



Analgesic Activity of Various Leaf Extracts of *Saraca indica* Linn

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Abstract

The nature has provided abundant plant wealth which possesses several medicinal values. It is necessary to explore their pharmacological properties to ascertain their therapeutic properties. Saraca indica is an important indigenous plant with a lot of traditional importance. The present study is an attempt to explore the analgesic activity of petroleum ether, chloroform, methanol and water of whole part of this plant. The analgesic activity of above extracts was evaluated by using tail immersion method and formaline induced pain method in albino mice. Analgesic activity of petroleum ether (PSI), chloroform (CSI), methanol (MSI) and water (WSI) extracts were investigated at a dose of 200 and 400 mg/kg. Extracts produce dose dependent analgesic activity. Methanol extract at a dose of 400 mg/kg produced highest activity. MSI produced 52.64 and 43.30% inhibition of formalin induced pain response in first and second phase respectively, whereas standard drug pentazocine (10 mg/kg) produced 61.3 and 52.38% pain response inhibition. Extracts also produced significant analgesic activity in tail immersion method also and produced highest activity after 90 min like that of standard drug. The result tends to suggest that the extracts of Saraca indica leaves possess analgesic activity.

Keywords: *Saraca indica*, analgesic activity, methanol extract, formalin, tail immersion.

INTRODUCTION

In spite of the advancement made in medical research for the past decades, the treatment of many serious diseases is still challenging [1]. Presently drugs which are available for the management of pain are either narcotics analgesics (eg: opioids), NSAIDs (eg. salicylates) and corticosteroids (eg. hydrocortisone). These synthetic drugs are expensive and also possess serious side and toxic effects. Non-steroidal anti-inflammatory drugs (NSAID) causes gastric lesions and opiates

induced tolerance and dependence, the use of these drugs as analgesic agents have not been successful in all cases [2, 3]. Since time immemorial people has been using plant to protect themselves against several diseases and also to improve his health and life-style. Plant and phytomedicine symbolized safety and are serving several purposes whether health, protection from disease or nutrition. Attention is being focused on the investigation of drugs from plant origin which are used traditionally [4].

Saraca indica L. coming under the family Leguminosae commonly known as asoka. It is evergreen, medium sized in height tree with numerous spreading and drooping glabrous branches. The color of the bark is dark brown to grey or black; flowers are fragrant, numerous, dance and orange or red color; leaves are pinnate, 15-25 cm long having 4-6 pairs of oblong-lanceolate leaflets [5]. *Saraca indica* is one of the important indigenous medicinal plants and found throughout India. In India this plant is popularly known as Asok or Asoka. *Saraca indica* widely used in Ayurveda for treat number of disease like to treat painful conditions, improves complexion of the body, improves digestion and assimilation, alleviates excessive thirst, to kills all infectious agents, in blood disease, inflammation. Bark of the plant having enormous traditional value like astringent, anthelmintic, demulcent, emollient, stomachic and in blood disease, biliousness, colic, piles, ulcers, fractures, menorrhagia, metropathy, dyspepsia, visceromegaly. Stem bark of *Saraca indica* is astringent, antileucorrhoeic, antibilious and uterine sedative; flowers are used as uterine tonic, antidiabetic and antisymphilitic traditionally. Leaves are used in stomachalgia and flowers are use in vitiated condition of pitta, syphilis, hyperdipsia, inflammation, dysentery, haemorrhoids and scabies in children [6, 7, 8]. Plant is also important for CNS depressant activity as aerial part is important for hypothermic, CNS depressant and diuretic activity [9, 10]. Different parts of the plant possess antimicrobial activity, cytotoxic [11], oxytotoxic [12], antiulcer [13], antidiabetic [14] and antioxidant activity [15].

The lack of potent analgesic drugs with fewer side effects in use prompted the present study. So far no systematic study has been reported for analgesic properties of *Saraca indica* leaf extracts. In the present study, our aim was to evaluate the analgesic potential of the different extracts of the leaves of *Saraca indica* Linn.

MATERIALS AND METHODS

Plant material

Saraca indica Linn. leaves were collected in August 2009 from Gonda region of Uttar Pradesh, India. Plant was identify by Kamala Nehru Krishi Vigyan Kentra, Sultanpur, Uttar Pradesh. The herbarium was prepared and a voucher specimen (Sample No 01, Ref no KVK/Gen/2009-10/3012) was deposited to the Department of Pharmacognosy, AND College of Pharmacy, Gonda, Uttar Pradesh, India.

