Analgesic and Anti-Inflammatory Activities of Extracts and Fatty Acids from *Celtis australis* L

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**Abstract:** Context: *Celtis australis* L. (Ulmaceae) is a deciduous tree distributed to mountainous and sub-mountainous Himalaya. This plant has been used as traditional medicine in bone fracture, pimples, contusions, sprains and joint pains in India.

**Objectives:** This is the first evaluation of *C. australis* extracts (bark and fruits) and fatty acids (fruits) for acute toxicity, analgesic, and anti-inflammatory activities.

**Materials and methods:** The ethanol extracts of air dried stem bark and fruits were prepared at 30-50°C with 95% EtOH for 15h. The solvent was evaporated under reduced pressure and the powdery extracts so obtained were used for present study along with crude fatty acids obtained from column elution of *n*-C₆H₁₂-CHCl₃ (6:4) of EtOAc fruit extract. Crude extracts and fatty acids were screened for analgesic and anti-inflammatory activities by oral administration of three different doses at 100, 250 and 500 mg/kg of each test sample on Swiss albino mice and Sprague-Dawley rats, respectively.

**Results:** All doses (i.e. 100, 250, and 500 mg/kg) of test samples were found active when compared with negative control. Crude extracts and fatty acids at higher concentration (i.e. 500 mg/kg) showed analgesic activity protection of 59.28, 63.22, and 45.79%, respectively, whereas at the same concentration, the anti-inflammatory inhibition was 44.26, 45.90, and 42.62%, respectively. Paracetamol and phenylbutazone were used as positive controls for analgesic and anti-inflammatory activities, respectively.

**Discussion and conclusion:** Present study concludes that extracts (stem bark and fruits) and fatty acids (fruits) of *C. australis* have significant (p<0.05) analgesic and anti-inflammatory activities.

**Keywords:** Ulmaceae, acetic acid, carrageenan, triterpenoid, phenolic, phenylbutazone, traditional medicine, analgesic and anti-inflammatory.

**INTRODUCTION**

Man’s existence on earth has been made possible only because of the vital role played by the plants in sustaining life. In recent years, there has been an increased and immense interest in the revival of herbal and homeopathic system of medicine, both of which are obviously based on the plants. Large numbers of plants are constantly being screened for their pharmacological value particularly analgesic, anti-inflammatory, hypotensive, hypoglycemic, anti-fertility, cytotoxic, antibiotic etc. Analgesics are primary need of patients to get rid of any kind of pain. The pain is one of the basic symptoms of almost all human ailments which is a sensorial modality and primary protection. Analgesics only relieve pain in a particular complaint without affecting its cause [1]. The most eminent analgesics including opiates and NSAIDs are not helpful in all cases due to their adverse effects. Consequently, the new compounds with potent painkiller action and no side effects are being required to investigate. Beside pain, the inflammation involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease conditions [2, 3]. At present, the natural analgesics and anti-inflammatory of plant origin are being appreciated due to possible toxicity of synthetic drugs.

*Celtis australis* L. vern. Kharik (Ulmaceae) is a deciduous tree distributed in montane and submontane Himalaya. It is about 25 m high, bark pale-ashy grey or brown, often with white specks, branchlet drooping, leaves ovate-elliptical, flowers greenish, polygamous, 4, 5 merous, drupes ellipsoid, glabrous, purplish black etc [4]. The bark of the plant gives yellow dye and wood used for making small articles. The paste obtained from the bark is an effective remedy for bone fracture and is also applied on pimples, contusions, sprains and joint pains [5]. Previously, three phenolics, acacetin 7-O-glucoside, isovitexin and cytisoside have been isolated from the leaves of *C. australis* [6]. The analysis of fatty acids from the fruits and their

