Analgesic and Antipyretic activity of whole parts of *Sphaeranthus indicus* Linn.

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Abstract

The aim of the present study is to investigate the analgesic and antipyretic activity of whole parts of the plant *Sphaeranthus indicus* Linn. (Compositae) on albino rats by Eddy's hot plate, Tail immersion and Brewer's yeast induced pyrexia method respectively. Dried powder plant materials were subjected to sucessesive solvent extraction taking Petroleum ether, Benzene, Chloroform, Ethanol and triple distilled water. The different extracts at a dose of 200mg/kg and 400mg/kg body weight were subjected to analgesic and antipyretic activity. The petroleum ether, chloroform and ethanol extracts showed significant analgesic activity in both doses ($p<0.001$ & $p<0.01$) from 1 hour onwards as compared to the standard drug diclofenac sodium. The chloroform and ethanol extracts showed potential significant antipyretic activity ($p<0.05$) from 1 hour onwards where as aqueous extracts exhibit activity from 2 hours onward as compared to the standard drug paracetamol amongst various extracts.

Keyword: Analgesic, Antipyretic, *Sphaeranthus indicus* Linn.

Introduction:

The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Pain, inflammation and fever are very common complications in human beings. Several plants and their products are claimed and proved to posses analgesic and antipyretic property. *Sphaeranthus indicus* Linn. (Compositae) is an aromatic herb, 30-60cm. tall, found abundantly all over India, ascending to an altitude of 1500m, especially as a weed in the rice fields [1]. The plant has long been used in the treatment of skin infection, bronchitis, jaundice and nervous depression [2]. The various parts of the plant are used in the treatment of cough, chest pain, bowel complaints, anthelmintic and tuberculosis [1].

Experimental Section

Plant materials

The mature whole plants were collected from the rural belt of Bhubaneswar, Khurdha District of Orissa in early morning during late winter (Dec-Jan) and authenticated by Central Herbarium, Botanical Survey of India, Shibpur, Howrah, West Bengal. After authentication, plant material were washed under running tap water to remove adhering dust, dried under shade and pulverized to get course powder.

Preparation of extract [3]

The powder plant material was successively extracted with petroleum ether, benzene, chloroform and ethanol in a soxhlet apparatus for 72 hrs, the marc left behind was cold macerated with triple distilled water. All extracts were filtered through Whatmann paper and concentrated by vacuum evaporation. The yield of the different extracts as per the solvents used were 5.1%w/w, 3.65%w/w, 3.13%w/w, 4.96%w/w and 12.2%w/w. The preliminary phytochemical screening of different extracts showed the presence of carbohydrate, amino acids & proteins, fixed oil, flavonoids, terpenoids and alkaloids. The dried extracts were suspended in 2% gum acacia solution and used for animal experiments.

Adult albino rats (150-200 g) of either sex were supplied by M/S Chakraborty Enterprises, Kolkota were used for the study. The selected animals were maintained under standard diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethical Committee prior to the conduct of the animal experiments (1200/ac /08 /CPCSEA).

Analgesic Activity

A) Eddy's Hot Plate Method [4]:

Glass man's method was used. Albino rats of either sex were selected, weighed and divided into six groups of six animals each. The time of reaction to pain stimulus of the rat placed on the plate heated at 55^0C $\pm$ 0.5^0C was recorded at 1,2 and 3 hrs, after the administration. The increase in reaction time against control group was calculated (Table-I).

Table – 1 Analgesic Activity (Hot Plate Method)

