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Anti-inflammatory activity of whole parts of *Sphaeranthus indicus* Linn.

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Abstract

The aim of the present study is to investigate the anti-inflammatory activity of whole parts of the plant *Sphaeranthus indicus* Linn. (Compositae) on albino rats by carrageenan induced acute inflammation, Formalin induced chronic inflammation and Cotton Pellet-Induced Granuloma respectively. Dried powder plant materials were subjected to sucessesive solvent extraction taking Petroleum ether, Benzene, Chloroform, Ethanol and triple distilled water. The preliminary phytochemical screening of different extracts revealed the presence of carbohydrate, amino acids & proteins, fixed oil, flavonoids, terpenoids and alkaloids. The different extracts at a dose of 200mg/kg and 400mg/kg body weight were subjected to anti inflammation activity. The ethanol and petroleum ether extracts showed significant ($p<0.05$) anti inflammatory activity in both doses from 1 hour onwards as compared to the standard drug diclofenac sodium, where as aqueous extracts exhibit activity from 2 hours onward as compared to the standard drug amongst various extracts.

Keyword: Anti inflammatory, *Sphaeranthus indicus* Linn. Carrageenan & Formalin induced paw oedema, Cotton Pellet-Induced Granuloma.

Introduction

Medicinal plants have been used since times immemorial as remedies for human diseases because they contain components of therapeutic value. Plants have been important sources for providing many pharmacological agents with novel structures and unique mechanisms. Plants with a history of use in traditional medicine constitute an obvious starting point in the search for new therapeutically active drugs. A scientific evaluation of medicinal plants according to their traditional claims could be incorporated into the complementary and alternative medicine system. Pain, inflammation and fever are very common complications in human beings. Several plants and their products are claimed and proved to posses anti inflammatory 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].

Materials and Methods

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.

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).

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.

Methods for Anti-inflammatory activities studies

Acute Inflammation: Carrageenan induced paw oedema [4]:-

The male healthy albino rats were selected, weighed and divided into twelve groups of six animals each. All these animals were fasted for 18 hrs before the beginning of the experiment and water was given *ad libitum*. In animals of all the groups acute inflammation was produced by sub plantar injection of 20µl of freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of rat. The paw thickness was measured using plethysmometer before and at an interval of 0, 1,2,3,4 and 5 hours after carrageenan challenge in each group. Animals were pre medicated with drug/extracts one hour before carrageenan injection as depicted in Table No.1. The increase in paw volume were measured inhibition were calculated by comparing with control group and represented in Table–2.

Chronic Inflammation

Formalin induced paw oedema [5]:-

The male healthy albino rats were selected, weighed and divided into twelve groups of six animals each. All these animals were fasted for 18 hrs before the beginning of the experiment and water was given *ad libitum*. In animals of all the groups chronic inflammation was produced by sub plantar injection of 20µl of freshly prepared 2% suspension of formalin in normal saline in right hind paw of rat was used as the oedematogenic agent. Animals were treated with drugs for 6 consecutive days as follows:

The paw volume was measured using a plethysmometer before and 6 days after formalin challenge in each group. The increase in paw volume and percent of inhibition are present in the Table – 3.

In the above model the degree of edema formation was assayed as increase in paw volume. The increase in paw volume and percent of inhibition were calculated as follows:

Increase in paw volume in control / treatment

$$PC /PT = Pt – Po$$

Percentage of inhibition = PC – PT x 100 / PC

Where
Pt = paw volume at time't', Po = initial paw volume, PC = Increase in paw volume of control group, PT= Increase paw volume of the treatment groups, Sub-Acute Inflammation

Table No.1: Experimental groups and drug/extracts treatment with dose

Group	Drug/Extract	Carrageenan induced paw edema	Formalin induced paw edema	Granuloma pouch
		Dose (mg/kg) p.o	Dose (mg/kg) p.o	Dose (mg/kg) p.o
Group-1	2% acacia solution	0.3ml	0.3ml	0.3ml
Group-2	Diclofenac sodium	100	100	100
Group-3	Petroleum ether	200	200	200
Group-4		400	400	400
Group-5	Benzene	200	200	200
Group-6		400	400	400
Group-7	Chloroform	200	200	200
Group-8		400	400	400
Group-9	Ethanol	200	200	200
Group-10		400	400	400
Group-11	Aqueous	200	200	200
Group-12		400	400	400

Cotton Pellet-Induced Granuloma in Rats [6]:-
This model is based on the foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets of compressed cotton. After several days, histologically gaint cells and undifferentiated connective tissue can be observed beside the fluid infiltration. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. More intensive granuloma formation has been observed if the cotton pellets have been impregnated with carrageenan.