Animals

Albino mice (18-25 g) of either sex were used in these experiments. Animals were maintained at a temperature of $25\pm 2^{\circ}\text{C}$, humidity of $55\pm 5\%$ and with 12 h light - dark cycle and provided with standard food and water *ad libitum*. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Preparation of extracts

Fresh leaves of *Saraca indica* were collected, washed thoroughly and dried under shade and then made into a coarse powder using dry grinder, passed through sieve no. 40 and stored in an air tight container at 25°C, used it for further study. Powdered plant material (1.2 kg) were successively extracted using soxhlet apparatus using petroleum ether (60-80°C), chloroform, methanol and water in order of increasing polarity. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by distilling the solvent in a rotary vacuum evaporator and evaporated to dryness. The yield was found to be 7.99, 1.46, 12.15 and 12.90% w/w respectively with reference to the dried plant material.

Preliminary phytochemical investigation

All the leaf extracts like petroleum ether (PSI), chloroform (CSI), methanol (MSI) and water (WSI) were investigated for phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins, triterpenoids. Phytochemical screening of the extracts was performed using the standard procedures [16, 17].

Analgesic activity

a) Formaline Test

Albino mice are divided into 10 groups randomly, each having six animals. Group I served as control and treated with vehicle (0.5% carboxy methyl cellulose). Group II-IX treated with PSI (200 and 400 mg/kg), CSI (200 and 400 mg/kg), MSI (200 and 400 mg/kg) and WSI (200 and 400 mg/kg) respectively. Group X served as standard and received pentazocine (10 mg/kg, i.p.). Extracts are suspended in 0.5% carboxy methyl cellulose (CMC) solution and given orally. After one hour of treatment each animal received 0.1 ml of 1% formalin injection by sub-plantar route. After formaline injection the number of paw licking noted for 0-5 min (first phase) and then 25-30 min (second phase) for each animal. Licking and biting of paw indicates the pain response, mean pain response and percentage inhibition was calculated by comparing with control [18].

b) Tail immersion method

The tail immersion method as described by Kumar and Shankar (2009) was used to evaluate the analgesic activity of the extract. In this method pain reactions were produced in animals by dipping the tip of the tail in hot water to evaluate central analgesic mechanism of the extract [19].

Mice were divided in ten groups of six each. Animals were fasted for 18 hrs with water ad libitum. Animals were kept in position in a suitable restrainer with the tail extending out. Tail (1-2 cm) was marked and immersed in the water bath thermo-statistically maintained at 55±1°C. Tail withdrawal time from hot water was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 180 seconds to avoid the injury of the tissues of tail. Group I treated with 0.5% CMC and served as control. Group II-IX administered with PSI (200 and 400 mg/kg), CSI (200 and 400 mg/kg), MSI (200 and 400 mg/kg) and WSI (200 and 400 mg/kg) respectively. Group X treated with pentazocine (10 mg/kg, i.p.). After administration of above drug, the basal reaction time was measured after regular interval of 30 min. Tail flick response was calculated and compare with control group.

Statistical analysis

Values are expressed as mean \pm standard error mean (S.E.M) and analyzed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test, $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In this present study analgesic activity of different extracts of *Saraca indica* leaves investigated using formalin test method and tail immersion method. Different extract of *Saraca indica* leaves produces dose dependent analgesic activity in both the models, though petroleum ether extract of *Saraca indica* leaves did not found effective as analgesic activity.

In formalin induced pain model, MSI produced highest analgesic activity at a dose of 400 mg/kg followed by the effect of WSI. MSI (200 and 400 mg/kg), WSI (200 and 400 mg/kg) and CSI (200 and 400 mg/kg) produce 31.15, 52.64, 37.28, 55.87, 19.58, 40.43% inhibition of pain response in first phase and 22.10, 43.30, 27.28, 49.21, 15.89, 33.19% inhibition of pain response in second phase respectively in formaline test method (Table 1).

Formaline test is one of the most important analgesic models to correlate with clinical pain. In the early phase of formalin test pain occur due to the direct stimulation of the sensory nerve fibres by formalin while in the late phase pain was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins [20, 21, 22]. Early and late phases of pain in this model can reveal the mechanism of pain and analgesia. It is believed that narcotic analgesics can act in both phases equally and peripherally acting drugs can act only on second phase to reduce the pain [23]. In this study, the extracts caused a dose-dependent decrease in pain response in both phases by the animals injected with formalin signifying the analgesic effect of the extract and it was suggested that extract might relieve pain by acting both peripheral and central mechanism.

