PHARMACOLOGICAL EVALUATION OF SPHAERANTHUS INDICUS AND CINNAMOMUM ZEYLANICUM FOR ANTI-INFLAMMATORY ACTIVITY IN RATS.

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ABSTRACT

The present study was designed to investigate the Anti-inflammatory activity of alcoholic extracts of Sphaeranthus indicus L. flower heads and Cinnamomum zeylanicum bark in Carrageenan induced paw oedema in rats. Carrageenan–induced paw oedema in rats was induced essentially as described by Winter et al. (1962). An injection was made of 0.1ml of 1% Carrageenan suspension into the right hind foot of each rat under the subplantar aponeurosis. The test groups of rats were treated intraperitoneally with 250 and 500 mg/kg of Cinnamomum zeylanicum and Sphaeranthus indicus extract 1hour before carrageenan injection. The control group received only the vehicle (0.2ml normal saline) and the reference group received Diclofenac sodium (5 mg / kg p.o.). The evaluation of anti-inflammatory activity carried out by inducing Paw oedema by injecting 0.1ml of 1% Carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat. The extracts were administered orally 30 min prior to Carrageenan administration. The paw volume was measured at 60, 120, 180, 240 minutes by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the control group was used as reference standard. The alcoholic extracts of Sphaeranthus indicus and Cinnamomum zeylanicum were found to possess good anti-inflammatory activity as compared to other extracts.

Keywords: Anti-inflammatory, Carrageenan, albino rats

1. INTRODUCTION

Sphaeranthus indicus Linn. (Mundi, Family compositae) is a small annual herb about 1ft. high, with spreading branches, long, divaricated, ascending, with toothed wings, glandular tomentose or villous. Stem cylindrical, strongly winged with the sharp – toothed decurrent bases of the leaves. Leaves 1 – 2 inch sessile, decurrent, oval, slightly tapering at the base, obtuse or subacute, sharply spinous-serrate, very glandular, and also with long white hair on both sides, glaucousgreen; compound heads, ovoid, globose, on winged peduncles, heads very numerous, densely packed, purple bracts, linac acuminate rather shorter than flower-heads, ciliate at the end, achenes stalked, smooth. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia. (Ambavade et al., 2006)

Medicinal plants are an indispensable part of the traditional medicine practiced all over the world due to low costs; easy access & ancestral experience. Traditional ayurvedic grantha like Bhavaprakash Nighanta described. Sphaeranthus indicus Linn. commonly known as ‘Gorakhmundi’ is distributed throughout the plains in India in wet places. The plant is reported to be useful for epilepsy, anemia, diabetes, gout etc. Extract of flower contain the principal essential oil, alkaloid, tannin, glycoside, reducing sugar, semidrying fatty oil and albumin. (Chaddha YR, 1976)

All parts of the plant found medicinal uses. The juice of the plant is styptic and said to be useful in liver and gastric disorders. (Dhar ML, 1968) The paste of the herb made with oil is applied in itch. The herb has a bitter sharp flavor with bitter taste. (Gupta MB, 198) It increases the appetite, enriches the blood, cools the brain & gives luster to the eye. (Kirtikar KR, 1987) The free radical scavenging potential of Sphaeranthus indicus Linn is also reported. (Shirwaikar et al., 2006)
Cinnamon bark is widely used as spices & condiments obtained from *Cinnamomum zeylanicum* belong to the family lauraceae. Traditional medicine literature describes use of cinnamon bark in the treatment for diarrhea, stomach upset against respiratory ailments & externally as skin antiseptic & rubefacient. Research also showed the antioxidant potential of cinnamon bark contains mainly cinnamaldehyde, eugenol, cinnamic acid & proanthocyanidins.

Cinnamon is native to India and Sri Lanka (Ceylon). It is now cultivated in many tropical countries including Mexico. This plant has been used in Ayurvedic and other medicinal traditions in Asia. In the American continent, most of the original uses are still prevalent; mainly as a treatment for diarrhea, stomach upset, against respiratory ailments and externally as a skin antiseptic and rubefacient (it stimulates capillary circulation). (Dhuley J. N, 1999)

A vast amount of circumstantial evidence implicates oxygen-derived free radicals (especially superoxide and hydroxyl radical) and high-energy oxidants (such as peroxynitrite) as mediators of inflammation, shock, and ischemia/reperfusion injury. (Salvatore Cuzzocrea et al., 2001) As mentioned above both of plants possess antioxidant potential and based on this information present study was designed to investigate the anti-inflammatory activity of *Sphaeranthus indicus* flower head extract and *Cinnamomum zeylanicum* bark extract.

**2. MATERIALS & METHODS**

**Plant materials & chemicals**

The flowers of *Sphaeranthus indicus* collected at Wardha (M.S.) and *Cinnamomum zeylanicum* bark directly purchased from Botanical Survey of India were authenticated from Regional Research Institute, Kothrud, Pune. The plant specimen is available in Regional Research Institute. Specimen voucher No. is 812 for future reference. Diclofenac sodium was obtained as gift sample from Aarti Bulk Drugs, Boisar, Mumbai.

