

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *STRYCHNOS POTATORUM* L.f. SEED EXTRACTS

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Summary

The successive ethanolic and aqueous seed extracts of *Strychnos potatorum* L.f. (family: Loganiaceae) were investigated for analgesic and anti-inflammatory activities at 500 mg/ kg body weight administered intraperitoneally (i.p.). Routine preliminary toxicity studies of these extracts were carried out in mice. For analgesic activity eddy's hot plate in swiss mice and for anti-inflammatory activity carrageenan induced oedema techniques in albino rat model were used. Moderate to significant analgesic activity of ethanolic extract of seed were observed, which is comparable to pentagocine, the standard analgin included especially at 120 min. Further, moderate to significant anti-inflammatory of the extracts were observed and this is reported to be long-lasting which can be comparable to the standard, diclofenac sodium. In conclusion, the results indicate analgesic and anti-inflammatory activities for the seed extracts of *S. potatorum* studied.

Key words: *Strychnos potatorum*, Loganiaceae, Analgesic and Anti-inflammatory activities.

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Introduction

The genus *Strychnos* of the family: Loganiaceae is consisting of 200 tropically distributed species, although known for dart and ordeal poisons since time immemorial, also have several species of medicinal importance been reported for several pharmacological properties [1-5]. *Strychnos potatorum* L.f., a medium sized deciduous tree, commonly known as Clearing Nut (English) or Kataka (*Sanskrit*) is a well known medicinal plant used in treating of various human ailments both in the Indian traditional systems (Ayurveda, Unani & Siddha) and also folk medicine since ancient times [6,7]. It occurs both in Asia (central and south India, Sri Lanka and Burma) and Africa (north and south-east Africa). The data on this plant with regard to pharmacological activities such as anticonvulsant, hypotensive [8,9], antidiarrhoeal [10], antimicrobial [11,12], anthelmintic [13] reported owing to the presences of several phytochemicals chiefly indole alkaloids [14,15] besides terpenoids, iridoid glycosides, flavonoids and phenols [16,17]. Therefore, the present study deals with the analgesic and anti-inflammatory activities of *S. potatorum* seed extracts.

Materials and Methods

Plant material: The seed material of *Strychnos potatorum* was collected from Karpakpalli Reserve Forest, Bidar District, Karnataka, India during December 2002 and identified [18]. A voucher specimen of the same was deposited in the Herbarium, Gulbarga University, Gulbarga (HGUG-214).

Extraction of crude extracts: Five hundred grams of the powdered plant material viz., seed were subjected to the Soxhlet successive extraction method (60-80°C) using about 2.5 liters of ethanol (95% v/v) for a period of 18 h. However, the aqueous extracts of seed were obtained by cold extraction owing to its jelly nature. The extracts obtained were concentrated to dryness *in vacuo* at 40°C and stored at 4°C in the refrigerator until further use.

Toxicity studies: The acute toxicity of both the ethanolic and aqueous extracts of *S. potatorum* seed were carried out in mice on a graded dose. Group – I animals received gum acacia (negative control) and Group – II to IV animals received ethanolic extract as 500, 1000, 1500 and 2000 mg/kg body weight (p.o.), were normal in their behaviour upto 48 h after treatment. However, two animals are killed out of the four from the Group - IV (2000 mg/kg) 48 h after treatment. Therefore, the lethal dose (LD₅₀) of the ethanolic extract of *S. potatorum* in mice was 2000 mg/kg b.w. Where as, the Group – V to VIII animals received the aqueous extract at the dose of 500, 1000, 1500 and 2000 mg/kg b.w. (p.o.). The treated mice of all groups were normal and healthy even seven days after treatment. Thus, the aqueous extract of *S. potatorum* is safer at the dose of 2000 mg/kg b.w.

Analgesic activity: In the present study, the analgesic activity of *S. potatorum* extracts was studied using Eddy's hot plate method for thermal stimuli induced pain [19]. Albino mice of either sex weighing 25-35 g were selected and divided into 4 groups of six animals each. The animals were not provided food for 18 h prior to the experiment with water *ad libitum*. The animals were placed on Eddy's hot plate maintained at a constant temperature 55 ± 1°C. The reaction time i.e. time taken by the animal to lick its hind paw or jump response was considered as a reaction time. Group – I animals served negative control given distilled water. Where as, Group – II animals received pentagocine, a standard analgin (10 mg/kg b.w., ip) and Group – III and IV received ethanolic and aqueous extracts of *S. potatorum* (500

mg/kg b.w., ip), intraperitoneally. The reaction time for each mouse was recorded at 0, 1, 2 and 4 h after the administration of drugs.

