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(Research Article)

Evaluation of Anti-inflammatory Activity of *Saraca asoca* Seeds using Cotton Pellet Induced Granuloma Method in Rats

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ABSTRACT

Saraca asoca has been used since ancient times for its spasmogenic, oxytocic, utero-tonic, antibacterial and anti-dysenteric pharmacological actions. The present research evaluates the anti-inflammatory action of aqueous, methanol and acetone extracts of Saraca asoca seeds by cotton pellet granuloma method in rats. Preliminary phytochemical screening revealed the presence of flavonoids, tannins and carbohydrates in all extracts and there was no sign of mortality up to the dose of 1000 mg/kg during acute toxicity study. The present study demonstrates the potential sub-acute anti-inflammatory effect of extracts of seeds of Saraca asoca (300 mg/kg and 500mg/kg body weight) as compared with the standard drug Indomethacin (2.5mg/kg). The dose of 500 mg/kg of test drug was found to result in higher and more significant anti-inflammatory action as compared to 300 mg/kg dose in all extracts. Among the three extracts, the methanolic one exhibited the highest inhibition of granulation tissue formation, the acetone extract having a slightly lower impact while the lowest effect was observed in case of the aqueous extract.

Key Words*: Saraca asoca*, Seed, Cotton-pellet method, Anti-inflammatory Activity, Caesalpiniaceae.

INTRODUCTION

Inflammation is a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or sequester both the injurious agent as well as the injured tissue. It is a complex reaction usually necrotic, involving cells that consist of vascular response, migration and activation of leucocytes and systemic reaction. The inflammatory response is closely related to the process of repair. During repair, the injured tissue is replaced through the regeneration of native parenchymal cells, by filling of the defect with fibrous tissue. Non- Steroidal Anti-inflammatory Drugs (NSAIDs) are used for the treatment for inflammation[1]. As a result of the inherent problems associated with the current anti-inflammatory agents, there is continuous search especially from natural sources for alternative agents. A good number of plants are employed in the treatment of inflammatory disorders by natural healers[2]. Herbal products symbolize safety in contrast to the synthetics that are regarded as unsafe to man and his environment[3]. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world[4].

Ashoka tree, universally known by its binomial Latin name *Saraca asoca* (Roxb.) or *Saraca indica* belongs to the *Caesalpiniaceae* family. It is found throughout India, especially in Kerala, Bengal, the whole south region and in Himalayas up to the altitude of 750 m. The legumes of *Saraca asoca* are 6-10 inches long and contain 4-8 grey dicotyledonous seeds like a chest nut. The seeds are 3-5 cm long with average diameter of 8-9 cm, smooth surface, ellipsoid-oblong and compressed. The stem bark of this plant has been extensively used in the Ayurvedic therapy mainly as a spasmogenic, oxytocic, uterotonic, antibacterial and antidysentric agent. It has also exhibited anti-progestational and anti-oestrogenic activity against menorrhagia[5]. The present study aims to evaluate the anti-inflammatory activity of the different extracts (Aqueous, methanol and acetone) of the seeds of Ashoka tree by using the cotton pellet induced granuloma tissue formation method in rats after assessing its acute toxicity effect.

MATERIALS AND METHODS

The chemical analysis and experimental study were done in the laboratory and CPCSEA registered animal facility of Dravyaguna department of the Institute of Post Graduate Ayurvedic Education and Research (IPGAEandR), Kolkata.

Collection and Identification of Plant material

The fresh seeds of *Saraca asoca* were purchased from the medicinal plant garden, Narendrapur Ramkrishna Mission, Kolkata and State Government Herbal Garden at Kalyani, West Bengal, India. All the seeds were cleaned and dried under sunlight. These seeds were authenticated at the

Botanical Survey of India, Howrah, India vide Ref No-BSI/CNH/AD/Tech/2010, dated- 21.07.2010. An authentic herbarium specimen was deposited in the herbarium museum of Department of Dravyaguna, IPGAEandR, Kolkata, India for future reference.

Chemicals

All the chemicals used for different chemical and pharmacological studies were of analytical grade. The standard drug Indomethacin was purchased from M/s Jagsonpal Pharmaceuticals Ltd., Rudrapur, Uttarakhand.

Experimental Animals

All animal experiments were performed in the animal house of IPGAEandR, registered by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India vide Reg. No. 1180/ac/08/CPCSEA. Swiss albino mice of either sex, weighing about 20-25 g and albino (Wistar) rats of either sex, weighing about 120-150 g were used for experimental study. All the animals were procured from the Govt. of West Bengal approved breeder, M/S Ghosh Scientific, Kolkata. The animals used for different studies were housed in standard polypropylene cages under standard environmental conditions with fixed 12 h light/dark cycles and provided with food and water ad libitum. These animals were acclimatized for a period of 14 days prior to performing any experiments. All experimental protocols were approved by Institutional Animal Ethical Committee (IAEC).