Name of Extract/ Drug	Reaction Time (Sec.)		
	1 hr.	2 hr.	3 hr.
Control	16.7± 0.39	17.0 ± 0.45	17.2 ± 0.22
Diclofenac Sodium (50 mg/kg)	18.5± 0.19[b]	20.8 ± 1.1[a]	23.9± 0.67[a]
Petroleum Ether (200 mg/kg)	17.5± 0.29	18.7± 0.12[b]	18.9± 0.35[b]
Petroleum Ether (400 mg/kg)	17.6± 0.09	18.9± 0.15[b]	19.1± 0.02[b]
Benzene (200 mg/kg)	16.5± 0.25	17.0± 0.21[b]	18.7± 0.33[b]
Benzene (400 mg/kg)	16.8± 0.43	17.9± 0.14[b]	20.1± 0.03[b]
Chloroform (200 mg/kg)	18.0 ± 1.1	19.7 ± 0.96[a]	22.9 ± 0.59[a]
Chloroform (400 mg/kg)	18.07 ± 0.52	20.1 ± 0.21[a]	23.3 ± 0.19[a]
Ethanolic (200mg/kg)	17.3 ± 0.68	18.7 ± 0.08[b]	18.9 ± 0.25[a]
Ethanolic (400mg/kg)	16. ± 0.35	18.4 ± 0.02[b]	19.7 ± 0.41[a]
Aqueous (200 mg/kg)	17.8 ± 0.89	17.9 ± 0.21	17.5 ± 0.01
Aqueous (400 mg/kg)	17.2 ± 0.51	17.4 ± 0.09	17.3 ± 0.16

Each value is Mean ± S.E.M (n=6), *Denotes significance difference when compared to control values at [a]p< 0.001, [b]p < 0.01.

All animals were fasted for 18 hrs. before the beginning of the experiment and access to water *ad libitum*. Animals of group I received 2% gum acacia solution (0.3 ml.), group II received diclofenac sodium (50 mg/kg) orally. Group III to VII received Petroleum ether, benzene, chloroform, ethanol and aqueous extracts at dose levels of 200 mg/kg and 400 mg/kg body weight per oral.

B) **Tail Immersion Method [5]**

Healthy albino rats weighing about 150-200gm were taken. They were divided into 7 groups having 6 each, numbered and placed into individual restraining cages leaving the tail hanging out freely. The animals are then allowed to adapt in the cages for 30 minutes before testing. The lower 5cm portion of the tail was marked and immersed in a cup of freshly filled warm water of exactly 55^{o}C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded by a stop watch. After each determination the tail was carefully dried. The reaction was determined before oral feeding of the drug and various extracts (200 mg/kg and 400 mg/kg body weight p.o) which was recorded as zero minutes reading. The control, standard and test substances were given to the animals by gastric tube. After the drug was administered the reaction time was recorded at an interval of ½ h, 1h, 1½, 2h and 2½h. The cut off time of the immersion is 15 seconds. The mean reaction time was recorded for each group and compared with the value of standard drug diclofenac sodium. (Table 2)

Table 2Analgesic Activity (Tail Immersion Method)

Name of the Extract/ Drug	Tail Immersion Response (Sec.)					
	0hr.	**1/2 hr.**	**1 hr.**	**1 1/2 hr.**	**2 hr.**	**2 1/2 hr.**
Control	2.5 ± 0.25	2.3 ± 0.28	2.0 ± 0.0	2.3 ± 0.24	2.0 ± 0.0	2.2 ± 0.12
Diclofenac Sod. (50 mg/kg)	2.3 ± 0.69	4.1 ± 0.29^{b}	4.4 ± 0.60^{b}	4.8 ± 0.25^{a}	5.5 ± 0.25^{a}	5.6 ± 0.41^{a}
Petroleum Ether (200 mg/kg)	2.3 ± 0.25	3.7 ±0.23^{b}	3.9 ± 0.41^{b}	4.1 ± 0.25^{b}	4.0 ± 0.40^{b}	4.3 ± 0.24^{b}
Petroleum Ether (400 mg/kg)	2.4 ± 0.03	3.8 ±0.12^{b}	4.0 ± 0.03^{b}	4.3 ± 0.02^{b}	4.2 ± 0.40^{b}	4.7 ± 0.24^{a}
Benzene (200 mg/kg)	2.3 ± 0.28	2.5 ±0.25	2.3 ± 0.31	2.6 ± 0.42	2.5 ± 0.02	2.9 ± 0.24
Benzene (400 mg/kg)	2.1 ± 0.18	2.4 ±0.05	2.2 ± 0.31	2.9 ± 0.42	2.5 ± 0.02	3.4 ± 0.24^{b}
Chloroform (200 mg/kg)	2.5 ± 0.28	3.2 ± 0.35	3.7 ± 0.28^{b}	4.6 ± 0.22^{a}	5.2 ± 0.70^{a}	4.2 ± 0.21^{b}
Chloroform (400 mg/kg)	2.4 ± 0.02	3.5 ± 0.35^{b}	4.2 ± 0.28^{a}	4.7 ± 0.22^{a}	5.3 ± 0.70^{a}	5.2 ± 0.21^{a}
Ethanolic (200mg/kg)	2.3 ± 0.01	3.9 ± 0.31^{a}	4.0 ± 0.34^{a}	4.1 ±0.25^{a}	4.2 ± 0.15^{a}	4.7 ± 0.02^{a}
Ethanolic (400mg/kg)	2.4 ± 0.52	4.0 ± 0.11^{a}	4.2 ± 0.19^{a}	4.6 ±0.04^{a}	4.7 ± 0.18^{a}	5.1 ± 0.13^{a}
Aqueous (200 mg/kg)	2.5 ± 0.85	2.5 ± 0.02	2.6 ± 0.13	2.6 ± 0.01	2.5 ± 0.45	2.7 ± 0.02
Aqueous (400 mg/kg)	2.3 ± 0.47	2.4 ± 0.29	2.5 ± 0.21	2.4 ± 0.38	2.3 ± 0.25	2.5 ± 0.32

