

ANTI-INFLAMMATORY ACTIVITY OF DODONAEA VISCOSA LEAVES

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Abstract: Inflammation is a response of vascularized living tissue to the local injury. Due The severe side effects of steroidal and non-steroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory drugs from the indigenous source. Hence, the present work was scrutinized to explore the anti-inflammatory activity of whole plant of *Dodonaea viscosa*. The powdered leaves of *D. viscosa* were extracted with hydroalcohol and n-hexane successively. The anti-inflammatory activity was studied using carrageenan-induced rat paw edema at the dose of (100 and 200 mg/kg b.w. p.o.) of hydroalcoholic and n-hexane extract respectively. The hydroalcoholic extract of *D. viscosa* exhibited significant anti-inflammatory activity at the dose of 100 & 200 mg/kg than the n-hexane extract. Indomethacin (10 mg/kg b.w. p.o) had exhibited significant anti-inflammatory activity.

Keywords: *D. viscosa*; anti-inflammatory; carrageenan; Indomethacin

I. INTRODUCTION

Inflammation is a pathological condition associated with local tissue damage, cell death, ischemic of organs and degeneration. However, it also arises due to various microbial infections with release of various inflammatory mediators like endotoxin, etc by the infectious agents ¹. Globally, traditional medicines orchestrate a cardinal role in the treatment of various diseases and recently there is a substantial shift towards herbal medicine due to noxious adverse effect in the allopathic treatment. Thus, the development of new drugs from traditional medicine offers new dimension in modern healthcare. *Dodonaea viscosa* Linn. (Sapindaceae) commonly known as 'virali' is an evergreen perennial shrub widely distributed in Western Ghats and Tamilnadu. The folklore claim reveals that the leaves have been used for the treatment of headaches and backaches by the Muthuvan tribes of the Kerala region. Hot water decoction of leaves is used to reduce swellings, backaches and steam inhalation is used to alleviate cold and ². Further, in traditional medical practice *D. viscosa* is used to alleviate stomach pain, piles and ulcer ³. Previous studies have reported the anti-inflammatory, antimicrobial, local anesthetic and smooth muscle relaxing activity of *D. viscosa* ⁴⁻⁶. In this scenario, the present study was scrutinized to evaluate the anti-inflammatory effect of hydroalcoholic and n-hexane extract of *D. viscosa* leaves on carrageenan induced rat paw edema model.

II. MATERIALS AND METHODS

Plant Material

The leaves of *D. viscosa* were collected in the month August 2018, from Trichy, Tamilnadu, India. The plant material was identified and authenticated by the botanist. The plant materials were dried under shade, sliced into small pieces, pulverized using a mechanical grinder and passed through 40 mesh sieve and stored in an airtight container for further use.

Extraction of Plant Material

The powdered leaves of *D. viscosa* were extracted with hydro alcohol and n-hexane successively at room temperature. After exhaustive extraction, the solvent was collected and filtered. The solvent was concentrated under reduce pressure at 50-55°C. The concentrated n-hexane and hydro alcohol extracts were kept in desiccators for further use.

Qualitative Phytochemical Analysis

The crude hydro alcoholic and n-hexane extract of *D. viscosa* leaves were analyzed for the presence of various phytoconstituents by following standard phytochemical protocols ⁷. The presence of alkaloid (Dragendorff reagent, Mayer's reagent, Hagers reagent and Wagner's reagent), flavonoids (Shinoda-Paw test), steroids (Lieberman Burchard test and Salkowski's reaction), terpenes (Vanillin sulfuric acid reagent) and carbohydrates (Fehlings test and Molisch test) were analyzed.

Drugs and Chemicals

Indomethacin and Carrageenan was purchased from Sigma Aldrich, USA. Carboxy methyl cellulose (CMC) and other reagents of analytical grade were purchased from S. D. Fine Chem. Ltd, India.

Animals

Swiss Albino mice (25-30 g) and Wistar albino rats (150-200g) of either sex were used for experimental study. The animals were housed in colony cages at $25 \pm 2^\circ\text{C}$, and relative humidity ($50 \pm 5\%$) with 12 h light, and 12 h dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water *ad libitum*. The animals were cared and used in accordance with the CPCSEA guidelines and experimental protocols approved by IAEC.

Acute Toxicity Studies

Healthy adult Swiss albino mice of either sex weighing between 20 and 25 g were subjected to the oral acute toxic class method set out in OECD guidelines 420 was adopted [8]. Groups of six mice each were administered orally fixed doses (5, 50, 300 and 2000 mg/kg, b.w; p.o.) of *D. viscosa* n-Hexane (DVH) and *D. viscosa* hydro alcoholic (DVHA) and extracts were suspended in 1% (w/v) aqueous CMC. After oral administration animals were observed individually after at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days for toxic symptoms and mortality.

Carrageenan-Induced Paw Edema

The carrageenan induced rat paw edema is the most reliable preclinical model to evaluate the anti inflammatory potential of therapeutic agents.

Wistar rats of either sex weighing 150-200g were divided into group of six animals each,

Group 1 - Received carrageenan alone (0.1ml of a 1% solution)

Group 2 - Received standard drug Indomethacin 10 mg/kg b.w; p.o.

