PHARMACOLOGICAL EVALUATION OF OXALIS DEHRADUNENSIS RAIZADA (LEAF EXTRACT) FOR ANALGESIC AND ANTIPYRETIC ACTIVITY ON WISTAR RATS

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ABSTRACT

*Oxalis dehradunensis* Raizada (oxalidaceae) is one of the important medicinal plants used traditionally for the treatment of fever, pain, inflammation and wound healing. The present study was aimed to validate folk use of *oxalis dehradunensis* Raizada as analgesic and antipyretic remedy. The analgesic activity of methanolic extract of *oxalis dehradunensis* Raizada was performed by hot plate, tail flick and tail immersion methods, while antipyretic activity was performed by Brewer’s yeast induced pyrexia method. The extract was screened at the dose of 200mg/kg and 400mg/kg by P.O. route. The drugs diclofenac sodium (10mg/kg P.O.) and paracetamol (150mg/kg P.O.) were taken as standard. Pyrexia was induced by 10ml/kg of 15% suspension of brewer’s yeast in 0.9% saline S.C. route. The study therefore, supports its use in folk medicine both as analgesic and antipyretic agent and calls for further investigations to elucidate its mechanism of action.

KEYWORDS: *Oxalis dehradunensis* Raizada analgesia, pyrexia, diclofenac, PCM.

INTRODUCTION

Many plants conveniently available in India are used in traditional folk medicine for the treatment of fever, pain, wound healing and inflammation. The plant selected for present studies is *oxalis dehradunensis* Raizada (oxalidaceae). Very common weed found throughout warmer parts of India and other country.\[1\] *Oxalis dehradunensis* Raizada is a species of flowering plant in the woodsorrel family known by the common names garden pink-sorrel and brosdleaf woodsorrel. *Oxalis dehradunensis* Raizada is a herbaceous perennial herb in the oxalidaceae or geraniaceae family that is native to Mexico, South and Central America.
and widespread throughout the southeastern united states, central America, the Caribbean, and northern south America (Colombia, Peru, and Brazil).[2] It is also used as complimentary medicine in antiseptic, refrigerant, wound healing. Other alternative used are anthelminthic, anti-inflammatory, astringent, diuretic, urinary tract infections, fever.[3] Chemical characterization showed the presence of oxalic acid, fatty acids, vitexin and isovitexin, neutral lipid, glycolipids. Phytochemical investigation of oxalis dehradunensis Raizada have revealed the presence of tannins, palmitic acid, lenoleic, linolenic and stearic acids.[4] methanolic extract of this plant show the presence of carbohydrate, glycosides, phytosterols, phenolic compounds, flavonoids, proteins, amino acids, volatile oil. It also show the presence of calcium fiber and tannins. Leaves contain tartaric acid, citric acids and calcium oxalate, flavones (apigenin), flavonols (quercetin), glycoflavones (vitexin, orientin). This herb is well known to have an acid taste due to the high content of oxalate in its leaves.[5] Herbal medicine is a major component in all traditional medicine system and a common element in Ayurvedic, Unani, Homeopathic, Naturopathic, Traditional Chines medicine and native Amercan medicine. As per literature herbal medicines are assuming greater importance in the primary health care of individuals and communities in rural areas. Plants and their derivatives are invaluable source of therapeutic agents to treat various disorders. Herbal products are safe because they are natural and having less side effect. Considerable efforts have been directed towards the development of natural products from various plant source.[6] Substantial number of drugs are developed from plants which are active against various diseases and disorders.[7] Present study was designed to evaluate analgesic and antipyretic potential of methanolic leaf extract of oxalis dehradunensis Raizada.

MATERIALS AND METHODS

Plant material
Leaves of oxalis dehradunensis Raizada were collected in the month of October 2016 from Bhimtal in the Nainital district of uttrakhand. It was authenticated by Dr. Ambrish Kumar (Scientist-D) Botanical Survey of India, Dehradoon. Voucher specimen No. is (117452). The leaves (5.6 kg) were air dried and pulverized using a mechanical grinder.

