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Studies on the Anti-Inflammatory and Analgesic Efficacy of *Saraca asoca* in Laboratory Animals

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Abstract

Objective: This research was focused on the qualitative and quantitative evaluation of anti-inflammatory and analgesic effects of *Saraca asoca* in laboratory animals and whether these effects were of any statistical significance.

Materials and methods: Carrageenan-induced Hind Paw Edema test in long evans rat was the experiment for anti-inflammatory activity of the ethanolic extract of *Saraca asoca* while hot plate test was carried out to assess its analgesic activity in swiss albino mice.

Result: The experimental activities for the ethanolic extract of *Asoca saraca* exhibited statistically significant ($p<0.05$) anti-inflammatory activity in Carrageenan-induced Hind Paw Edema in long evans rat.

Conclusion: In conclusion, these observations provide evidence and possible mechanisms of action for the anti-inflammatory and probable analgesic properties of bark of *Saraca asoca* claimed in Ayurveda medicine. Further studies should be undertaken to correlate the pharmacological activities with the chemical constituents of the bark of *Saraca asoca*.

Key words:

Analgesic, Carrageenan, Anti-inflammatory

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Introduction

Saraca asoca (Roxb.), De.wild or *Saraca indica* belonging to family *Caesalpinaceae* is a small evergreen plnt 7-10cm high.

Leaves are narrowly lanceolate, cork like at the base and with a shot pestistipules which are intra-petiolar and completely united. The bark is dark brown or grey or almost black with warty surface[1]. It mainly contains glycosidic principles, non-phenolic, sapogenetic glycoside, sterols and aliphatic alcohols. Catechol, (-) epicatechol and leucocyanidin has been isolated from pods and wood contains quercetin.[2-5] It also contains flavonoids and sterols [6,7]. It is extensively used in the Ayurvedic system of medicine for a variety of ailments [8, 9] as a blood purifier, in stomach ache [10], and as a hypothermic and diuretic [11]. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer.[1]

So far no information is available for the analgesic and anti inflammatory activity of the ethanol extract of *Saraca asoca.* So, the present study has been undertaken to evaluate the analgesic and anti inflammatory activity of the ethanolic extract of *Saraca asoca* using hot plate method in swiss albino mice and Carrageenan-induced Hind Paw Edema methods in long evans rat respectively.

Material and Method

Hot Plate (Model – 35100, UGO BASILE, ITALY), Balance, Refrigerator, Beakers, Petri dishes & glass wrought, Safety rat handling gloves, Mortar & pestle., Hypodermic , Syringes, Holder & test tube, Hot water bath., Plethysmometer

Medicinal plants (extracts)

Extract were examined in two concentrations of 500mg/kg and 250mg/kg body weight of animal

Control & Positive Control

Analgesic activity

1. Control – distilled water
2. Positive control – ketorolac

Administered dose – 2.5mg/kg body weight animal

Anti-inflammatory activity

1. Control – Normal saline

2. Positive control – Diclofenac sodium
Administered dose – 300mg/kg body weight animal

Experimental animal

Swiss albino mice (male and female), weighing 25-27g bred in International Centre for Diarrheal Diseases and Research, Bangladesh(ICDDR,B) and grown in the Animal House of the Department of Pharmacy, North South University (NSU). Long Evans rats (male and female), weighing 180-200g of either sex, bred in NSU and ICDDR,B and grown in the animal house of the Department of Pharmacy NSU. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2^{0}C, and 12 hours light dark cycle). The animals were fed with standard diet from ICDDR, B and had free access to filtered water.

Plant Extraction method

Collection

The plant sample of *Saraca asoca*, was collected from Jahangirnagar University, Bangladesh, during 20.07.2010 in the form of bark shavings. The bark of the plant was collected and washed with water several times.

Drying and grinding

The collected plant bark was washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried bark was then grinded to a powdered form and stored in the refrigerator at +4°C for a few days.

Cold extraction (Ethanol extraction)

457.46 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 80% ethanol, sealed and kept for a period of 2 days with occasional shaking and stirring. It was then filtered first by cotton material and twice through filter paper to obtain a finer filtrate. The separated filtrate was found to be a precipitate of dark brown chocolate color and designated as the crude ethanolic extract of the bark of *Saraca asoca*. It was then dried in the freeze drier and preserved at +4°C for two weeks.