The cotton pellet granuloma was produced in rats by the method of Winter and Porter with slight modification [7]. The pellets, weighing exactly 10 mg each, were made from 5mm sections of cotton rolls. The cotton pellets were sterilized in an autoclave for 30 minutes at 121^0 C and 15 lb pressure. Four pellets were inserted s.c into the ventral region, two on either side, in each rat under light ether anesthesia. Control (2% gum acacia solution), various extracts of *Sphaeranthus indicus* Linn as suspension in 2% gum acacia solution and Diclofenac Sodium (100 mg/kg) were administered daily for 7 days subcutaneously in the neck region. The cotton pellets (along with the granular tissue formed around) were removed surgically and freed from extraneous tissue. The pellets were weighed immediately for wet weight. Then, pellets were dried in an incubator at 60 ^{0} C until a constant weight was obtained.

Table: 2-Anti-inflammatory effect of whole parts *Sphaeranthus indicus* Linn. extracts and ibuprofen in Carageenan induced Rat Paw Edema

Drug/ Extract	Dose (mg/kg)	Paw Volume(ml.)					
		0 h	1 h	2 h	3 h	4 h	5 h
Control		4.13± 0.13	4.98± 0.16	5.93± 0.06	6.84± 0.13	7.68± 0.16	7.94± 0.09
Diclofenac Sodium	100	3.57± 0.06	3.94± 0.11* 56.47 %	3.96± 0.08* 78.33%	4.13± 0.09* 79.33%	4.32± 0.04* 78.87%	4.58± 0.06* 73.49%
Petroleum ether extract	200	3.43± 0.03	3.97± 0.02* 36.47%	4.56± 0.06* 37.22%	5.01± 0.08* 41.69%	5.87± 0.03 31.26%	6.19± 0.16 27.55%
	400	3.51± 0.05	4.01± 0.17* 41.17%	4.53± 0.08* 43.33%	4.94± 0.06* 47.23%	5.32± 0.16* 49.01%	5.3± 0.18* 53.01%
Benzene extract	200	3.88± 0.05	4.7± 0.03 3.52%	5.39± 0.18 16.11%	5.98± 0.99 22.5%	6.72± 0.04 20%	7.03± 0.06 17.32%
	400	3.76± 0.04	4.54± 0.06 8.23%	5.2± 0.02 20%	5.72± 0.02 27.67%	6.58± 0.09 20.56%	6.81± 0.07 19.94%
Chloroform extract	200	3.58± 0.06	4.39± 0.02 4.7%	5.26± 0.09 6.66%	6.08± 0.07 7.74%	6.72± 0.03 11.54%	6.85± 0.07 14.17%
	400	3.67± 0.03	4.45± 0.38 8.23%	5.29± 0.05 10%	5.93± 0.02 16.6%	6.57± 0.18 18.3%	6.63± 0.05 22.3%
Ethanolic extract	200	3.58± 0.04	4.11± 0.06* 37.64%	4.62± 0.05* 42.22%	5.28± 0.07 37.26%	5.79± 0.08* 36.61%	6.11± 0.02* 33.59%
	400	3.53± 0.06	3.98± 0.08* 47.05%	4.41± 0.05* 51.11%	4.72± 0.04* 56.08%	4.69± 0.06* 67.32%	4.68± 0.08* 69.81%
Aqueous extract	200	3.98± 0.08	4.78± 0.19 5.88%	5.73± 0.11 2.77%	6.57± 0.08 4.42%	7.12± 0.02 11.54%	7.75± 0.14 1.04%
	400	3.74± 0.05	4.51± 0.16 9.41%	5.42± 0.12 6.66%	6.18± 0.06 9.96%	6.8± 0.08 13.8%	7.43± 0.03 3.14%

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

Table-3 -Anti-inflammatory Effect of whole parts Extract of *Sphaeranthus indicus* Linn. (Formalin induced paw edema in albino rats)