Extraction and isolation
Dried and powdered leaves (50g) of the plant were Defating with 100ml Petroleum ether and extracted with 100ml Methanol for 24 h. using soxhlet apparatus. The extracts were dried using a rotary vacuum evaporator and stored in a refrigerator until further use.
Animals
Wistar rats (150-200g) weighing 150-200g were housed under laboratory condition, in groups of six each and used for analgesic and antipyretic activity. The animal had free access to water and food *ad libitum*. The ethical committee of the institute approved the protocol of the study having Protocol no. KUDOPS/60.

Analgesic activity by hot plate method
Central nociceptive activity was evaluated using the hot plate method.[8] Wistar rats were divided into 4 groups of six animal each. The first group served as control and received only vehicle (distilled water) and the second group was administered standard drug diclofenac sodium 10mg/kg (p.o.). The animal of third and forth group T₁ and T₂ were treated with extract of *oxalis dehradunensis* Raizada dose 200mg/kg and 400mg/kg respectively. The extracts, standard drugs were dissolved into the vehicle. The rats were placed individually on the hot plate maintained at 55⁰c and latency of nociceptive response such as licking, flicking, of the hind limbs or jumping was noted. The reading were taken at 0,30,60,90,120,180 min after treatment. The cut off time was taken as 15 second on the hot plate to avoid damage to the paws.[9]

Analgesic activity by tail flick method
Central nociceptive activity was evaluated using the Tail flick method.[10] Wistar rats were divided into 4 groups of six animal each. The first group served as control and received only vehicle (distilled water) and the second group was administered standard drug diclofenac sodium 10mg/kg (p.o.). The animal of third and forth group T₁ and T₂ were treated with extract of *oxalis dehradunensis* Raizada dose 200mg/kg and 400mg/kg respectively. The tail of rats were placed individually on the analgesiometer maintained at 55⁰c and latency of nociceptive response time flicking of tail was noted. The reading were taken at 0,30,60,90,120,180 min after treatment. The cut off time was taken as 12 second to avoid damage to the tail.[11]

Analgesic activity by Tail immersion method
Central nociceptive activity was evaluated using the Tail immersion method. Wistar rats were divided into 4 groups of six animal each. The first group served as control and received only vehicle (distilled water) and the second group was administered standard drug diclofenac sodium 10mg/kg (p.o.). The animal of third and forth group T₁ and T₂ were treated with extract of *oxalis dehradunensis* Raizada dose 200mg/kg and 400mg/kg respectively. The tail
of rats use at mark 5cm and dip individually in water beaker temperature maintained at 55±
0.20c and latency of nociceptive response, such as withdrawal time of tail was noted. The
reading were taken at 0,30,60,90,120,180 min after treatment. The cut off time was taken as
15 second in the hot water beaker to avoid damage to the tail.\textsuperscript{[12]}

**Antipyretic activity by Brewer’s yeast induced pyrexia method**

Wistar rats (male) of constant rectal temperature for a week were selected for the experiment. Rectal temperature (T\textsubscript{R}) were recorded by inserting a lubricated digital thermometer (external diameter: 3mm, 0.10c precision) 2.8 cm into the rectum of rats. Animal presenting initial rectal temperature between 36 and 370c were selected for the antipyretic tests. The antipyretic activity of the extracts was evaluated based on Brewer’s yeast induced pyrexia in rats. Pyrexia was induced by subcutaneous injection of 10ml/kg of 15% w/v brewer’s yeast suspension below the nape of neck. The rectal temperature of each rat was measured at time, 0 hour, using a lubricated thermometer and before injection of the yeast. At 18 h following yeast injection, the different groups were treated with the vehicle, extracts (200and 400mg/kg p.o.) and standard drug, Paracetamol (150mg/kg p.o.). The rectal temperatures were recorded at 1, 3 and 5 hour after treatment.\textsuperscript{[13]}

**Percentage reduction in rectal temperature** = \frac{Y-X}{Y-Z} \times 100

Where Z = Initial rectal temperature 0C, Y = Rectal temperature 18 hour after yeast administration, X =Rectal temperature after extract administration.

