

Effect of Ethanolic Extract of *Semecarpus Anacardium* Fruit on Carrageenan Induced Paw Edema in Albino Rats

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Abstract: *Background:* *Semecarpus anacardium* plant derivatives has been used since antiquity, The fruit and nut extract of *Semecarpus anacardium* shows various activities like antiatherogenic, anti-inflammatory, antioxidant, antimicrobial, antireproductive, CNS stimulant, hypoglycemic, anticarcinogenic. The present study is an attempt to explore the anti-inflammatory activity of ethanolic extract of fruits of *Semecarpus anacardium* plant in albino rats by employing carrageenan induced rat hind paw edema model. *Methods:* Acute anti-inflammatory model performed on wistar male albino rats (150-200gm) were divided into 4 groups, each group containing 6 albino rats, group 1 serving as control, group 2 as standard, group 3 receiving 300mg/kg.b.wt of ethanolic extract of *Semecarpus anacardium* fruit and group 4 receiving 600 mg/kg.b.wt of ethanolic extract of *Semecarpus anacardium* fruit. The anti-inflammatory property was assessed by Plethysmograph. *Results:* Ethanolic extract of *Semecarpus anacardium* fruit demonstrated a significant anti-inflammatory property at different levels when compared to control and standard ($p < 0.05$). *Conclusion:* Ethanolic extract of *Semecarpus anacardium* fruit exhibited a dose dependent anti-inflammatory activity in carrageenan induced rat paw edema model of inflammation and shows that this is a potent source of phytomedicine development in the future.

Keywords: *Semecarpus anacardium*, ethanolic extract, anti-inflammatory, carrageenan, plethysmograph.

1. Introduction

Inflammation is a very complex, multifactorial and dynamic process involving many systems which is closely associated with the process of repair. Inflammation is defined as local response of living mammalian tissue to injury due to any agent and manifests usually in form of painful swelling associated with some changes in skin covering the site. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury, edema, leukocyte infiltration and granuloma formation represents the components of inflammation. Inflammation can be classified either as acute or chronic, though it is a defense mechanism and the complex event mediators involved in the inflammatory reaction can induce or aggravate many reactions. The clinical treatment of inflammatory diseases is dependent on non steroidal or steroidal chemical therapeutics¹. Non-steroidal anti inflammatory drugs (NSAID) reduce the pain and inflammation by blocking the metabolism of arachidonic acid by inhibiting cyclooxygenase enzyme (COX), and thereby the production of prostaglandin². Since long term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non-selective inhibition of both COX isoforms^{3,4,5}. On the other hand, fully selective and reversible COX-2 inhibitors with reduced gastro-intestinal toxicity have been associated with adverse cardiovascular effects⁶. Furthermore, the use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects⁷. Therefore, developing new agents with more powerful anti-inflammatory activity and with lesser side effects is at present, of great interest. Plant based traditional medicine system continues to play an essential role in health care.

2. Review of Literature

Semecarpus Anacardium is commonly known as 'Marking nut', 'Oriental cashew', 'Bhilawa' or 'Bhallataka', Is a moderate sized semi-deciduous tree with grey bark that exfoliates in small irregular flakes. The leaves are simple, alternate, obovate-oblong rounded at the apex, glabrous above pubescent below. The greenish fruits are ovoid to oblong drupes that has attached to a swollen, fleshy receptacle that sit below it and turns yellow when ripe. The fruits are useful in Leucoderma, Scaly skin, Allergic dermatitis, Poisonous bites, leprosy, Cough, Asthma, Dyspepsia^{8,9}.

According to WHO report, about 70-80% of the world's population rely on non conventional medicine mainly from herbal sources in their primary health care^{10,11}. Especially its demand is increasing day by day in developing countries where the cost of consulting a physician and price of Medicine are beyond the limit of most people¹². These herbal medicines having anti-inflammatory activity are used to ease pain in various conditions including arthritis, muscle and ligament pains¹³. In the present study, acute anti-inflammatory activity of ethanolic extract of *Semecarpus anacardium* fruit have been evaluated by comparing with the standard drug Diclofenac sodium.

Objective: Evaluation of effect of ethanolic extract of *Semecarpus anacardium* fruit on male albino rats using carrageenan induced rat hind paw edema model

3. Materials and Methods

The experimental study was done in the Department of Pharmacology, Bidar Institute of Medical Sciences, Bidar District, Karnataka. The experimental protocol was approved by Institutional Animal Ethical Committee.

