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Evaluation of anti-inflammatory activity of ethanolic extract of *Sphaeranthus indicus*

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ABSTRACT

Sphaeranthus indicus had been widely used for its reported biological activity in indigenous system of medicine. The present investigation was carried out to find the anti-inflammatory effect of ethanolic extract of *Sphaeranthus indicus* in albino rats. The anti-inflammatory activity was evaluated using acute inflammatory model like carrageenan induced paw edema and chronic inflammatory model like cotton pellet induced granuloma respectively. The ethanolic extract in different doses (100,200, and 400mg/kg, p .o) exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan induced hind paw edema, $p < 0.05$) and chronic (cotton pellet granuloma formation, $p < 0.05$) model of inflammation.

Keywords: Anti-inflammatory, *Sphaeranthus indicus*, Carrageenan induced paw edema, Cotton pellet granuloma.

INTRODUCTION

Since time immemorial indigenous plants have been a major source of medicine because of the different components they contain have immense therapeutic value. The history of inflammation is as old as man's existence in this planet. It is one of the most fundamental response of the cells and tissue to injury. It is essentially a defense reaction but sometimes it over do itself either in intensity or in duration and cause lot of suffering and pain. Steroidal and Non steroidal anti-inflammatory drugs are available but their prolonged administration is known to be associated with various adverse effects. Herbal drugs have lesser side effects and are largely replacing synthetic drugs.

Sphaeranthus indicus (Hindi-Gorakhmundi) is a much branched herb widely distributed in India and belongs to family asteraceae [1]. It is widely used in the treatment of diverse disease conditions namely convulsion, bronchial asthma, dysentery [2]. Essential oil obtained from leaves possess antifungal activity [3] and ethanolic extract showed an anxiolytic activity [4]. There is paucity of study available for its anti-inflammatory activity, Hence the present study is an attempt to assess the anti-inflammatory activity of ethanolic extract of *Sphaeranthus indicus* using various model of inflammation.

EXPERIMENTAL SECTION

Chemicals and drugs

Aspirin, Carrageenan were used in the study

Collection of Plant

The leaves of *Sphaeranthus indicus* were collected from rural area of Sambalpur, Orissa. They were authenticated by faculty of botany Sambalpur University.

Preparation of the extract

The powdered plant material was extracted with ethanol in a Soxhlet apparatus for 48 hrs. The extracts were filtered through Whatman filter paper (No.1) and concentrated by vacuum evaporation. The yield of extract as per solvent used was 4.25% w/w. The dried extracts were suspended in 2% gum acacia and used for experiments.

Phytochemical studies

Preliminary phytochemical study was performed [5]. The presence of phytoconstituents such as flavonoids, terpenoids, carbohydrates and glycosides were confirmed.

Test Animals

Adult Swiss male albino rats (150-200gms) were obtained from animal house, Department of Pharmacology, V.S.S Medical College, Burla and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature $25 \pm 2^\circ\text{C}$ and 12hr dark/light cycle) with standard laboratory diet and water *ad libitum*. All experimental procedures and protocol used in this study were reviewed and approved by institutional animal ethical committee, V.S.S. Medical College, Burla.

Anti-inflammatory activity

Carrageenan Induced Rat Paw Edema

The method of Winter et al (1962) was used to study acute inflammation [6]. Seven groups of six rats in each group were treated with vehicle, EESI (100,200 and 400mg/kg, p. o.), Aspirin (50 and 100mg/kg, p .o.) and combination of Aspirin (50mg/kg) and EESI (100 mg/kg, p .o.) one hour prior to Carrageenan injection. 0.1ml of 1% Carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of Carrageenan injected feet were measured at 0, 1, 2, 3,4hr using Plethysmometer (Ugo Basile, Italy).

Cotton pellet granuloma in rats

The method of Winter and Porter with slight modification was used to study chronic inflammation [7]. Seven groups of six animals in each group were taken, anaesthetized with ether. The axillary skin was shaved and disinfected with 70% ethanol. An incision was made and by a blunt forcep subcutaneous tunnels were formed and a sterilized cotton pellet ($50 \pm 1\text{mg}$) was placed in both axillas. The vehicle, test drug, EESI (100, 200, and 400 mg/kg, p.o.), standard

drug, Aspirin (50,100mg/kg) and combination of Aspirin (50mg/kg) and EESI (100mg/kg) were administered for 7 consecutive days starting from day of cotton implantation. At 8th day rats were anaesthetized again and the cotton pellet (along with 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° C until a constant weight was obtained.