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antimicrobial activity was reported earlier [7]. Recently, a novel sulphonated phenolic celtisanin [8] and a bacteriohopanoid \( \beta \)-hydroxy-35-(cyclohexyl-5'-propan-7'-one)-33-ethyl-34-methyl-bacteriohopane [9], along with three known compounds apigenin, quercetin and quercetin glucoside have been isolated from the plant, in addition to four triterpenoids (9\( \beta \),31R)-9,25-cyclo-30-propylhopan-31-ol; (9\( \beta \))-3-hydroxy-30-propylhopan-31-one; (3\( \beta \))-oleanan-3-ol and (3\( \beta \),9\( \beta \))-9,25-cycloolean-12-en-3-yl \( \beta \)-D-glucosylfuranoside; a steroid (3\( \beta \),9\( \beta \),14\( \beta \))-14-hydroxy-9,19-cyclocholan-3-yl \( \beta \)-D-glucopyranoside, and an anthraquinone 6-hydroxy-5,7,8-trimethoxy-9,10-dioxo-9,10-dihydronaphthacene-2-yl acetate [10]. The chemical structures of isolated compounds are shown in Fig. (1). The plant has been used in the treatment of inflammation in traditional medicine but no scientific evidences are yet available. The plant contains various triterpenoids along with other phenolics and since the triterpenoids as well as fatty acids (synergistic effect) exhibited potent anti-inflammatory and analgesic activities [11, 12]. The extracts (bark and fruits) and fatty acids from the plant were assayed for their analgesic and anti-inflammatory activities for the first time in the present study.

**MATERIAL AND METHOD**

**Plant Material**

Fresh bark (5 kg) and fruits (3 kg) of *C. australis* were collected during the month of November, 2009 from Village Bhatwara, District Tehri Garhwal, Uttarakhand and identified by Taxonomical Laboratory, Department of Botany, H.N.B. Garhwal University Srinagar. A voucher specimen (GUH-17595) of the plant has been deposited in Departmental Herbarium for future records.

**Preparation of Plant Extracts**

Air dried powder of bark and fruits was exhaustively extracted with 95% ethanol at 30-50°C (for 15h, 3 times) on a heating mantle, separately. The extraction mixtures were filtered and solvent evaporated to dryness under reduced pressure to yield black-brown powder in each case. The
powdery extracts of bark and fruits were used to evaluate analgesic and anti-inflammatory activities.

**Isolation of Fatty Acid**

The dry powder of fruit extract was further fractionated with EtOAc through Soxhlet apparatus yielding EtOAc soluble and insoluble fractions. The EtOAc soluble fraction was concentrated, pre-adsorbed with silica gel and applied on the top of silica gel packed column. The elution was first started with $n$-C$_6$H$_{12}$ and the polarity was increased by CHCl$_3$. The fractions were collected 50ml each and combined by monitoring on TLC. The elution of $n$-C$_6$H$_{12}$-CHCl$_3$ (60:40) afforded viscous liquid (3 ml) as a separate layer with CHCl$_3$. The viscous liquid (characteristic properties of fatty acid confirmed by FT-IR) was separated out by separating funnel to carry out the present study.

**Drugs**

Phenylbutazone and paracetamol were supplied from Allied Chemicals & Pharmaceuticals (P) Ltd., New Delhi, India and Ind-Swift Ltd, Parwanoo, India, respectively.

**Animals**

Swiss albino mice of both sexes (25-30 g) were used for the evaluation of analgesic activity, whereas adult female Sprague-Dawley rats (150-180 g) were used in anti-inflammatory testing. Experiments were performed according to the guidelines for the care and use of laboratory animals, from the CPCSEA, Ministry of Environment and Forest, Govt. of India (Reg. No. 107/1999/ CPCSEA). Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. All the animals have been divided into five groups in each case and placed in separate cages, each consisting of 6 animals. The animals were acclimatized to the laboratory condition (room temp. 22 ± 1°C with a 12h light/dark cycle) for one week before the onset of experiment. All the selected animals were kept fasted throughout the experimental period, but allowed free access to water *ad libitum*.

**Acute Toxicity Studies**

The crude extracts and fatty acid were administered at doses of 500, 1000 and 2000 mg/kg, p.o., body weight to the experimental animals (overnight fasted). The animals were observed at regular intervals for 24h up to 7 days, for any toxic sign including increased motor activity, sedation, acute convulsion, coma and death [13]. No lethality or other behavior changes were observed up to the dose of 1000 mg/kg, p.o., b.w. The animals treated with a dose of 2000 mg/kg, p.o., exhibited decreased motor activity and consequently, sedation. Therefore, the dose of 2000 mg/kg was recognized as a toxic dose.

**Statistical Analysis**

Results are expressed as the mean ± S.E.M. of 6 independent experiments. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey’s Multiple Range Test. The value, $p<0.05$ is considered as statistically significant.