3.1 Plant Material Collection and Identification

The fruits of *Semecarpus Anacardium* were collected from the local market of Bidar. The fruits were identified and authenticated by Prof.Sajjan, Department of Botony, B.V.B.College,Bidar,Karnataka,India. The fruits were collected in the month of December 2011 and the plant material was washed under running water to remove all the dust, soil particles and finally rinsed with distilled water and shade dried at room temperature, dried material was then pulverized separately into coarse powder by a mechanical grinder , the resulting coarse powder was then used for extraction .

3.2 Preparation of Extract

The course powdered fruits of *Semecarpus anacardium* was subjected to ethanolic extraction.The dried fruits were powered(100g) was extracted with 50% ethanol using soxhlet apparatus and filterate obtained is weighed, given weights of the extract were reconstituted in appropriate volume of distilled water and the appropriate volumes administered to the animals based on their body weights as indicated and ethanolic extract was used to evaluate the anti-inflammatory action using two doses 300 mg/kg and 600mg/kg. b.wt.

Animals: Albino rats (150-200grams) were procured from NIN, Hyderabad, India. Albino rats of wistar strain having good health conditions and weighing between 150-200g were included in this study. Male rats were of age more than 10weeks and were housed in cage 10x10.5x8 cm size. All animals were housed in clean environment. The animals were acclimatized for 10 days under standard husbandry conditions; room temperature (27+ or -3 degree C)relative humidity(65= or -10%) and 12 hours of light and dark cycle. They were allowed free access to standard dry pellet diet and water *ad libitum* under strict hygienic conditions. All the described procedure were reviewed and approved by the Institutional Animal Ethical Committee (Reg No:1216/a/08/CPCSEA).

3.3 Drugs and Chemicals Used

Ethanol and carrageenan was purchased from Hi Media, Mumbai, India . Standard anti-inflammatory drug Diclofenac Sodium was purchased from Recon , Bangalore , India.

3.4 Acute Oral Toxicity Studies

Male albino rats (150-200g weight) were used for acute oral toxicity study. They were randomly divided into three groups of five rats each. Animals in group 1 received a single dose of the extract (500mg/kg) orally. Groups 2 and 3 were similarly

treated with 1g/kg and 2g/kg of the extract respectively. The study was carried out as per the guidelines set by the OECD and no adverse effects or mortality were detected in the rat during 24h observation period. Based on the results obtained from this study, and according to the study of “ Effect of *Semecarpus anacardium* fruits on reproductive function of male albino rats “ done by Arti Sharma, Pramod Kumar verma, V.P.Dixit . The dose for the study was fixed to be 300mg/kg b.w. and 600mg/kg b.w. for dose dependent study.

3.5 Carrageenan induced Acute Inflammatory Model

Experiment was done in January 2012 in the experimental pharmacology laboratory , department of pharmacology, BRIMS, Bidar. The study was done on Wistar male albino rats weighing 150-200 gms and were divided into 4 groups each group containing 6 rats. Anti-inflammatory activity was measured using carrageenan induced rat paw edema model^{13,14} . edema was induced by subplantar injection of 100µl of 1% freshly prepared solution of carrageenan in distilled water into the right hind paws of each rat of all the groups except the group 1 rats ,all group rats were treated with the single dose of vehicle , and drug respectively . 1 hour prior to carrageenan injection paw thickness were measured just before the carrageenan injection that is at 0 hour and then at 1,2,3,4,5,6 hours. Diclofenac sodium(5mg/kg) used as standard drug, group 1 served as control receiving normal saline orally, group 2 received standard drug Diclofenac sodium, Group 3 served as test received ethanolic extract of *Semecarpus Anacardium* fruit at dose of 300 mg/kg b.w.per oral , Group 4 served as test received ethanolic extract of *Semecarpus Anacardium* fruit at dose of 600 mg/kg b.w. per oral ,All the above drugs were administered orally 1hr prior to injection of 0.1ml, 1% carrageenan in sub plantar region of paw to induce edema. The volume of paw was measured at 0, 1,2,3,4,5,6th hr by using Plethysmograph. Reduction in the paw volume was compared with control & standard.

4. Statistical Analysis:

The Values for edema volumes are expressed as mean ± SEM and ANOVA, p<0.05 were considered to be statistically significant.

