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Analgesic and Anti-Inflammatory Activity of *Argyreia speciosa* and *Sphearanthus indicus* in the Experimental Animals

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Abstract: *Argyreia speciosa* (Convolvuceae) is regarded as a 'Rasayan' drug in the ayurvedic system of medicine to cure diseases of nervous system. *Sphearanthus indicus* is known as nervine tonic and used for relief of pain in Indian traditional system of medicine. In the present study an attempt was made to evaluate the analgesic and anti-inflammatory activity of hydroalcoholic extract of *Argyreia speciosa* root (ASE) and *Sphearanthus indicus* herb (SIE) using various experimental models. ASE and SIE at the doses of 100, 200 and 500 mg/kg (orally) were tested for its analgesic effect using tail flick test in rats and acetic acid induced writhing test in mice. The anti-inflammatory effect was evaluated for both plant extracts (100, 200 and 500 mg/kg, orally) using acute inflammatory model, carrageenan induced paw oedema in rats. ASE and SIE exhibited significant ($p<0.05$) and dose dependent analgesic activity compared with the control as evidenced by increased escape latency in the tail flick test and reduction in abdominal writhing induced by acetic acid. Furthermore, ASE and SIE were significantly ($p<0.05$) reduced rat paw edema induced by subplantar injection of carrageenan. This study provides scientific basis for the traditional medicinal uses of these plants for analgesic and anti-inflammatory activity.

Key words: *Argyreia speciosa* · *Sphaeranthus indicus* · Tail flick method · Acetic acid induced writhing · carrageenan induced paw oedema · Analgesic activity · anti-inflammatory activity

INTRODUCTION

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history [1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources. According to various medical literatures, several adverse reactions are known to be associated with the conventional nonsteroidal anti-inflammatory drugs, thereby limiting the widespread application of these agents. The study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and anti-inflammatory drugs.

Argyreia speciosa (L.f.) Sweet (convolvulaceae) commonly known as 'Elephant creeper' is a woody climber distributed throughout the India up to an altitude of 300 meters [2]. The root part of this plant is regarded as a tonic and useful in rheumatism and diseases of nervous system [3].

Sphaeranthus indicus Linn. (Asteraceae) commonly known as 'Gorakhmundi' is a highly branched herb distributed throughout the plains in India in wet places. The herb is regarded as a nervine tonic and is commonly employed in folk medicine for relieving pain in many disorders [4]. The present study was designed to evaluate the analgesic and anti-inflammatory activity of hydroalcoholic extracts of *Argyreia speciosa* roots and whole herb of *Sphaeranthus indicus* using various experimental animal models.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts: The fresh roots of *Argyreia speciosa* were collected from the forests of Balasinor, Gujarat. The flowering herbs of

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Sphaeranthus indicus were collected from Vallabh Vidyanagar, Gujarat. The materials collected were authenticated by a Taxonomist, Department of Bioscience, Sardar Patel University, Vallabh Vidyanagar, Gujarat. Their voucher specimens as ARGH 8 and ARGH 7, respectively, were preserved in Department of Pharmacognosy, A. R. College of Pharmacy, Vallabh Vidyanagar. The powdered materials were extracted exhaustively with 50% ethanol by maceration for 2 days at room temperature with occasional shaking. The hydroalcoholic extracts were filtered and dried under reduced pressure at 40°C. The yield of hydroalcoholic extract of *Argyreia speciosa* (ASE) and *Sphaeranthus indicus* (SIE) were 9.3% w/w and 11.1% w/w of dried plant materials, respectively. Aqueous solutions of dried extracts were used for pharmacological testing.

Preliminary Phytochemical Screening: The hydroalcoholic extracts of the *Argyreia speciosa* roots and *Sphearanthus indicus* herbs were studied for its preliminary phytochemical screening for the detection of various plant constituents [5].

