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Research Article

ANTI – INFLAMMATORY POTENTIAL OF AQUEOUS EXTRACT OF *SESBANIA SESBAN* (L) MERR

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ABSTRACT

Objective: To evaluate the anti-inflammatory potential of aqueous extract of *Sesbaniasesban* (L) Merr. seeds in carrageenan induced paw edema model in rats.

Methods: The seeds of *Sesbaniasesban* (L) Merr. were powdered and aqueous extract prepared were used for the evaluation of its anti-inflammatory potential. Paw thickness of the experimental animals were measured for assessment of potency of the extract and percentage inhibition of the paw edema was calculated. Levels of protein and nitric oxide were analysed in the serum. Histopathological analysis were also done in the hindpaw tissue for assessing the anti-inflammatory potential of the extract.

Results: The paw thickness of treatment group animals showed a significant reduction upon treatment with the extract. During inflammation, the levels of protein were found to be declined and nitric oxide levels were found to be elevated. The histopathological analysis showed the potential of treatment delivered by the extract in the tissues of experimental animals after inflammation.

Conclusion: The assessment of paw thickness, levels of protein & nitric oxide and histopathological analysis revealed the anti-inflammatory potential of the aqueous extract of *Sesbaniasesban* (L) Merr. seeds.

Keywords: *Sesbaniasesban* (L) Merr., Aqueous extract, Carrageenan, Anti-inflammatory, Paw thickness.

INTRODUCTION

Pain and inflammation is a common complaint in most patients suffering from disease conditions [1]. Inflammation is a protective physiological response of an organism to chemical, physical, infectious agents, environmental toxins, ischemia or an antigen-antibody interaction. However, prolonged or overactive inflammation may cause tissue damage. Inflammation is very commonly manifested as body temperature change, edema, itch and pain, occasionally as serious as septic shock, tissue cirrhosis, necrosis or cancer [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) make up one of the largest groups of drugs used for pain and inflammation[3]. Currently available anti-inflammatory agents are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclooxygenase enzyme in gastric mucosa[4]. The added advantages of indigenous medicinal treatment would include its complementary nature to the conventional treatment making latter safer, well tolerated and economical remedy for acute and chronic inflammatory conditions [1].

Antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects. Antioxidant agents are well known to retard the progress of many chronic diseases as well as lipid peroxidation [5].

Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals in the organism. Phenolic plant compounds have attracted considerable attention for being the main sources of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activities of phenolics play an important role in the adsorption or neutralization of free radicals [6]. Several synthetic antioxidants are commercially accessible but have been reported to be toxic [7]. Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolics, proanthocyanidins and flavonoids [8].

Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae(aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. *Sesbaniasesban* belongs to fabaceae family has a long history of use in India, grows in a wide range of soils from loose sands to heavy clays. It is found widely in tropical Asia and Africa upto an altitude of 1200m[3]. *Sesbaniasesban* has synonyms such as Common sesban, Sevari, Shewari, Jayanti, Jait, Jaya, Jayantika etc [9,10]. *Sesbaniasesban* is a soft, slightly woody, 1-6 m tall perennial nitrogen fixing small tree. The leaves are compound 12-18 cm long and made up of 6-27pairs of leaflets. The raceme has 2-20 flowers whichare yellow with purple or brown streaks on the corolla. Pods are subcylindrical, straight or slightly curved up to 30 cm long and 5 mmwide containing 10-50 seeds. [11]

It has been used as a live support for black pepper, grapes, cucurbits and betel vine and as a shade tree for coffee and turmeric. Root and bark used as bitter tonic used in debility nervous disorders and act as a CNS stimulant. Root of plant used as dysuria, retention of urine, hepatoprotective activity. Leaves are used as antihelmintic agent [12].

MATERIALS AND METHODS

Reagents and Chemicals

Carrageenan used in the study were purchased from Himedia, Banglore, India. The standard drug diclofenac sodium was purchased from Shreya Life Sciences Pvt. Ltd. All other chemicals used in this study were of analytical grade.

Preparation of plant extract

The healthy, mature seeds of *Sesbaniasesban* were purchased from the local market, Coimbatore and authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore. The seeds were shade dried and ground well to a coarse powder. 10g of the powdered sample was extracted in 300 ml of water by heating at 80°C. The extract that was obtained was condensed in an oven and was preserved in an air tight container and stored at 4^{0}C for further use.

Experimental animals

Female Sprague Dawley rats weighing approximately 180-200g obtained from Small Animal Breeding Station, Thrissur, Kerala, were

used for the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2ºC) and light (12 h light/dark). They were acclimatized to animal house conditions and were fed on a commercial pelleted rat chow (AVM Cattle Feeds, Coimbatore, Tamil Nadu) and water *ad libitum.* Permission were obtained for performing in*vivo* experiments and Experimental animals were handled according to the university and institutional legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Experimental design

The animals were divided into five groups of six animals each. Inflammation was induced by sub plantar injection of Carrageenan(CG) (1%) [13].