**RESULTS**

The results from the present study show that the crude extract of *oxalis dehradunensis* Raizada exhibit activities in varying degrees against pain and fever.

**Effect of the extracts on hot plate test**

ODRME were found to cause time- dependently prolongation of the hot plate latency. The longest latency was obtained at 180 min post administration of the extract or the reference drug. The standard drug diclofenac (10mg/kg) was more potent than the extracts. The leaves extract showed significant result at 60, 90,120 and 180 min in compare to control and standard drugs.
Table 1: Effect of *oxalis dehradunensis* Raizada leaves extracts on analgesia by hot plate method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Reaction time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>3.716±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2088</td>
</tr>
<tr>
<td>Standard Diclofenac sod.</td>
<td>10 mg/kg</td>
<td>4.95±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1147*</td>
</tr>
<tr>
<td>Test drug (T1)</td>
<td>200mg/kg</td>
<td>3.98±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2136</td>
</tr>
<tr>
<td>Test drug (T2)</td>
<td>400 mg/kg</td>
<td>4.466±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2155*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM (N=6). In standard p<0.05 significant with respect to the control, and in test 2 p<0.05 significant with respect to the control group by one way ANOVA test.

![Fig 1: Effect of *oxalis dehradunensis* Raizada leaves extracts on analgesia by hot plate method.](image)

**Effect of the extracts on tail immersion test**

The treatment of rats with the plant extract at dose of 200 mg/kg and 400mg/kg exhibit a significant increase in reaction time while compared with control. Diclofenac and ODRME showed highly significant increase in reaction time at 90 min after drug administration. Standard drug was more potent than both of the extracts.
Table 2: Effect of leaves extracts andDiclofenac sodium on analgesia by tail immersion test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Reaction time in min</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
<td>120 min</td>
<td>180 min</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>3.11±0.153</td>
<td>3.133±0.133</td>
<td>3.15±0.067</td>
<td>3.883±0.1815</td>
<td>4.066±0.1783</td>
<td>3.805±0.2121</td>
</tr>
<tr>
<td>Standard Diclofenac sod.</td>
<td>10 mg/kg</td>
<td>4.466±0.042**</td>
<td>5.6±0.073**</td>
<td>6.333±0.066**</td>
<td>7.966±0.323**</td>
<td>8.786±0.498**</td>
<td>8.825±0.507***</td>
</tr>
<tr>
<td>Test drug (T1)</td>
<td>200 mg/kg</td>
<td>3.616±0.1537*</td>
<td>4.683±0.2301*</td>
<td>5.933±0.1145*</td>
<td>6.733±0.224*</td>
<td>7.433±0.095*</td>
<td>7.695±0.320*</td>
</tr>
<tr>
<td>Test drug (T2)</td>
<td>400 mg/kg</td>
<td>4.2±0.233**</td>
<td>4.683±0.330**</td>
<td>6.216±0.238**</td>
<td>7.3±0.188**</td>
<td>8.383±0.279**</td>
<td>8.566±0.236**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM (N=6). In standard p<0.01 significant with respect to the control, and in test 1 p<0.05 with respect to the control and test 2 p<0.01 significant with respect to the control group by one way ANOVA test.

Fig 2. Effect of leaves extracts and Diclofenac sodium on analgesia by tail immersion test.

Effect of the extracts on analgesia by Tail flick method

ODRME were found to cause time- dependently prolongation of the tail flick latency. The longest latency was obtained at 180 min post administration of the extract or the reference drug. The standard drug diclofenac was more potent than the extracts at all time levels. The leaves extract showed significant result at 60, 90,120 and 180 min in compare to control and standard drugs.
Table 3: Effect of the extracts and diclofenac sodium on analgesia by Tail flick method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Reaction time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>3.61±0.200</td>
</tr>
<tr>
<td>Standard Diclofenac sod.</td>
<td>10 mg/kg</td>
<td>4.57±0.170*</td>
</tr>
<tr>
<td>Test drug (T1)</td>
<td>200 mg/kg</td>
<td>3.73±0.202</td>
</tr>
<tr>
<td>Test drug (T2)</td>
<td>400 mg/kg</td>
<td>4.538±0.1684*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM (N=6), p<0.05 significant with respect to the control group by one way ANOVA test.

