Anti-inflammatory and Analgesic activities of root and stem of *Cissus rependa* vahl

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**ABSTRACT**

Anti-inflammatory and analgesic activity of root and stem of *Cissus rependa* Vahl.(Vitaceae), a folklore medicinal plant of Gandhamardana hill ranges of Orissa, was carried out on albino rats and mice at the dose of 180 mg/kg and 260 mg/kg respectively. The effect on inflammation was studied on carrageenan and formalin induced paw oedema models and compared with phenylbutazone and diclofenac sodium standards respectively. Analgesic activity was evaluated in formalin induced paw licking and tail flick method where indomethacin and pentazocine were used as standard drugs respectively. The *C. rependa* root (CRR) significantly inhibited carrageenan induced paw oedema, formalin induced paw licking and prolonged tail flick response, however it was failed to suppress the formalin induced paw oedema, whereas *C. rependa* stem (CRS) significantly inhibited carrageenan induced paw oedema and formalin induced paw oedema at 24h, however it failed to inhibit formalin induced pain response. Further, CRS prolonged tail flick response significantly only at 180minutes. The results suggest that the root of *C. rependa* has significant analgesic and anti-inflammatory potential as reflected by the parameters investigated, while stem has weak anti-inflammatory and analgesic activity. Thus it can be concluded that roots of *C. rependa* have significant anti-inflammatory and analgesic activity and can be preferred in the treatment of pain and inflammation.

**Keywords:** *Cissus rependa*, folklore, Gandhamardan hill, analgesic, anti-inflammatory.

**INTRODUCTION**

Plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities, for the control, management and treatment of a variety of human and animal ailments. The use of leaves, stems, barks, flowers, seeds or roots is common among people, especially by the tribal people to manage various ailments. *Cissus rependa* Vahl.(Vitaceae) is a medicinal plant distributed from Kuman to Arunachal Pradesh, Tripura, Assam, Bihar, Orissa, Madhya Pradesh, and Western Ghats region up to 1350 meters.[1] The root and stem of this plant has been used among the tribal peoples of Gandhamardana hill ranges, Bolangir, Orissa for its properties of alleviation pain from cutting wound, and bone fractures.[2,3,4] In spite of its reputation in treating these ailments, till date no pharmacological work to support these claims has been reported. Hence the present study was under taken to evaluate the anti-inflammatory and analgesic activities of root and stem of *Cissus rependa* Vahl. in albino rats and mice.

**MATERIALS AND METHODS**

**Plant material**

Fresh plant material was collected from its natural habitat of Gandhamardana hill ranges, Bolangir, Orissa, India. The plant was authenticated by pharmacognosist. The verified specimen was preserved in the departmental herbarium museum (vide no. 6001/2009) for future references. The root and stem were cutout separately and washed thoroughly. The cleaned material was shade dried and powdered (Mesh 80). The *C. rependa* root was coded as CRR and *C. rependa* stem as CRS and stored in air tight container for experimental study.

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Experimental animals

Wistar strain albino rats of either sex weighing 200 ± 20g and Swiss albino mice of either sex weighing 30 ± 06g were procured from the animal house attached to our institute (Registration No.548/2002/CPCSEA). They were housed in polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*. The animals were acclimatized for at least one week in lab conditions before the commencement of experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60 % humidity. Before the test, the animals were fasted for at least 12 hours. Institutional animal ethics committee had approved the experimental protocol (Approval number; IAEC 03/08-10/Ph.D-06) and the care of animals was taken as per the CPCSEA guidelines.

Dose selection

The dose fixation for the experimental animals was done on the basis of body surface area ratio by referring to the standard table of Paget and Barnes (1964)\[8\]. The adult human dose (2g per day) was converted to animal dose. On this basis the rat dose was found to be 180 mg/kg and for mouse 260 mg/kg. The test drug was suspended in distilled water with suitable concentration depending up on body weight of animals and administered orally with the help of gastric catheter inserted to syringe. The drugs were administered to overnight fasted animals.

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced rat paw oedema

The Wistar strain albino rats of either sex were weighed and randomly divided in to four groups of six each. First group received distilled water and served as control group. The second and third groups received test drugs CRR and CRS respectively. Fourth group was administered with standard anti-inflammatory drug phenylbutazone (Wilson Laboratories, Mumbai) in the dose of 100mg/kg. The vehicle and test drugs were administered to the respective groups for five consecutive days; whereas standard drug was given only once i.e., one hour before the carrageenan injection\[8\].

Initially left hind paw volumes up to the tibio-tarsal articulation were recorded prior to Carrageenan injection by using plethysmograph\[7\]. The plethysmograph employed, consisted of 10 ml glass vessel (25mm × 65mm) fixed to 2 ml glass syringe through pressure tubing. About 4 ml of Mercury was filled in the syringe and the mercury level was adjusted to zero mark on the micropipette. The space between the zero mark and the fixed mark on the glass vessel was filled with water and few drops of teepol. The initial level of fluid was adjusted and set at zero. The paw was immersed in water exactly up to the tibio-tarsal articulation. The increased level of water in the glass vessel was adjusted to the prefixed mark by releasing the pressure of the connected syringe. The level where water and mercury interface in the micropipette was recorded as paw volume.

