Research Article

An Experimental Study to Evaluate the Analgesic, Anti-Inflammatory and Antipyretic Activities (Vednasthapan Karma) of the Aqueous Extract of *Saraca asoca* Seeds

***Mradu Gupta[1], Saumyakanti Sasmal[2], Arup Mukherjee[3]**

[1]*Mradu Gupta, Dravyaguna Department, Institute of Post Graduate Ayurvedic Education and Research, Kolkata, West Bengal, India.
[2]Saumyakanti Sasmal, Dravyaguna Department, Institute of Post Graduate Ayurvedic Education and Research, Kolkata, West Bengal, India.
[3]Arup Mukherjee, Department of Chemical Technology, University College of Science and Technology, Calcutta University, Kolkata, India.
***Corresponding author's E-mail:** mradu_gupta@hotmail.com

Accepted on: 23-11-2013; **Finalized on:** 31-01-2014.

ABSTRACT

Saraca asoca has been well-known for its therapeutic pharmacological properties and mentioned in the Ayurvedic texts as a cure for pain, fever and inflammation. The aqueous extract of its seeds was pharmacologically evaluated in this study for its acute anti-inflammatory, antipyretic and peripheral analgesic effect on rodents. Phyto-chemical analysis revealed the presence of tannins, flavonoids, carbohydrates, saponins and high concentrations of phenolic compounds. No significant toxic symptoms, morbidity or mortality were observed in animals during acute toxicity study up to 1500 mg/kg dose. Peripheral analgesic effect assessed from inhibition in writhings after drug administration was found to be significant and comparable, measuring 38.15 % at 300 mg/Kg drug dose and 49.19 % at 500 mg/kg drug dosage while the standard drug Aspirin resulted in 61.38% inhibition after 15 minutes as compared to the control group. The aqueous extract showed significant antipyretic effect at both drug doses as compared to the standard Aspirin (100 mg/Kg), the pharmacological activity being more sustained and persistent up to 5 hours possibly due to the presence of flavonoids. The reduction in rectal temperature after 4 hours was 3.32% in case of standard, 2.83% in 300 mg/kg test drug, 3.19% in 500 mg/kg test drug and only 1.08 % in the control group up to 5 hours. The Carrageenan-induced paw edema model was used to evaluate the acute anti-inflammatory effect using rats. The circumference of paw oedema induced by the Carrageen agent at 300 mg/kg and 500 mg/kg drug dose showed significant decrease over 4 hours of 76.74% and 93.02% as compared to standard drug Indomethacin, which resulted in 97.67% inhibition when compared with control group. The results confirm the significant anti-inflammatory, analgesic and antipyretic pharmacological actions of aqueous extract of *Saraca asoca* seeds.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, *Saraca asoca*.

INTRODUCTION

Saraca asoca belonging to the family *Caesalpinaceae* is a small evergreen tree which occurs up to the altitude of 750 meters in the western coastal zone of the Indian subcontinent. Its leaves are parpinnate 15-20 cm long and the leaflets 6-12, oblong and rigidly sub-coriaceous. The stem bark is dark brown or grey with warty surface due to the presence of rounded or projecting lenticles. Its fragrant flowers are yellowish orange while the seeds are 4-8, ellipsoid-oblong and compressed. The stem bark of this plant is chiefly used in medicines and has been reported to contain chemicals such as glycoside, flavanoids, tannins, saponins, alkanes, esters and primary alcohols. The primary phytochemical constituents in the stem bark include epicatechin, procyanidin p2,11'-deoxyprocyanidin B, catechin, leucopelargonidin and leucocyanidin. The flowers contain Oleic, linoleic, palmitic and stearic acids, P-sitosterol, quercetin, kaempferol, leucocyanidin and gallic acid. The seed and pod contains oleic, linoleic, palmitic and stearic acids, catechol, epicatechol and leucocyanidin.[1-4]

Saraca asoca, locally called Ashoka, is known for the many pharmacological activities of its stem bark like anti-cancer, anti-menorrhagic, anti-oxytoxic and anti–microbial properties and has been traditionally used to treat skin infections and genitor-urinary ailments. [5 – 11]