**Analgesic Activity**

The test samples were selected for investigating their analgesic activity in acetic acid induced writhing response in mice by following the method described earlier [14, 15]. The mice were divided into eleven groups (I-XI) and placed in separate cages, each group consisting of 6 mice. The mice were acclimatized to the laboratory condition for 7 days before the onset of experiment. All the selected mice were kept fasted throughout the experimental period, but allowed free access to water *ad libitum*. Group I (Negative control) received distilled water only, the test groups II-IV were administered with different doses of aqueous suspension of bark extract, groups V-VII received aqueous suspension of fruits extract, groups VIII-X treated with fatty acid (100, 250 and 500 mg/kg, p.o. in each case) and group XI (positive group) received paracetamol. All mice were administrated 0.7% of aqueous acetie acid (10 ml/kg b.w.) after 30 minutes of sample/drug administration and finally placed in transparent boxes for the observation of writhing. The writhes were counted for 20 min after the injection of acetic acid. The number of writhes in each group was compared to that of the negative and positive control groups.

**Anti-inflammatory Activity**

The anti-inflammatory activity was performed by following the modified methods described earlier [15, 16]. The rats were divided into eleven groups of six animals each, named negative control (I), test group 1-9 (II-X) and positive control (XI). The induction of edema in the left hind paw of all animal groups was performed by a subcutaneous injection of 0.1 ml aqueous carrageenean (1%, m/V) into their footpads. Group I was given 1 ml 10% (V/V) Tween-80, test groups were orally administered the extracts (suspended in 10% Tween-80) in three difference doses of 100, 250 and 500 mg/kg, b.w., respectively and last group (XI) was treated with phenybutazone at the dose of 100 mg/kg, b.w. The paw volume of each rat was measured by mercury plethysmometer, just prior to carrageenean injection and then hourly up to 3h post treatment.

**RESULTS AND DISCUSSION**

The results of analgesic activity showed that all the test samples including the fatty acid from fruits of *C. australis* exhibited significant activity (Table 1). The test samples were taken orally in three different concentrations of 100, 250 and 500 mg/kg, b.w. It was observed that the higher concentration (i.e. 500 mg/kg) of each sample was found most significant ($p<0.05$) when compared with negative control (distilled water). Paracetamol at the dose of 100 mg/kg b.w. was used as positive control. Individually, the bark extract, fruit extract and fatty acid at the dose of 500 mg/kg exhibited the analgesic activity with protection percentage of 59.28, 63.22 and 45.79%, respectively, whereas the positive control showed 80.12% protection against acetic acid induced writhes.

The anti-inflammatory activity data (Table 2) indicated that all test samples protected rats from carrageenean induced inflammation. The treatment with different doses of the crude extracts and isolated fatty acid orally, showed
Table 1. Analgesic Activity in Acetic acid Induced Writhes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose mg/kg, b.w.</th>
<th>No. of Writhes in 20 min ± SEM</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>33.50 ± 1.68</td>
<td>00.00</td>
</tr>
<tr>
<td>CA (b)</td>
<td>100</td>
<td>26.16 ± 1.86</td>
<td>21.91</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>17.83 ± 1.19*</td>
<td>46.78</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>13.64 ± 1.82*</td>
<td>59.28</td>
</tr>
<tr>
<td>CA (f)</td>
<td>100</td>
<td>26.50 ± 1.31</td>
<td>20.89</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>19.66 ± 1.11*</td>
<td>41.31</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>12.32 ± 1.66*</td>
<td>63.22</td>
</tr>
<tr>
<td>CA (fa)</td>
<td>100</td>
<td>26.33 ± 1.84</td>
<td>21.40</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>23.83 ± 1.46</td>
<td>28.86</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>18.16 ± 1.14*</td>
<td>45.79</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100</td>
<td>06.66 ± 0.45</td>
<td>80.12</td>
</tr>
</tbody>
</table>

Vehicle: Distilled water (1 ml); Abbreviations: CA = Celtis australis; b = bark; f = fruits; fa = fatty acids; * = significant (p<0.05) using Tukey's Multiple Range Test