Table 1. Effect of different extract of *Saraca indica* leaf on formaline induced pain response in mice

Treatment	First Phase (0-5 min)		Second Phase (25-30 min)	
	Mean pain response	Percentage inhibition	Mean pain response	Percentage inhibition
Control	36.21 \pm 2.11	-	16.42 \pm 1.10	-
PSI (200 mg/kg)	33.05 \pm 3.90	8.72	15.27 \pm 3.31	7.00
PSI (400 mg/kg)	28.82 \pm 3.72	20.41	13.07 \pm 3.06	20.40
CSI (200 mg/kg)	29.12 \pm 2.84	19.58	13.81 \pm 2.01	15.89
CSI (400 mg/kg)	21.57 \pm 3.33*	40.43	10.97 \pm 1.66*	33.19
MSI (200 mg/kg)	22.71 \pm 3.03*	37.28	11.94 \pm 1.51*	27.28
MSI (400 mg/kg)	15.98 \pm 2.51**	55.87	8.08 \pm 1.03**	49.21
WSI (200 mg/kg)	24.93 \pm 3.72*	31.15	12.79 \pm 1.32*	22.10
WSI (400 mg/kg)	17.15 \pm 1.97**	52.64	9.31 \pm 1.11**	43.30
Pentazocine (10 mg/kg)	14.01 \pm 1.13**	61.30	7.82 \pm 0.76**	52.38

All values are mean \pm SEM; Statistical analysis by one-way ANOVA followed by Dunnett's multiple comparison test; * $P < 0.05$ ** $P < 0.01$; N = 6.

Where in tail immersion method mean tail flick latency time was increased in extract treated groups. Results of extracts of *Saraca indica* leaf on tail immersion method were tabulated in Table 2. Highest analgesic effect of extract and standard drug were observed after 90 min and MSI produced highest activity. MSI increased the tail flick time to 6.74, 7.48 and 6.28 after 60, 90, 120 sec compare to control which was 1.32, 1.60, 1.69 sec. Tail immersion method usually employed to investigate those compounds which may act through opioid receptor [24]. Analgesic activity against tail immersion method suggesting that extracts may also have central analgesic activity.

Table 2. Effect of different extract of *Saraca indica* leaf on formaline induced pain response in mice

Treatment	Tail flick latency (sec)				
	30 min	60 min	90 min	120 min	180 min
Control	1.53±0.12	1.32±0.09	1.60±0.11	1.69±0.16	1.49±0.13
PSI (200 mg/kg)	2.13±0.15	2.76±0.17	2.72±0.33	2.25±0.32	1.58±0.27
PSI (400 mg/kg)	3.21±0.29*	4.42±0.19*	4.33±0.36*	3.47±0.26*	2.45±0.12
CSI (200 mg/kg)	2.73±0.31	3.60±0.27*	4.70±0.41*	3.72±0.13*	1.88±0.24
CSI (400 mg/kg)	4.11±0.19**	5.20±0.23**	5.63±0.46**	5.17±0.33**	3.15±0.28*
MSI (200 mg/kg)	3.92±0.30*	4.81±0.44**	6.04±0.52**	4.96±0.62**	2.51±0.22*
MSI (400 mg/kg)	5.21±0.41**	6.74±0.53**	7.48±0.76**	6.28±0.61**	4.14±0.17**
WSI (200 mg/kg)	3.32±0.23*	4.48±0.49**	5.80±0.61**	4.49±0.25**	2.28±0.11*
WSI (400 mg/kg)	5.05±0.50**	6.13±0.33**	7.11±0.61**	5.87±0.39**	3.76±0.17**
Pentazocine (10 mg/kg)	5.44±0.22**	7.43±0.31**	8.16±0.40**	6.59±0.25**	5.04±0.19**

All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; * $P < 0.05$ ** $P < 0.01$; N = 6.

