Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbial agents (1). An uncontrolled and persistent inflammation may act as an etiologic factor for many of chronic illnesses such as cancer, autoimmune diseases, cardiovascular diseases, obesity, and diabetes (2, 3). Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages, and mast cells. Cyclooxygenase (COX), a crucial enzyme in the inflammatory process, plays a key role as a rate-limiting enzyme in the production of potent pro-inflammatory prostaglandins (PGs) biosynthesis, exists in at least two isoforms, designated as COX-1 and COX-2 (4). The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses (5). Hence, the development of anti-inflammatory drugs with lesser adverse effect is needed to be designed. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (6). The plant derived drugs are considered to be associated with less adverse effects as compared to synthetic compounds. These are still being used in several countries as alternative medicines. Many plants like roots of Glycyrrhiza glabra, L, the seeds of Trigonella foenum-graecum, L, and the fruits of Coriandrum sativum L are used to treat inflammatory conditions (7).

Berberis calliobotrys belongs to Berberidaceae, a family of three genera flowering plants commonly called barberry. Berberis calliobotrys is widely distributed in Afghanistan and Pakistan. The species
include trees, shrubs and perennial herbaceous plants. This is a shrub, 1-2 m tall, glabrous; stems are red-brown to pale-brown. *Berberis calliobotrys* has folkloric claim to be used in the treatment of jaundice, fever, backache, pharyngitis and relief of intestinal colic by the local people of Chitral Valley, Pakistan (8). The present study was carried out to evaluate the analgesic, anti-inflammatory and anti-pyretic activity of *Berberis calliobotrys* to validate its folkloric claim.

**MATERIALS AND METHODS**

**Plant material**

Stem part of *Berberis calliobotrys*, common name chownij, was collected from hilly areas of Quetta, Balochistan. Plant, authenticated by Dr. Rasool Bux, Taxonomist, Department of Botany, University of Balochistan, was used in this study. The dried and coarsely powdered stem part of *B. calliobotrys* (1.7 kg) was extracted by method of cold maceration by adding 9 L (70 : 30, v/v ratio of methanol to distilled water) solvent. The powder was soaked for 72 h at room temperature with occasional shaking. Then, it was filtered through muslin cloth and Whatman qualitative Grade 1 filter paper. The final filtrate was evaporated with the help of rotary evaporator at 50 OC and then, lyophilized to give a yield of 4.8% of extract. For administration, the extract was dissolved in distilled water (9, 10).

**Animals used**

Albino mice of either sex weighing 20-30 g were used in this study. The animals were housed in propylene cages under controlled conditions of 24 ± 1°C and 12 h light – 12 h dark cycles and ventilation. Standard feed and water were provided *ad libitum* unless otherwise specified. All animals were treated according to the standard procedures guided by National Research Council (11).

**Anti-inflammatory activity**

**Carrageenan induced paw edema in mice**

Anti-inflammatory activity of aqueous methanolic extracts in the dose of 250 and 500 mg/kg was evaluated against carrageenan induced paw edema model in mice. Twenty animals were divided into four groups consisting of five animals each. Group I served as control group and received distilled water (2 mL/kg b.w.). Groups II and III received orally aqueous methanolic extract of *Berberis calliobotrys* at 250 and 500 mg/kg, respectively. Group IV received standard drug ibuprofen at a dose of 40 mg/kg p.o. The linear diameter of the injected paw was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Vernier calipers. The increases in the right hind paw diameters induced by the injections of fresh egg albumin were compared with those of the contra-lateral, non-injected left hind paw diameters. The percentage inhibition was calculated by the above mentioned formula (1, 15).

**Egg albumin-induced paw edema in mice**

Adopting the method prescribed by Muko and Ohiri (14), the paw edema was induced by subplantar administration of 0.1 mL of fresh egg albumin into the right hind paw of mice. Twenty animals were divided into four groups consisting of five animals each. Group I served as control group and received distilled water (2 mL/kg b.w.). Group II and III received orally aqueous methanolic extract of *Berberis calliobotrys* at 250 and 500 mg/kg, respectively. Group IV received standard drug ibuprofen at a dose of 40 mg/kg p.o. The linear diameter of the injected paw was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Vernier calipers. The increases in the right hind paw diameters induced by the injections of fresh egg albumin were compared with those of the contra-lateral, non-injected left hind paw diameters. The percentage inhibition was calculated by the above mentioned formula (12, 13).