Analgesic activity of Saraca Asoca

Study design

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 6 mice in each group Individual weighing was done to adjust individual doses. Here, distilled water was given to group I, 2.5 mg/kg ketorolac for group II, 250 mg/kg for group III and 500 mg/kg for group IV of the crude extract of *Saraca asoca.*

Mice Screening

Mice Screening was performed before Hot plate test. In that experiment mice with significant response action (Licking, Shaking and Jumping) and response time (at the range of 9-15 seconds) were selected.

Hot plate test method

A comparison of Hot plate test was made between positive control (ketorolac), control and test sample given orally 30 minutes after hot plate induction. Positive analgesic activity was shown when sample animal gave longer number of stimuli than the control, or the sample. The temperature of the metal surface of the hot plate was maintained at 55 ± 0.2°C. Latency to a discomfort reaction (licking, shaking or jumping) was determined before and after drug administration. The cut-off time was fixed at 15s to avoid the damage to the animal paw. The latency was recorded at 0, 30, 60, 120, 180 min following oral administration of the agents. The prolongation of the sample latency time compared with that of control was used for statistical comparison. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time). Each mouse served as its own control. Before treatment, its reaction time was taken once. The mean of these values on determination constituted initial reaction time before treatment of the mouse. This was pooled for the mice in each treatment group and the final test mean value (Ta) for each treatment group was calculated. This final test mean (Ta) value represented the after treatment reaction time (Ta) and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the formula. [12]

% protection against thermal stimulus = (V_c - V_t) / V_c x 100

Where, V_C = Value of control animal
V_t = Value of test animal

Anti-inflammatory Effect of Saraca Asoca

Preparation of inflammatory agent

Carrageenan was used as inflammatory agent in this experiment. It was obtained from Jahangirnagar University. Carrageenan powder was suspended in 5 ml saline to make 1% suspension and kept in water bath for proper homogenization. The tube was kept in hot water (50±2˚c) containing beaker to prevent transformation into a jelly like compound.

Carrageenan-induced Rat Hind Paw Edema test

The ethanolic extract of *Saraca asoca* on carrageenan induced inflammation in rat paw was investigated by following the method of Winter *et al* (1962) with minor modifications. Rats were randomly divided into three groups, each consisting of six animals. Group I (control) was given only water. Group II & group III were given the test material and positive control orally, respectively. Half an hour after oral administration of the test materials, 0.1ml 1% carrageenan suspension was injected subcutaneously in left hind paw of each animal leading to the formation of edema *in situ* (localized inflammation). The volume of paw edema was measured at ½, 1, 2, 3, 4, 8 and 24 hours using water plethysmometer after administration of carrageenan. The right hind paw served as a reference non inflamed paw for comparison [13,14]. The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

% Inhibition of paw edema = [1- (Vt / Vc)] X 100

where V_c and V_t represent average paw volume of control and treated animal respectively.

Statistical analysis

All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. P values <0.05 were considered as statistically significant.

Results

Anti-inflammatory Activity

The ethanolic extract of *Saraca asoca* exhibited statistically significant (p<0.05) anti-inflammatory activity in Carrageenan-induced Hind Paw Edema of rat. This was determined by analyzing data using one way ANOVA followed by Dunnett's test. In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity four hours after the injection of the phlogistic agent. Ethanol extract of *Saraca asoca* showed a significant dose depended reduction at 300 mg/kg body weight. However maximum inhibition of *edema* was found to be 64.14% at fifth hour of study at a dose of 300 mg/kg body weight respectively.

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Analgesic activity

The ethanolic extract of *Saraca asoca* did not exhibit any statistically significant (p > 0.05) analgesic effect in hot plate test of white albino mice. This was determined by analyzing data using one way ANOVA followed by *Dunnett's post hoc* test. However, the data shows that the dose dependent effect reached 72.57% at 180 minutes and 37.67% at the 60 minutes at the doses of 500 and 250 mg/kg-body weight respectively.

