Anti-inflammatory, anti-pyretic and analgesic activities of
*Tamarix dioica*

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**Abstract:** Medicinal and aromatic plants contribute to major portion of the flora. The plant materials obtained from these plants are used in the pharmaceuticals, cosmetics, and drug industries. *Tamarix dioica* is locally used in the management of splenic and hepatic inflammation as well as diuretic and carminative. It also possesses cytotoxic, antimicrobial, antifungal and anti-inflammatory activity. The present study investigates the anti-inflammatory, anti-pyretic and analgesic activities of the crude methanolic extract from *Tamarix dioica*. Anti-inflammatory activity was measured by Carrageenan Induced Paw Edema and Xylene Induced Ear Edema methods. Pyrexia induction with Brewer’s yeast assay was used to determine antipyretic activity and analgesic activity was estimated by acetic acid induced writhing and hot plate methods. The data indicated that anti-inflammatory, anti-pyretic and analgesic activities of the crude methanolic extract from *Tamarix dioica* was dose and time dependent when measured by different assays. Exposure of model animal to increasing concentrations of the plant extract for longer period increased their anti-inflammatory, anti-pyretic and analgesic activities. Significantly highest anti-inflammatory, anti-pyretic and analgesic activities were noted at highest doses of the crude methanolic extract for longer exposure compared with their respective controls.

**Keywords:** Anti-inflammatory, anti-pyretic, analgesic, *Tamarix dioica*

**INTRODUCTION**

Medicinal and aromatic plants contribute to major portion of the flora. The plant materials obtained from these plants are used in the pharmaceuticals, cosmetics, and drug industries. Approximately 20% of the world flora has been tested for their pharmacological or biological activities (Suffredini et al., 2004). It is estimated that 80% of the population in developing countries relies on traditional plant based medicines for their health requirements (WHO, 1991). Even many of the modern medicines are based on raw materials obtained from medicinal plants due to their least side effects, low prices, and lasting curative property. Extracts of many plants possesses potent antimicrobial activities (Madiha et al., 2018; Khaleeq et al., 2018). Different medicinal plants extracts, infusions, decoctions, powders are used to treat different illness in humans, animals and plants (Chaun et al., 2015).

*Tamarix dioica* Roxb. locally known as ghaz or jhau or khagal belongs to the family tamaricaceae. It is an evergreen shrub or small tree about 6 meters having reddish bark. Flowers are about 3 mm in diameter. These flowers are unisexual, pink or purple colored. The spikes are cylindrical in shape and closely or compactly arranged. Fruit is a capsule shaped about up to 5mm in length, cone like in shape and consists of 3 valves (Khan et al., 2013). *Tamarix dioica* is locally used in management of splenic and hepatic inflammation, also used as diuretic and carminative. The leaves of *Tamarix dioica* possess cytotoxic, antimicrobial and antifungal activity in their crude extracts (Khan et al., 2013). The plant of this specie is also used as an astringent for symptoms such as leucorrhea (Samejo et al., 2013). *Tamarix dioica* also possess anti-inflammatory activity of Jigrine (Karunakar et al., 1997). Different parts of the plant have been investigated for chemical constituents analysis of the data indicated that various phytochemicals like tannins, phlobatannins, flavonoids, phenols, steroids, saponins and terpenoids were found present, while glycosides, alkaloids, amino acid and protein were absent (Samejo et al., 2013a). From the leaves, many other agents have also been obtained such as D-mannitol, Kaempferide and tamarixetin (Rastogi et al., 1990). Keeping in view the medicinal importance of *Tamarix dioica*, the present research project was initiated to study in vivo anti-inflammatory, anti-pyretic and analgesic activities of the crude methanolic extract.

**MATERIALS AND METHODS**

**Plant material**

*Tamarix dioica* plants (about 8kg) were collected at flowering stage from different areas of district Mardan, Khyber Pukhunkhwa Pakistan. The plant specimen was identified by plant taxonomist, allotted voucher number of
AI-002-ICP and deposited in the Herbarium of Botany Department, Islamia College Peshawar, KPK Pakistan.

**Crude extraction**
The collected plant part identified and (stem bark) was shade dried for three weeks at room temperature. The dried stem bark was processed with electric grinder for powder formation. Six gm powder was soaked in analytical grade methanol (4.3L) for 15 days at room temperature with occasional shaking. After filtration the methanol soluble residue was concentrated with rotary evaporator at 40°C.

**Experimental animals**
The present research project was approved by the Institutional Ethics Committee. During pharmacological experiments, mice of either sex were obtained from the Department of Pharmacy, University of Peshawar. To keep these animals healthy, recommended guidelines were followed throughout the experiments (Muhammad et al., 2008).