The term *Vednasthapan Karma* has been mentioned in the ancient Ayurvedic texts such as Charka Samhita and Susruta Samhita as the property of any plant/herb to reduce or mitigate the pain and its associated parameters in the different body parts on the basis of aetiological factors. Ashoka is one of the important plants mentioned in this category of herbs which have been used in the form of single drug or compound drug for the treatment of pain, inflammation, fever and uterine bleeding, etc.[12-14] While a lot of research has been done on the pharmacological properties of the stem bark of the plant, there was no detailed study of therapeutic actions of its seeds, which are more easily available in abundance. [15-17] Therefore, in this study the aqueous extract of its seeds was pharmacologically evaluated for its acute anti-inflammatory, antipyretic and peripheral analgesic effect on rodents.

MATERIALS AND METHODS

The pharmacognostical and experimental studies were done in the laboratory and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India registered animal house facility of Dravyaguna Department of the Institute of Post Graduate Ayurvedic Education and Research, Kolkata.

Collection and identification of plant materials

The seeds of *Saraca asoca* were collected from the reputed supplier of pharmacy department of the Institute and identified by the botanist at the Botanical Survey of India, Howrah, India vide Ref. No. BSI/CNH/AD/Tech./2010 and Sample Reg. No (AS-01). The authentic herbarium specimen was deposited in the herbarium museum of the department of Dravyaguna at IPGAE&R, Kolkata for future reference.

Chemicals

The analytical chemicals were procured from M/s Merck Ltd throughout the study. The Carrageenan was purchased from M/s Sigma Aldrich, USA while Aspirin was purchased from M/s NICE Chem. Pvt. Ltd., India. Indomethacin was purchased from M/s Jagsonpal Pharmaceuticals Ltd., India.

Pharmacognostical study of crude drug powder

The macroscopic and microscopic study of the powder of test drug was performed according to the standard procedures. The final crude drug powder was mounted in glycerine, observed and photographed under optical microscope (40×) of Dewinter, Italy.

Physiochemical analysis

The analysis of the physiochemical parameters such as moisture content, extractive value, acid insoluble ash, water soluble ash and total ash content of the test drug powder was performed according to the standard procedures.

Estimation of total phenolic content

The total phenolic content of the drug was determined by Folin Ciocalteu method using Gallic acid as standard compound at different concentrations (100 to500 µg/ml). The assessed total phenol values were expressed in terms of Gallic acid equivalent as mg/g of dry mass.

Extraction of research sample

The aqueous extract of the coarse powder of dried seeds of *Saraca asoca* was prepared by using the soxhlet apparatus after proper cleaning and drying, and powdered into coarse form for 48 hours. Thereafter it was concentrated by rotatory evaporator in the form of powder which was used throughout the chemical and pharmacological activities on rodents. This was further dried under a vacuum oven drier to give a solid residue and preserved in refrigerator below 10°C for subsequent experiments.

Phyto-chemical screening

Preliminary phyto-chemical screening of the different constituents like alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, fats and oils, proteins and amino acids was performed following standard procedures.

Experimental studies

The acute toxicity and adjuvant anti-arthritis studies of acetone extract of the seeds of *Saraca asoca* were done on rodent animals after getting approval from the Institutional Animal Ethical Committee (IAEC) in the animal house of IPGAE&R (registration number 1180/ac/08/CPCSEA dated 27.03.2008 of CPCSEA) according to the guidelines of CPCSEA.

Experimental animals

Swiss albino mice of either sex, weighing about 20-30 gm and albino (Wistar) rats of either sex, weighing about 120-150 gm were used for different *in-vivo* evaluation.[18] All animals were procured from the Government of West Bengal approved breeder, M/s Satyacharan Ghosh, Kolkata and housed under standard environmental conditions with fixed 12 h light/dark cycles and at a temperature of approximately 25°C in animal house of IPGAE&R. The animals were kept in standard polypropylene cages and provided with food (standard pellet diet) 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 IAEC.

