Anti-inflammatory activity of methanolic extracts of *Pseudarthria viscida* and *Uraria picta* against carrageenan induced paw edema in albino rat

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**Abstract:** *Pseudarthria viscida* and *Uraria picta* are the major constituents of Ayurvedic Dashmool formulation. They belong to the family *Fabaceae*. Both plants are reported for their important biological activities in indigenous system of medicine. This study was carried out to evaluate anti-inflammatory activities of the whole plant methanolic extract of *P. viscida* and *U. picta* on carrageenan induced paw edema in albino rat. The methanolic extract of *P. viscida* and *U. picta* were administered orally at dose levels of 200 and 400 mg/kg body weight. Level of inhibition by the extracts was compared with Indomethacin standard reference drug. However *P. viscida* had higher inhibitory activity compare to *U. picta*. This signifies that methanolic extract from *P. viscida* is more potent than *U. picta* in the treatment of paw edema in albino rat. Present study provides information on pharmacological evidences that *Pseudarthria viscida* and *Uraria picta* could be used as agent against inflammation.

**Keywords:** *Pseudarthria viscida; Uraria picta; anti-inflammatory; wistar albino rat*

**INTRODUCTION**

Inflammation is a complex process which is generally characterized by redness on external plane and swelling on skin which leads to pain and perhaps it can also result in loss of function at sight of injury.(Leelaprakash and Mohan Dass, 2011) Tissue response to injury causes
inflammation. Numbers of Indian medicinal plants are available which are rich in source of active phyto-substances that can stimulate para-immunity. Plant derived substance with immunomodulatory properties had occupy an important position in different pharmacopoeias. *Pseudarthria viscida* (*Shalparni*) is an important plant diffuse under shrub, belonging to the family *Fabaceae*. Major chemical compounds present in the roots are gallic acid, caffeic acid, rutin, quercetin and ferulic acid as phenolic compounds (Rajan and Muthukrishnana 2013; Suriyavathana and Rajan 2011). There are reports of presence of flavanoids, protein and tannins in whole plant (Kuppusamy et al. 2012). This plant is getting vanished from its natural habit and its number is highly reduced in the wild due to its excessive collection (Sangeetha et al. 2014). That is why it is included under red listed category (Prakasa and Venkateswara 2014). The decoction of plant is useful in cough, fever, inflammation, asthma etc and it also posses analgesic activity (Shantha kumar et al. 2012; Vijayabaskaran et al. 2010). *Uリアria picta* (*Prishnaparni*) is important constituent of Dashmool formulation from family *Fabaceae*. Chemical constituents of *U. picta* are flavanoids, steroids, amino acids and fatty acids (Nitesh et al. 2014). Its content flavanoids are known against inflammation and also as antithrombotic, antioxidant activity and oxide scavenging effect (Kale et al. 2012; Rahman et al. 2007). There are reports on other therapeutic properties like wound healing activity of *U. picta* (Lalitha et al. 2012). Root of *U. picta* is used originally for Dashmool formulation but due to less availability *U. lagopodoides* is being used as substitute species. Many of the important constituents like flavanoids, alkaloids and tannins from these plants have been identified. Many pharmacological investigations on these plants have already reported its antimicrobial, antioxidant, wound healing effect (Baskar et al., 2012; Rahman et al., 2007). Therefore this study was aimed to provide scientific evidence for anti-inflammatory activity of both plants.

**MATERIALS AND METHODS**

**Plant material:** The well grown and healthy whole plant of *P. viscida* and *U. picta* were collected from waghai Botanical garden, Ambapada, Gujarat, NH360, India. Herbarium sheet of both plant species (voucher number: - UTU/CGBIBT/16-17/01 and UTU/CGBIBT/16-17/02) were submitted to Navsari Agricultural University, Navsari,
Gujarat, India. For identification and authentication which was done by Dr. Bimal Desai (Assistant Professor) at Navsari Agricultural University.

**Chemicals:** Carrageenan (Himedia, India), Indomethacin (Astron Chemical pvt. ltd.), 0.9% Normal saline (Aculife Healthcare Pvt. Ltd.) chemicals used for the study were of analytical grade. Other chemicals were also of analytical grade.