**Analgesic activity**

**Acetic acid induced writhing in mice**

Analgesic activity of aqueous methanolic extract of *Berberis calliobotrys* against acetic acid induced writhing (0.6%, v/v in saline, 10 mL/kg, i.p.) in doses of 250 and 500 mg/kg p.o. was conducted in mice (16). The various groups of the animals were orally administered with the extracts (250 and 500 mg/kg b.w.), ibuprofen (40 mg/kg b.w.) and distilled water. After 60 min of treatment, mice were administered with acetic acid (0.6%, v/v in normal saline, 10 mL/kg, i.p.). The number of writhings (characterized by contraction of the abdominal musculature and extension of the hind limbs) exhibited by each animal, started 5 min after acetic acid injection and was counted for 10 min.

\[
\% \text{ Inhibition} = \frac{\text{No. of writhings (control)} - \text{No. of writhings (treated)}}{\text{No. of writhings (control)}} \times 100
\]

**Formalin induced paw licking in mice**

The procedure described by Bhalke (17), was used for the determination of response to pain
induced by formalin. Twenty µL of a 2.5% solution of formalin (0.9% formaldehyde) was injected subcutaneously under the surface of the left hind paw of each mice. The times spent in licking and biting responses of the injected paw, indicative of pain, was recorded for each animal. The animals were pre-treated with distilled water (10 mL/kg body weight p.o.), ibuprofen (40 mg/kg body weight p.o.) and extracts (250 & 500 mg/kg body weight p.o.), 60 min before the administration of formalin. The responses of mice were observed for the first 5 min (first phase) and then 15–30 min (second phase) post-formalin injection. The % inhibition is calculated by the following formula.

% Inhibition = \frac{\text{Reaction time (control) – Reaction time (treated)}}{\text{Reaction time (control)}} \times 100 \hspace{1cm} (3)

**Anti-pyretic activity**

**Yeast induced pyrexia in mice**

Albino mice either male or female were divided into four groups of five mice each. The animals were fasted 24 h before the commencement of the experiment but deprived of water only during experiment. Initial rectal temperatures were recorded using a digital thermometer. The pyrexia was induced by subcutaneously injecting 20% (w/v) brewer’s yeast suspension (10 mL/kg) into the mouse’s dorsum region. When the temperature was at peak, 18 h after yeast injection, only mice that showed an increase in temperature of at least 0.7°C were used for the experiment. Group I (control group) was treated with distilled water (10 mL/kg) p.o. Groups II and III received AMEBC in doses of 250 and 500 mg/kg, p.o., respectively. Group IV was treated with standard drug - ibuprofen 40 mg/kg, p.o. The rectal temperature was measured at 0, 1, 2, 3 and 4 h after each agent administration (18).

**Statistical analysis**

The results are expressed as the means ± SEM. Parametric data were compared to control group and were assessed by the method of one-way ANOVA and two-way ANOVA followed by Bonferroni post hoc tests. Values p < 0.05 were considered as statistically significant.

### RESULTS

Aqueous methanolic extract of *Berberis calliobotrys* significantly (p < 0.001) reduced the edema formation of the paw induced by carrageenan at all assessment times. At 0 min, aqueous methanolic extract at dose of 250 mg/kg showed non-significant effect but aqueous methanolic extract at a dose of 500 mg/kg showed significant (p < 0.01) effect. At 30 min, aqueous methanolic extract at both doses, showed highly significant (p < 0.001) reduction in paw edema, that showed its potent anti-inflammatory effect. This result demonstrated dose-time related significant reduction by aqueous methanolic extract. Ibuprofen (40 mg/kg), similarly produced significant inhibitory effect of the paw edema as compared to normal control group (Table 1).