Prior to experiments, the animals were fasted overnight, but allowed free access to water. The following standard methods were used for evaluation of toxicity, analgesic, antipyretic and anti-inflammatory activities using rodent animals.

Acute toxicity study

Acute toxicity study of the aqueous extract was carried out on healthy Swiss *albino* mice following OECD guideline 423.[19] The animals of both sexes were selected by random sampling technique and divided into 5 groups of 3 animals each. A single oral dose of the extract was administered orally at the level of 100, 300, 500, 700, 1000 and 1500 mg/kg body weight respectively. All the animals were observed for appearance of toxic symptoms including muscle spasm, loss of righting reflex, tremors, behavioral changes, locomotion, convulsions and mortality for 1, 2, 4, 8 and 24 h. The supervision was continued for a period of 14 days for observing any occurrence of toxic symptoms and mortality.[20]

Assessment of peripheral analgesic effect

The peripheral analgesic activity of the extract of test drug was assessed in acetic acid induced writhing experiments using mice. The standard procedure prescribed by Veerappan *et al.*[21] was used for observing the abdominal constriction writhing resulting from intra-peritoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in water). Saline (10 ml/kg) was orally administered to group A (control group) whereas standard Aspirin (100 mg/kg) was prescribed for group B and 300 mg/kg & 500 mg/kg test drug extract was orally administered to Groups C & D respectively. Acetic acid solution was then administered to each animal after 30

minutes and the number of writhes counted for the next 15 minutes.[22, 23]

Evaluation of antipyretic activity

The assessment of antipyretic activity was carried out using Brewer's yeast induced pyrexia in Wistar rats by the method as described by Hajare *et al.*[22] Rats were fasted overnight with water *ad libitum* before the experiment. The normal body temperature of each animal was measured by digital tele-thermometer (IMCORP, Ambala, India) and recorded. Pyrexia was induced by subcutaneously injecting 20% w/v Brewer's yeast (10 ml/kg), suspended in normal saline, into the animal's dorsum region. The peak pyrexia was observed to be at 18 hours after yeast administration by conducting trial experiments. The animals that showed an increase in rectal temperature of at least 1°C were used for the study. The drugs were administered orally at the time of peak pyrexia. The control group was administered normal saline (10 ml/kg), the standard group received aspirin (100 mg/kg) and the research groups were given the aqueous extract of research drug at doses of 300 and 500 mg/kg respectively. The rectal temperature was recorded at time intervals of 1 hr, 2 hr, 3 hr, 4 hr and 5 hours after drug administration.

Evaluation of acute anti-inflammatory activity (Carrageenan- induced paw oedema in rats)

During anti-inflammatory studies, paw oedema was induced by injecting 0.1 ml of 1% (w/v) Carrageenan suspension into the sub planter region of the right hind paw of the rats.[24,25] The control group was orally administered saline (10 ml/kg) while the standard group was given Indomethacin (5 mg/kg) and Test drug groups were given 300 mg/kg & 500 mg/kg of the test drug extract 1 hour before Carrageenan injection. The measurement of paw oedema was carried out by displacement technique using Plethysmometer to find out the circumference of paw oedema immediately before and after at 1 hr, 2 hr, 3 hr and 4 hours following the Carrageenan injection.[26, 27]

The inhibitory activity was calculated according to the formula

$$\% \text{ Inhibition} = \frac{(C_t\text{-}C_o) \text{ control} - (C_t\text{-}C_o) \text{ treated}}{(C_t\text{-}C_o) \text{ control}} \times 100$$

Where C_t is the paw circumference at time t, C_o is the paw circumference before Carrageenan injection and (C_t - C_o) is oedema or change in paw size after time t [36].

Statistical analysis

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

RESULTS

Pharmacognostical study

The seed coating is brown or slightly black in colour while sun-dried seeds are dark brown colored having a smooth surface with hard texture. Coarse powder of the seed is light brown in colour with an aromatic odor and having a slightly sweet taste. The fine powder was mounted in glycerin as well as stained with different reagents. Observation under microscope (Dewinter, Italy) showed presence of cells containing tannins, stone cells, crystals, endospermic cells, starch grains, vessels, etc.