Anti-inflammatory activity: Anti-inflammatory activity of *S. potatorum* extracts was carried out by adopting carrageenan induced rat paw oedema method [20]. Wister rats of either sex weighing between 150-200 g were selected and divided into four groups containing six animals each. Animals were fasted for 18 h prior to the experiment with water *ad libitum*. 0.1 ml (1% (w/v) carrageenan is injected subcutaneously into the right hind paw of all animals of every group. Group – I animals received distilled water, to serve as negative control, and Group-II animals received diclofenec sodium, as positive control (30 mg/kg b.w.). Whereas, Group III and IV animals received ethanolic and aqueous extracts of *S. potatorum* (500 mg/kg b.w.) respectively by intraperitoneal route (i.p) 30 min before carrageenan injection. The anti-inflammatory activity was studied by mercury displacement method using plethysmometer. The reduction in paw volume is measured with the plethysmograph at ½, 1, 2, 4 and 6 h after drug administration. The percentage reduction in paw oedema volume was calculated using the following formula

$$\text{Percentage inhibition of oedema (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where,

V_c : Volume of paw oedema of negative control

V_t : Volume of paw oedema of treated rats.

Statistical analysis: The data of analgesic and anti-inflammatory activities of *S. potatorum* extracts are represented as the mean value \pm standard error of six animals ($N = 6$), and the significance was derived using Student's paired *t*-test and Tukey Post-Hoc multiple variance comparison by one-way ANOVA with the help of SPSS package version 10.0 [21].

Results and Discussion

Analgesic activity: The aqueous extract of *S. potatorum* seed has shown significant ($P < 0.01$) analgesic activity compared to the standard analgin, pentagocine. Further, its activity was more significant at 120 min. after drug administration (5.33 ± 0.21 min.) and similar to that of pentagocine (5.5 ± 0.43) (Table – 1). On the other hand, the ethanol extract did not show considerable activity except at 120 min. after drug administration (4.5 ± 0.42 min). Thus the exerted analgesic activity by the aqueous extract is long - lasting when compared to the standard drug. Further, its activity may be attributed to the presence of secondary metabolites like alkaloids, flavonoids, glycosides and saponins [14-17].

Several plants are possessing analgesic properties. Tits *et al.* (1991) have evaluated isoretuline, *O*-acetylretuline and *N*-desacetyl-isoretuline isolated from *Strychnos henningsii*, for analgesic activity [1]. Similarly, Yin *et al.* (2003) have studied significant analgesic activity of brucine of unprocessed seed and brucine *N*-oxide of processed seed of *Strychnos nux-vomica* and further their long lasting action compared to the standard analgin, pethidine, was observed [5]. The analgesic stimulates stereospecific opioid receptors located in certain parts of the central nervous system. The receptors probably modulate the appreciation of pain. Attempts to isolate these receptors have led to the identification of a group of peptides known as endorphins. There are two simplest members of the endorphins, methioxine enkephalin and

leucine enkephalin, probably neurotransmitters having pharmacological actions of analgesics. The analgesics are best known for altering psychological response to pain and thereby suppress anxiety and apprehension, and enable the subject to be more tolerant to discomfort and pain [22].

Table – 1: Analgesic activity of *S. potatorum* seed extracts in mice

Drug	Dosage (mg/kg b.w)	Reaction time (in seconds) after				
		0	30 min	60 min	120 min	240 min
Control	5 ml	2.67 ± 0.33 ^a	3.0 ± 0.37	3.33 ± 0.21	3.5 ± 0.22	3.33 ± 0.21
Pentagocine (standard)	10	2.5 ± 0.22 ^{NS}	3.83 ± 0.75 ^{NS}	5.17 ± 0.65 ^{**}	5.5 ± 0.43 ^{***}	3.17 ± 0.31 [*]
Ethnolic Extract	500	2.5 ± 0.34 ^{NS}	3.17 ± 0.40 ^{NS}	3.5 ± 0.62 ^{NS}	4.5 ± 0.42 [*]	4.17 ± 0.98 ^{NS}
Aqueous Extract	500	2.67 ± 0.21 ^{NS}	3.33 ± 0.21 ^{NS}	4.5 ± 0.22 [*]	5.33 ± 0.21 ^{***}	4.17 ± 0.17 [*]

^aData represents the mean value of 6 (N) animals ± SE

* (P > 0.1); ** (P > 0.5); *** (P < 0.001) Significance derived from Student's *t*-test verses control followed by Tukey Post-Hoc multiple comparisons by one-way ANOVA.