**Anti-inflammatory activity**
Crude methanolic extract was subjected for evaluating anti-inflammatory activity by two different methods (Carrageenan Induced Paw Edema and Xylene Induced Ear Edema Methods).

**Carrageenan induced paw edema model**
Tested animals (mice) of either sex (25-30g body weights) were used for this assay. The animals were distributed into 5 different groups. In every group, there were 6 animals (n=6). Two groups (I & II) were selected as negative and positive control respectively.

Group I was administered with normal saline by the dose of 10ml kg⁻¹ (body weight). Indomethacin (standard drug) was administered at a dose of 10mg/kg (body weight) to another group (group II). Remaining groups such as group III, IV and V were subjected with crude extract with doses of 50, 100 and 200mg/kg (body weight). Carrageen (1%) was administered in sub planter tissue of the hind paw (right) of every animal (mouse), 30 minutes after, of the above mentioned treatments. Anti-inflammatory potential was recorded for 5 hrs (0, 1, 2, 3, 4 and 5 hours by using Plethysmometer (LE 7500 plan lab S.L) after the administration of Carrageen an (Khan et al., 2009).

The % inhibition of edema was measured with the help of formula given below

\[
\text{% Inhibition} = \frac{A - B}{B} \times 100
\]

A is for edema volume in negative control
B for paw edema in tested groups.

**Xylene-Induced ear edema**
All the tested organisms consisted of five groups (six mice in each group) were administered orally with distilled water (10ml/kg), plant extracts (50, 100 and 200 mg/kg) and Dexamethasone (1mg/kg) in Xylene induced ear edema study. After 30 min, Xylene (0.03ml) was applied to the inner surface of the right ear for the induction of edema. The left ear was used as control. After 15 min, the mice were killed with the help of ether anesthesia. Both ears were cut and weighed. For each group, the mean of the difference between the left and right ears was calculated (Nunez-Guillen et al., 1997).

The percent inhibitory effect was measured with the help of formula given below:

\[
\% \text{Inhibition} = \frac{Vc - Vt}{Vc} \times 100
\]

Vc is for difference in weight of ear in control
Vt is for difference in weight of ear in group treated with standard & extract.

**Antipyretic activity**

Pyrexia induction with brewer’s yeast
Antipyretic effect of crude methanolic extract of each plant was screened by mice (30-35g) of either sex. Before screening, mice were provided with water only for 12 hours. The tested animals were separated into 5 different groups (n=6). The group I was called negative and group II was treated as positive control. The animals of group I were administered with normal saline at a dose of 10 ml/kg (body weight). Group II was treated with Paracetamol at 150mg/kg dose (body weight). Groups III, IV and V were subjected with crude extracts at doses of 100, 200 and 300mg/kg (Body weight). Normal body temperature of every animal was recorded with the help of digital thermometer. To induce pyrexia, aqueous suspension of Brewer’s (15%) was subcutaneously injected with a dose of 10ml/kg to mice. Digital thermometer was used to note the rise in body temperature 24 hours after the treatment. The mice were selected for further study that showed at least 0.5°C increase in body temperature while those animals were excluded who showed rise in body temperature less than 0.5°C (Khan et al., 2011).

Through ip route all doses were injected to all groups. The temperature of rectum was noted regularly at 1st, 2nd, 3rd, 4th and 5th hour of each mouse in all treated groups. Reduction in body temperature (%) was measured by the formula given below:

\[
\% \text{Reduction in body temperature} = \frac{100 - \frac{B - Cn}{B - A} \times 100}{100}
\]

A is for normal body temperature
B indicates body temperature after 24 hrs
C is for temperature at 1st, 2nd, 3rd, 4th and 5th hr of treatment.

**Analgesic activity**

Acetic acid induced writhing
The tested animals (mice) of either sex (18-22g body weights) were separated into 5 different groups (n=6). Among these groups, group I was called as negative
control while group II was treated as positive control. The normal saline at a dose of 10 ml/kg (body weight) was administered to group I (negative control), while group II was subjected to Diclofenac sodium (standard drug) at the dose of 10 mg/kg (body weight). They were fed according to the guidelines recommended. Two hours before the start of activity, the food supply was stopped (Muhammad et al., 2008). Groups III, IV and V (remaining groups) were subjected with crude extracts at 100, 200 and 400 mg/kg doses (body weight) respectively and waited for 30 minutes. After 30 minutes, acetic acid (1%) was injected to all tested groups through intra-peritoneal route. The abdominal writhing (constrictions) were started 5 minutes after acetic acid injection, which were counted for next 10 minutes. The analgesic potential (percent) was calculated with the help of the following given formula:

\[
\text{% Analgesic effect} = 100 - \frac{\text{No. of writhing in tested animals}}{\text{No. of writhing in control animals}} \times 100
\]