Physiochemical analysis

The results obtained during the physiochemical analysis are listed in table 1.

Table 1: Results of physiochemical analysis

Parameter	Result
Foreign matter	6.5%
Moisture content	6.5%
Total ash value	6.7%
Water soluble ash	6.0%
Acid insoluble ash	0.7%
Total phenolic content	3.7 mg of dry mass as calculated from the standard curve of Gallic acid
Extractive value	1.63%

Phyto-chemical analysis

The aqueous extract of the seeds of *Saraca asoca* tested positive for the presence of tannins, flavonoids, carbohydrates and saponin by using standard chemical tests.

Acute toxicity studies

The results of acute toxicity experiments showed that there were no significant toxic symptoms like sedation, convulsion, diarrhoea, irritation, etc and also no mortality up to 1500 mg/kg dose in Swiss albino mice in both sexes. The observation of the animals was first done up to 24 hrs and later up to 14 days for recording their diet, weight, behavior and symptoms, etc.

Assessment of peripheral analgesic effect using writhing analysis

Writhing analysis was done on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of mice during the test. The number of writhes was recorded for the different treatment groups and compared with the results of control group. The aqueous extract of the research drug at the dose level of 500 mg/kg showed 28.34 ±1.667 number of writhes which was higher than that in case of the standard drug Aspirin (21.54 ±1.229) and more than the observed values (table 2) at 300 mg/kg dose of research drug (34.50 ±1.258). The average number of writhes (55.78 ±1.764) was very high in case of the

control group as compared to all other groups (figure 1). Therefore, the research drug at higher dose exhibited significantly higher peripheral analgesic effect as compared to the lower dose in this test.

Table 2: Peripheral analgesic activity of aqueous extract by acetic-acid induced writhing in mice

Treatment Group	No. of writhes (Mean ± S.E.M)	Percentage inhibition compared with control
Control (10 ml/kg)	55.78±1.764	--
Standard ASA (100 mg/kg)	21.54±1.229	61.38%
Aqueous extract (300 mg/kg)	34.50±1.258	38.15%
Aqueous extract (500 mg/kg)	28.34±1.667	49.19%

ASA: Acetyl Salicylic Acid, $p < 0.05$, n= 6

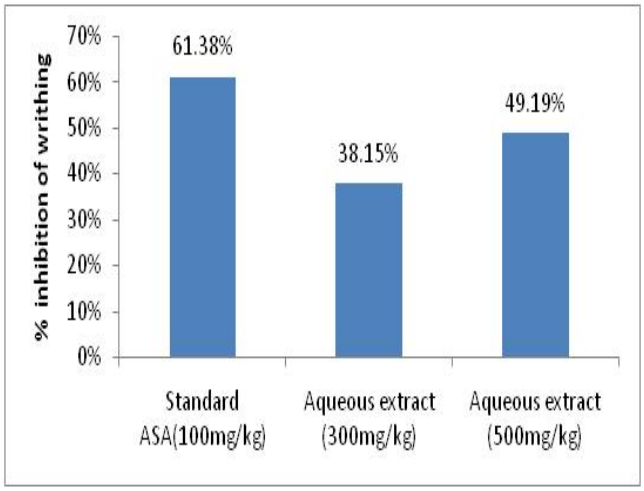

Figure 1: Percentage inhibition during acetic acid induced writhing in mice.

Table 3: Antipyretic effect of aqueous extract of *Saraca asoca*

Groups	Initial Rectal Temp. in °C	Rectal Temperature in ^{0}C after 18hrs of Yeast Injection						% Reduction of rectal temp. after 4 hours
	Before 18 hrs	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	
Control (Normal saline)	36.38 ± 0.291	37.79 ±0.138	37.72 ±0.179	37.62 ±0.197	37.51 ±0.206	37.38 ±0.184	37.31 ±0.243	1.08 %
Standard Aspirin (100 mg/kg)	36.34 ± 0.117	37.63 ±0.158	37.39 ±0.239**	37.13 ±0.174**	36.78 ±0.206**	36.38 ±0.174***	36.49 ±0.109**	3.32%
Aqueous extract 300 mg/kg	36.32 ± 0.309	37.73 ±0.236	37.61 ±0.193*	37.39 ±0.188*	37.02 ±0.287**	36.66 ±0.228***	36.49 ±0.191*	2.83%
Aqueous extract 500 mg/kg	36.19 ± 0.194	37.58 ±0.178	37.38 ±0.238	37.19 ±0.135**	36.81 ±0.189*	36.38 ±0.155*	36.31 ±0.257**	3.19%