**Plant Extracts**

The flower heads of *Sphaeranthus indicus* and bark of *Cinnamomum zeylanicum* were dried in shade, under normal environmental condition and then subjected to size reduction to get coarse powder. Such powdered material was charged into the Soxhlet apparatus, and extraction was carried out successively with the following solvents like Benzene, carbon tetrachloride, petroleum ether, chloroform, ethanol and water. Each time before extracting with the next solvent, the powdered material was air dried below 50°C and then each extract was concentrated by distilling off the solvent to obtain the crude residue. The drug was extracted with each solvent till complete extraction was effected (about 30 cycles). All the extracts were stored in desiccators over phosphorous pentoxide and self indicating silica gel G.

About 125 g of the powder of both crude drugs was taken in Soxhlet extractor & extracted with Benzene (2000 ml) for 72 hrs. The solvent recovered by distillation was concentrated to yield a residue. The process of extraction was repeated with used marc & same volume of Chloroform, petroleum ether, ethanol etc. The aqueous extract was prepared after ethanol extraction by same procedure & evaporated at 40°C to give dark brown color solid mass.

**Animals**

Albino rats of Wistar strain (150-200 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20C; relative humidity 60-70%) in a 12 hours light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee. (650/02/c/ CPCSEA)

**Carrageenan–induced paw oedema**

Carrageenan–induced paw oedema in rats was induced essentially as described by Winter *et al* (1962). An injection was made of 0.1ml of 1% Carrageenan suspension into the right hind foot of each rat under the subplantar aponeurosis. The test groups of rats were treated intraperitoneally with 250 and 500 mg/kg of *Cinnamomum zeylanicum* and *Sphaeranthus indicus* extract 1 hour before carrageenan injection. The control group received only the vehicle (0.2ml normal saline) and the reference group received Diclofenac sodium (5 mg / kg p.o.). The evaluation of anti-inflammatory activity carried out by inducing Paw oedema by injecting 0.1ml of 1% Carrageenan in physiological saline into
the sub plantar tissues of the left hind paw of each rat. The extracts were administered orally 30 min prior to Carrageenan administration. The paw volume was measured at 60, 120, 180, 240 minutes by the mercury displacement method using a plethysmograph.

**Acute toxicity Studies**

Acute toxicity studies were study performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg / kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight. The alcoholic extracts of *Sphaeranthus indicus* and *Cinnamomum zeylanicum* did not cause any mortality up to 2000 mg/kg and were considered as safe.

**3. DISCUSSION**

In the present study, the anti-inflammatory activities of the ethanolic extract of *Sphaeranthus indicus* and *Cinnamomum zeylanicum* has been established in acute inflammation. Carrageenan induced rat paw oedema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products. Development of oedema in the paw of rat after injection of Carrageenan is a biphasic event. The result of present study indicates that alcoholic extracts of *Sphaeranthus indicus* and *Cinnamomum zeylanicum* (500 and 250 mg/kg, p.o.) and diclofenac sodium play a crucial role as protective factors against the carrageenan induced acute inflammation.

The percentage inhibition of paw volume in drug treated group was compared with the control group was used as reference standard. The alcoholic extracts of *Sphaeranthus indicus* and *Cinnamomum zeylanicum* were found to possess good anti -inflammatory activity as compared to other extracts.

**Table 1: - Anti-inflammatory effect of Sphaeranthus indicus and Cinnamomum zeylanicum on Carrageenan induced rat paw edema in rats.**

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Dose (mg/kg)</th>
<th>1 h</th>
<th>Oedema volume (ml)</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Distilled water)</td>
<td>5 ml</td>
<td>0.62 ± 0.05</td>
<td>0.68 ± 0.05</td>
<td>0.74 ± 0.05</td>
<td>0.69 ± 0.05</td>
<td>0.67 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>5 (61.30)</td>
<td>0.24±0.03b (72.06)</td>
<td>0.19±0.01c (89.09)</td>
<td>0.14±0.02c (69.57)</td>
<td>0.21±0.02c (65.68)</td>
<td>0.23±0.02c</td>
<td></td>
</tr>
<tr>
<td>CZ alcoholic extract</td>
<td>250 (32.26)</td>
<td>0.42±0.04a (44.12)</td>
<td>0.38±0.03b (63.52)</td>
<td>0.27±0.03c (40.58)</td>
<td>0.41±0.03b (37.83)</td>
<td>0.26±0.04c</td>
<td></td>
</tr>
<tr>
<td>SPH alcoholic extract</td>
<td>500 (43.55)</td>
<td>0.35±0.05b (66.18)</td>
<td>0.23±0.04c (79.73)</td>
<td>0.15±0.03c (65.22)</td>
<td>0.24±0.03c (61.20)</td>
<td>0.26±0.03c</td>
<td></td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td>P&lt;0.001</td>
<td>F=11.15</td>
<td>P&lt;0.001</td>
<td>F=22.59</td>
<td>P&lt;0.001</td>
<td>F=42.86</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

CZ; Cinnamomum zeylanicum, SPH; Sphaeranthus indicus

Each value is the mean ± SEM of 6 rats.
Figures in parentheses indicate the % anti-inflammatory activity.

a p < 0.05; b p<0.01; c p<0.001 compared to control.
REFERENCES