**Preparation of Plant Extracts for anti-inflammatory activity:** Whole plant of *P. viscida* and *U. picta* were shade dried and milled into powder. About 150 gm powder of whole plant was used for methanolic extraction using Soxhlet apparatus. Then the methanolic extracts were concentrated in rotary evaporator at 60°C. The concentrated methanolic extracts were dissolved in 0.9% normal saline and used for anti-inflammatory activity.

**Experimental Animals:** Approval for animal study was obtained from Institutional Animal Ethical Committee (MPC/IAEC/12/2016) before starting of the experiment. All the protocols were followed as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animals. Wistar albino rat of either sex (180 gm in weight) were used for this study. Animals were maintained under standard environmental condition and supplementation of standard pellet diet and water were given *ad libitum*.

**Acute toxicity study:** Acute oral toxicity test was performed according to Organization for Economic Co-operation and Development-423 guidelines. A total of twelve rats used for this study were starved overnight and provided only with water. According to OECD 423 guidelines, oral administration of methanolic extracts at 50 mg/kg weight were given to three animals and observed for 14 days. If mortality was observed in any two animal then the given dose was consider as toxic dose. In case of mortality in single animal, same dose should be repeated. If no mortality was observed, the protocol was repeated for higher doses as 100, 200 and 2000 mg/kg body weight. All animal were observed for behavioral changes like tail movement, hair loss and change in body weight.

**Carrageenan induced paw edema:** For the evaluation of anti-inflammatory potential of methanolic extract of both plants, carrageenan induced rat paw edema model was used. Total thirty six rats were randomly divided into 6 different groups as Control, Standard (drug- Indomethacin 10 mg) and
four different test groups. Control group received 0.9% normal saline orally. 10 mg/kg Indomethacin per body weight was administrated orally to Standard group. Test group III and IV received 200 mg/kg and 400 mg/kg dose of extract of *P. viscida* respectively via oral route. Test group V and VI orally received 200mg/kg and 400 mg/kg dose of extract of *U. picta* respectively. After 1 hour of drug administration, 0.1ml of 0.1% carrageenan was injected in left hind paws of the rats on plantar side. The paw volume was measured at interval of 30 minutes till the end of 240 minutes with digital caliper. The normal paw volume and 0 minute readings were measured after carrageenan injection. Percentage increases in paw volume of rats were compared to evaluate inhibitory effect of the drug. The potential of test samples were calculated considering the percentage inhibition rate of inflammation. Percentage inhibition was calculated using formula,

\[
\%\text{increase} = \frac{V_c - V_t}{V_c} \times 100.
\]

Where, \( V_c \) = paw volume of rat in control group, \( V_t \) = paw volume of rat in treatment group

**Statistical analysis:** All the results were expressed in the form of mean±SD. Statistical analysis were made using One-way ANOVA. Dunnett’s test was followed to determine significant differences between means.

**RESULTS**

The powdered yields of the extract after extraction were 18.92 gm and 15.78 gm from 150 gm of milled of whole plant of *P. viscida* and *U. picta* respectively. These yielded powders were sticky and dark greenish brown in colour. For dose selection and to avoid adverse effect of both plants extracts, acute toxicity test were performed as single exposure. All the tested animals were observed for behavioral changes, tail movement, hair loss and change in body weight for period of 14 days, but there was not any considerable changes observed when compared to control group. Results of acute toxicity study clearly shows that whole plant methanolic extract of *P. viscida* and *U. picta* have superior safety concern, as no mortality and behavioral changes observed after oral doses of 50, 100, 200 and 2000 mg/kg in rats. According to OECD toxicity guidelines one fifth of highest dose should be selected for further testing. Hence 200 mg/kg was selected as minimum dose and 400 mg/kg as higher dose for each of the plants. Whole plant methanolic extract of *P. viscida* at 200 mg/kg showed 33.80% (p<0.01) inhibition and dose 400mg/kg showed 35.43%
(p<0.001) inhibition, while methanolic extract of *U. picta* at 200 and 400 mg/kg dose levels showed 29.62% (p<0.01) and 32.71% (p<0.01) inhibition respectively at 240 minutes after the injection of carrageenan (Graph 1).