Group I: Control

Group II: Inflammation was induced by subcutaneous injection of freshly prepared 0.1ml Carrageenan in saline into the right hind paw

Group III: Treated with 150mg/kg body weight of aqueous extract of *S.sesban* (SSAE)orally, 1 hour prior to Carrageenan induction as group II rats.

Group IV: Treated with 300 mg/kg body weight of aqueous extract of *S. sesban* (SSAE)orally, 1 hour prior to Carrageenan induction as group II rats.

Group V: Treated with 10mg/kg body weight of Diclofenac sodium orally, 1 hour prior to Carrageenan induction as group II rats.

The edema was measured after 1, 2, 3 and 4 h. After 4 h, the rats were killed by cervical dislocation, and then the whole spleen, thymus and hindpaw were removed and washed with ice-cold saline. The tissues were homogenized using 0.1 M Tris- HCl buffer (pH 7.4) to give 10% homogenate. The serum was prepared from blood and used for various analysis.

Carrageenan induced inflammation model

Paw thickness of the experimental animals

Paw thickness was measured using vernier calipers before and after carrageenan induction in each group. Increase in paw thickness was calculated using the formula P_t-P_o, where, Po is the initial paw thickness at time t_o and P_t is the thickness at time t (4 h). Percentage inhibition of the increase in paw thickness was calculated by the formula, (1-P_t/P_c) x 100, where P_t is the increase in paw thickness of treated and P_c is that of carrageenan induced control.

Estimation of Proteinby Lowry *et al*[14]

The amount of protein present in the sample was measured by blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components. The colour developed by the Biuret reaction of the protein with the alkaline cupric tartarate measured at 660 nm. Pipetted out 0.2 to 1.0 ml working standard solution of BSA. 0.1 ml of the sample was taken. The volumes in all the tubes were made up to 1.0 ml with distilled water. Added 5.0 ml of alkaline copper reagent to each tube. Mixed well and allowed to stand for 10 min. Added 0.5 ml of Folin-Ciocalteau reagent. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes the blue colour developed were read at 660 nm.

Estimation of Nitric oxide by Green *et al* [15].

Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions that is estimated spectrophotometrically at 540 nm. Serum samples were diluted four times with distilled water and deproteinized by adding 1/20 volume of zinc sulfate (300 mg/ml) to a final concentration of 15 mg/ml. After centrifugation at 10000 *g* for 5 min at room temperature, 100 µl supernatant was collected. 0.1 ml of supernatant was mixed with 0.1 ml of Griess reagent at room temperature for 5 min. Absorbance was measured at 540 nm.

Histopathology of the experimental animals

Histopathological Examination

The hindpaw samples were preserved in 10% commercial formalin immediately on removal from animal.

Tissue Processing

The tissues were placed in 10% normal saline (10% formalin in 9% sodium chloride) for one hour to rectify shrinkage due to higher concentration of formalin. The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in 80% overnight, 100% for one hour and second change of 100% isopropyl alcohol for one hour. The dehydrated tissues were cleared in two changes of xylene, one hour each. Then the tissues were impregnated with histology grade paraffin wax at 60°C for 2 changes of one hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with rotary microtome at 3 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C and after 5 min the sections were allowed to cool.

Tissue Staining

The sections were deparaffinised by immersing in xylene for 10 minutes in horizontal staining jar. The deparaffinised sections were washed in 100% Isopropyl alcohol and stained in Ehrlich's hematoxylin for eight minutes in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (8.3% HCl in 70% Alcohol). The sections were then placed in running tap water for 10 minutes for slow alkalization. The sections were counter stained in 1% aqueous eosin (1.0 g in 100 ml water) for one minute and the excess stain were washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections were ensured by placing the sections in the incubator at 60°C for five minutes. When the sections were cooled, they were mounted in DPX mount having the optical index of glass (the sections were wetted in xylene and inverted on to the mountant placed on cover slip). The architecture was observed at low power objective. The tissue injury and other aspects were observed under high power dry objective.

Statistical analysis

Statistical comparison was done at significance level, $P<0.05$ using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

RESULTS

The paw thickness of the experimental animals of different groups was analysed upon induction with the drug carrageenan. The initial paw thickness of all the experimental animals was measured. The paw thickness of group I animals was found to be 0.59 ± 0.003 cm and upon induction with carrageenan the paw thickness was found to increase to 0.89 ± 0.008cm. This increase is due to the inflammation induced by carrageenan. Upon treatment with the aqueous extracts of *S. sesban*there was a decrease in the paw thickness indicating, reduction of edema. The results obtained were similar to those evidenced in the group V animals that were treated with diclofenac sodium. Thus it could be said that our extract was able to reduce inflammation in comparison to the standard drug. The values are shown in table. 1.