**Hot plate method**
In this experiment, analgesic effect was measured by Eddy’s Hot Plate method (Turner, 1965). For this purpose, hot plate was maintained at a temperature of 55±1°C and tested animals (mice) were placed on hot plate individually. Every mouse was observed for reaction on hot plate in the form of licking or jumping. The animals (of both sexes) used in this experiment were randomly selected. These selected mice were separated into five groups, group I, group II, group III, group IV and group V. Each group was consisted of six mice for both control and test samples. All of the above mentioned groups were given a particular treatment i.e. positive control with Aspirin (100 mg/kg) and the test sample (plants extract) of 200 and 400 mg/kg respectively. The animals were placed on Eddy’s Hot Plate maintained at a temperature of 55±1°C. The time of 15 seconds was a cut off period to observe animals on hot plate. After the treatment, the reaction time in treated and control animals was recorded at 0, 30, 60, 90 and 120 minutes (Janssen and Eddy, 1959).

**STATISTICAL ANALYSIS**
Data are shown as mean values of three replications, MSTATC computer software was used for statistical analysis (Russel and Eisensmith, 1983). The data was analyzed by ANOVA followed by Dunnett’s test. *P<0.05, **P<0.01 in comparison to control (Steel et al., 1997).

**RESULTS**

**Acute toxicity activity**
Crude methanolic extract of the selected plant was observed safe at all the tested doses of 500, 1000 and 2000 mg/kg i.p. In 24 hrs evaluation period, all the tested animals were found normal. No considerable difference was found between saline group and test groups in moving, eating, respiration and others behaviors.

**Anti-inflammatory activity**
Analysis of the data reveals that the tested plant showed the activity at both phases of 3 and 5 hours when tested by Carrageenan Induced Paw Edema Model (table 1). Results indicated that the inhibitory activity was time and dose dependent. Maximum anti-inflammatory activity was shown by 200 mg/kg. This dose inhibited the inflammation by 54.12 and 34.98% after 5 and 3 hours respectively. Anti-inflammatory activity shown by 100 mg/kg after 3 and 5 hours was 18.34 and 33.89% respectively. Similarly, at the dose of 50 mg/kg, the inhibitory activity measured was 6.46% at 3 hours and 28.12% at 5 hours. Significant (p<0.05) inhibitory effect was demonstrated by different doses of Tamarix dioica when assayed through Xylene-induced Ear Edema Model (table 2). The anti-inflammatory activity was dose and time dependent. Maximum anti-inflammatory activity was shown by 200 mg/ both at 15 and 60 minutes. The data indicated that the inhibitory effect of the said dose was 60.98% and 56.99% at 60 and 15 minutes respectively. Similarly, the inhibitory effect of 100 mg/kg was also significant (p<0.05) measuring 53.65% and 45.29% at 60 and 15 minutes respectively. The tested plant also demonstrated good inhibitory activity even at low dose of 50 mg/kg which was 32.29 and 45.92% at 15 and 60 minutes respectively.

**Antipyretic activity**
Antipyretic activity of Tamarix dioica of crude methanolic extract when tested by Pyrexia induction with Brewer’s yeast is shown in table 3. Analysis of the data revealed that different doses of the crude extract showed significant (p<0.05) antipyretic activity. In the 1st hour of the treatment, the percent antipyretic effect produced was 0.88, 0.37 and 1.14% at 100, 200 and 300 mg/kg respectively. In 2nd hr, 100, 200 and 300 mg/kg reduced the body temperature by 0.29, 1.20 and 1.64% respectively. At 3rd hour, the antipyretic effect measured was statistically significant antipyretic activity (p<0.05) for all test doses. Highly significant (p<0.01) effects were observed for the dose of 300 mg/kg. Percent inhibition demonstrated by all test doses at 3rd hr at the doses of 200 and 300 mg/kg was 1.87 and 2.44% respectively. Similarly, 1.43, 2.07 and 2.41% antipyretic activity was recorded for 100, 200 and 300 mg/kg doses respectively at 4th hr. Reduction in temperature at 5th hr was 1.42, 2.07 and 2.43% respectively for test doses.