Total percentage inhibition covered by 10 mg/kg standard Indomethacin drug was 36.39% (p<0.001) after the 240 minute of carrageenan injection (Graph 1). Whole plant extract of *P. viscida* at dose 200 mg/kg and 400 mg/kg showed similar response when compare to standard group and more significant than the control group. While methanolic extract of *U. picta* at dose 200 mg/kg and 400 mg/kg showed better inhibition compare to control group. Though, on comparison of both plants, *P. viscida* showed higher inhibition than *U. picta*. Gradual increase in paw size of group of untreated rat was observed for particular time course, while other group treated with extract and standard drug, showed significant control on paw swollen, as a result of anti-inflammatory response (Graph 2). According to result of all statistical analysis, extract of both plant had significant anti-inflammatory activity at dose level 200 mg/kg and 400 mg/kg after 240 minutes against carrageenan.

**DISCUSSION**

Carrageenan induced paw edema model in rat was used to study anti-inflammatory activity of compound which involves chemical mediators like, histamine, serotonin and prostaglandins (Dimo *et al.* 2006). Whole plant extract of *P. viscida* and *U. picta* were prepared at two different doses and tested for their anti-inflammatory activity against carrageenan induced paw edema. Since the extracts were prepared by Soxhlet apparatus and concentrated by open fire heat at 60°C, the active phyto-constituents are considered to be heat resistant and thus extract were prepared for both the plants. 2000 mg/kg dose of extracts of *P. viscida* and *U. picta* were the highest dose for toxicity study and extract of both plants showed no mortality at this higher dose. According to OECD toxicity guidelines one fifth of highest dose should be selected for further testing. Therefore 200 mg/kg and 400 mg/kg dose were selected as sub-maximal and maximal cutoff dose for entire *in vivo* experimental study. The result of this experiment study showed, extract of *P. viscida* and *U. picta* possess acute anti-inflammatory activity against phlogistic agent carrageenan. Carrageenan induced inflammation includes three different stages of release of mediator like, first stage includes serotonin and histamine, second
stage include kinins and third stage includes prostaglandin (Anto and Brito 1998; Dimo et al. 2006). Anti-inflammatory drug inhibit only the third stage because anti-inflammatory drug is capable of inhibiting prostaglandin synthesis (Winter et al. 1980). In the present experimental study, Indomethacin was used as standard anti-inflammatory drug against acute inflammatory response in terms of swelling. In comparison with control group, treatment with *P. viscida* and *U. picta* reduces swelling significantly at 240 minutes after carrageenan injection. The extract of *U. picta* at dose level 200 and 400 mg/kg inhibit carrageenan induced paw edema in the first and third stage indicating effective inhibition on serotonin and/or histamine and prostaglandin. *U. picta* extract at 400 mg/kg dose showed effective inhibition in comparison to its lower dose 200 mg/kg. Inhibitory activity of higher dose of *U. picta* is comparable to inhibition by Indomethacin standard drug. While the extract of *P. viscida* at dose levels 200 and 400 mg/kg significantly inhibit carrageenan induced paw edema in the first and third stage indicating capability of inhibition over serotonin and/or histamine and prostaglandins. That simply means that tested extract do not have inhibition control on production of kinins in second stage, and so swelling is greatly higher in second stage, a middle phase of inflammation. *P. viscida* extract at 400 mg/kg dose showed higher inhibition over 200 mg/kg dose of same plant extract. Moreover the effectiveness of 400 mg/kg dose of extract of *P. viscida* is very similar to effectiveness of standard Indomethacin drug. For both of the plants, the high dose showed superior inhibition that is possibly because the higher dose contains higher amount of alkaloids and flavanoids which are responsible for inhibitory activity against released mediator in inflammation (Anto and Brito, 1998; Yesilada and Kupeli 2002). Furthermore both doses of *P. viscida* extract showed higher amount of inhibition than both doses of *U. picta* extract. These results clearly indicate that phyto-constituents responsible for anti-inflammatory activity, are present in higher concentration in *P. viscida* than in *U. picta* (Rahman et al. 2007; Vijayabaskaran et al. 2011). Previous report supported the present investigation which confirmed *P. viscida* could be used as potent agent against inflammation (Suriyavathana and Rajan 2011). There is separate report on anti-inflammatory activity of Dashmool plant which supports present investigation...
conforming that *U. picta* could be used as anti-inflammatory agent (Nagarkar et al. 2013). According to other literature, many active fractions do not showed considerable inhibition on synthesis of inflammatory mediators even at the higher dose. Despite the fact that this statement is hardly conclusive, the observed effectiveness of the agent in the acute stage of inflammation indicates the inhibition of histamine and/or serotonin as possible mechanism of action (Osadebe and Okoye 2003). In this experimental study it was noted that extracts of *P. viscida* and *U. picta* inhibit inflammation by inhibiting the synthesis of histamine, serotonin and prostaglandin which are the main stages of inflammation. In another report different fractionation of extract of *Turnera ulmifolia* were tested for same activity and concluded that alkaloid and isoflavonoids are responsible for the activity (Anto and Brito, 1998; Yesilada and Kupeli 2002). Active phyto-constituents present in *P. viscida* and *U. picta* may be involved in inhibition of inflammatory mediators. Phyto-constituents like saponins, terpenoids, flavonoids have not only be found present but also responsible for anti-inflammatory activity and also possessed wound healing, anti-oxidant, fracture healing, anti-diabetic etc activity (Rahman et al. 2007; Pathak et al. 2015; Vijayabaskaran et al. 2011). Future work should target these responsible phyto-constituents for further fractionation and isolation.

