Evaluation of anti-inflammatory activity of Ethanolic whole plant extract of Desmodium gangeticum L.

Nusrath Yamseen1, K. Sujatha2

Abstract
The present study was designed to investigate the anti-inflammatory activity of the ethanolic extract of whole plant of Desmodium gangeticum. Investigations were done by Carrageenan-induced paw oedema method in rats. Here the extract at the 100 & 200mg/kg dose level showed 36.68% (p<0.001) inhibition of edema volume at the end of 4hr .The extract reduced significantly the formation of oedema induced by carrageenan. Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Therefore the search for a better tolerated anti-inflammatory agent appears to be a necessity. Desmodium gangeticum is used as a folk medicine for the treatment of inflammation in India. Present study revealed that the plant Desmodium gangeticum possesses a significant anti-inflammatory activity as evidenced in carrageenan induced paw edema method, which supports the folkloric claim of the anti-inflammatory activity of the plant.

Keywords: Desmodium gangeticum, anti-inflammatory activity, Carrageenan, Diclofenac Sodium.

Introduction
Despite the progress made in medical research during the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's major health problems [1-3]. Inflammation, clinically, causes, as shown by Cornelius Celsius of Rome 2000 years ago, rubor (redness), calor (heat), dolor (pain) of the affected region [4] and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells [5]. It is defensive mechanism of the body to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if unchecked, leads to onset of diseases such as vasomotor rhinorrhea, rheumatoid arthritis, and atherosclerosis [6]. It is believed that current drugs available such as Opioids and NSAIDs drugs are not useful in all cases of inflammatory disorders, because of their side effects, economy and potency [7, 8]. As a result, a search for other alternatives is necessary. The use of plants to treat ailments is as old as antiquity. Records of humans using plants to treat diseases have been dated as far back as 6000 to 4000 years ago when Ayurvedic physicians started treating tumors with extracts from Vinca roseae [9, 10]. Desmodium gangeticum (L.) DC. (Family Leguminosae) is a small shrub, which has been used in Indian system of medicine (Ayurveda) as a bitter tonic, febrifuge, digestive, anticatarhal, antiemetic, in inflammatory conditions of chest and various other inflammatory conditions due to 'vata' disorders[11]. It has been reported to contain alkaloids, flavone and isoflavonoid glycosides. Total alkaloids of this species showed anticholinesterase, smooth muscle stimulant, CNS stimulant and depressant responses [12, 13]. The plant has been reported to contain gangetin, a pterocarpnoid shown to possess anti-inflammatory and analgesic activities. This study therefore seeks to examine Desmodium gangeticum for anti-inflammatory activity since pain is one of the cardinal signs of inflammation.

Materials and Methods
Collection of drug
The plant Desmodium gangeticum was collected from the forest area of Chittoor district in AP (India) during the month of October. Botanist of Sri. Venkateshwar University, Tirupathi, authenticated the plant.

Drugs and chemicals
Carrageenan was purchased from Merc Pvt.Ltd, and Diclofenac Sodium was obtained from Zydus Cadilla Ltd. All the solvents used were of analytical grade and were obtained from Sd. Fine Chemicals, Mumbai,India.

Preparation of the DGE
Dried and powdered whole plant material of Desmodium gangeticum was purchased from a commercial source (Madhavchetty). The powdered material was soaked with 70% ethanol overnight in Soxhlet thimble and extracted using soxhlet apparatus. The residue in the R.B flask was transferred into a beaker and was concentrated under reduced vacuum pressure to give an average yield of 70% (w/w). Solutions of the Desmodium

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gangeticum extract (DGE) were prepared freshly for the pharmacological studies.

Animals
The male wistar albino rats (150-200gms) were procured from Shadan Animal Husbandary and from Sai Animal Distributors, Musheerabad. The animals were acclimatized for 1 week. They were fed with commercial pelleted rats chow and were given free access to water ad libitum throughout the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output. All animal experiments strictly complied with the approval of institutional animal ethical committee.

Acute Toxicity Studies
The acute toxicity study was carried out in adult female albino rats by the ‘up and down’ method [14]. The animals were fasted overnight and next day extracts of the Desmodium gangeticum dissolved in normal saline was administered orally at different dose level. Then the animals were observed continuously for 3 hours for general behavioral, neurological and autonomic profiles and then every 30 minutes for next 3 hours and finally death after 24 hours [15].

Determination of Anti-Inflammatory Activity
Carrageenan induced rat paw oedema model
The rats were divided into four groups containing five rats in each group (one control, one standard & two test groups) acute inflammation was induced according to edema assay [16]. The extracts were suspended in 2% gum acacia & administered orally (100-200 mg/kg/b.w) to rats 1 hour before Carrageenan injection. Diclofenac Sodium (10 mg/kg b.w) is given to standard group. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl & injects 0.1 ml underneath the planter region.

Carrageenan + 2% Tween 80(10 ml /kg b.w)
Standard group 2:Carrageenan + Diclofenac Sodium (10 mg/kg b.w)
Test group 1:Carrageenan + Ethanol extract (100 mg/kg b.w)
Test group 2:Carrageenan + Ethanol extract (200 mg/kg b.w)
The paw circumference was measured with vernier calipers/cotton thread at 1, 2, 3, 4 and 5 hours after Carrageenan injection.

Results
Carrageenan induced paw oedema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator syleaves through a common trigger mechanism. The development of carrageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [17, 18] and the delayed phase is sustained by the leucotrienes and prostaglandins [19]. Flavonoids and tannins are reported to inhibit PG synthesis [20]. Most of the NSAIDs have well-balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity. The AEGP possesses a marked anti-inflammatory activity and hence may pose itself as very good anti-inflammatory drug. Still further investigation with respect to pharmacological and phytochemical profile of the drug needs to be carried out. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [21, 22]. Carrageenan oedema is a multi mediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1hr) involves the release of serotonin and histamine while the second phase (over 1hr) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins[23]. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [24, 25], the results of this study are an indication that Desmodium gangeticum can be effective in acute inflammatory disorders. Diclofenac sodium is a cyclooxygenase inhibitor, and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema [26], so inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme. Dextran induced edema is a well known experimental model in which the edema is a consequence of liberation of histamine and serotonin from mast cells [27].

This study demonstrates the efficacy of Desmodium gangeticum as an anti-inflammatory agent and also scientifically justifies the use of this plant as an anti-edematous agent.

In folk medicine, however, further studies are required to determine the constituents responsible for its anti-inflammatory activity and further authenticate its mechanism of action.

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