Extract Preparation

The seeds of *Saraca asoca* were washed properly with distilled water to remove dirt and soil. They were further dried in shade for three weeks until a constant weight was obtained, ground into a coarse powder using a domestic blender and passed through a #40 mesh sieve. The extraction was done after thorough pharmacognostical and physiochemical analysis of the powder of the test sample[5]. This coarse powder was sequentially extracted with petroleum ether (60°C-80°C), chloroform, acetone, methanol and water using Soxhlet apparatus. These extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper at room temperature and concentrated at reduced temperature and pressure using rotary evaporator. All obtained extracts were stored in refrigerator below 10°C for subsequent experiments.

Phytochemical Screening

The extracts were screened for various chemical constituents like carbohydrates, alkaloids, tannins, flavonoids, glycosides, saponins, fats, proteins and amino acids employing standard screening tests following conventional phytochemical screening protocols[6-8].

Experimental Methods

Acute toxicity study

The OECD guideline # 423 was followed for the acute oral toxicity study for fixing the dose[9]. Adult Swiss albino mice of both sexes having weight 20-25gm were randomly selected for acute toxicity tests. The animals were divided into control and test groups containing three animals each. The control group received the vehicle (normal saline) while the test groups got graded doses (200, 400, 600, 800 and 1000 mg/kg) of different extracts orally. The animals were observed carefully up to 4 hours and then occasionally up to 48 hours for seeing any toxic sign or symptom like behavioral changes, locomotion, loss of righting reflex, convulsions etc. and further supervised for a period of 14 days for occurrence of any significant changes in the autonomic or behavioral responses and mortality[10].

Cotton Pellet Induced Granuloma Inflammation

Employing an earlier described technique (Winter and Porter, 1957)[11], granulomatous lesions were induced by surgically implanting two autoclaved sterile pellets of cotton weighing 10±1 mg subcutaneously in the dorsal surface of the rats under light ether anaesthesia, one near each groin. All rats of both sexes were previously acclimatized and divided in to eight groups each having six animals. All extracts (aqueous, methanol, acetone) of *S. asoca* were administered orally in two different dosages (300 mg/kg b.w and 500 mg/kg b.w) for seven consecutive days. The rats of the control group were administered with the vehicle (5% gum acacia) and standard group was given Indomethacin for seven consecutive days. The first dose of each sample was given 20 minutes before the implantation of pellets. All the animals had free access to drinking water and food. On the eighth day, the pellets surrounded by granuloma tissue were dissected out carefully, freed from extraneous tissues and dried at 60°C. Mean weight of the granuloma tissue formed around each pellet was recorded. The pellets were weighed under both moist and dry conditions. The difference between the initial weight and the final weight of the cotton pellet is the amount of granulation tissue formed. The weight of the granulation tissue formed in standard and extract-administered rats was compared with the weight of the granulation tissue formed in the control group[11].

The percentage inhibition of Granuloma tissue formation in rats was calculated using the following formula:

$$\% \text{ Inhibition} = [(\text{Control} - \text{Test}) / \text{Control}] \times 100$$

Drug Treatment Protocol for Animal Experimentation

Group I - Vehicle treated control animals received gum acacia (10 ml/kg b.w.)

Group II - Animals received standard drug Indomethacin (2.5 mg/kg b.w. p.o.)

Group III - Animals received aqueous extract of *S. asoca* (300mg/kg b.w. p.o.)

Group IV - Animals received aqueous extract of *S. asoca* (500mg/kg b.w. p.o.)

Group V - Animals received methanolic extract of *S. asoca* (300mg/kg b.w. p.o.)

Group VI - Animals received methanolic extract of *S. asoca* (500mg/kg b.w. p.o.)

Group VII - Animals received acetone extract of *S. asoca* (300mg/kg b.w. p.o.)

Group VIII - Animals received acetone extract of *S. asoca* (500mg/kg b.w. p.o.)

Statistical Analysis

The data were statistically analyzed using one-way ANOVA test followed by Dunnet's *t* test for individual comparison of groups with control[10-12]. Results were expressed as Mean ± SEM. $p < 0.05$ was used to indicate statistical significance.

RESULTS

Phytochemical Screening

Phytochemical classes characterized in different extracts of the seeds of *Saraca asoca* have been presented in Table-1. Preliminary phytochemical screening of extract has revealed the presence of carbohydrates, flavonoids, polyphenols, tannins and saponins. However, no alkaloids, proteins and amino acids were found present in the extracts.