n=6; values are mean±SEM (***p<0.0001,**p<0.001,*p<0.01, when compared with control)

Table 4: Anti-inflammatory effect using Carrageenan induced paw oedema

Treatment group with dose	0 hour	1hour	2 hour	3 hour	4hour	% inhibition compared with control after 4hr
Control (10ml/kg)	0.48±0.279	0.64±0.318	0.78±0.239	0.93±0.335	0.91±0.473	00.00
Indomethacin (5mg/kg)	0.52±0.248	0.55±0.487	0.60±0.377	0.63±0.329	0.53±0.362	97.67%
Aqueous extract (300mg/kg)	0.49±0.145	0.55±0.288	0.64±0.357	0.72±0.394	0.59±0.422	76.74%
Aqueous extract (500mg/kg)	0.53±0.241	0.58±0.294	0.62±0.373	0.69±0.413	0.56±0.279	93.02%

n=6; values are mean ± SEM

Antipyretic effect using yeast induced pyrexia

The measurement of rectal temperature was done to find out the degrees of fever in different time periods (1hr, 2hr, 3hr, 4hr & 5hr) of all the groups as shown in table 3. While the standard, 300 mg/kg and 500 mg/kg drug groups showed significant decrease in temperature, the control group showed very little decrease in the elevated rectal temperature after 1 hr of drug administration. This was further followed in both doses of test drug by a gradual trend of decrease in a dose dependent manner up to 5 hours of oral administration of the extract (figure 2). The same trend is noticed in case of the standard group also, however, after 4 hours the temperature increases again. In case of the control group, there is a very low decrease of rectal temperature as compared to the other groups up to 5 hours.

Anti-inflammatory effect by Carrageenan-induced paws oedema

The diameter and circumference of the paw oedema was recorded by using the plethysmometer in different time periods, namely at 1st hr, 2nd hr, 3rd hr and 4th hr after inducing the Carrageenan suspension in the different

groups. As shown in fig. 3, the size of paw oedema in case of control group grew substantially during almost the entire experiment while this increase was noticeably lower in case of all the other groups. The diameter of paw oedema was the largest in case of the lower drug dose (300 mg/kg), followed by the higher drug dose of 500 mg/kg and comparatively lower in case of the standard group. After 4 hours, the calculated inhibition w.r.t. the control was found to be 97.67 % in the standard group, 93.02 % in the 500 mg/kg group and 76.74 % in case of the 300 mg/kg group (table 4).

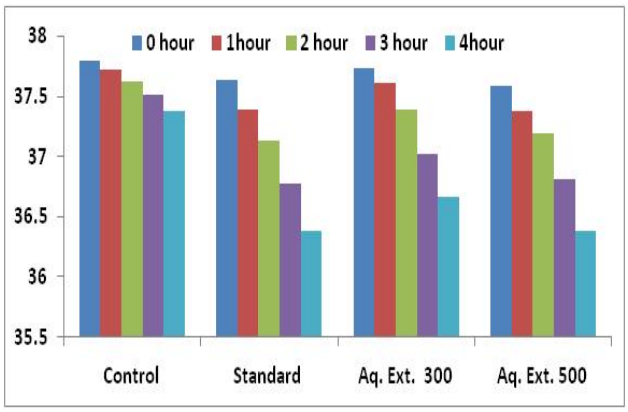

Figure 2: Rectal temperature of control, aspirin and aqueous extract groups over time during antipyretic studies