Acute toxicity

On oral administration of graded doses (250, 300 & 500mg/kg) of the ethanol extract of *Saraca asoca* to rats and mice, no mortality was recorded in any group after 24h of administering the extract to the animals

Discussion

As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all the cases [15]. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [15]. *Saraca asoca* is highly regarded as a universal *panacea* in the ayurvedic medicine. Specific studies have been carried out to assess the anticancer, antimicrobial and antioxytocic profiles of *Saraca asoca* as reported in scientific literature [1]. In absence of any verifiable scientific data, this study was carried out in order to assess the anti-inflammatory and analgesic profile of the ethanolic extract of *Saraca asoca* in laboratory animals.

The carrageenan-induced paw edema is commonly used as an experimental model of acute inflammation [16] .In the present study, an attempt has been made to evaluate the anti-inflammatory activity of *Saraca asoca* by use of the carrageenan-induced paw edema model. The results of this investigation indicate that the extract of *Saraca asoca* have a marked ability to counter acute inflammation induced by carrageenan. The development of carrageenan-induced paw oedema is believed to be biphasic [17]. First phase occurs within an hour of injection of phlogistic agent and is mediated through release of histamine, serotonin and kinins while the second phase (3 to 4 hours) is related to release of prostaglandins [18]. According to Vinegar *et al.* (1987) and Antonio and Brito (1998) in the carrageenan model, the early phase (1–2 h) is mainly mediated by histamine, serotonin and the increase of prostaglandin synthesis in the surroundings of the damaged tissues while the late phase is mainly mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced in tissue macrophages.

In the present study the ethanolic extract showed slight inhibition of inflammation in first phase and maximum inhibition in the second phase. The possible anti-inflammatory effect may be due to inhibition of cyclooxygenase enzyme which catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid. Saraca asoca is reported to contain flavanoids[6,7]. There are reports that flavonoids possess anti-inflammatory activity [19] and some of them also act as phospholipase inhibitors [20]. Such inhibitors are able to decrease the inflammatory response to Carragennan in rats [21].

The application of thermal noxious stimuli forms the basis of some widely used tests to detect either hyperalgesic or analgesic reactions. Among the various analgesic tests using thermal nociceptive stimulation, the most popular are the tail-flick and the hot plate test. In the hot plate test, animals are exposed only once to the heat stimulus, resulting in minimal tissue injury. The assay may be performed without any previous habituation and offers good reliability and reproducibility. In the classical hot plate test, mice react by licking their paws and/or jumping. In the hot plate test, in addition to analgesic responses, hyperalgesic responses can also be detected, usually by summarizing several withdrawal behaviors (licking feet, jumping or rapidly stamping paws) which are computed as a whole [21].

In the present study, the dose dependent effect of ethanolic extract of *Saraca asoca* in hot plate test for the evaluation of analgesic activity in mice was observed and it was found to be not statistically significant. This may be

indicative of various errors of mechanical or human origin. Alternatively, it is also possible that the analgesic activity of the ethanolic extract of *Saraca asoca* is simply not statistically significant. Safety aspect of the ethanolic extract of *Saraca asoca* bark was established since graded doses of the ethanolic extract of *Saraca asoca* (250, 300 and 500 mg) in laboratory animals did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 24h of administering the extract to the animals. This opens up the doors to the possibility of a new safe anti-inflammatory drug.

Conclusion

In conclusion, these observations provide evidence for the anti-inflammatory and probable analgesic properties of bark of *Saraca asoca* claimed in Ayurveda medicine. Further studies should be undertaken to correlate the pharmacological activities with the chemical constituents of the bark of *Saraca asoca* and uncover specific mechanisms of action so that we may find a viable natural alternative to the traditional NSAIDs.