NS: Non – significant

Anti-inflammatory activity: The ethanolic and aqueous extracts of *S. potatorum* were screened for carrageenan induced anti-inflammatory activity at the concentration of 500 mg/kg b.w. (i.p.) in albino rats (Table – 2). Both the extracts i.e. ethanolic and aqueous, have exhibited significant anti-inflammatory activity compared to the standard, diclofenac sodium. Further, both extracts have decreased the oedema volume significantly after 2 h of the carrageenan injection. The decrease in oedema volume in the ethanolic extract treated animals was recorded as 0.38 (± 3.07), 0.27 (± 2.12) and 0.33 ml (± 3.33) after 2, 4 and 6 h carrageenan injection. Further, the percent reduction in the oedema volume is 14.71% (1 h), 29.17% (2 h), 46.67% (4 h) and 37.5% (6 h), respectively. Similarly, the aqueous extract treated animals showed significant decrease in the oedema volume as 0.41 (± 3.07), 0.35 (± 2.24) and 0.42 ml (± 3.07) at 2, 4 and 6 hours after carrageenan being administered. The percent of reduction in the oedema volume is recorded as 11.76% (1 h), 19.35% (2 h), 30% (4 h) and 21.88% (6 h), respectively. Further, it is observed that the ethanolic extract of *S. potatorum* has shown long lasting anti-inflammatory activity that is comparable to the standard.

The anti-inflammatory activity of *S. potatorum* may be attributed to the presence of secondary metabolites like alkaloids [1, 5] and / or due to the synergism of other secondary metabolites [23]. Some species of *Strychnos* and their alkaloids have been evaluated for anti-inflammatories. Tits *et al.* (1991) have observed significant anti-inflammatory activity of retuline and isoretuline alkaloids of *S. henningsii* at the dose of 50 mg/kg b.w. On the other hand, holstine, acetyl isoretuline and brucine were devoid of activity. They believed that the structural change in the basic skeleton produces a loss of activity of holstiine and brucine compared to the earlier alkaloids. Sundari *et al.* (2001) have studied the significant anti-inflammatory activity of katakakhadiradi kasayam, an ayurvedic formulation of twelve plants

including *S. potatorum*, had 30% reduction in the paw oedema volume at the tested dose (0.5 mg/kg b.w.) [24]. Further, Hong *et al.* (2002) have screened several plants including *Strychnos icaja* methanolic extracts for anti-inflammatory activity. *S. icaja* is shown to have significant inhibition of cyclooxygenase-2 (COX-2) 48.8%, which is an inhibitor of prostaglandin biosynthesis [25].

Table – 2: Anti-inflammatory activity of *S. potatorum* seed extracts in albino rats

Drug	Drug Dose (mg/kg b.w.)	Oedema volume (ml) and % reduction in oedema volume									
		½ h		1 h		2 h		4 h		6 h	
		ml	%	ml	%	ml	%	ml	%	ml	%
Control (D. W)	0.4 ml	0.58 ± 3.07 ^a	-	0.57 ± 2.1	-	0.52 ± 1.67	-	0.5 ± 2.58	-	0.53 ± 2.12	-
Diclofenec Sodium (Standard)	30	0.57 ± 2.11 ^{NS}	2.86	0.47 ± 2.1 ^{**}	17.65	0.32 ± 3.07 ^{***}	38.71	0.2 ± 3.65 ^{***}	60	0.3 ± 3.65 ^{***}	43.75
Ethanollic Extract	500	0.58 ± 3.07 ^{NS}	-	0.48 ± 3.07 ^{NS}	14.71	0.38 ± 3.07 ^{***}	29.17	0.27 ± 2.12 ^{***}	46.67	0.33 ± 3.33 ^{***}	37.5
Aqueous Extract	500	0.60 ± 3.07 ^{NS}	-	0.5 ± 2.58 ^{NS}	11.76	0.41 ± 3.07 ^{**}	19.35	0.35 ± 2.24 ^{***}	30	0.42 ± 3.07 [*]	21.88

^aData represents the mean value of replicates (N = 6) ± S. E.

* (P > 0.1); ** (P < 0.5); *** (P < 0.001) Significance derived both by Student's *t*-test against control followed by Tukey Post Hoc multiple comparisons by one-way ANOVA.

NS: Non-significant

Inflammation is known to occur via a series of complex pathophysiological pathways, influenced by various mediators such as prostaglandins and leukotrienes. These mediators can cause oedema such as heat, pain, disturbed tissue function, reddening and swelling [26] Carrageenan – induced paw oedema as an *in vivo* model of inflammation has been frequently used to assess the anti-edematous effect of natural products. Di Rosa *et al.* (1971) showed that this system participates in the process from the very beginning, and that carrageenan – induced inflammation is divided into three steps according to the mediators released. In the initial step (first 90 min) the release of both histamine and 5-HT occurs and the second step (from 90 – 150 min.) is mediated by kinins, while in the third step (from 150 min onwards), prostaglandin release takes place [27]. All these mediators are dependent upon the complement system. Histamine and 5-HT are responsible for vasodilation and increase in vascular permeability in the initial phase of the inflammatory process. Bradykinin has been implicated in acute inflammatory processes due its ability to induce an increase in blood vessel permeability. Another characteristic carrageenan induced edema is the massive infiltration of poly morphonuclear leucocytes observed in the third step [28].

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