Treatment	Treatment	Initial paw Volume	Paw volume after 6 days	Increase in paw Volume	% of inhibition
Control(2%gum acacia solution)	0.3ml	3.93± 0.13	7.94± 0.09	4.01± 0.02	---------
Diclofenac Sodium	100mg/kg	3.57± 0.06	3.98± 0.06	0.41± 0.16	89.77
Petroleum ether	200mg/kg	3.56± 0.16	5.84± 0.42	2.28± 0.03	43.14
	400 mg/kg	3.76± 0.03	5.66± 0.66	1.9± 0.26	52.61
Benzene	200mg/kg	3.76± 0.06	6.85± 0.16	3.09± 0.02	22.94
	400 mg/kg	3.51± 0.16	6.56± 0.86	3.05± 0.15	23.94
Chloroform	200 mg/kg	3.58± 0.16	6.95± 0.06	3.25± 0.04	18.95
	400 mg/kg	3.65± 0.04	6.63± 0.03	2.98±0.17	25.68
Ethanolic	200 mg/kg	3.58± 0.03	5.43± 0.06	1.85± 0.18	53.86
	400 mg/kg	3.98± 0.26	5.24± 0.04	1.26± 0.11	68.57
Aqueous	200 mg/kg	3.63± 0.13	7.12± 0.38	3.49± 0.16	12.96
	400 mg/kg	3.71± 0.79	7.09± 0.04	3.38± 0.04	15.71

Results are expressed on mean ± SEM from four observations
Paw Volume was measured after 6 days.

The amount of exudate (weight of exudate in mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight of granuloma) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet and taken as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined as follows:

$$\text{Exudate inhibition (\%)} = \left[1 - \frac{\text{Weight of exudate in mg of drug treated group of rats}}{\text{Weight of exudate in mg of saline treated group of rats}}\right] X\ 100$$

$$\text{Granuloma inhibition (\%)} = \left[1 - \frac{\text{Weight of granuloma in mg of drug treated group of rats}}{\text{Weight of granuloma in mg of saline treated group of rats}}\right] X\ 100$$

Experimental groups
Rats were randomly allotted into twelve experimental groups each containing six animals (n=6). List of experimental groups and respective drug treatment along with the dose used are tabulated in the following tables.

The result of Anti-inflammatory activities were calculated by comparing with control group and represented in the Table–4.

Table 4: Effect of whole parts *Sphaeranthus indicus Linn* extracts and *Diclofenac Sodium* on Exudation and Granular Tissue formation in Cotton Pellet Granuloma in Rats

Drug/Extract	Dose (mg/kg. p.o)	Weight of Exudate (mg)	Exudate Inhibition (%)	Dry Weight of Granuloma (mg)	Granuloma Inhibition (%)
Control		101.24 ± 4.17		25.67 ± 0.24	
Diclofenac Sodium	100	65.12 ± 3.27	35.68	14.79 ± 1.21	42.39
Petroleum ether extract	200	75.05 ± 3.74	25.87	17.96 ± 0.88	30.04
	400	69.38 ± 2.16	31.47	17.22 ± 0.97	32.92
Benzene extract	200	81.95 ± 3.11	19.05	20.93 ± 1.04	18.47
	400	80.24 ± 2.75	20.75	20.68 ± 0.79	19.44
Chloroform extract	200	80.15 ± 3.11	20.84	20.3 ± 1.04	20.92
	400	79.34 ± 3.19	21.64	20.06 ± 0.74	21.86
Ethanolic extract	200	72.66 ± 2.16	28.23	18.02 ± 0.97	29.81
	400	70.05 ± 3.74	30.81	17.66 ± 0.88	31.21
Aqueous extract	200	81.95 ± 3.11	19.05	20.93 ± 1.04	18.47
	400	81.75 ± 3.11	19.26	20.38 ± 1.04	20.61

Result and Discussion

Anti-inflammatory Activity

The anti-inflammatory activity was studied in carrageenan induced acute inflammation and Formalin induced chronic inflammation and Cotton Pellet-Induced Granuloma Sub-Acute Inflammation. The anti-inflammatory activity of extracts of *Sphaeranthus Indicus*. Linn in three models are given in Table – 1, 2 & 3.The various extracts at a dose of 200 mg/kg and 400 mg/kg body weight showed comparable anti-inflammatory activity was studied in carrageenan induced acute inflammation, Formalin induced chronic inflammation and Cotton Pellet-Induced Granuloma Sub-Acute Inflammation in Rats

Carrageenan induced model

The results obtained as mean increase in paw volume (ml) and % inhibition are represented in table -1.The results indicate that in carrageena induced paw edema model Ethanolic and Petroleum ether extracts showed 42.22 % and 41.69% inhibition of increased paw thickness at the end of 1hr and 2hrs followed by benzene, chloroform and aqueous extract about 22.5, 14.17 and 11.54% inhibition of increased paw thickness respectively at the end of 2, 4 and 3 hours at a dose of 200 mg/kg body weight p.o. However at the dose of 400 mg/kg body weight Ethanolic and Petroleum ether showed 69.81 and 53.01% inhibition of inflammation control at the end 5hrs, followed by benzene 27.67 %, chloroform 22.3 % and aqueous extract 13.8 % inhibition of inflammatory increased paw thickness when compared to solvent control at the end of 2, 5 and 3 hours . At the same time Diclofenac sodium the standard drug produces 79.33% inhibition of inflammatory paw thickness after the end of 2nd hours of drug administration. This study revealed that in carrageenan induced inflammation model all extracts are ranking in the following order.

