EVALUATION OF ANALGESIC EFFECTS OF THESPESIA POPULNEA

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ABSTRACT
The objective of the present study was to investigate in vivo analgesic effects of thespesia populnea extract (TPE). The crude drug was screened for to identify the antinociceptive properties by reduction in the number of writhing’s and time difference in tail flick latency of mice. Methods: In chemical method acetic acid writhing test and in thermal method tail flick test were performed. The extract at all doses 100, 150 and 200 mg/kg/p.o inhibited the abdominal constrictions induced by acetic acid and also increased the pain threshold of mice towards the thermal source in a dose dependent manner. The standard drug was aspirin for the comparison of effects in each method.

Results: The result of chemical method (acetic acid writhing test) revealed that TPE significantly (p<0.01) reduced the acetic acid induced number of writhes (Control= 132 ± 2.50) in mice and in the tail-flick model TPE produced a marginal, increase in the tail flick latency as the results was statistically significant (p<0.05- 0.01).The herbal preparation exhibited inhibitory effect on acid induced abdominal contraction and tail flick method in both phases. Conclusion: On the basis of this study, it has been shown that thespesia populnea bark extract of TPE exhibited profound antinociceptive activity by central and peripheral mechanism(s).

KEY WORDS: Thespesia populnea, analgesic effects, tail flick test, writhing’s test.

INTRODUCTION
Thespesia populnea Soland ex Correa (Family: Malvaceae) is a large avenue tree found in the tropical regions and coastal forests in Subcontinent. The bark, leaves, flowers and fruits are useful in cutaneous infections, such as scabies, psoriasis, eczema, ringworm and guinea worm. Among several traditional claims, the usefulness of Thespesia populnea bark in
inflammation and pain has been emphasized more in literature\[1\]. Villagers have traditionally used the poultice prepared from the fruits of Thespesia populnea to treat a variety of skin ailments including wounds. The aqueous extract of Thespesia populnea fruit showed significant wound healing activity in the excision wound and incision wound models in rats following topical and oral administration, respectively \[2\]. The mechanism of the anti-inflammatory effect of Thespesia populnea extract in formaldehyde-induced edema in rats may depend on the neutralization of active globulins, which are non-steroidal anti-inflammatory \[3\]. TPE (200 and 400 mg/kg, p.o.) effectively suppressed the edema produced by the histamine and serotonin indicating that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz., histamine, serotonin and prostaglandin might be involved in inflammation\[4]\.

Antisteroidogenic activity of various extracts of Thespesia populnea was screened in female albino mice. The floral extract of Thespesia populnea exhibited antisteroidogenic activity in mouse ovary. The weight of the uterus and ovaries were reduced significantly and the cholesterol and ascorbic acid content in ovaries were significantly elevated due to the treatment with extract of Thespesia populnea \[5\]. Gossypol was found to be the major component of Thespesia populnea exerts significant anti implantation \[6\] and producing antifertility effects in rats \[7\] as well as in human beings \[8\]. Four naturally occurring quinines viz. thespone, mansonone-d, mansonone-H, thespone and thespesone have also been extracted from heartwood of Thespesia populnea \[9\]. Thespesia populnea possesses blood glucose lowering effect in normoglycemic and in alloxan induced hyperglycemic rabbits. The antidiabetic action of Thespesia populnea is probably due to enhanced insulin secretion or due to increase in peripheral glucose uptake. The results clearly indicated a significant antidiabetic activity of the fruit of Thespesia populnea and support the traditional usage of fruits by the Ayurvedic physicians for the control of diabetes \[10\]. Cost effective antimicrobial combination for multidrug resistant diseases based on the synergistic activity of oxytetracycline with methanolic extract of thespesia populnea (Malvaceae) a medicinal plant common in South India. The MIC of methanolic extracts in combination with oxytetracycline using 12 different both gram positive and gram negative bacteria was found to be around (62.5 μg/ml to 1000 μg/ml). The synergistic activity shows 83.3% effect against all 12 different bacteria both gram positive and gram negative species. The highest synergism rate was attained against Shigella boydii \[11\]. Thespesia populnea Leaf has therapeutic use in cough, headache, scabies, psoriasis, skin disease, dysentery, diabetes, gonorrhea, indigestion, ulcers, and worms. Amongst methanolic extracts, maximum antibacterial activity was shown
by Datura innoxia followed by Thespesia populnea [12]. Thespesia populnea barks flowers possess astringent, hepatoprotective and substantial antioxidative activity as determined by inhibition of lipid peroxidation level against carbon tetrachloride-induced liver injury in rats [13]. Ethanolic extract of Thespesia populnea has increased the volume of urine significantly at 400 mg/kg. The leaves possess diuretic effect, is justifiable [14]. Various parts of the Thespesia populnea plant have high tannin contents and plant extracts have been shown to have anti-bacterial and anti-viral activity [15].