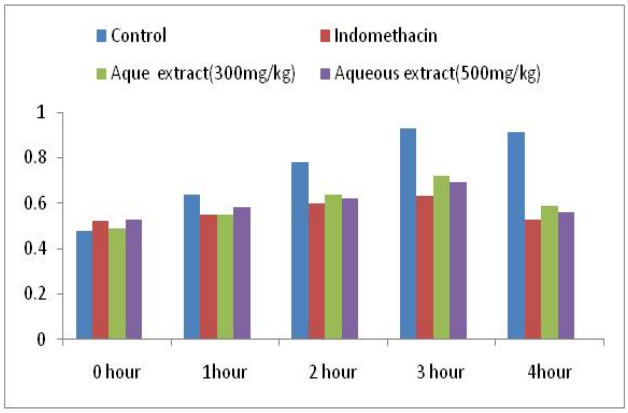

Figure 3: Diameter of paw oedema in different groups over time

DISCUSSION

The experimental study of the aqueous extract of seeds of *Saraca asoca* was done to evaluate its analgesic, anti-inflammatory and antipyretic pharmacological actions by using the standard methods on rodents. Phyto-chemical analysis revealed the presence of tannins, flavonoids, carbohydrates, saponins and high concentrations of phenolic compounds (up to 3.7%) in the aqueous extract of this sample which could be responsible for its pharmacological activities. Flavonoids are a large family of compounds synthesized by plants that have a common chemical structure. The flavonoidic phenolic compounds have been known to exhibit anti-inflammatory, anti-oxidant, analgesic and metal-chelating properties.[28]

The animals tested in acute toxicity study showed no significant toxic symptoms up to the dose of 1500 mg/kg such as sedation, convulsion, diarrhoea, irritation, etc. Some animals showed mild symptoms of irritation and minor behavioral changes but returned to normal condition during a few hours. Even at this high dose, no further toxic symptoms or mortality was observed for the next 24 hrs and subsequently up to 14 days, demonstrating the very low toxicity levels of the research drug.

The peripheral analgesic effect was evaluated on the basis of average number of abdominal constrictions indicated by the extension of hind paw of animals. The observed inhibition in writhes as a result of administration of the test drug was significantly higher ($p < 0.05$) at the dose of 300 mg/kg (38.15%) as well as at 500 mg/kg (49.19 %) when compared with the control group. Comparing the performance of the test drug with the standard drug, the observed peripheral analgesic effect was slightly lower at the higher test drug dose (500 mg/kg) as compared to the standard drug Aspirin which resulted in 61.38% inhibition after 15 minutes. The significant analgesic effect at the higher dose could be attributed to the presence of high concentration of flavonoidic compounds which inhibited the synthesis, release or receptor responses in prostaglandin mediated effects.[28-30]

Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. Mediators like interleukin 1β, α, β and TNF-α increase the synthesis of Prostaglandin E_2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature. The brewer's yeast-induced pyrexia test is of great relevance, as most NSAIDs inhibit the hyper-thermal response. The injection of Brewer's yeast causes release of pro-inflammatory cytokines which stimulate the synthesis of PGE_2 in the surroundings of the hypothalamic thermoregulatory centers.[31] The overall reduction in the rectal temperature of animals after 4 hours of oral drug administration was 3.32% in case of standard drug, 2.83% in 300 mg/kg test drug and 3.19% in 500 mg/kg test drug while the control group showed a decrease of only 1.08 % up to 5 hours. From the perspective of percentage reduction of rectal temperature after 4 hours, the 500 mg/kg dose of the test drug showed highly significant ($p < 0.05$) and almost similar results as compared to the standard drug Aspirin. The aqueous extract of the test drug extract showed significant antipyretic effect at both the doses (300 mg/kg and 500 mg/kg). The test drug also exhibited more sustained and persistent antipyretic effect lasting up to 5 hours during this test because flavonoids are known to target prostaglandins which are involved in pyrexia and also inhibit the effect of enzymes that are responsible for the inflammatory process.[28,32]