The levels of protein were found to decline in the induced group animals due to inflammation. Upon treatment with the plant extract, the levels were found to restore to near normal levels in a dose dependent manner. Serum NO was found to be increased significantly at $p<0.05$ in group II animals. After the treatment, the serum NO decreased in the treatment groups. This shows the effect of the *Sesbania sesban*seed extract. The standard drug, Diclofenac sodium effect was more or less similar to the extract concentration.

Table 1: **Effect of aqueous extract of *Sesbania sesban* on carra geenan induced paw edema in rats**

Groups	Initial paw thickness (cm)	Paw thickness after 4 h (cm)	Increase in paw thickness (cm)	Percentage inhibition (%)
Carrageenan Control	0.59 ± 0.003	0.89 ± 0.04	0.30 ± 0.01	-
SSAE (150 mg/kg b wt) +CG	0.57 ± 0.03	0.82 ± 0.05	0.25 ± 0.008	16.66
SSAE (300 mg/kg b wt) +CG	0.58 ± 0.01	0.71 ± 0.03	0.13 ± 0.009	56.66
Diclofenac sodium (10mg/kg b wt)+ CG	0.58 ± 0.02	0. 69± 0.04	0.11 ± 0.007	63.33

Values are expressed as mean ± SD (n=6)

Group comparison and statistical significance at p<0.05: [a]: Group II vs. II, III, IV, V

The effect of aqueous extract of *Sesbania sesban on* the levels of serum protein and nitric oxide in the experimental animals of various groups were analysed. The values are shown in fig. 1 and 2.

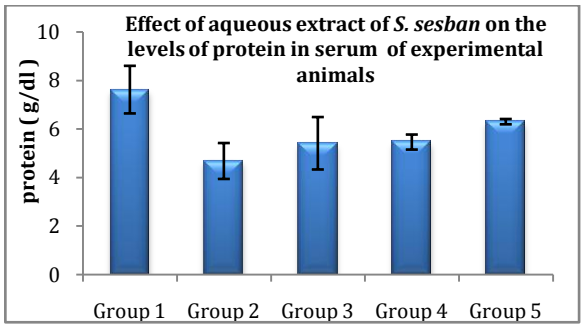


Fig.1

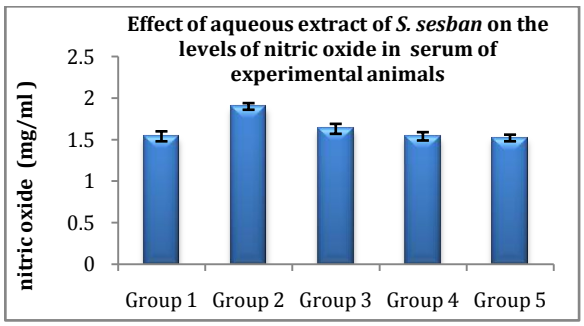


Fig. 2

Effect of aqueous extract of *Sesbaniasesban* on the histopathology of the hind paw tissue

The hind paw tissue of the experimental animals was analysed for histological changes. The results are presented in fig.3.

The histopathological investigations on the hindpaw tissue of the experimental animals revealed the protective nature of the aqueous extract of *Sesbania sesban*.

The various architectural changes in the tissue were depicted as follows.

Slide 1: Shows the section of hind paw tissue of Group I animals. The section reveals no obvious abnormality. The blood vessels appear normal.

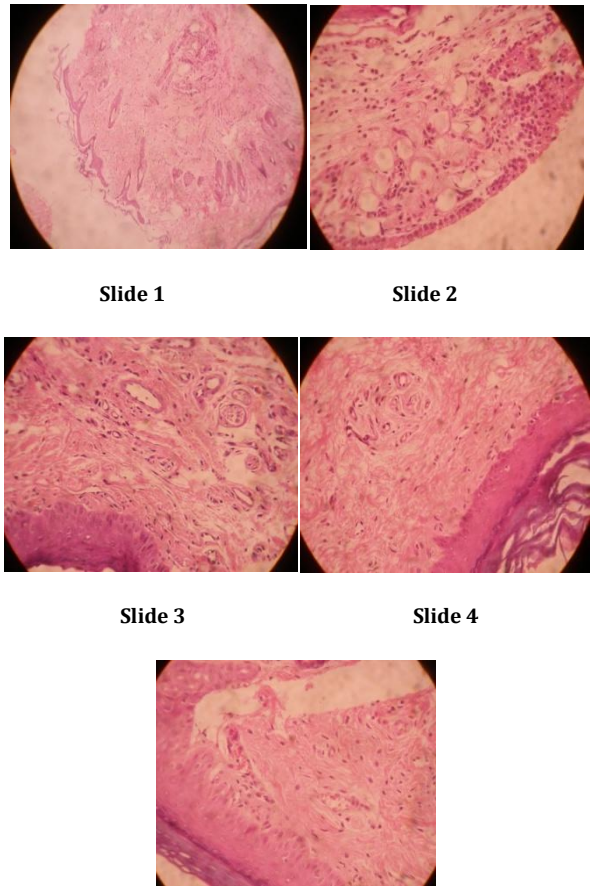
Slide 2: Shows the section of hind paw tissue of Group II (CG induced) animals. Inflammatory cell infiltration observed. Macrophages and histocytes seen.