Table 2. Anti-inflammatory Activity in Carrageenean Induced Paw Edema

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg, b.w.)</th>
<th>Carrageenean Induced Paw Volume (ml)</th>
<th>1h (inhibition %)</th>
<th>2h (inhibition %)</th>
<th>3h (inhibition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>1.10 ± 0.02 (00.00)</td>
<td>1.04 ± 0.02 (00.00)</td>
<td>1.22 ± 0.01 (00.00)</td>
<td></td>
</tr>
<tr>
<td>CA (b)</td>
<td>100</td>
<td>1.01 ± 0.01 (08.18)</td>
<td>0.99 ± 0.03 (04.81)</td>
<td>0.92 ± 0.02 (24.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.94 ± 0.02 (14.55)</td>
<td>0.84 ± 0.02 (19.23)*</td>
<td>0.87 ± 0.04 (28.69)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.78 ± 0.03 (29.09)*</td>
<td>0.72 ± 0.04 (30.77)*</td>
<td>0.68 ± 0.02 (44.26)*</td>
<td></td>
</tr>
<tr>
<td>CA (f)</td>
<td>100</td>
<td>0.99 ± 0.02 (10.00)</td>
<td>0.93 ± 0.03 (10.58)</td>
<td>0.89 ± 0.02 (27.05)*</td>
<td></td>
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<tr>
<td></td>
<td>250</td>
<td>0.91 ± 0.03 (17.27)</td>
<td>0.75 ± 0.03 (27.88)*</td>
<td>0.71 ± 0.04 (41.80)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.88 ± 0.02 (20.00)*</td>
<td>0.74 ± 0.02 (28.85)*</td>
<td>0.66 ± 0.02 (45.90)*</td>
<td></td>
</tr>
<tr>
<td>CA (fa)</td>
<td>100</td>
<td>1.05 ± 0.02 (04.55)</td>
<td>0.98 ± 0.03 (05.77)</td>
<td>0.92 ± 0.04 (24.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.96 ± 0.02 (12.73)</td>
<td>0.86 ± 0.04 (17.31)*</td>
<td>0.85 ± 0.03 (30.33)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.88 ± 0.02 (20.00)*</td>
<td>0.75 ± 0.02 (27.88)*</td>
<td>0.70 ± 0.02 (42.62)*</td>
<td></td>
</tr>
<tr>
<td>PBZ</td>
<td>100</td>
<td>0.77 ± 0.02 (30.00)</td>
<td>0.69 ± 0.02 (33.65)</td>
<td>0.62 ± 0.03 (49.18)</td>
<td></td>
</tr>
</tbody>
</table>

Vehicle: 10% (V/V) Tween 80 (1 ml); Abbreviations: CA = Celtis australis; b = bark; f = fruits; fa = fatty acids; PBZ: Phenylbutazone; * = significant (p<0.05) using Tukey’s Multiple Range Test.

A dose-dependent inhibition of swelling caused by carrageeen after 3h equivalent to 24.59 to 45.90% protection in higher concentrations (500 mg/kg). The results were found significant (p<0.05) when compared to negative control. The standard phenylbutazone at a dose of 100 mg/kg showed 49.18% anti-inflammatory activity. The bark and fruits extracts of C. australis has been found significant in all concentration i.e. 100, 250 and 500 mg/kg b.w.

Preliminary results showed that C. australis contains triterpenoids in major proportions together with phenolics [8]. The fatty acid has also been isolated and characterized from the fruits of the plant along with its antimicrobial activity [7]. The esterified fatty acids contain methyl oleate (25.7%), methyl palmitate (22.2%), methyl tricosanoate (13.3%), methyl lineolate (7.8%), methyl tetradecanoate (2.9%), octadecanoate (2.7%), 1,2-epoxy-1-venylcyclododecane (2.9%), methyl dotoaccentanoate (2.6%), methyl 2,4-dimethyl heneicosanoate (2.2%), methyl hexadecanoate (2.1%), methyl 14-acetylhydroxypalmitate (2.1%), teratriacontane (1.9%), 2-methylstearoate (1.8%), methyl 13-methyltetradecanoate (1.4%), methyl octadecanoate (1.3%), methyl 1-dotriacontanoate (1.1%) and methyl 1-tetradecanoate (1.1%).
out of the total composition of esterified fatty acid. In the present study, the triterpenoids, phenolics and other terpenes (in fatty acids) are perhaps responsible constituents for the analgesic and anti-inflammatory activities.

CONCLUSION

The study concludes that all the test samples obtained from *C. australis* exhibited significant analgesic and anti-inflammatory activities. The extracts from stem bark and fruits showed the activities superior to those of fatty acids. The plant is a rich source of triterpenoids and few phenolics, hence, the activity is due to these constituents. Fatty acids contain a mixture of many saturated and unsaturated constituents, and synergistically, these play the role in biological activities. The potency of these samples can be improved by the purification of crude extracts or by the isolation of pure constituents responsible for the activity from them which needs further studies in advanced level.

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CONFLICT OF INTEREST

The authors report no declaration of interest.

REFERENCES


