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Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of *Saraca asoca* Roxb.

Sasmal S[1], Majumdar S[1], Gupta M[1*], Mukherjee A[2], Mukherjee PK[3]

[1]*Department of Dravyaguna (Medicinal Pharmacology), Institute of Post Graduate Ayurvedic Education and Research, Kolkata, India*
[2]*Department of Chemical Technology, University of Calcutta, Kolkata, India*
[3]*Department of Health & Family Welfare, Government of West Bengal, India*

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ABSTRACT

Objective: To conduct a systemic evaluation of the medicinal value of seeds which include macroscopic and microscopic characterization, physiochemical evaluation, preliminary phytochemical screening and experimental antipyretic activity. **Methods:** *Saraca asoca* seed was studied for pharmacognostical, phytochemical and other recommended methods for standardizations. Also, the acetone extract of the seeds was evaluated for acute toxicity study and antipyretic activity using Brewer's yeast induced pyrexia in Wistar rats at oral doses of 300 mg/kg and 500 mg/kg. **Results:** After phytochemical screening, the acetone extract showed the presence of saponin, tannins and flavonoids which inhibit pyrexia. The therapeutic efficacy achieved at both the dose levels of the research drug and standard drug aspirin (100 mg/kg) showed significant ($P<0.01$) antipyretic activity when compared to the control group. The highly significant antipyretic effect exhibited at the dose of 500 mg/kg was also found to be sustainable in nature. **Conclusions:** The antipyretic effect of the acetone extract showed significant results in rats at the dose of 500 mg/kg after following the standard pharmacognostical and phytochemical methods.

1. Introduction

Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. In the Ayurvedic system of medicine, herbal extracts instead of purified compounds have been used since centuries because many constituents with more than one mechanism of action are considered essential for the required holistic therapeutic action. Ashoka is one of the most legendary and sacred trees of India which has been utilized from ancient times till date[1-3]. Ashoka tree, universally known by its binomial Latin name *Saraca asoca* (Roxb.) or *Saraca indica* belonging to the Caesalpiniaceae family[4], is found throughout India, especially in Kerala, West Bengal, regions of southern India and in the Himalayas up to an altitude of 750 m. It is a small, spreading evergreen tree of 7-10 m height whose bark is dark brown or almost grey with a warty surface. Its leaves are parpinnate, 15-20 cm long and the leaflets are 6-12 cm, oblong and rigidly subcoriaceous[5] while the flowers are fragrant and polygamous apetalous, yellowish orange turning to scarlet[6]. Stem bark of *S. asoca* is reported to contain glycosides, flavonoids, tannins and saponins[4,7]. It is used as a spasmogenic, oxytocic, uterotonic, antibacterial and antidysentric agent[5,8]. It has also been reported to possess antiprogestational and antioestrogenic activity against menorrhagia[4]. An extensive search of the literature reveals no proper studies on the pharmacological activity of the seeds of this plant although a large number of its seeds are readily found scattered near the trees without being put to any special use. Thus, the present investigation aims towards the pharmacognostical evaluation, determination of physiochemical parameters, preliminary phytochemical screening and assessment of the antipyretic efficacy of acetone extract of *S. asoca* seeds.

2. Materials and methods

*Corresponding author: Dr. Mradu Gupta, Reader, Department of Dravyaguna, (Medicinal Pharmacology) Institute of Post Graduate Ayurvedic Education and Research, Kolkata-700 009, India.
Tel: +91-9433665125
Fax: +91-33-23504159
E-mail: mradu_gupta@hotmail.com
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2.1. Collection and identification of plant material

The seeds of *S. asoca* were collected from the medicinal plant garden of Narendrapur Ramakrishna Mission, Kolkata and the State Government Herbal Garden at Kalyani, West Bengal, India. The identification of the seeds was done at the Botanical Survey of India, Howrah, India vide Ref. No. BSI/CNH/AD/Tech./2010 and Sample Reg. No (AS-01). An authentic herbarium specimen was deposited in the herbarium museum of Department of Dravyaguna, IPGAE&R, Kolkata, India for future reference.

2.2. Chemicals

Aspirin was purchased from NICE Chem. Pvt. Ltd., Cochin, India. Gallic acid, ferric chloride, sodium hydroxide and all other chemicals used in different studies were of analytical grade.

2.3. Processing and solvent extraction

Seeds were washed and cleaned thoroughly to remove any extraneous matter and dried under sun light for about twenty days. The sun-dried whole seeds were powdered with a grinding machine (Hammer mill) and passed through a #40 mesh sieve. Powdered material was stored properly in airtight containers for experimental purposes. The powder of research drug was subsequently extracted sequentially in petroleum ether (60-80 ℃), chloroform, acetone, methanol and water in a Soxhlet's extractor and then filtered. The extract was concentrated under vacuum in a rotary evaporator to yield semi-solid mass. This was further dried under a vacuum oven drier to give solid residue and preserved in refrigerator below 10 ℃ for subsequent experiments.

2.4. Animals

Swiss albino mice of either sex, weighing about 20-30 g and albino (Wistar) rats of either sex, weighing about 120-150 g were used for in-vivo evaluation. All animals were procured from the Government of West Bengal approved breeder, M/s Ghosh Scientific, Kolkata and housed under standard environmental conditions with fixed 12 h light/dark cycles in animal house of IPGAE&R, registered by CPCSEA (Reg. No. 1180/ac/08/CPCSEA dated 27.03.08). The animals were kept in standard polypropylene cages 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 the Institutional Animal Ethical Committee.

2.5. Pharmacognostic study

Fresh seeds authenticated from the Botanical Survey of India were taken for morphological and histological studies. Coarse powder (#40 mesh) was used to find out different pharmacognostical (macroscopic and microscopic) characteristics in the department of Dravyaguna of IPGAE&R according to established procedures[9,10].

2.6. Physiochemical parameters and preliminary phytochemical screening

Different physiochemical parameters (such as moisture content, ash values, extractive values, total phenolic content, saponification value, *etc.*) of the powdered seeds were estimated using standard methods[11-13]. The fluorescence analysis of the powdered material was done under visible and UV (254 and 365 nm) lights[14,15]. The acetone extract of the seeds was then subjected to different qualitative tests to determine the presence of various phytoconstituents[16,17].

2.7. Acute toxicity test

Acute toxicity study of acetone extract of the seeds of *S. asoca* was carried out on healthy Swiss albino mice following OECD guideline 423[18]. A single oral dose of the extract was administered orally at the level of 100 mg, 300 mg, 500 mg, 700 mg and 1 000 mg/kg body weight respectively to the 5 groups containing 6 mice each. These groups were observed for any signs of toxic symptoms, behavioral changes, locomotion, convulsions and mortality for 1, 2, 4, 8 and 24 h and further for a period of 14 days. During this period, their activity levels and behavior patterns were closely watched and meticulously noted[19].

2.8. 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 Loux *et al*[20]. 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 h after yeast administration by conducting trial experiments. The animals that showed an increase in rectal temperature of at least 1 ℃ were used for the study. The drugs were administered orally at the time of peak pyrexia. The control group (group I) was administered 5% gum acacia, the standard group (group II) received aspirin (100 mg/kg) and the research groups (group III and IV) was given the research drug at doses of 300 mg/kg and 500 mg/kg respectively. The rectal temperature was recorded at a time interval of 1, 2, 3, 4 and 5 h after drug administration.

2.9. Statistical analysis

The data were statistically analyzed using one-way ANOVA followed by Dunnet's *t* test for individual comparison of the various groups with the control group[19,21].