Slide 3: Shows the section of hind paw tissue of Group III (CG + *S.sesban* 150 mg/kg b wt) animals. The tissue shows mild hyperplasia and hypertrophy with evidence of infiltration of few eosinophils.

Slide 4: Shows the section of hindpawtissue of Group IV(CG + *S.sesban* 300 mg/kg b wt) animals. The sections present no obvious abnormality.

Slide 5: Shows the section of hindpawtissue of Group V(CG + Diclofenac sodium 10 mg/kg b wt) animals. Normal architecture observed with mild inflammation seen.

Effect of aqueous extract of *Sesbania sesban* on the histology of the hind paw tissue of experimental animals



Slide 1 **Slide 2**

Slide 3 **Slide 4**

Slide 5

Fig. 3

DISCUSSION

Oedema which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and the second phase is due to kinin like substances[16]. The second phase is said to be promoted by prostaglandin like substances. It has been reported that the second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and indometacin[17]. The results of carrageenan induced rat paw oedema model indicated the dose dependent anti-inflammatory activity i.e the dose effect of 300 mg/kg b.wt of the aqueous extract of *Sesbania sesban* seeds was more active than 150 mg/kg b wt which were found to be statistically significant.

Free radicals and reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are normal by-products of aerobic metabolism produced in vivo during oxidation. [18]Overproduction of ROS can result in oxidative damage to various biomolecules including lipids, proteins, DNA and cell membranes[19]. They also lead to the development of a variety of diseases such as coronary heart diseases, cancer, diabetes, hypertension and neuro degeneration.[20, 21]. The generation of ROS by phagocytic leukocytes (neutrophils, monocytes, macrophages, and eosinophils) is one of the most important hallmarks in the inflammatory process. The ROS are mediators of cellular injury and are involved in the onset of cellular damage during endotoxemia [22,23].The propagation of free radicals can bring many adverse reactions leading to extensive tissue damage. Lipids, proteins, DNA are very susceptible to attack by free radicals. Nitric oxide (NO) is a signaling molecule that plays a key role in the pathogenesis of inflammation. It gives an anti-inflammatory effect under normal physiological conditions. On the other hand, NO is considered as a pro-inflammatory mediator that induces inflammation due to over production in abnormal situations. NO is believed to induce vasodilatation in cardiovascular system and furthermore, it involves in immune responses by cytokine-activated macrophages, which release NO in high concentrations. NO is involved in the pathogenesis of inflammatory disorders of the joint, gut and lungs. Therefore, NO inhibitors represent important therapeutic advance in the management of inflammatory diseases. Selective NO biosynthesis inhibitors and synthetic arginine analogues are proved to be used for the treatment of NO-induced inflammation. Finally, the undesired effects of NO are due to its impaired production, resulting in vasoconstriction, inflammation and tissue damage[24]. The histopathological investigation revealed the protective nature of the aqueous extract of S. sesban against carra geenan induction. The seeds of S. sesban were found to possess various primary and secondary metabolites and the in-vitro antioxidant activities were reported for the seed extracts. The anti inflammatory activity of the seeds of S. sesban may be attributed to the presence of the primary and secondary metabolites which carry out a diverse vital functions in the biological system.

CONCLUSION

The aqueous seed extract of *Sesbania sesban* were assayed for its anti-inflammatory activity. The measurement of percentage inhibition of the paw thickness in all experimental animals showed the curative nature of the plant extract. The levels of protein and nitric oxide analysed in the serum of experimental animals were found to deviate from the normal levels to abnormal in the inflammated state, where it showed near normal levels in the treatment received animal groups. The histopathological results reveal the structural damage occurred in the hind paw tissue due to inflammation and the restoration of the damaged tissue upon receiving the treatment. As a whole, the results indicate that the aqueous seed extract of *Sesbaniasesban* is potent enough in curbing inflammation and can act as an anti-inflammatory agent.

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