MATERIAL AND METHODS

MATERIAL

Extract: Extract of Thespesia populnea bark obtained from the laboratory of pharmacognosy, university of Karachi. A brownish-black colored of solution, which was kept in a desiccator. This ethanolic extract of Thespesia populnea bark (TPE) was used in experiment.

Drugs: The entire drug used in this study was of pharmaceutical grade. Aspirin (acetylsalicylic acid), gum tragacanth and acetic acid.

Animals & Analgesic Activity

Albino mice of either sex weighing (25-30 gm) were bred in the animal house of university of Karachi, Karachi. They were kept in colony cages with raised mesh bottoms and allowed free access to food, standard laboratory mice chows and water. They were maintained in a temperature and light controlled room (25 ± 1°C, 12/12- hrs. light/dark cycle) at least 7 days before testing or administration of test or standard drugs. They were divided in different groups of five each per dose.

METHODS

Acetic acid-Induced Writhing Method: Analgesic activity was evaluated by the acetic acid-induced writhing method was that of Koster (17). When acetic acid is injected intraperitoneal it causes abdominal contractions or writhing, a syndrome characterized by a wave of contractions of the abdominal musculature followed by extension of hind limbs. Mice (25-30 gm.), five mice per dose and at least three doses of each drug, were employed in writhing assay. Drugs were administered orally 30 minutes prior to an intra-peritoneal injection 1% acetic acid in the dose of 10 ml/kg. Immediately after administering acetic acid, the number of writhing were counted and recorded for 20 minutes after acetic acid injection. Analgesic potency was taken as difference between a test group mean and its control.
Criterion of Analgesia
Reduction in the number of writhing’s as compared to the control group was considered as analgesia and expressed as percent inhibition of writhing. All results were compared to controls without drug treatment.

\[
\text{Inhibition (\%)} = \frac{\text{Mean Number of writhes (control)}}{\text{Mean Number of writhes (test)}} \times 100
\]

Statistical Analysis
Data are presented as the mean ± SEM writhing’s per group. significant difference means determined by Dunnett’s t-test. Values of p <0.05 were considered as significant and P< 0.01 as highly significant.

Tail Flick Method
In present study analgesia was assessed according to the method of Luiz (18). Mice (25-30 gm.), divided in seven groups having 5 mice in each and were held in a suitable container with the tail extend out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 30 seconds to avoid the injury of the tissues of tail. The test compound was administered as a suspension in 1% gum tragacanth. The control animals received only the vehicle, plant extracts in doses of 100, 150, and 200 mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and then 30, 60, 90 and 120 minutes after administration.

Analgesia Tail Flick Latency Difference (TFLD)
Analgesia was defined as a loss of sensitivity to noxious stimuli without a loss of conscious awareness of the environment or loss of the ability to respond. In experimental animals, two different types of noxious stimuli were used to induce pain such as thermal and chemical.

Criterion for Analgesia
The criterion for analgesia was post drug latency was two times greater than pre-drug average latency as reported by Janssen [19]. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.
Analgesia TFLD was calculated as follows.
Analgesia TFLD = signifies post-drug tail flick - latency pre-drug tail flick latency.

**Statistical Analysis**

Values for analgesic activity were expressed as “mean increase in latency after drug administration ± SEM (s) or tail flick latency difference” in terms of seconds and significance of difference between means determined by Dunnett’s t-test. Values of p <0.05 were considered as significant and P< 0.01 as highly significant. All statistical procedures were performed according to the method of Alcaraz (20).

**RESULTS**

**Effect of Acetic Acid Induced Writhing**

TPE significantly (p<0.01) reduced the acetic acid induced number of writhes (Control= 132 ± 2.50) in mice. The analgesic effect of the extract revealed maximum (67.52%) at a dose of 200 mg/kg, whereas at the dose of 100 mg/kg aspirin produced (70.0%) of the response, thus plant preparation showing the superior potency and efficacy.

**Effect of Tail Flick Method**

In the tail-flick model TPE produced a marginal, though statistically significant, increase in the tail flick latency. The tail withdrawal reflex time following administration of the extract of thespesia populnea extract was found to increase with increasing dose of the sample. The result was statistically significant (p<0.05-0.01) and was comparable to the reference drug Aspirin (Table. 2).