Preliminary phytochemical screening of all extracts was performed separately. Primarily methanolic extract showed the presence of tannins, triterpenoids, saponin, flavonoids and glycosides; aqueous extract contain alkaloids, saponin, tannins, flavonoids and glycosides; chloroform extract contains alkaloids, glycosides, tannin and steroids; petroleum ether contain glycosides, steroids and triterpenoids. Phytochemical screenings also showed the presence of tannins, triterpenoids, saponin, flavonoids and glycosides in the extracts of *Saraca indica*, which may responsible for its analgesic activity. Study showed the effectiveness of the extract as potent analgesic agent.

CONCLUSION

In conclusion, the results showed the analgesic activity of *Saraca indica* leaf extract and indicated that leaf extract of *Saraca indica* might contain the active constituents capable of relieving or modifying responses to pain. Though a details work is needed to isolate active constituents and pharmacodynamics studies should be undertaken to establish the mechanism of action of the plant extracts.

REFERENCE

- [1] AA Adedapol; MO Sofidiya; V Maphosa; Busani; Moyo; PJ Masika; AJ Afolayan. *Rec. Nat.* **2008**, 2, 46-53.
- [2] JR Dharmasiri; AC Jayakody; G Galhena; SSP Liyanage; WD Ratnasooriya. *J. Ethnopharmacol.*, **2003**, 87, 199-206.
- [3] S Sen; R Chakraborty; B De; J Mazumder. *Pharmacog. Rev.*, **2009**, 3, 270-279.
- [4] F Ahmad; RA Khan; S Rasheed. *J. Islam. Acad. Sci.*, **1992**, 5, 111-114.
- [5] KR Kirtikar; BD Basu; ICS An. Indian Medicinal Plants, vol 2, International Book Distributors, Derhadun, **2005**; pp. 883-4.
- [6] Anonymous. Indian Medicinal Plants, A Compendium of 500 Species, vol 5, Orient Longman Pvt Ltd, Chennai, **2006**; pp. 66.
- [7] AK Nadkarni. Dr. K.M. Nadkarni's Indian Materia Medica, vol 1, Bombay Popular Prakashan, Mumbai, **2005**; pp. 1104-6.
- [8] K Kashyapa; R Chand. The Useful Plants of India, National Institute of Science Communication and information Resources, New Delhi, **2006**; pp. 549-50.
- [9] PP. Joy; J Thomas; S Mathew; BP Skaria. Medicinal Plants, Kerala Agricultural University, Ernakulam, **1998**.
- [10] CK Kokate; AP Purohit; SB Gokhale. Pharmacognosy, Nirali Prakashan, Pune, **2007**; pp. 122-135.
- [11] JD Kaur; K Misra. *J. Indian Chem. Soc.*, **1980**, 57, 1243.
- [12] MJ Bhandary; KR Chandrasekhar; KMK Averiappa. *J. Ethnopharmacol.*, **1995**, 47, 149-158.
- [13] V Maruthappan; KSJ Shree. *J. Pharm. Res.* **2010**, 3, 17-20.
- [14] F Preethi; J Fernandes; K Pricilla. *J. Pharm. Res.* **2010**, 3, 491-493.
- [15] JK Sandhu; A Khatun; P Phattanawasin. *J. Nat. Med.*, **2007**, 61, 480-482.
- [16] S Yarnalkar. Practical Pharmacognosy, Nirali Prakashan, Pune, **1991**; pp. 38-47.
- [17] KR Khandelwal. Practical Pharmacognosy, Techniques and Experiments, 11th ed., Nirali Prakashan, Pune, **2004**; pp. 149-156.
- [18] C Arkesh, S Parth; J Dilpesh; S Rahul. *Int. J. Pharmacol. Biol. Sci.*, **2009**, 3, 25-32
- [19] JP Kumar; NB Sankar, *Asian J Pharma. Clin. Res.*, **2009**, 2, 61-63
- [20] A Tjolsen; DG Berge; S Hunskaar; JH Rosland; K Hole. *Pain*, **1992**, 51, 5-17.
- [21] A Ghannadi; V Hajhashemi; H Jafarabadi. *J. Med. Food*, **2005**, 8, 488-493.
- [22] AK Dhara; V Suba; T Sen; S Pal; AKN Chaudhuri. *J. Ethnopharmacol.*, **2000**, 72, 265-268.
- [23] CO Okoli; PA Akah; ON Egbuniwe. *Indian J. Exp. Biol.*, **2006**, 44, 422-424.
- [24] SK Kulkarni. Hand Book of Experimental Pharmacology, 2nd ed., Vallabh Prakashan, New Delhi, **1999**; pp. 125.