<table>
<thead>
<tr>
<th>Treatment (dose, mg/kg)</th>
<th>Inflammation induced at 0 min (mm) (% inhibition)</th>
<th>Inflammation induced at 30 min (mm) (% inhibition)</th>
<th>Inflammation induced at 60 min (mm) (% inhibition)</th>
<th>Inflammation induced at 90 min (mm) (% inhibition)</th>
<th>Inflammation induced at 120 min (mm) (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 mL/kg)</td>
<td>3.46 ± 0.051</td>
<td>3.58 ± 0.058</td>
<td>3.7 ± 0.071</td>
<td>3.86 ± 0.051</td>
<td>4.0 ± 0.045</td>
</tr>
<tr>
<td>Aqueous methanolic extract (250 mg/kg)</td>
<td>3.02 ± 0.086 ph s (12.7%)</td>
<td>2.74 ± 0.07 *** (23.4%)</td>
<td>2.72 ± 0.18 *** (26.4%)</td>
<td>2.72 ± 0.31 *** (29.5%)</td>
<td>2.76 ± 0.11 *** (31%)</td>
</tr>
<tr>
<td>Aqueous methanolic extract (500 mg/kg)</td>
<td>2.8 ± 0.12 ** (19.0%)</td>
<td>2.74 ± 0.04 *** (23.4%)</td>
<td>2.52 ± 0.02 *** (31.8%)</td>
<td>2.42 ± 0.05 *** (37.3%)</td>
<td>2.34 ± 0.05 *** (41.5%)</td>
</tr>
<tr>
<td>Ibuprofen (40 mg/kg)</td>
<td>2.9 ± 0.025 % (16.1%)</td>
<td>2.8 ± 0.34 *** (21.7%)</td>
<td>2.69 ± 0.13 *** (27.2%)</td>
<td>2.54 ± 0.22 *** (34.1%)</td>
<td>2.32 ± 0.14 ** (42%)</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 5). Key: *** = (p < 0.001) highly significant, ** = (p < 0.01) * = (p < 0.5) and n.s = non-significant as compared to control.
The aqueous methanolic extract (250 and 500 mg/kg) significantly (p < 0.001) reduced the edema formation of the paw induced by fresh albumin injection from 30 min to onward at all assessment times. The maximum inhibition was observed at 120 min. Ibuprofen at the dose of 40 mg/kg produced time-related significant (p < 0.05-0.001) inhibitory effect of the paw edema as compared to normal control group (Table 2).

The animals which were pretreated with aqueous methanolic extract at the doses of 250 and 500 mg/kg showed significant reductions (p < 0.001) in the number of writhings with % inhibition of 24.48 and 37.9%, respectively. Whereas intra peritoneal injection of acetic acid to the control group elicited (72.5 ± 1.19) writhings counted in 10 min. Ibuprofen at a dose of 40 mg/kg orally showed a marked inhibition (68.5 %) of writhings (Table 3).

Aqueous methanolic extract produced a significant (p < 0.001) dose-dependent inhibition of nociceptive reaction induced by formalin with peak effect (87% inhibition) produced at the highest dose of 500 mg/kg. Ibuprofen at the dose of 40 mg/kg produced marked inhibition (89%). In the second phase, the total duration of nociceptive reaction in the control group was 203.0 ± 12.3 s. The effect of aqueous methanolic extract in inhibiting the biting and licking response was also significant (p < 0.001). Ibuprofen at the dose of 40 mg/kg produced marked inhibition (92%) of formalin induced paw licking (Table 4).

### Table 2. Effect of aqueous methanolic extract of Berberis calliobotrys at 250 and 500 mg/kg against egg albumin induced paw edema in mice.

<table>
<thead>
<tr>
<th>Treatment (dose, mg/kg)</th>
<th>Inflammation induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at 0 min (mm) (% inhibition)</td>
</tr>
<tr>
<td>Control (2 mL/kg)</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Aqueous methanolic extract (250 mg/kg)</td>
<td>3.38 ± 0.15 (6.1%)</td>
</tr>
<tr>
<td>Aqueous methanolic extract (500 mg/kg)</td>
<td>3.14 ± 0.09 (12.7%)</td>
</tr>
<tr>
<td>Ibuprofen (40 mg/kg)</td>
<td>3.0 ± 0.12 (16.6%)</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 5). Key: *** = (p < 0.001) highly significant, ** = (p < 0.01) * = (p < 0.5) and n.s = non-significant as compared to control.

### Table 3. Effect of aqueous methanolic extract of Berberis calliobotrys against acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment (Dose, mg/kg)</th>
<th>No. of writhings</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 mL/kg)</td>
<td>72.5 ± 1.19</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous methanolic extract (250 mg/kg)</td>
<td>54.75 ± 0.854 ***</td>
<td>24.48%</td>
</tr>
<tr>
<td>Aqueous methanolic extract (500 mg/kg)</td>
<td>45 ± 2.345 ***</td>
<td>37.90%</td>
</tr>
<tr>
<td>Ibuprofen (40 mg/kg)</td>
<td>22.83 ± 1.14 ***</td>
<td>68.50%</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 5). Key: *** = (p < 0.001) highly significant as compared to control.
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The *Berberis calliobotrys* extract (500 mg/kg) produced the highly significant (p < 0.001) inhibition of pyrexia after induction. Whereas, 250 mg/kg of extract produced significant (p < 0.01) inhibition of pyrexia after 1 h of induction of pyrexia but becomes less significant (p < 0.05) after 3 h of post induction (Table 5).