Fig 3: Effect of the extracts and diclofenac sodium on analgesia by Tail flick method.

Effect of the extracts on brewer’s yeast suspension induce pyrexia

Standard drug Paracetamol 150 mg/kg and test drugs (t2) 400 mg /kg orally showed statistically significant (P<0.05) difference in 1hr, 2hr, 3hr, and 4hr respectively.

Table 4: Effect of *oxalis dehradunensis* Raizada leaves extracts and paracetamol on pyrexia.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Reaction in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
<td>1 hr</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg</td>
<td>38.666±0.305</td>
</tr>
<tr>
<td>Std PCM</td>
<td>150mg/kg</td>
<td>37.616±0.246*</td>
</tr>
<tr>
<td>Test drug (T1)</td>
<td>200mg/kg</td>
<td>39.366±0.231</td>
</tr>
<tr>
<td>Test drug (T2)</td>
<td>400mg/kg</td>
<td>39.6±0.3724*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM (N=6), p<0.05 significant with respect to the control group by one way ANOVA test.
DISCUSSIONS
Currently available drug regimens for management of Analgesic and antipyretic activity have certain drawbacks and therefore there is a need for safer and more effective drugs. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health has been widely observed.

The study indicated that *oxalis dehradunensis* Raizada leaves extract has both analgesic as well as antipyretic property. The analgesic effect of the extract was determined from hot plate, Tail flick and Tail immersion method. The antipyretic activity of the extract was determined from brewer’s yeast induce pyrexia method.

The analgesic activities were also studied using thermal tests. The thermal test (hot plate, Tail flick and tail immersion test) was selected because the test is sensitive to strong analgesics and limited tissue damage because of a cutoff point that is usually applied to limit the amount of time the animal spends on the hot plate. The extracts produced a prolongation of time latency in all the three tests. These tests are supraspinally mediated and therefore a test of central activity. The groups treated with test extracts did not show any toxicity. Thus the test extracts are safe and may be used as an analgesic agent.

It is an established fact that any agent that causes a prolongation of the latency using this test may be acting centrally. This is however, subject to its not having any effect on motor activity and not causing any sedative action. From the present work, the mechanism (s) of the
analgesic effects of the *oxalis dehradunensis* Raizada tested is not readily apparent. It can, however, be spectulated that it may be linked to processes involved in the prevention of sensitization of the nociceptor, down regulation of the sensitized nociceptors and/or blockade of the nociceptor at peripheral and/or central levels. One of the well characterized signaling systems believed to participate in this mechanism(s) is the arachidonic acid metabolic pathway.\[^{17}\] In this regard, it inhibits the production of prostaglandins and thromboxanes similar to acetylsalicylic acid and thus reduces pain libration.

It is well known that most of the analgesic drugs possess antipyretic activity. The leaves extract of *oxalis dehradunensis* Raizada revealed marked antipyretic activity in brewer’s yeast-induced pyrexia in rats. In the present study the initial rise of temperature after 18 hrs of subcutaneous yeast injection was above 38°C. There was significant difference between the initial mean basal temperature of the different groups and the mean temperature between the groups of pyrexia rats, after 18 hours of yeast injection. Rectal temperature of pyrexia rats were lowered significantly with the test drug and standard drug when compared with control group. Standard drug Paracetamol is more potent than the test extracts.\[^{18}\] No toxicity was seen in the test drugs treated groups after 24 hours of experiment so the test drug is a safe antipyretic agent.

**CONCLUSION**

From the above discussion it can be concluded that the leaves extract of *oxalis dehradunensis* Raizada possess both analgesic and antipyretic activity. So we can say that herbal extract may be remunerative effect with lower side effect as compare to standard drug (Diclofenac sodium and Paracetamol) for the management of analgesia and pyretic respectively.

**ACKNOWLEDGEMENT**

I am thankful to Dr. Tirath Kumar Asst Prof. Kumaun University, Bhimtal campus for guiding me and providing laboratory facilities for my research work.

**REFERENCES**