Group 3 & 4 - Received DVHA 100 & 200 mg/kg b.w; p.o respectively

Group 5 & 6 - Received DVH 100 & 200 mg/kg b.w; p.o respectively

All the treatments were given orally 1 h prior to the injection of carrageenan. Then the edema was induced by sub plantar injection of 0.1ml of a 1% freshly prepared suspension of carrageenan into the right hind paw of each rat. The paw volume was measured before (0) h and 3 h after the injection of carrageenan using a plethysmometer [9]. The percentage decrease in paw edema volume was calculated using the formula $[\text{Paw edema volume in control} - \text{Paw edema volume in test} / \text{Paw edema volume in control}] \times 100$.

III. STATISTICAL ANALYSIS

All data were expressed as the mean \pm SEM. The results were analyzed for statistical significance ($P < 0.001$, 0.01 and 0.05) by One-way (ANOVA) followed by Dunnett's test using computerized SPSS software v 18.

IV. RESULTS

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis for hydro alcoholic and n-hexane extract of *D. viscosa* leaves were displayed in Table: 1.

Acute Toxicity Studies

DVHA and DVH when orally administered in the dose range of 5-2000mg/kg to mice did not produce any significant changes in the autonomic or behavioral response during the observation period. The body weight was not significantly altered. No mortality was observed up to 14 days of monitoring. So, the extracts were safe for administration upto the dose of 2000mg/kg.

Carrageenan-Induced Paw Edema

DVHA and DVH (100 & 200 mg/kg) displayed a significant inhibition of paw volume when compared to the carrageenan control. In this study hydro alcoholic extract of *D. viscosa* (DVHA) (100 & 200mg/kg) had elicited maximum inhibition of paw volume by 34.34 and 56.01% as that of DAH, whereas indomethacin displayed 80.84% inhibition of paw volume (Table 2).

V. DISCUSSION

The preliminary anti-inflammatory activity of *D. viscosa* extracts was evaluated against carrageenan-induced paw edema. The carrageenan-induced paw edema model is a valid preclinical test to evaluate the potency of cyclooxygenase (COX) inhibitors and to underscore the effect of non-steroidal anti-inflammatory agents in the inhibition of COX pathway during prostaglandin biosynthesis¹⁰. Carrageenan mediated paw edema is an effective animal model to study the process of acute inflammation. In this model the edema developed during the various time courses is displayed by a biphasic curve¹¹. After one hour of carrageenan injection the first phase of inflammation starts and it might be due to the injury at the site of injection as well histamine and serotonin secretion¹². The second phase of inflammation involved in the release of various inflammatory mediators like bradykinin, protease, prostaglandin, and lysosome. Further, prostaglandins (PGs) orchestrate a cardinal role in the inflammation process during the second phase. Treatment with DVHA and DVH at the doses of 100 & 200 mg/kg had displayed significant decrease in paw volume. In this study, DVHA effectively suppressed the inflammation induced by carrageenan than the DVH. Phytochemical studies on *D. viscosa* extracts had revealed the presence of flavonoids, steroids, triterpenoids and carbohydrates. In this study hydroalcoholic extract of *D. viscosa* showed the presence of maximum phytochemicals and thus effectively mitigate the inflammation induced by carrageenan than the n-Hexane extract of the plant. Previous studies have shown that Hautriwaic Acid, a diterpene isolated from *D. viscosa* leaves has anti-inflammatory activity in 12-O-tetradecanoylphorbol 13-acetate induced mice ear edema¹³.

VI. CONCLUSION

Qualitative phytochemical analysis of *D. viscosa* n-hexane and hydroalcoholic extract contains steroids, triterpenoids and flavonoids. The prolific anti-inflammatory activity of hydroalcoholic extract of *D. viscosa* may probably be due to the presence of several bioactive anti-inflammatory principals. However, it needs isolation, structural elucidation, and screening of any of the above-mentioned active principle/s to pin point activity of drug.

Table:1 Qualitative phytochemical analysis of various extracts of *D. viscosa*

Treatment	Dose	% Increase in paw volume	% inhibition
Carrageenan Control	(0.1ml of 1% solution)	80.08 ± 0.96	-
Indomethacin	10mg/kg	15.15 ± 1.40 ^{c*}	80.84
DAH	100mg/kg	70.50±2.32 ^{b*}	10.84
	200mg/kg	57.27±1.45 ^{c*}	27.57
DAHA	100mg/kg	72.74±1.74 ^{a*}	8.01
	200mg/kg	65.87±1.34 ^{c*}	16.70

Table 2: Effect of *D. viscosa* extracts on Carrageenan Induced Paw Edema

Type of Constituent	n-Hexane	Hydro alcohol
Alkaloids	-	-
Flavonoids	-	+
Steroids	+	+
Triterpenoids	+	+
Carbohydrates	-	+

Each value represents the mean ± S.E.M., n = 6. ^{a*}P < 0.05, ^{b*}P < 0.01 and ^{c*}P < 0.001 compared with control, Dunnett's *t*-test after analysis of variance.

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