Table1: Physiochemical and phytochemical analysis of different extracts of *Saraca asoca*

Physiochemical and Phytochemical Tests	Aqueous Extract	Methanolic Extract	Acetone Extract
Extractive Value (in % w/w)	1.09	3.22	1.27
Flavonoids	Present	Present	Present
Tannins	Present	Present	Present
Alkaloids	Absent	Absent	Absent
Saponins	Present	Present	Present
Carbohydrates	Present	Present	Present
Glycosides	Absent	Absent	Present
Proteins and Amino acids	Absent	Absent	Absent
Fixed oils and Fats	Absent	Present	Present

Acute Toxicity

Oral administration of different extracts of seeds of *Saraca asoca* up to 1000 mg/kg did not produce any toxic effects in mice during the first 48 hours and even up to the next 14 days. No mortality was observed and all extracts were found to be safe at the given doses.

Evaluation of Cotton Pellet Induced Granuloma Test

All extracts of the seeds of *Saraca asoca* was able to reduce the inflammatory process of granuloma formation in rats after the treatment period in comparison with control rats (Table-2). This was evident from the reduction of both wet and dry weights of the cotton pellets. Treatment with Indomethacin also effectively reduced the granuloma. Pus was observed in control groups, but was not found in treated groups. The results showed significant ($p < 0.01$) inhibition of cotton pellet granuloma in a dose-dependent manner when compared to the control group. In this assay, all extracts displayed marked anti-inflammatory effect at a dose of 500 mg/kg but decrease in inflammation by methanol extract in this dose was comparable with standard drug Indomethacin (2.5 mg/kg) which showed highest inhibition (56.34%) of granuloma tissue formation. The inhibition of test extracts (aqueous, methanol and acetone) at a dose of 500 mg/kg b.w was found to be 43.68%, 49.50% and 46.76% respectively. Similarly this inhibition at a dose of 300 mg/kg b.w was found to be 32.71%, 40.00% and 38.86% respectively during the study (Figure-1).

Table 2: Effect of seeds of *Saraca asoca* on cotton pellet induced granuloma in rats.

Group	Treatment	Dose (mg/kg, p.o.)	Granuloma wt. (dry) mg	% inhibition
I	Control (5% Gum acacia)	10 ml/kg	48.97 ± 0.812	---
II	Indomethacin	2.5	21.38 ± 0.576	56.34
III	Aqueous Extract	300	32.95 ± 0.927	32.71
IV	Aqueous Extract	500	27.85 ± 1.497	43.68
V	Methanolic Extract	300	29.38 ± 0.846	40.00
VI	Methanolic Extract	500	24.73 ± 1.233	49.50
VII	Acetone Extract	300	29.94 ± 1.148	38.86
VIII	Acetone Extract	500	26.07 ± 1.275	46.76

Values are expressed as mean ± SEM (n =6), p <0.01 as compared to control group

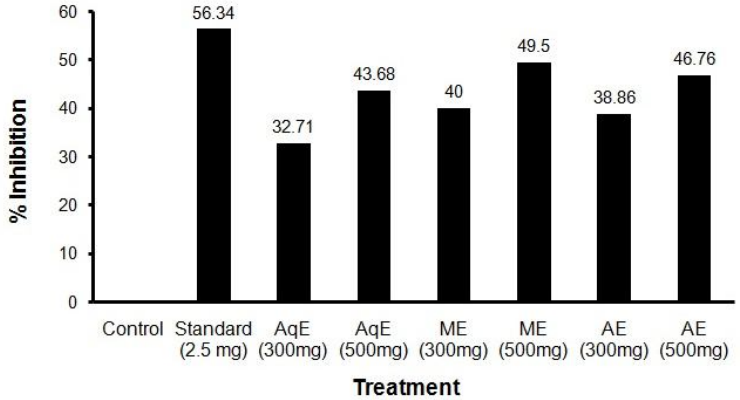

Figure-1: Percentage inhibition in mean weight of cotton pellet in different groups *(Standard- Indomethacin, AqE- Aqueous Extract, ME- Methanolic Extract, AE- Acetone Extract).*

DISCUSSION

The response to a subcutaneously implanted cotton pellet in rats is divided into at least three phases - transudative phase, exudative phase and proliferative phase[13]. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation[11]. Moreover, the implanted material induces a host inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granular formation. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue[14-16].

Anti-inflammatory drugs can reduce transudative weight probably via their ability to inhibit the permeability response of the blood vessels around the cotton pellet implantation. They can also effectively inhibit the granuloma formation probably due to interference with proliferative components of inflammatory process. NSAIDs such as aspirin elicit a slight inhibition whereas steroidal anti-inflammation drugs have a strong inhibition on both transudative and proliferative phases of inflammation[13].

During the study, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by all the seed extracts of *Saraca asoca*. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with better therapeutic index.

CONCLUSION

In the present study, oral administration of the extracts of *Saraca asoca* seeds has been observed to inhibit the weight of granuloma tissue in a dose dependent manner and the higher dose of all extracts exhibited inhibition of inflammation very close to the inhibitory effect of indomethacin (56.34%). Among the three extracts, the highest activity was found in the higher dose (500 mg/kg b.w) of methanolic extract (49.50%).

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