Ethanolic > Petroleum ether > Benzene> Chloroform > Aqueous extract

The percentage inhibitions of inflammation of all extracts are dose dependent. The development of carrageenan induced edema is biphasic, the 1st. phase is attributed to release of histamine, 5-HT, Kinin, while 2nd. Phase is related to the release of Prostaglandin [8] [9]. Carrageenan induces paw

oedema by inducing protein-rich exudates containing a large number of neutrophils [10]. Therefore it is proposed that the anti inflammatory property of extracts is due to inhibition of one of the pain mediators like histamine, 5-H.T., Kinin or Prostaglandin or both.

Formalin induced model

Formalin induced paw edema is one of the most suitable test procedure to screen chromic anti-inflammatory agents as it closely resembled human arthritis [11]. The results obtained as mean increase in paw volume (ml) and % inhibition are represented in table -2,in this model at 200 mg/kg dose level of Petroleum ether, benzene, chloroform, ethanolic and aqueous extracts showed 43.14, 22.94, 18.95, 53.86, and 12.94 % inhibition of increase in paw thickness after 6 days, whereas at the 400 mg/kg dose level of Petroleum ether, benzene, chloroform, ethanolic and aqueous extracts showed 52.61, 25.68, 23.94, 68.57, and 15.71 % inhibition of increase in paw thickness after 6 days. However at 200 mg/kg dose level Ethanolic and Petroleum ether extracts showed 53.86 and 43.14 % inhibition of increase in paw thickness after 6 days, whereas at the 400 mg/kg dose level ethanolic and Petroleum ether extracts showed 68.57% and 52.61% inhibition of inflammatory paw thickness after 6 days. All the results were compared with solvent control and diclofenac sodium reference drug control, the anti-inflammatory activity of all extracts in this model are ranking in the following order.

Ethanolic extract> Petroleum ether extract> Benzene extract > Chloroform extract > Aqueous extract.

The result showed that the anti-inflammatory activities of all extracts are dose dependent. The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by a later tissue mediated response[12]. The results suggest the usefulness of *Sphaeranthus indicus* Linn in the treatment of inflammation associated diseases like arthritis.

Cotton Pellet-Induced Granuloma in Rats

In cotton pellet induced granuloma and in glass rod granuloma, the foreign body like cotton or glass rod when implanted in the skin of animal is producing undifferentiated connective tissue around it indicating state of inflammation. The amount of newly formed connective tissue is measured by weighing the dried pellet after removal as an index of the extended severity of the inflammation. This model is the indication of the proliferative phase of the inflammation of the microphages, neutrophils, fibroblasts and collagen formation which are basic source for the granuloma formation; therefore decrease in the granuloma formation indicates the suppression of the proliferative phase **[**13**]**.

Conclusion

The present experimental protocols showed that the *Sphaeranthus indicus* (Whole Plant) individually elicited a significant anti-inflammatory activity in carrageenan (acute), cotton pellet granuloma (sub-acute) and Formalin (Chronic) induced paw oedema rat models.

The anti-inflammatory activities of all extracts of *Sphaeranthus indicus* Linn are validated through acute, sub-acute and chronic inflammatory models in rats. In all the three models of the anti-inflammatory effects against carrageenan, cotton pellet and Formalin induced paw edema in rats are in a dose dependent manner. The activities of all extracts are comparable with Diclofenac sodium and hence it is useful in the treatment of inflammation associated disease like arthritis.

The anti-inflammatory activity possessed by the *Sphaeranthus indicus* Whole Plant extracts is being reported for the first time. The phytochemical investigation of the plant revealed the presence

of flavonoids, terpenoids, and carbohydrates. The flavonoids are known to possess anti-inflammatory activity by inhibiting the cyclooxygenase responsible for synthesis of inflammatory prostaglandin [14].Thus it can be concluded that, the anti-inflammatory activity of the *Sphaeranthus indicus* extracts is attributed to the kinin and prostaglandin biosynthesis enzyme inhibiting property of flavonoids present.

Through a series of studies, there is no doubt that the *Sphaeranthus indicus* Linn is very useful in the treatment of inflammation and inflammation associated diseases like arthritis.

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