Results are expressed as Mean ± SEM from four observations, $^{a}p< 0.001$, $^{b}p < 0.01$.

Antipyretic Activity [6]

The albino rats were randomly distributed in control and test groups of six animals each. They were fed with standard laboratory diet ad libitum and allowed free access to drinking water. The animals were kept in 12/12 hours dark – light cycle. One hour after starvation, the rectal temperature was recorded and animals having temperature between 37.0^0 and 37.5^0 C were selected for the test. Pyrexia was induced by injecting subcutaneously 12% w/v suspension of Brewer's yeast in 0.9% NaCl (1ml/100gm. Body weight) and allowed to feed. The animals were divided into 7 groups of six each and numbered. 10 hours later rectal temperature was recorded using a clinical thermometer by introducing 1 inch into the rectum and keeping it inside for 1 minute. The temperature first recorded after 10 hours of yeast administration was taken as zero hour reading. The control, standard and test substances were given to the animals by gastric tube. After the drug was administered the temperature of all the rats in each group was recorded at an interval of 1h, 2h, 3h and 4h. The mean temperature was found out for each group and compared with the value of standard drug paracetamol. The findings were shown in table 3.

Results and Discussion

The various extracts at a dose of 200 mg/kg and 400 mg/kg body weight showed comparable analgesic activity in Tail flick method (Table 2). Chloroform extract showed a higher analgesic activity (5.2 sec. $P<0.001$ at 2 hr) followed by aqueous extract (5.1 sec. at 2 hr $p< 0.001$) then followed by Ethanolic extract (4.2 sec at 2 hr $p<0.01$) and Petroleum ether extract (4.1 sec at 2 hr, $p<0.01$) as compared to control at both the doses. The significant and nearly equal activity was observed in chloroform and aqueous extracts as compared to Diclofenac sodium (5.5 sec at 2 hr, $p<0.001$).

The Hot plate method also showed analgesic activity (Table-1) in both chloroform and aqueous extract followed by Ethanolic and Petroleum ether extract. The reaction time of treated animals after the treatment of one hour were significantly higher when compared with solvent control and slightly more after 2 and 3 hrs, in chloroform and aqueous extract ($p < 0.001$) where as at the same time Petroleum ether and ethanolic extracts showed significant activity($p < 0.001$) at both doses.

The antipyretic activity (Table-3) of all the extracts also showed good response. The experimental rats showed a mean increase of about 1.5^0C in rectal temperature 10 hrs. after the yeast injection. The fall in elevated body temperature of experimental animals by Petroleum ether and Ethanolic extract showed that the temperature reduced upto 2 hrs after that there is a gradual increase in temperature, which enforces to validate the activity by still higher dose; However the antipyretic activity is carried out by two dose level i.e. 200 and 400 mg/kg. body weight. The results revealed that by increasing the dose (400 mg/kg) fall in temperature is uniform upto 4 hr. in both Petroleum ether and Ethanolic extract. (0.94^0C:1.0^0 C by 4 hrs. of Petroleum Ether: Ethanolic). On the other hand on both dose level the Chloroform and aqueous extract show continuous fall in temperature up to 4 hrs. (1.25^0C: 1.19^0C, by 4 hrs. of chloroform extract: aqueous extract at 400 mg/kg body weight). The average temperature was reduced with the administration of Petroleum ether, chloroform, ethanolic and aqueous extracts in dose dependent manner. The significant and maximum antipyretic activity was observed in chloroform and aqueous extracts which is dose dependent and followed by Ethanolic and Petroleum ether extract when compared with control.