**Analgesic activity**
Stem bark extracted samples of Tamarix dioica showed significant (p<0.05) analgesic effect at all tested doses (100, 200 and 400 mg/kg) measured by acetic acid induced writhing method (table 4). Analysis of the data revealed that the percent writhing inhibitory result was
Anti-inflammatory, anti-pyretic and analgesic activities of Tamarix dioica

Table 1: Anti-inflammatory activity of methanolic crude extract of Tamarix dioica by Carrageenan-Induced Edema method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>3 hours</th>
<th>5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>% Inhibition</td>
<td>Difference</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>5.89 ± 0.54</td>
<td>-</td>
</tr>
<tr>
<td>TD (Extract)</td>
<td>50</td>
<td>5.51 ± 0.54</td>
<td>6.46</td>
</tr>
<tr>
<td>TD (Extract)</td>
<td>100</td>
<td>4.81 ± 0.55*</td>
<td>18.34</td>
</tr>
<tr>
<td>TD (Extract)</td>
<td>200</td>
<td>3.83 ± 0.51**</td>
<td>34.98</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>3.07 ±0.49**</td>
<td>47.88</td>
</tr>
</tbody>
</table>

TD = Tamarix dioica; Values are reported as mean ± (SEM), n=5, **p<0.01, compared to saline group, analyzed by t-test

Table 2: Anti-inflammatory activity of crude methanolic extract of Tamarix dioica on by Xylene-Induced Ear Edema method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>% Inhibition</td>
<td>Difference</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>31.78± 2.91</td>
<td>-</td>
</tr>
<tr>
<td>TD Extract</td>
<td>50</td>
<td>21.52 ± 2.92*</td>
<td>32.29</td>
</tr>
<tr>
<td>TD Extract</td>
<td>100</td>
<td>17.39 ± 2.43*</td>
<td>45.29</td>
</tr>
<tr>
<td>TD Extract</td>
<td>200</td>
<td>13.67 ± 2.71*</td>
<td>56.99</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.5</td>
<td>9.61±2.77**</td>
<td>69.77</td>
</tr>
</tbody>
</table>

TD = Tamarix dioica; Values are reported as mean±(SEM.), n=5., *p<0.05; **p<0.01, compared to saline group (analyzed by t-test) (TD = Tamarix dioica)

Table 3: Antipyretic activity of crude methanolic extract of Tamarix dioica

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose mg/kg</th>
<th>Rectal temperature (°C)</th>
<th>After administration of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal after 24h</td>
<td>1h</td>
</tr>
<tr>
<td>Saline</td>
<td>10mL</td>
<td>36.69 ±0.52</td>
<td>39.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.26</td>
<td>±0.31</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150 mg</td>
<td>37.05 ±0.32</td>
<td>39.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.32</td>
<td>±0.32</td>
</tr>
<tr>
<td>TD Extract</td>
<td>100</td>
<td>37.11 ±0.27</td>
<td>39.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.35</td>
<td>±0.35</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>37.21 ±0.21</td>
<td>39.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.33</td>
<td>±0.33</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>37.04 ±0.03</td>
<td>39.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.27</td>
<td>±0.56</td>
</tr>
</tbody>
</table>

TD = Tamarix dioica; Values are reported as mean±S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett’s test. *P<0.05, **P<0.01 in comparison to control

dose dependent. Maximum analgesic activity was demonstrated by 400mg/kg (64.33%) Similarly, the dose of 200mg/kg also significant (p<0.05) inhibited the writhing (56.60%). At a dose of 100mg/kg the percent writing inhibition was 19.18% (table 4).

Analgesic activity measured by hot plate method showed that the dose of 200mg/kg was found significant (p<0.05) only at 120 minutes. The analgesic effect of 400mg/kg was significant (p<0.05) both at 30 and 60 minutes, however, it was more significant (p<0.01) at 90 and 120 minutes. The activity observed for 200 and 400mg/kg at 30 minutes was 13.32 and 39.50% respectively. After 60 minutes, the percent reduction in analgesia for the same doses was 38.09 and 39.02% respectively. At 90 minutes, the nociceptive response observed for 200 and 400mg/kg was 36.52 and 52.64%. At 2 hours, the inhibitory effect demonstrated by 400mg/kg was 48.05% (table 5).
The results indicated that mechanism of action of crude extract resembles those of NSAID group of the anti-inflammatory drugs. These drugs have anti-inflammatory activities both in central and peripheral tissue (Okokon, 2011).