On fifth day one hour after drug administration oedema was produced by injecting 0.1 ml freshly prepared 1% carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administrated tap water in the dose of 2 ml per 100 g body weight to ensure uniform hydration and hence to minimize variations in oedema formation. Paw volume was recorded three hour after carrageenan injection. Results were expressed as an increase in paw volume in comparison to the initial paw volumes and also in comparison with control group.

Formaldehyde induced paw oedema

The test conditions and groupings were similar to carrageenan induced paw oedema as mentioned above, except the standard anti-inflammatory drug used (diclofenac sodium - 5mg/kg - Novartis India Limited). Pedal inflammation was induced by injecting 0.1 ml of 3 % formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 24 and 48 h after formaldehyde injection\[8\]. Results were expressed as an increase in paw volume in comparison to the initial paw volumes and also in comparison with control group.

ANALGESIC ACTIVITY

Tail flick test

Mice were placed on the tail flick unit so that constant heat intensity was applied to the lower third of the animal’s tail. When the animal flicked its tail in response to the noxious stimulus both the heat source and timer were stopped. A cut off time of 10 seconds was set to avoid tail damage. Thus basal reaction time of each mouse to radiant heat was recorded and those having TFL (tail flick latency) less than 10 seconds were selected. Thus selected mice were randomly divided in to four groups of six each. First group received similar volume of vehicle as test drug and served as normal control. Mice in group two and three were treated with CRR and CRS respectively. To the group four standard analgesic drug pentazocine (20mg/kg i.p. - Ranbaxy laboratories) was administered. The vehicles test drug and
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reference standard were administered to the respective groups one hour prior to experiment. The TFL was recorded at 30, 60, 90, 120, 180 and 240 minutes[9].

**Formalin induced hind paw licking**

Animal grouping and test drug administration are similar to carrageenan induced paw oedema model. Indomethacin (10mg/kg orally - Cipla) was used as standard analgesic drug. Pain was induced by injecting 0.1 ml of 3% formalin in distilled water in subplantar region of right hind paw and the duration of paw licking as an index of nociception was counted in periods of 0 to 10 minutes (Early phase) and 20 to 30 minutes (Late phase)[10].

**Statistical analysis**

Data are expressed as mean ± SEM. Statistical evaluation was carried out by one way analysis of variance (ANOVA followed by Dunnnett’s multiple ‘t’ test) and also by unpaired Student ‘t’ test. The level of significance was set at P<0.05 and the level of significance was noted and interpreted accordingly.

**RESULTS**

Both CRR and CRS at the dose of 180mg/kg, significantly inhibited paw oedema at both 3 and 6 hours after carrageenan injection. The observed inhibition is almost equal to standard anti-inflammatory drug Phenylbutazone (Table-1).

The result (Table - 2) shows that CRR at the dose of 180 mg/kg did not suppress the formalin induced paw oedema at both 24 and 48 hour intervals. CRS at the dose of 180 mg/kg has significant reduction in the formalin induced paw oedema at 24 hour, where as it did not suppress the paw oedema at 48 hour intervals. Diclofenac sodium suppressed the formalin induced paw oedema in significant manner at both 24 and 48 hours comparison to control group.

Table - 3 shows the results of the formalin induced paw licking response. The CRR significantly decreased the paw licking response at both early phase and late phase. However the observed effect is comparatively less to indomethacin treated group. Administration of CRS unable to inhibit formalin induced pain at both early phase and late phase of pain.

Mice, pre-treated with Pentazocine (20mg/kg) significantly increased TFL (P<0.01) after 30 minute and non-significantly at 60 minutes onwards (Table - 4). CRR significantly increased TFL in time dependant manner i.e., P<0.01 at 90 min, P<0.05 at 120 min and P<0.01 at 180 minute after drug administration. In contrast to this CRS shows significant increase in TFL only at 180 minutes in comparison to control group (Table-4).

<table>
<thead>
<tr>
<th>Table 1: Effect on carrageenan induced paw edema.</th>
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<td><strong>Treatment</strong></td>
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<tr>
<td>Control</td>
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<tr>
<td>CRR</td>
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<tr>
<td>CRS</td>
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<td>Diclofenac sodium (100mg/kg,po)</td>
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Data: Mean ± SEM; ↓ – Decrease
*One way ANOVA – F value 23.340; P<0.001: DMTT – P<0.05 for root, stem, phenyl butazone Vs normal control.