The Carrageenan-induced paw edema model has frequently been used to evaluate the anti-edematogenic effect of most anti-inflammatory drugs in routine clinical use.[33] Reports describe that several mediators are

released through the injection of Carrageenan in the rat's paw; while histamine, serotonin and bradykinin are released in the initial phase (0-1h), in the later phase (1-6 h) there is an increase in the production levels of prostaglandins (PGs) through the activation of cyclooxygenase-2 (COX-2) and release of nitric oxide (NO) (Silva *et al*., 2005). Inflammation can result in the locally increased production of free radicals by inflammatory enzymes, as well as the release of inflammatory mediators that promote cell proliferation and angiogenesis and inhibit apoptosis.[30] The diameter and circumference of the paw oedema was recorded by using the plethysmometer in different time periods after inducing the Carrageenan suspension in the different groups. The circumference of paw oedema in rats induced by the Carrageen agent at 300 mg/kg and 500 mg/kg drug dose showed significant decrease from the initial reading at the 1st hr to the 4th hours of 76.74% and 93.02% respectively ($p < 0.05$) as compared to the control group. However, the acute inflammatory effect of the test drug was found to be lower in the 500 mg/kg of test drug dosage as compared to the standard drug Indomethacin, which resulted in 97.67% inhibition after 4 hours of treatment when compared with control group.

CONCLUSION

The significant results obtained in respect of the anti-inflammatory, analgesic and antipyretic pharmacological actions of the aqueous extract of the seeds of Ashoka plant have validated its therapeutic properties mentioned in the ancient texts of Ayurveda. Its low toxicity and efficacy as an herbal analgesic, antipyretic anti-inflammatory drug could be due to the high concentrations of flavonidic phyto-chemicals in its extract, although the responsible exact chemical compound needs to be ascertained by further studies.

REFERENCES

1. Middelkoop TB, Labadie RP, Proanthocyanidins in the bark of *Saraca asoca* Roxb., Naturforsch., 40B(6), 1985, 855-857.
2. Indrani N, Balasubramanian K, Isolation of condensed tannins from *Saraca asoca*, Part-1, Leather Sci., 31(12), 1984, 349-351.
3. Warrier PK, Nambier VPK, Ganpathy PM, Some important medicinal plants of the western ghats, India: A Profile. International Development Research Centre, New Delhi, 2000, 343-360.
4. Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, Verma R, Bajpai A, *Saraca asoca* (Ashoka): A Review, Journal of Chemical and Pharmaceutical Research, 1(1), 2009, 62-67.
5. Satyavati GV, Prasad DN, Sen SP, Das PK, Investigations into the uterine activity of Saraca indica Linn. (Ashoka), J. Res. Indian Med., 4 (1), 1969, 37.
6. Satyavati GV, Prasad DN, Sen SP, Das PK, Oxytocic activity of a pure phenolic glycoside (P2) from Saraca indica Linn. (Ashoka): a short communication, Indian J. Med. Res., 58(5), 1970, 660-663.
7. Satyavati GV, Prasad DN, Sen SP, Das PK, Further studies on the uterine activity of Saraca indica Linn., Indian J. Med. Res., 58 (7), 1970, 947-960.
8. Varghese CD, Nair SC, Panikkar KR, Chemoprotection of Saraca asoka extracts on cisplatrin induced toxicity in mice, Amla Res. Bull., 12, 1992, 86-91.
9. Varghese CD, Nair SC, Panikkar KR, Potential anticancer activity of Saraca asoka extracts towards transplantable tumours in mice, Indian J. Pharm. Sci., 1, 1992, 37-40.
10. Pal SC, Maiti AP, Chatterjee BP, Nandy A, Antibacterial activity of flowers and flowers buds of Saraca indica linn., Indian J. Med. Res., 82, 1985, 188-189.
11. Annapurna J, Bhalerao UT, Iyengar DS, Antimicrobial activity of *Saraca asoca* leaves, Fitoterapia, 70 (1), 1999, 80-82.
12. Sharma PC, Yelne MB, Dennis TJ, Database on medicinal plants used in Ayurveda. Central Council for Research in Ayurveda & Siddha, Department of Indian system of medicine, Govt. of India, New Delhi, 3, 2001, 76-87.
13. Sharma PV, Dravyaguna Vijnana, Chaukhamba Bharati Academy, Varanasi, 2, 1995, 617-619.
14. Shastri SN, Charak Samhita (Sutra Sthanam), Chaukhamba Bharati Academy, Varanasi, Sut.4., Sloka 18, 1988, 47.
15. Chatterjee A, Pakrashi SC, The treatise on Indian Medicinal Plants, Publications and Information Directorate, CSIR, New Delhi, 2, 1992, 45.
16. Kirtikar KR, Basu BD, Indian Medicinal Plants, Allahabad, 2, 1989, 883.
17. Sen SP, Chemical study of indigenous plant (asoka), Curr. Sci., 32, 1963, 502.
18. Ghosh MN, Fundamentals of Experimental Pharmacology, Scientific Book Agency, Kolkata, 1984, 153-157.
19. OECD, Acute Oral Toxicity – Acute Toxic Class Method, Paris: OECD Guideline 423 for Testing of Chemicals, 2001, 1-14.
20. Litchfield JT, Wilcoxon F, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther., 96(2), 1949, 99-133.
21. Veerappan A, Miyazaki S, Dhananjayan R, Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos,* Corr. J. Ethnopharmacol., 96(1-2), 2005, 159-163.
22. Hajare SW, Chandra S, Tandon SK, Sharma J, Lal J, Telang AG, Analgesic and antipyretic activities of Dalbergia sissoo leaves, Indian J. Pharmacol., 32(6), 2000, 357-360.
23. Ibironke GF, Ajiboye KI, Studies on the anti- inflammatory and analgesic properties of Chenopodium Ambrosioides leaf extract in rats, International Journal of Pharmacology, 3(1), 2007, 111-115, ISBN 811-7775.
24. Winter CA, Rusley EA, Muss CW, Carageenan–induced oedema in hind paws of rats as an assay of anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med., 111, 1962, 544-547.
25. Bamgbose SOA, Noamesi BK, Studies on Cryptolepine inhibition of Carrageenan-induced oedema, Plant. Med., 42, 1981, 392-396.