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Conflict of Interests:

Authors have no Conflict of Interest

Table 1. Anti-inflammatory Activity for Control and Extract at Different Periods of Time

Treatment	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	24 hr
Control	.8233±.017	1.0075±.027	1.0575±.056	1.0500±.042	1.0792±.044	1.0875±.06	1.0725±.084
Extract 300mg/kg	.7025±.027*	.7725±.033*	.8000±.024*	.7950±.023*	.7808±.017*	.7900±.014*	.7800±.009*

One way ANOVA. (Values in the results are expressed as mean ± SEM, n=6) * Significant p<0.05

Table 2. Analgesic Activities for Control, Standard and Extract (250 mg/kg and 500mg/kg)

Treatment	0 min	30 min	60 min	120 min	180 min
Control	7.233±1.81	8.183±2.29	7.550±2.30	6.500±1.65	7.633±2.12
Extract 250mg/kg	4.183±1.04	5.016±1.07	6.816±1.85	6.083±1.90	7.450±2.11
Extract 500mg/kg	6.183±.85	7.850±1.50	8.783±1.90	7.700±1.64	7.450±1.30
Standard	4.766±1.10	6.433±1.82	6.016±1.64	8.500±1.99	6.667±1.56

One way ANOVA. (Values in the results are expressed as mean ± SEM, n=6) * Significant p<0.05

Table 3. Percentage of Change in Reaction Time for Standard and Extract at Different Periods of Time

Treatment	0 min	30 min	60 min	120 min	180 min
Extract 250/kg	0	6.779692	58.56256	55.55605	72.57163
Extract 500mg/kg	0	13.82678	37.66809	34.66912	14.96146
Standard	0	21.84267	21.84475	88.48073	34.35649

Figure 1: Percentage of Inhibition of Inflammation for Standard and Extract Versus Observation Time

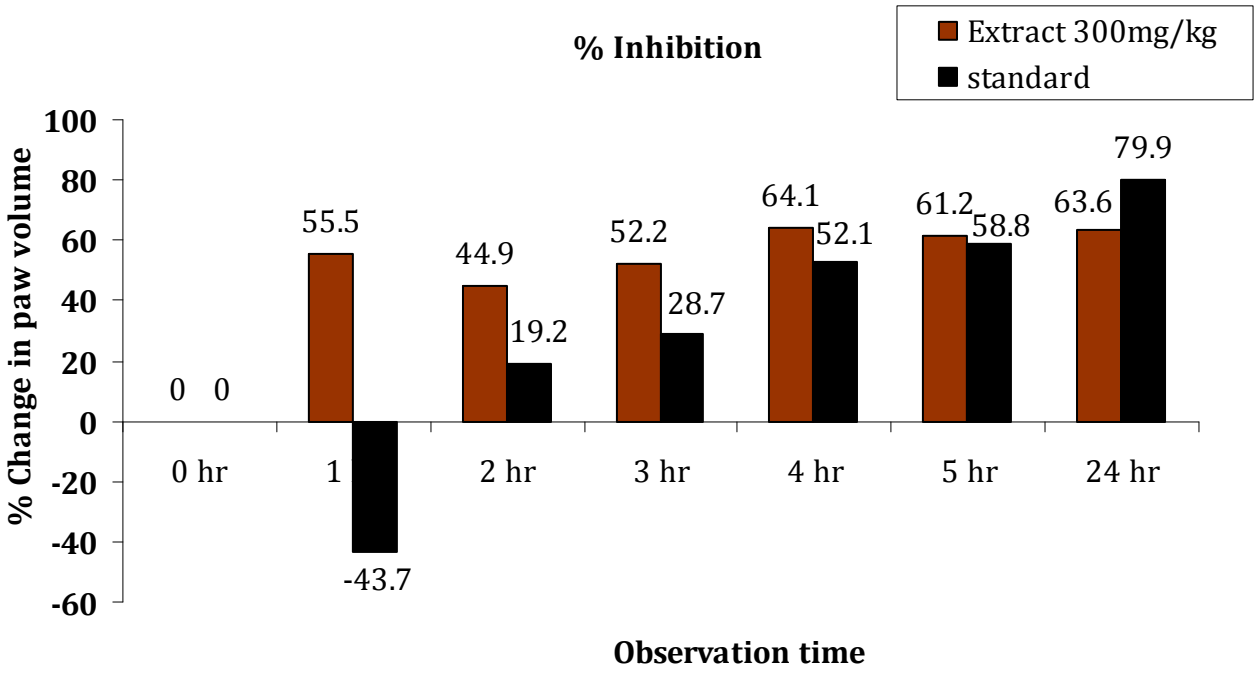

Figure 2: Percentage of Change in Reaction Time for Standard and Extract Versus Time