**DISCUSSION**

The anti-inflammatory activity of *Berberis calliobotrys* was studied by using carrageenan and albumin induced paw inflammation. The aqueous methanolic extract exhibited significant anti-inflammatory activity. Carrageenan-induced edema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever. The aqueous methanolic extract may produce its anti-inflammatory effect by blocking the release of these inflammatory mediators. Our study agrees with the previous study conducted by Bhalke (17).

The result of this study suggests that aqueous methanolic extract of *Berberis calliobotrys* has anti-inflammatory effect comparable to those of the standard drug, ibuprofen. Carrageenan-induced inflam-

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### Table 4. Effect of aqueous methanolic extract of *Berberis calliobotrys* against 2.5% formalin induced paw licking in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First phase</th>
<th>Second phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Licking time (s)</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Control (10 mL/kg)</td>
<td>173.0 ± 19.70</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous methanolic extract (250 mg/kg)</td>
<td>32.6 ± 0.92 ***</td>
<td>81%</td>
</tr>
<tr>
<td>Aqueous methanolic extract (500 mg/kg)</td>
<td>22 ± 1.14 ***</td>
<td>87%</td>
</tr>
<tr>
<td>Ibuprofen (40 mg/kg)</td>
<td>19.67 ± 1.68 ***</td>
<td>89%</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 5). Key: *** = (p < 0.001) highly significant, as compared to control.

### Table 5. Effect of aqueous methanolic extract of *Berberis calliobotrys* on yeast induced pyrexia in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temp. before induction</th>
<th>Temp. after 18 h of induction (°C)</th>
<th>Temp. at 1 h (°C)</th>
<th>Temp. at 2 h (°C)</th>
<th>Temp. at 3 h (°C)</th>
<th>Temp. at 4 h (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 mL/kg)</td>
<td>36.32 ± 0.85</td>
<td>36.24 ± 0.19</td>
<td>36.27 ± 0.21</td>
<td>36.21 ± 0.31</td>
<td>36.56 ± 0.09</td>
<td>36.03 ± 0.13</td>
</tr>
<tr>
<td>N/S + Yeast (0.9% + 20%)</td>
<td>36.29 ± 0.9</td>
<td>39.1 ± 0.1 ***</td>
<td>39.3 ± 0.5 ***</td>
<td>39.5 ± 0.8 ***</td>
<td>39.6 ± 0.3 ***</td>
<td>39.9 ± 0.01 ***</td>
</tr>
<tr>
<td>Aqueous methanolic extract (250 mg/kg)</td>
<td>36.76 ± 0.63</td>
<td>36.8 ± 0.2 n.s.</td>
<td>36.8 ± 0.06 **</td>
<td>37.9 ± 0.24 *</td>
<td>38.54 ± 0.1 *</td>
<td>38.26 ± 0.3 *</td>
</tr>
<tr>
<td>Aqueous methanolic extract (500 mg/kg)</td>
<td>36.40 ± 0.36</td>
<td>37.9 ± 0.1 **</td>
<td>37.3 ± 0.3 ***</td>
<td>37.92 ± 0.2 *</td>
<td>38.22 ± 0.4 *</td>
<td>38.14 ± 0.4 **</td>
</tr>
<tr>
<td>Ibuprofen (40 mg/kg)</td>
<td>36.00 ± 0.72</td>
<td>36.8 ± 0.2 **</td>
<td>36.5 ± 0.04 ***</td>
<td>36.1 ± 0.5 ***</td>
<td>36.2 ± 0.01 ***</td>
<td>36.0 ± 0.05 ***</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 5). Key: *** = (p < 0.001) highly significant, ** = (p < 0.01), * = (p < 0.05) significant and n.s. = non-significant as compared to control.
matory process is believed to be biphasic. The initial phase seen at first hour is attributed to the release of histamine and serotonin. The second accelerating phase of swelling is due to release of prostaglandin (PGs), bradykinin and lysozyme. It has been reported that the second phase of inflammation is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents (19). Aqueous methanolic extract produced significant (p < 0.001) inhibition of paw edema by carrageenan injection in mice after 30 min and continued during all phases of inflammation in both groups. This activity may be assumed probably due to the inhibition of different aspects and chemical mediators of inflammation as established for ibuprofen.