26. Farsam H, Amanlou M, Dehpour AR, Jahaniani F, Anti-inflammatory and analgesic activity of *Biebersteinia multifida* DC. root extract, J. Ethnopharmacol., 71 (3), 2000, 443-447.

27. Regilane Matos da Silva et al., Anti inflammatory, anti nociceptive and antipyretic effects of hydroethanolic extract from *Macrosiphonia velame* (A. St.-Hil.) M. Arg. in animal models, Braz. J. Pharm. Sci., 46(3), São Paulo July/Sept. 2010.

28. Pan MH, Lai CS, Ho CT, Anti-inflammatory activity of natural dietary flavonoids, Food Funct., 1, 2010, 15-31.

29. Gupta M, Shaw BP, Mukherjee A, Studies on antipyretic, analgesic and ulcerogenic activity of polyherbal preparation in rats and mice, Int. J. Pharmacol., 4(2), 2008, 88-94.

30. Sasmal S, Majumdar S, Gupta M, Mukherjee A, Mukherjee PK, Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb., Asian Pacific Journal of Tropical Biomedicine, 2(10), 2012, 782-786.

31. Chan GH, Fiscus RR, Exaggerated production of nitric oxide (NO) and increases in inducible NO-synthase mRNA levels induced by the pro-inflammatory cytokine interleukin- beta in vascular smooth muscle cells of elderly rats, Exp. Gerontol., 39(3), 2004, 387-394.

32. Binny K, Kumar SG, Thomas D, Anti-inflammatory and antipyretic properties of the rhizome of Costus Speciosa (Koen.), J. Basic Clin. Pharm., 1(3), 2010, 177-181.

33. Carvalho JCT, Ferreira LP, Da Silva SL, Correa MJC, Oliveira CLM, Bastos JK, Sarti SJ, Anti-inflammatory activity of flavone and some of its derivates from *Virola michelli* Heckel, J. Ethnopharmacol., 64 (2), 1999, 173-177.

Source of Support: Nil, **Conflict of Interest:** None.