Animals: Albino mice (25-30 g) and rats (200-250 g) of either sex bred in Central Animal House facility of the institute were used (80 rats and 80 mice). The animals were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1 h before the experiments. Animals were randomly distributed into groups of 10 animals each. Each animal was used only once. All experiments were conducted during the light period (08.00-16.00 h). All the protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Treatment: Two models, viz. tail flick assay (thermal method) in rats and acetic acid induced writhing response (chemical method) in mice were used to study the analgesic effect. Carrageenan induced rat paw oedema was performed for evaluation of anti-inflammatory action. For all the methods, the animals were divided into eight groups of ten animals each. Group I served as normal control and received per oral (p.o.) distilled water (1 ml/kg), group II served as reference group and received standard drug, groups III-VIII served as treatment groups in which groups III, IV and V received SIE at the doses of 100, 200 and 500 mg/kg, p.o. respectively and groups VI, VII and VIII received ASE at the doses of 100, 200 and 500 mg/kg, p.o. respectively. Pentazocine (10 mg/kg, i. p.) was used as a standard drug for tail flick method. Aspirin (150 mg/kg, p.o.) was used as a standard drug for acetic acid induced writhing and carrageenan induced paw oedema method. Carrageenan (SD Fine Chem, Mumbai, India) was dissolved in saline solution and 0.1 ml of 1% solution is injected in paw oedema method.

Analgesic Activity

Tail Flick Method: The tail flick latency of rats was assessed by the analgesiometer [6]. The pretreatment latency of tail flick response (initial reaction time) was noted down for each rat and cut-off time of 15 s was fixed. Tail flick latency was measured at 30, 60, 90 and 120 minutes after treatments. Result of tail flick latency was expressed in terms of reaction time in seconds.

Acetic Acid Induced Writhing: The abdominal constriction test was performed in the group of mice by the method of Koster *et al.* [7]. One hour after treatment, writhing was induced by an intraperitoneal injection of 0.4% acetic acid (0.4 ml/20g b.w.). The number of writhing movements in 20 minutes of observation immediately after the acetic acid injection was recorded. Percentage of protection against acetic acid induced writhing was calculated using the formula:

- Percentage protection = (Wc-Wt)/ Wc) × 100
- Where, Wc = Mean values of number of writhing in control group
- Wt = Mean values of number of writhing in the test groups.

Anti-Inflammatory Activity: Inflammation in rats was produced by carrageenan according to the method described by Winter *et al.* [8]. After 1 h of the treatment, acute inflammation was produced by sub-planter injection of 0.1 ml of 1% carrageenan in normal saline in the right hind paw of the rat. Mean increase in paw size was measured with dial caliper [9] at 0, 1 and 3 h after carrageenan injection to each group. 0 h reading was considered as initial paw size of the animals. Data was shown as an increase in paw thickness and percentage inhibition of paw oedema produced by treated groups. Percentage inhibition of paw oedema was calculated at 1 and 3 h by using the formula:

Anti inflammatory activity (%) = (1–D/C) × 100

Where D is the change in paw diameter in treated group and C is the change in paw diameter in control group.

Statistical Analysis: The data was expressed as mean ± S.E.M. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered significant at $p<0.05$.

RESULTS

Preliminary Phytochemical Screening: Preliminary phytochemical screening revealed the presence of tannins, steroids, triterpenes, coumarins and flavonoids in the both plant extracts (ASE and SIE).

Analgesic Activity

Tail Flick Method: ASE and SIE increased the tail flick latency of rats towards the thermal source in a dose-dependent manner (Fig.1). Oral administration of 100 mg/kg of ASE showed significant analgesic activity at 90 and 120 min of drug administration. While, ASE 200 mg/kg (p.o.) dose produced analgesic effect at 60, 90 and 120 min of drug administration. However, ASE in the dose of 500 mg/kg (p.o.) produced significant analgesic effect at 60 and 90 min of drug administration. Thus, significant analgesic effect of ASE was observed in tail flick test, which started at 60 min and attained the peak effect at 90 min of time intervals.