The anti-inflammatory activity of aqueous methanolic extract was also evaluated by using egg albumin induced paw edema in mice. The aqueous methanolic extract significantly (p < 0.001) reduced inflammation in 250 and 500 mg/kg doses. It indicates that aqueous methanolic extract may inhibit inflammation by blocking the release of histamine and 5-HT, which are released by egg albumin. Histamine is an important mediator of inflammation, a potent vasodilating substance and is also involved in increasing vascular permeability (20). The edema produced by egg albumin was effectively suppressed by the aqueous methanolic extract in both doses. The present experimental findings tend to suggest that the extract might demonstrate anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin, cytokines and prostaglandins (21).

Furthermore, the abdominal constriction response induced by acetic acid is a sensitive process to evaluate peripherally acting analgesics (22). Acetic acid has been reported to cause hyperalgesia by liberating endogenous substances such as prostaglandins, leukotrienes, 5-HT, histamine and kinins, which have been implicated in the mediation of pain perception. The dose-dependent inhibition of writhings induced by acetic acid in this study suggests a peripherally mediated analgesic activity based on the association of the model with stimulation of peripheral receptors especially the local peritoneal receptors at the surface of cells lining the peritoneal cavity (23, 24). The aqueous methanolic extract in 250 and 500 mg/kg doses produced highly significant (p < 0.001) reduction in abdominal constrictions and stretching jerk of the hind limb. The significant reduction in acetic acid-induced writhes by aqueous methanolic extract at the dose of 250 and 500 mg/kg suggests their analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances as already proposed by Koster (25).

For analgesic activity, the formalin-induced paw licking test was conducted. This test possesses two distinctive phases: first phase measures direct chemical stimulation of nociceptors (neurogenic phase), whereas second phase is dependent on peripheral inflammation and central processing (inflammatory phase). The early phase is due to a direct effect on nociceptors and a consequent C-fibre activation, whereas second phase involve histamine, serotonin, PGs, NO and bradykinin (26). Thus, both centrally and peripherally mediated actions can be measured. An immediate and intense increase in impulsive activity of C afferent fiber, caused by formalin injection, evokes a diverse quantifiable behavior indicative of pain demonstrated as paw licking by the animals (27). The aqueous methanolic extract in 250 and 500 mg/kg doses produced highly significant (p < 0.001) reduction in paw licking in mice indicating that its activity may be because of its central as well as peripheral action as previously reported by Begum (28) and Ghannadi (29).

The yeast-induced hyperthermia in mice was employed to investigate the antipyretic activity of extract in 250 and 500 mg/kg doses. It was found that extract caused a significant reduction in rectal temperature in a dose dependent manner. The yeast induces pathogenic fever by enhancing the production of prostaglandins, mainly PGE\textsubscript{2}, which elevates the set point of the thermoregulatory centre in hypothalamus (30). The hypothalamus regulates body temperature with a fragile balance between heat production and heat loss through the set-point control (31). In this approach, the body matches the brain blood temperature with the new set point made by the hypothalamus. The infected or damaged tissue initiates the improved formation of pro-inflammatory mediators (cytokines like interleukins and TNF-\textalpha), which increase the synthesis of prostaglandin E\textsubscript{2} near the pre-optic hypothalamic area, thereby triggering the hypothalamus to raise body temperature (32). Antipyretic activity is commonly stated as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis (33). The possible mechanism of antipyretic activity of AMEBC might be through inhibition of prostaglandin E\textsubscript{2} concentration especially in hypothalamus by its action on newly reported isoenzyme cyclooxygenase 3 (COX 3), present in cerebral cortex and some other tissues that has possible role in pyrexia (34). It has been previously studied that
COX 3 is inhibited by acetaminophen and some other NSAIDs.

CONCLUSIONS

It is conceivable from this study that aqueous methanolic extract of *Berberis calliobotrys* exhibited analgesic, anti-inflammatory and antipyretic effects through different mechanisms. It also validates the traditional use of this plant in the treatment of various types of pain, fever and inflammation. However, further studies are required to isolate active compounds from the potent extracts and to elucidate their exact mechanism of action.

REFERENCES


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