Table – 3 Antipyretic Effect of *Sphaeranthus indicus* L Leaf Extract

Treatment	Initial Temp.(0^0C)	Temperature (0^0C) after 10hrs of yeast administration	Temperature (0^0C) in hour ± SEM			
			1 hr.	2 hr.	3 hr.	4 hr.
Control (2% gum acacia sol.)	37.6 ± 0.43	39.57 ± 0.28	39.20 ± 0.13	39.40 ± 0.21	39.13 ± 0.32	39.03 ± 0.11
Paracetmol (50 mg/kg)	37.37 ± 0.65	39.94 ± 0.42	37.86 ± 0.24*	37.72 ± 0.21*	37.66 ± 0.12*	37.09 ± 0.13*
Pet. ether (200 mg/kg)	37.15 ± 0.12	39.35 ± 0.13	39.53 ± 0.22	39.66 ± 0.19	39.25 ± 0.36	39.44 ± 0.28
Pet. ether (400 mg/kg)	37.35 ± 0.25	39.46 ± 0.32	39.50 ± 0.09	39.41 ± 0.38	39.02 ± 0.15	38.94 ± 0.51
Benzene (200 mg/kg)	37.33 ± 0.25	39.76 ± 0.09	39.26 ± 0.11	39.1 ± 0.16	39.05 ± 0.12	39.03 ± 0.15
Benzene (400 mg/kg)	37.25 ± 0.21	39.29 ± 0.22	39.03 ± 0.15	38.90 ± 0.34	38.62 ± 0.19	38.45 ± 0.62
Chloroform (200 mg/kg)	37.08 ± 0.31	39.06 ± 0.23	38.96 ± 0.13*	38.83 ± 0.21*	38.12 ± 0.16*	37.65 ± 0.13
Chloroform (400 mg/kg)	37.21 ± 0.12	39.48 ± 0.24	39.25 ± 0.19*	38.73 ± 0.33*	38.42 ± 0.36*	37.62 ± 0.43*
Ethanolic (200 mg/kg)	37.45 ± 0.23	39.74 ± 0.32	39.66± 0.11*	39.06 ± 0.13*	38.53 ± 0.24*	37.59 ± 0.31*
Ethanolic (400 mg/kg)	37.15 ± 0.12	39.74 ± 0.13	39.58± 0.43*	39.28± 0.19*	38.29± 0.28*	37.35± 0.11*
Aqueous (200 mg/kg)	37.67 ± 0.34	39.56 ± 0.14	39.44 ± 0.03	39.35 ± 0.22*	38.8 ± 0.16*	37.91 ± 0.15*
Aqueous (400 mg/kg)	37.55 ± 0.45	39.41 ± 0.12	39.62 ± 0.11	39.23 ± 0.3*	38.6 ± 0.08*	37.56 ± 0.05*

Each value is Mean ± S.E.M (n=6), *Denotes significance difference when compared to control values at $p<0.05$

Conclusion

The various extracts of whole plant of *Sphaeranthus indicus* at a dose of 200mg/kg and 400mg/kg body weight were investigated for analgesic and antipyretic activity. The petroleum ether, chloroform and ethanol extracts showed significant analgesic activity in both doses ($p<0.001$ & $p<0.01$) from 1 hour onwards as compared to standard drug diclofenac sodium. The chloroform and ethanol extracts showed potential significant antipyretic activity ($p<0.05$) from 1 hour onwards where as aqueous extracts exhibit activity from 2 hours onward as compared to the standard drug paracetamol amongst various extracts. The significant analgesic and antipyretic activity may be due to the presence of flavonoids. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception [7].

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