Analysis of the data revealed that different doses of the crude extract of *Tamarix dioica* showed significant (*p*<0.05) antipyretic activity. The percent antipyretic effect produced was significantly highest at 300mg/kg during the entire period of the treatment when compared with other doses and control. The results indicated that anti-pyretic activity showed dose dependent decrease in the temperature of mice. These results showed that the

### DISCUSSION

Acute toxicity activity revealed that the crude methanolic extract of *Tamarix dioica* was safe during 24 hrs evaluation period at all the tested doses. No significant difference was observed between the control (saline group) and test groups in normal life activities. The toxic agents usually bind to vital organs like kidneys and liver and produce toxic effects. This becomes very important to evaluate the toxic properties of an agent in respect to public health protection, because exposure to such chemical substances can be hazardous and leads to harmful effects on human beings. This evaluation of toxic agents usually includes acute, sub-chronic, chronic, reproductive and carcinogenic effects (Asante-Duah, 2002; Johy *et al.*, 2011).

Results indicated that the inhibitory activity when tested by Carrageenan Induced Paw Edema Model was time and dose dependent. Maximum time dependant anti-inflammatory activity was shown by the highest dose of 200mg/kg compared with other concentrations. The in-vivo anti-inflammatory activity of *Tamarix dioica*, revealed that Carrageenan produced biphasic inflammatory events was significantly (*p*<0.05) controlled by plant extracted samples and hence might be used as active anti-inflammatory agents. Certain chemical substances such as serotonin, histamines and other related compounds in the first phase (90-180 minutes) of inflammation was increased in the paw characterized the second phase of inflammation (270-360 minutes). This increase in volume is due to presence of certain inflammatory mediators (Khan *et al.*, 2011). Non-

**Table 4:** Analgesic activity of crude methanolic extract of *Tamarix dioica* by acetic acid induced method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg i.p.)</th>
<th>Number of writhing (10min)</th>
<th>% Analgesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>10 ml/kg</td>
<td>63.38±2.79</td>
<td>-</td>
</tr>
<tr>
<td>TD Extract</td>
<td>100</td>
<td>51.23±2.77*</td>
<td>19.18</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>27.51±1.83**</td>
<td>56.60</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>22.61±1.46**</td>
<td>64.33</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>10</td>
<td>10.17±1.27**</td>
<td>83.96</td>
</tr>
</tbody>
</table>

TD = *Tamarix dioica*; Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett’s test. *P*<0.05; **P*<0.01 in comparison to control

**Table 5:** Analgesic activity of crude methanolic extract of *Tamarix dioica* by hot plate method

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Latency of nociceptive response in min (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>8.22±0.23</td>
</tr>
<tr>
<td>TD Extract</td>
<td>200</td>
<td>8.36±0.36</td>
</tr>
<tr>
<td>TD Extract</td>
<td>400</td>
<td>8.49±0.46</td>
</tr>
<tr>
<td>Tramadol</td>
<td>20</td>
<td>8.51±0.37</td>
</tr>
</tbody>
</table>

TD = *Tamarix dioica*; Values are reported as mean ± S.E.M. for group of 5 animals. The data was analyzed by ANOVA followed by Dunnett’s test. *P*<0.05; **P*<0.01 in comparison to control
subjected extracts acted both centrally and peripherally like aspirin (Ferreira et al., 1978). Aspirin reduces the fever by decreasing prostaglandin E2 brain concentration, especially through its action on COX-3 in the hypothalamus too (Vane, 1971).

Acetic acid induced abdominal constriction assay is commonly used model. It is sensitive, easy and rapid technique used to measure peripheral analgesic effect (Atta and Alkofahi, 1998; Gupta et al., 2005). Extracted samples of Tamarix dioica showed significant ($p<0.05$) analgesic effect at all tested doses when measured by acetic acid induced writhing method. Analysis of the data revealed that the percent writhing inhibitory result was dose dependent. Maximum analgesic activity was shown by the highest dose of 400mg/kg when compared with other concentrations and control. Analgesic activity measured by hot plate method showed that the dose of 200mg/kg was found significant ($p<0.05$) only when treatment was carried out for maximum duration of 120 minutes. The analgesic effect of 400mg/kg was significantly ($p<0.05$) higher both at 30 and 60 minutes, however, it was more significant ($p<0.01$) at 90 and 120 minutes. This test is specifically applied to understand the analgesic effect of drugs and chemicals that act centrally such as morphine and its analogues. Anti-nociceptive drugs that act peripherally are found to be inactive on temperature induced hyperalgesia (Coutaux et al., 2005).

CONCLUSION

It can be concluded from these results that crude extracts of Tamarix dioica possesses anti-inflammatory, anti-pyretic and analgesic activity.

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