5. Results

The effect of ethanolic extract of *Semecarpus Anacardium* fruit on carrageenan induced paw edema in rats is shown in table. The result obtained indicates that the fruit ethanolic extract with 600 mg/kg found to have significant (p<0.05) anti-inflammatory activity in rats as compared 300 mg / kg. Both the different dose extracts compared with the control and standard diclofenac sodium. The interplantar injection of carrageenan in rats led to time-dependent increase in paw thickness; this increase was observed at 1hr and was maximal at 4hr after administration. The extract at the test doses 300 & 600 mg/kg.b.wt reduced the edema induced by carrageenan by 52.66% and 77.63% respectively, whereas the standard drug,

diclofenac, showed 86.03% of inhibition as compared to the control group. The plant extract significantly reduced the paw

volume ($P < 0.05$) as compared to the control rats, Diclofenac sodium showed similar type of reduction ($P < 0.05$)

Acute anti-inflammatory activity of <i>Semecarpus anacardium</i> fruit ethanolic extract in carrageenan-induced rat hind paw edema								
	0 hr	1hr	2hr	3hr	4hr	5hr	6hr	% inhibition of oedema
Group 1	0.865±0.008	0.881±0.004	0.893±0.006	0.912±0.003	0.945±0.006	0.952±0.006	0.961±0.006	0
Group 2	0.852±0.006	0.748±0.004	0.605±0.007	0.424±0.008	0.352±0.009	0.262±0.005	0.202±0.007	86.03
Group 3	0.847±0.005	0.775±0.008	0.663±0.007	0.612±0.003	0.508±0.008	0.445±0.004	0.348±0.005	52.66
Group 4	0.863±0.005	0.757±0.004	0.626±0.006	0.473±0.006	0.343±0.006	0.308±0.008	0.238±0.004	77.63

6. Discussion

Carrageenan induced rat hind paw edema model is a suitable test for evaluating acute anti-inflammatory drugs, which has frequently been used to assess the acute anti-inflammatory effect of the drug, carrageenan is a strong chemical used for the release of inflammatory and pro-inflammatory mediators prostaglandins, leukotrienes, histamine, bradykinin, TNF α etc.¹⁶ the course of acute inflammation is biphasic. first phase starts with the release of histamins, serotonin and kinins after the injection of phlogistic agent in the first few hours¹⁷. While the second phase is related to the release of prostaglandins like substances in 2-3 hours, second phase is sensitive to both the clinically useful steroidal and non-steroidal anti-inflammatory agent¹⁸ Prostaglandins are the main culprit for acute inflammation. *Semecarpus anacardium* fruit might be containing some anti-inflammatory agent which is responsible for the blockage of prostaglandins and inflammatory pathway. In this model of inflammation, ethanolic extract of *Semecarpus anacardium* fruit had very consistent anti-inflammatory activity and thus showed significant decrease in the paw thickness of group 4 although, the cyclooxygenases and lipoxygenase pathways play a pivotal role in the inflammatory process the inhibition of cyclooxygenase is more effective in inhibiting carrageenan induced inflammation than lipoxygenase inhibitors¹⁹.

Ethanolic extract of *Semecarpus anacardium* fruit might have inhibited the cyclooxygenase that synthesizes prostaglandins. Oral administration of the extract significantly down regulated the pro-inflammatory cytokines while up-regulated the anti-inflammatory cytokines. Prostaglandins play essential role in inflammation Prostaglandins synthesis is down regulated by anti-inflammatory cytokines like IL-10, IL-4 and IL-13 which also check cyclooxygenase-2 synthesis, cyclooxygenase-2 is responsible for the increased production of prostaglandins and hence extracts overcome the inflammation induced by carrageenan^{20,21} studies have shown that IL10 has been found as a potent macrophage deactivators which blocked TNF α IL-1, IL-6, IL-8 and GM-CSF by human monocytes²². The present results suggest that *Semecarpus anacardium* fruit extract suppresses the first phase of carrageenan induced paw edema thus confirming an NSAID like property. The present study showed that ethanolic extract of *Semecarpus anacardium* fruit have acute anti-inflammatory properties.

7. Conclusion

The results of this study show that ethanolic extract of *Semecarpus Anacardium* fruit has anti-inflammatory activity against early phase of inflammation. Further, pharmacological studies can be done on the basis of the results obtained. This relevant information is helpful for further investigation.

8. Conflict of Interest

There are no financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this paper.

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