**Table 1: Analgesic Effect of Thespesia Populnea Extract on Acetic Acid Induced Writhing Response in Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>No. of writhes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 10mg/kg</td>
<td>----</td>
<td>132±2.50</td>
<td></td>
</tr>
<tr>
<td>Thespesia Populnea Extract (TPE)</td>
<td>100 mg</td>
<td>62.5 ± 0.87**</td>
<td>52.65%</td>
</tr>
<tr>
<td></td>
<td>150 mg</td>
<td>48±1.077**</td>
<td>63.63%</td>
</tr>
<tr>
<td></td>
<td>200 mg</td>
<td>43±0.74**</td>
<td>67.52%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100mg</td>
<td>40±0.27***</td>
<td>70.0%</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of five mice. Symbol represent statistical significance, *p<0.05, **p<0.01, (n =5) as compared with the control group.
Table 2: Analgesic Effect of Thespesia Populnea Extract In Mice Tail Immersion Method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose/kg Orally</th>
<th>Analgesia TFLD or mean increase in latency after drug administration + SEM (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+30</td>
</tr>
<tr>
<td>Control</td>
<td>-------</td>
<td>1.72±0.07</td>
</tr>
<tr>
<td>Thespesia Populnea</td>
<td>100mg</td>
<td>0.81±0.09*</td>
</tr>
<tr>
<td>Extract (TPE)</td>
<td>150mg</td>
<td>1.74±0.19**</td>
</tr>
<tr>
<td></td>
<td>200 mg</td>
<td>2.34±0.14**</td>
</tr>
<tr>
<td>Aspirin</td>
<td>150 mg</td>
<td>2.71±0.12*</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM of five mice. Symbol represent statistical significance, *p<0.05, **p<0.01, (n =5) as compared with the control group.

Acute Oral Toxicity Test

Thespesia populnea extract did not produce any mortality. All the doses (100, 150 and 200 mg/kg, p.o.) of TPE were thus found to be non-toxic. Three doses (100, 200 and 400 mg/kg, p.o.) of TPE were selected for pharmacological studies.

DISCUSSION

In the present study, the ethanolic extract of thesperia popluea bark was investigated for their analgesic activity by the abdominal writhing and the tail-flick methods. These tests are very common and perfectly in results for the evaluation of analgesic effects of drugs/crude extracts in rodents. The abdominal constriction response induced by glacial acetic acid is a sensitive procedure and the writhing test allows us to identify central and peripheral analgesic compounds. The thermal model of the tail flick test is considered to be a spinal reflex, but could also involve higher neural structures and this method identifies mainly central analgesic effects.[21] The effectiveness of analgesic agents in the tail flick pain model is highly correlated with relief of human pain.[22] We used the tail-flick and the writhing method to assess the analgesic activity. The ethanolic extract of T. populnea possessed a significant analgesic activity by increase of delayed onset of writhing and decrease of the number of writhes as compared with the control group. In acetic acid induced writhing model, compounds with percentage analgesia of 70% are considered to have minimal analgesic activity.[23] Percentage analgesia by T. populnea extract was nearly of 70%, and it was only more than 70% in the aspirin treated animals (Table 1). The extract doses at (100, 150 and 200 mg/kg), administered orally, significantly inhibit acetic acid induced writhing in mice. There writhing are related to increase in the peritoneal level of prostaglandins and leukotrienes.[24] The result
strongly suggests that the mechanism of action of extract may be linked to lipooxygenase, cyclooxygenase and/or endogenous substances including serotonin, histamine, PGs, bradykinin and substance P which also stimulate pain nerve endings \[25\]. The analgesic effect of any plant extract may therefore be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful message \[26\].

The T. Populnea extract preferably 200 mg/kg dose showed significant activity after the 120 minute and the data (Table 2) suggest that the extract dose-dependently increased pain threshold. However, the pain threshold tail flick latency was less than the effects of aspirin (standard drug). Phytochemical investigation reveals that ethyl alcohol extract contains carbohydrates, steroids, alkaloids, terpenoids, flavanoids, tannins, polyphenols while aqueous extract contains carbohydrates, alkaloids, flavanoids, tannins, poly phenols \[27\]. The analgesic effects of the tespesia poplunea extract is due to of these classes of chemical namely flavonoids such as quercetin are known to be effective in acute inflammation \[28\]. There are also reports on the analgesics effects of alkaloids, essential oils and saponins \[29\]. There are also few reports on the role of tannins in antinociceptive and anti-inflammatory activities \[30\].

CONCLUSION
In conclusion, the results of present study demonstrate that T. populnea preparation possesses profound analgesic activity. The herbal preparation exhibited inhibitory effect on acetic acid induced abdominal contraction and tail flick method.

Apparently analgesic effect of T. poplunea preparation is probably mediated via inhibition of the prostaglandin synthesis whereas central inhibitory mechanisms cannot be ruled out. These results also validate the traditional use of the plant as analgesic and other conditions associated with pain such as trauma, burns, rheumatism and neuralgia.

During the present work, it was discovered that compound possesses significant pharmacological activity and hence work should be extended to study toxicological effects of the compound.

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REFERENCES