SIE in the dose of 100 mg/kg, p.o. did not produce any significant change in tail flick latency of rats indicate absence of analgesic activity. SIE in the dose of 200 mg/kg, p.o. significantly increased the time of tail withdrawal at 90 min of drug administration. While, SIE 500 mg/kg (p.o.) dose showed significant analgesic activity after 30, 60 and 90 min of drug administration. Thus, significant analgesic effect of SIE was observed in tail flick test, which started at 30 min and attained the peak effect at 90 min of time intervals. Pentazocine (10 mg/kg, i.p.) as positive control was significantly increased the tail flick latency of rats at all observed time intervals.

Acetic Acid Induced Writhing: Effects of ASE and SIE on acetic acid induced writhing were demonstrated in Table 1. Oral administration of ASE in the 100, 200 and 500 mg/kg dose showed significant ($P<0.05$) and dose dependent protection of acetic acid induced writhing in mice, as indicated by 26.72, 32.76 and 44.83% of writhing inhibition, respectively. SIE also showed significant and dose dependent analgesic action as indicated by 15.81, 26.83 and 44.51% writhing inhibition in mice with 100, 200 and 500 mg/kg doses, p.o. respectively. Similarly positive control, Aspirin (150 mg/kg, p.o.) also exhibited significant writhing inhibition in mice (67.38%).

Anti-Inflammatory Activity: The results of anti-inflammatory activity of ASE and SIE are shown in Table 2. ASE in the highest tested dose (500 mg/kg, p.o.) was found to inhibit rats paw oedema significantly after 1 h (60.15 %) and 3 h (44.79 %) of carrageenan injection.

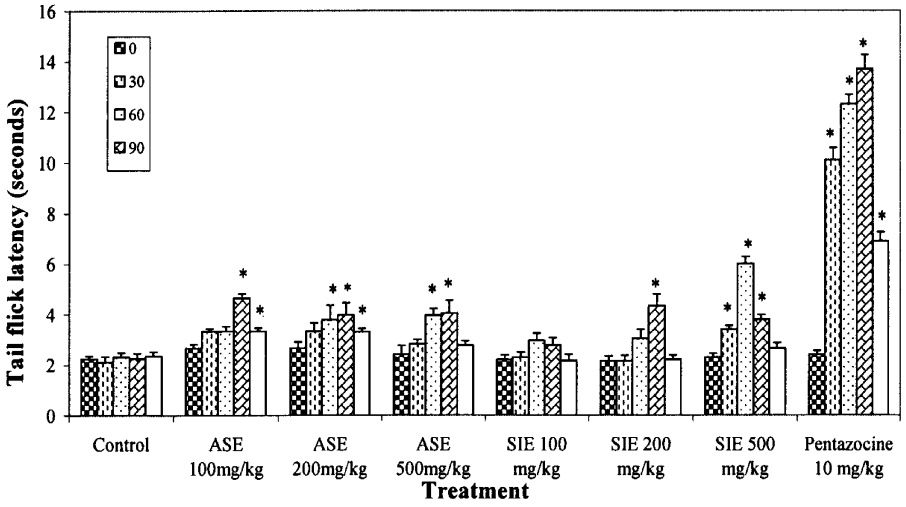

Fig. 1: Effect of ASE and SIE on tail flick test in rats(n = 10). Each bar expressed as mean ± SEM. One way ANOVA followed by Dunnett's test, *$p<0.05$ when compared with control group.

Table 1: Effect of ASE and SIE on Acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhes	Inhibition %
Control	-	34.8 ±1.69	-
ASE	100	25.5 ± 0.83*	26.72
ASE	200	23.4 ± 1.72*	32.76
ASE	500	19.2 ± 0.89*	44.83
SIE	100	29.3 ± 0.94*	15.81
SIE	200	24.0 ± 0.92*	26.83
SIE	500	18.2 ± 0.69*	44.51
Aspirin	150	10.7 ± 0.98*	67.38

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

Table 2: Effect of ASE and SIE on Carrageenan induced rat paw oedema.