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<th>Table 2: Effect on formalin induced paw edema.</th>
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<td><strong>Treatment</strong></td>
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<tr>
<td>Control</td>
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<tr>
<td>CRR</td>
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<td>CRS</td>
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<tr>
<td>Diclofenac sodium (5mg/kg, po)</td>
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</table>

Data: Mean ± SEM; ↓ – Decrease
*One way ANOVA – F value 11.0; P<0.001: DMTT – P<0.05 for stem and diclofenac sodium Vs normal control.
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Investigated by adopting formalin induced pain and tail flick response methods. Formalin injection to plantar aponeurosis of rats shows pain response in two phases viz., initial and late phases. The initial phase lasts for 0-10 min. of formaldehyde injection, it is supposed to be mediated through modulation of neuropeptides \[13\]. The second phase, which is observed 20-30 minutes of formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin etc. The root of test drug (CRR) significantly decreased the paw licking episodes at both the phases so as the indomethacin which is a non-selective cyclooxygenase inhibitor. This suggests that the antinociceptive effect of the CRR was mediated by both neurogenic and inflammatory mechanisms. In this model stem part of test drug (CRS) failed to antagonize the formalin induced algonic effect.

Tail flick model which is thermal induced nociception indicates narcotic involvement which is sensitive to opioid μ receptors\[^{12}\].\[^{12}\]. The ability of the CRR to prolong the reaction latency to thermally induced pain in mice further suggests central analgesic activity. The effect observed with pentazocine was short lived where as the effect observed in CRR is long lasting. This indicates that the CRR exhibit analgesic effect by central action. The mechanism through which this effect is brought about may be due to modulation of opioid receptors or by release of endogenous analgesic factors like endorphin etc. Further CRS is also having TFL prolonging effect, but the magnitude of prolongation is comparatively less.

<table>
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<th>Table 3: Effect on formalin induced paw licking response.</th>
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<tr>
<td>Treatment</td>
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<td>Control</td>
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<tr>
<td>CRR</td>
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<tr>
<td>CRS</td>
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<tr>
<td>Indomethacin (10mg/kg,po)</td>
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Data: Mean ± SEM; ↓ Decrease
\[^{1}\]One way ANOVA – F value 6.565; P < 0.001: DMTT – P < 0.05 for root, Indomethacin Vs normal control.
\[^{2}\]One way ANOVA – F value 16.837; P < 0.001: DMTT – P < 0.05 for root, Indomethacin Vs normal control.

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<th>Table 4: Effect on tail flick response</th>
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<tr>
<td>Treatment</td>
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<td>Control</td>
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<td>CRR</td>
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<td>CRS</td>
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<td>Pentazocine (20mg/kg, ip)</td>
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Data: Mean ± SEM; *P < 0.05, ** P <0.01 (unpaired t test)

DISCUSSION

Carrageenan induced inflammation in rats is one of the most suitable acute model to screen anti-inflammatory agents. The intraplantar injection of carrageenan in rats leads to paw edema. Its first phase (Up to 3 hours after injection of carrageenan) results from the concomitant release of mediators: histamine, serotonin and kinins on the vascular permeability. The second phase is correlated with leukotrienes\[^{11}\]. In present study, both root and stem parts of test drug produced a considerable suppression of carrageenan induced paw oedema at both the phases. The inhibitory effect on carrageenan induced inflammation in the rats observed could be due to inhibition of enzyme cyclo oxygenase , leading to inhibition of prostaglandins.

The formalin-induced inflammation in the rats foot may be conveniently divided into two parts, the first involving 5-hydroxytryptamine as mediator and the second mediator which is unrelated to 5-hydroxytryptamine\[^{13}\]. In contrast to results obtained in carrageenan induced paw oedema, root of test drug failed to suppress the paw oedema at both time intervals, where as stem part able to suppress oedema at on 24 hour after formalin injection. Thus shows root part is not having 5-HT suppression activity while stem is having capacity to suppress 5-HT mediated paw oedema.

The mechanism for testing analgesic was selected such that both centrally and peripherally mediated effects were investigated by adopting formalin induced pain and tail flick response methods. Formalin injection to plantar aponeurosis of rats shows pain response in two phases viz., initial and late phases. The initial phase lasts for 0-10 min. of formaldehyde injection, it is supposed to be mediated through modulation of neuropeptides\[^{13}\]. The second phase, which is observed 20-30 minutes of formaldehyde injection, is supposed to be mediated through release of inflammatory mediators like prostaglandin etc. The root of test drug (CRR) significantly decreased the paw licking episodes at both the phases so as the indomethacin which is a non-selective cyclooxygenase inhibitor. This suggests that the antinociceptive effect of the CRR was mediated by both neurogenic and inflammatory mechanisms. In this model stem part of test drug (CRS) failed to antagonize the formalin induced algonic effect.
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It has been reported that the plant *C. repanda* is having alkaloids, tannins, glycosides and flavonoids\[^{15}\]. Presence of these phyto-constituents in *C. repanda* supports the claim that these compounds have antinociceptive and anti-inflammatory properties since alkaloids, flavonoids and saponins have been found in other natural products with analgesic and anti-inflammatory properties\[^{16, 17}\].

CONCLUSION

From the present study, it can be conclude that, roots of *Cissus rependa* have significant anti-inflammatory and analgesic activity in comparison to stem. Hence, it can be preferred in the treatment of pain and inflammation. However studies to systematically extract the active metabolites from the roots will be needed to ascertain their analgesic and anti-inflammatory properties separately.

REFERENCES