**CONCLUSION & RECOMMENDATION**

This investigation noted that, *Pseudarthria viscida* and *Uraria picta*; both showed good anti-inflammatory response in comparison to control group. Anti-inflammatory activity of *P. viscida* is similar to activity of standard drug. Hence, we recommend extract of *P. viscida* and *U. picta* as an alternative anti-inflammatory agent. However further study for characterization, identification of active compound and mechanism of action responsible for said activity is required.

**ACKNOWLEDGEMENT**

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**REFERENCES**


Graph 1: Percentage Inhibition of different concentration of methanolic extract of P. viscida and U. picta against standard drug at 240 minutes

Graph 2: Paw size of rats of all treated groups after the injection of carrageenan at time interval of 30 minute

n=6. ***p<0.001, **p<0.01, *p<0.05. One way ANOVA followed by Dunnett’s test.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose mg/kg</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>% inhibition after 240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Group-I</strong></td>
<td>Normal saline 0.9 %</td>
<td>5.15±0.04</td>
<td>7.44±0.07</td>
<td>8.54±0.3</td>
<td>8.27±0.12</td>
<td>8.10±0.30</td>
<td>-</td>
</tr>
<tr>
<td><strong>Indomethacin Group-II</strong></td>
<td>10 mg</td>
<td>5.04±0.05</td>
<td>5.50±0.12</td>
<td>5.90±0.7</td>
<td>5.65±0.05</td>
<td>5.16±0.03</td>
<td>36.29</td>
</tr>
<tr>
<td><em>Pseudarthria viscida</em> Group III</td>
<td>200 mg</td>
<td>5.08±0.01</td>
<td>5.78±0.08</td>
<td>6.33±0.0</td>
<td>5.81±0.10</td>
<td>5.36±0.04</td>
<td>33.80</td>
</tr>
<tr>
<td><em>Pseudarthria viscida</em> Group IV</td>
<td>400 mg</td>
<td>5.10±0.02</td>
<td>5.61±0.01</td>
<td>6.12±0.0</td>
<td>5.71±0.11</td>
<td>5.23±0.10</td>
<td>35.43</td>
</tr>
<tr>
<td><em>Uraria picta</em> Group-V</td>
<td>200 mg</td>
<td>5.12±0.03</td>
<td>6.14±0.04</td>
<td>6.55±0.0</td>
<td>6.34±0.11</td>
<td>5.70±0.13</td>
<td>29.62</td>
</tr>
<tr>
<td><em>Uraria picta</em> Group-VI</td>
<td>400 mg</td>
<td>5.09±0.03</td>
<td>5.88±0.04</td>
<td>6.18±0.1</td>
<td>6.29±0.28</td>
<td>5.45±0.03</td>
<td>32.71</td>
</tr>
</tbody>
</table>

For all 6 observation each value represents mean±SEM.*p<0.05; **p<0.01, ***p<0.001
Data was analyzed using One-way ANOVA followed by Dunnett’s test
Table- 1: Comparison of percentage inhibition by *Pseudarthria viscida* and *Uraria picta* against carrageenan induced paw edema at different dose level against Indomethacin