \text{absent } (\pm) \text{ traces}\)
Inflammation was significantly (p<0.001) reduced in all control group observed at 4 h after formaldehyde administration to the increase in paw thickness where the maximal increase was caused a time dependent increase in paw thickness where the maximal increase was observed at 4 h after formaldehyde administration to the control group (Table 4). However, formaldehyde induced inflammation was significantly (p<0.001) reduced in all phases of the experiment for treatment with *T. aegyptiaca* extract at 500 mg kg\(^{-1}\) and reference anti-inflammatory drug diclofenac sodium. The oral administration of *T. aegyptiaca* extract at 500 mg kg\(^{-1}\) caused maximum inhibition of 42.65 % that was nearly close to diclofenac sodium (47.06 %) at a dose of 50 mg/kg (Table 5). Also, it is clear that the anti-inflammatory activity of *T. aegyptiaca* extract was potent. The injection of formaldehyde into rat paw increases the release of an inflammatory mediator bradykinin, which causes paw edema. Prostaglandins cause hyperalgesia at lower doses and pain at higher doses. They produce edema by increasing the effects of histamine and bradykinin. It is known that formaldehyde-induced inflammation usually involves two distinct phases. It has been proposed that the early or first phase reflects the direct stimulation of nociceptors, while the later or second phase may be associated with inflammation mediators. Some studies have shown that substance P receptor antagonists inhibit the later phase of formaldehyde-induced edema, and substance P has a role in this response. *T. aegyptiaca* contains terpenes and flavonoids that have been proved to possess analgesic anti-inflammatory activities.

Table 2: The effect of the hydro ethanolic extract of *Taverniera aegyptiaca* on the number of acetic acid-induced writhes in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose in mg kg(^{-1})</th>
<th>Mean ± SE of writhes</th>
<th>protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>51.70 ± 3.41</td>
<td>19.43</td>
</tr>
<tr>
<td><em>T. aegyptiaca</em></td>
<td>250</td>
<td>39.80 ± 2.55*</td>
<td>42.10</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>28.60 ± 2.77**</td>
<td>58.70</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>50</td>
<td>20.40 ± 1.87**</td>
<td></td>
</tr>
</tbody>
</table>

\(^{*P<0.005} \quad ^{**P<0.001}\) as compared to control

Table 3: The effect of the hydro ethanolic extract of *Taverniera aegyptiaca* on the latency of the tail flick test in mice (n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose in mg kg(^{-1})</th>
<th>Mean ± SE of Time (seconds)</th>
<th>protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>3.27 ± 0.26</td>
<td></td>
</tr>
<tr>
<td><em>T. aegyptiaca</em></td>
<td>250</td>
<td>3.93 ± 0.29</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>4.80 ± 0.30*</td>
<td>46.79</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>50</td>
<td>5.20 ± 0.31**</td>
<td>59.02</td>
</tr>
</tbody>
</table>

\(^{*P<0.005} \quad ^{**P<0.001}\) as compared to control

Table 4: Mean ± S.E. of paw thickness of the hydro ethanolic extract of *Taverniera aegyptiaca* by formaldehyde induced rat paw edema (n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose in mg kg(^{-1})</th>
<th>Mean ± S.E. of paw thickness in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Pos control</td>
<td>250</td>
<td>0.52 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.48 ± 0.02*</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50</td>
<td>0.41 ± 0.02**</td>
</tr>
</tbody>
</table>

\(^{*P<0.005} \quad ^{**P<0.001}\) as compared to control positive

Table 5: Anti-inflammatory activity of the hydro ethanolic extract of *Taverniera aegyptiaca* by formaldehyde induced rat paw edema (n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose in mg kg(^{-1})</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td><em>T. aegyptiaca</em></td>
<td>250</td>
<td>10.34</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>17.24</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>50</td>
<td>29.31</td>
</tr>
</tbody>
</table>
Therefore, it can be suggested that the pharmacological effects of the extract may be due to their content of the preceding active constituents. Pretreatment of rats with *T. aegyptiaca* extract suspension at both doses reduced the ulcer index and produced protection in ethanol induced ulceration model. The protection percentage was statistically significant compared to standard drug ranitidine (Table 6). There was a significant (P<0.001) dose-dependent reduction in the ulcer indices relative to control. Administration of ethanol has been known to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa. It was observed in this study that the extract reduced significantly ethanol-induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion. Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting prostaglandin (PGF2α).

**Conclusion and recommendations**

In conclusion, according to the preliminary phytochemical and pharmacological screening results of the hydro ethanolic extracts of the aerial parts of *T. aegyptiaca* possess promising peripheral & central analgesic, anti-inflammatory and anti-ulcerogenic effects. Further investigations and more detailed phytochemical studies including isolation and structure elucidation of the active compounds are necessary to elucidate the exact mechanism of the anti-inflammatory activity of this extract.

**CONFLICTS OF INTEREST**

There is no conflict of interest to be declared.

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