		Carrageenan induced paw oedema			
		Increase in paw oedema (mm)	Inhibition%	Increase in paw oedema (mm)	Inhibition%
Treatment	Dose (mg/kg)	(After 1 h)		(After 3 h)	
Control		1.38 ± 0.21	-	1.92 ± 0.14	-
ASE	100	1.29 ± 0.17	6.52	1.43 ± 0.19	25.5
ASE	200	1.18 ± 0.13	14.49	1.02 ± 0.12*	46.87
ASE	500	0.55 ± 0.08*	60.15	1.06 ± 0.1*	44.79
SIE	100	1.06 ± 0.11	23.19	0.6 ± 0.08*	68.75
SIE	200	1.03 ± 0.16	25.36	0.62 ± 0.05*	67.71
SIE	500	0.78 ± 0.12*	43.48	0.67 ± 0.05*	65.11
Aspirin	150	0.96 ± 0.13	30.43	0.41 ± 0.1*	71.64

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

ASE was also significantly inhibited rat paw oedema in oral dose of 200 mg/kg after 3 h (46.87 %) of carrageenan injection. ASE at the dose 100 mg/kg, p.o. did not produce significant anti-inflammatory activity.

Similarly, 500 mg/kg oral dose of SIE was found to inhibit rats paw oedema significantly after 1 h (43.48 %) and 3 h (65.11 %) of carrageenan injection. SIE in other two tested doses (100 and 200 mg/kg, p.o.) was found to inhibit rat paw oedema formation to the extent of 68.75% and 67.71%, respectively at 3 h of carrageenan injection. Aspirin (150 mg/kg, p.o.) as a reference standard inhibited the oedema formation due to carrageenan to an extent of 30.43 and 71.64 % at 1 h and 3 h, respectively.

DISCUSSION

The present study demonstrated central and peripheral analgesic activity of hydroalcoholic extracts of *Argyreia speciosa* and *Sphaeranthus indicus* using tail flick test and acetic acid induced writhing. Tail flick test is most sensitive to centrally acting analgesics whereas acetic acid induced writhing test is used for detection of peripheral analgesics. Increase in tail flick latency by ASE and SIE indicated possible involvement of a higher center [10]. Acetic acid causes an increase in peritoneal fluid levels of prostaglandins (PGE_2 and $PGF_{2\hat{a}}$) and releases other algesic mediators such as bradykinin, histamine and 5-hydroxytryptamine [10, 11]. Significant inhibition of writhing response by ASE and SIE indicated peripheral analgesic activity. Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable criteria for evaluation of anti-inflammatory agents [8]. The time course of oedema development in carrageenan-induced paw oedema model in rats is generally represented by a biphasic curve [12]. The first phase, which occurs between 0 to 2.5 h after injection of the phlogistic agent, has been attributed to the release of histamine or serotonin [13]. The oedema volume reaches its maximum approximately 3 h post treatment and then begins to decline. The second phase of inflammatory reaction which is measured at 3 h is caused by the release of bradykinin, protease, prostaglandin and lysosome [13, 14]. The inhibition of rat paw oedema at 1 h and 3 h by ASE and SIE indicated anti-inflammatory activity via action on both phases of carrageenan induced inflammation. The anti-inflammatory action of hydroalcoholic extracts of *A. speciosa* and *S. indicus* observed in this study support previous work reported

[15, 16]. There is also evidence that inflammation processes are accompanied by increase in free radical activity [17]. Previous investigations showed that hydroalcoholic extracts of *A. speciosa* [18] and *S. indicus* [19] have appreciable levels of antioxidant activity. On the basis of this function, the extracts may exert their anti-inflammatory effects at least partially through the relative antioxidant activity.

The efficacy of most herbal remedies is attributed to various active constituents in combination. The analgesic and anti-inflammatory activity of ASE and SIE may be due to the presence of phyto-constituents like flavonoids, tannins, saponins, triterpenes and coumarins as reported in phytochemical investigation. Since, antinociceptive and/or anti-inflammatory activity of many plants has been attributed to their flavonoids [20, 21], tannins [22], triterpenes [21, 23] and coumarins [25]. It is, therefore, possible that the antinociceptive and anti-inflammatory effects observed with both plant extracts in the present study may be attributable to the components that are present in abundance in the extracts. This study provides scientific basis for the traditional medicinal uses of these plants for analgesic and anti-inflammatory activities.

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