Statistical Analysis

All values were shown as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dun net's t test. P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table -1 – Anti-inflammatory activity (Carrageenan Induced Paw Edema Method)

Group	Treatment Dose(mg/kg, p.o)	Increase in paw volume (mean±SEM) in ml	% inhibition of paw edema
I	Control	0.67 ±0.18	-
II	EESI(100)	0.28 ±0.08*	57.22%
III	EESI(200)	0.21 ±0.07*	67.65%
IV	EESI(400)	0.16 ±0.04**	77.61%
V	Aspirin(50)	0.28 ±0.09*	58.31%
VI	Aspirin (100)	0.13 ±0.03**	80.59%
VII	Aspirin(50) + EESI (100)	0.23± 0.07*	65.67%
<i>One Way ANOVA</i> $f:6.3$ $df: 6,35$ <i>N=6 in each group</i> $p * 0.01$ $p^{**} 0.001$ compared to control			

Table – 2 Anti-inflammatory activity (Cotton Pellet Granuloma Method)

Group	Treatment (mg/kg)	Weight of granuloma (in mg)	% inhibition of granuloma formation
I	Control	74.28 ± 2.56	-
II	EESI (100)	59.85 ± 1.40*	14.43%
III	EESI (200)	50.85 ± 1.22*	23.43%
IV	EESI (400)	38.42 ± 0.99**	45.29%
V	Aspirin (50)	44.71 ± 0.74*	39.80%
VII	Aspirin (100)	33.85 ± 1.12**	54.21%
VIII	Aspirin(50)+EESI(100)	36.00 ± 1.04**	51.72%
<i>One Way ANOVA</i> $f:25.52$ $df: 6,42$ <i>N=6 in each group</i> $p * 0.01$ $p^{**} 0.001$ compared to control			

The results obtained as mean increase in paw volume and %age inhibition are shown in table -1. The results shown %age inhibition of paw edema by EESI in dose (100, 200, 400 mg/kg) were 57.22%, 67.77% and 77.61% respectively. The values were 58.32% and 80.59% for aspirin in 50 and 100 mg/kg doses respectively. Whereas for aspirin (50) and EESI (100mg/kg) combination it is 65.67%.

The results obtained as percentage inhibition of granuloma formation are shown in table-2. The results shown percentage inhibition of granuloma formation with 100,200 and 400 mg/kg dose EESI are (14.43%, 23.43%, and 47.29%) respectively. The percentage inhibitions with aspirin

(50, 100 mg/kg) are (39.80% and 54.21%) respectively where as for combination dose (aspirin, 50 and EESI 100mg/kg) it is 51.72%.

In the present study EESI demonstrated a significant anti-inflammatory activity in both model of inflammation. Carrageenan induced rat paw edema has been a popular inflammatory model. It shows a biphasic response [8]. The first phase is due to release of histamine and serotonin and second accelerating swelling is due to release of PG [9] [10][11]. In our study, EESI (100,200,400 mg/kg) significantly reduced edema induced by Carrageenan in all phases. Cotton pellet granuloma is a model of non immunological types of inflammation and edema is due to mainly due to proliferative phase of inflammation [12]. Efficacy of EESI in this model might be due to an increase in the synthesis of collagen and mucopolysaccharides and increase in the number of fibroblasts during granuloma tissue formation.

CONCLUSION

The present experimental study protocol showed that ethanolic extract of *Sphaeranthus indicus* elicited significant anti-inflammatory activity in carrageenan induced paw edema and cotton pellet granuloma model. In both model they exhibited anti-inflammatory effect in a dose dependent manner which can be comparable with that of aspirin. The phytochemical analysis revealed the presence of terpenoids and flavonoids. The flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis [13]. So anti inflammatory activity of *Sphaeranthus indicus* can be attributed to bradykinin and PG synthesis inhibition property of flavonoids.

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