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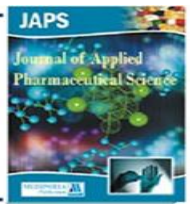

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The analgesic and anti-inflammatory activities of the hydroalcholic extract of *"Shirishadi compound"* in animal model

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ABSTRACT

The main objective of the present investigation is to evaluate the anti-inflammatory & analgesic activity of ethanolic extract of Shirishadi polyherbal compound on rats. Shirishadi compound consist of three herbal drugs namely- *Shirisha* (Albizzia lebbeck), *Nagarmotha* (Cyprus rotandus) & *Kantakari* (Solanum xanthocarpum).In Ayurveda (ancient Indian system of medicine) all these herbs alone or in combination with other herbs are commonly used in the managmant of bronchial asthma. In the carrageenan-induced rat paw edema test for acute inflammation, the extract of *Shirishadi* compound in doses of 50mg, 200 mg and 500 mg/kg body weight showed 77% and 79% and 81% inhibition of edema, respectively, at the end of 4h which is comparable to that of standard (endomethacin) i.e. 92%. In the acetic acid induced writhing test the extract of *Shirishadi* compound (200 and 500 mg/kg body weight) showed a significant (p<0.001) reduction in the number of writhes with 65.6% and 70.9% of inhibition, respectively. In radiant heat tail-flick test the crude extract produced 58.1% (p<0.001) and 61.1% (p<0.001) elongation of tail flicking time 30 minutes after oral doses of 200 and 500 mg/kg body weight respectively . After 60 minutes the extract showed 56.3% (p<0.001) and 59% (p<0.001) elongation of tail flicking time. Experimental results showed that *Shirishadi* compound has persuasive anti-inflammmatory property along with significant analgesic activity.

Keywords: *Shirisadi* polyherbal compound, Anti-inflammatory activity, Analgesic activity, Radiant heat tail-flick test, Writhing test.

INTRODUCTION

Asthma is a common disease that is rising in prevalence worldwide with the highest prevalence in industrialized countries. Asthma affects about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025(1-2). Asthma is defined as disorder characterized by chronic airway inflammation and increased airway responsiveness resulting in symptoms of wheezing, cough, chest tightness, and dyspnea. It is characterized functionally by the presence of airflow obstruction which is variable over short period of time or is reversible with treatment. It is not a uniform disease but rather a dynamic clinical syndrome which has a number of clinical patterns. Current asthma therapy lack satisfactory success due to adverse effect, hence patients are seeking complementary and alternative medicine to treat their asthma. Medicinal plant used for the treatment of asthma should have anti-inflammatory, immunomodulatory,antihistaminic, smooth-muscle relaxants and allergic activity. The basic pathology of Asthma starts with the process of inflammation so to show the antiasthmatic activity of drug first step involve to demonstrate the anti- inflammatory activity of drug. Inflammation is a defensive and normal response of the body to any noxious stimulus, varying from acute, transient and highly localized response to a minor mechanical injury to a complex sustained response

involving the whole organism. It has been stressed that inflammation is a process and not a state (Florey, 1970). Acute inflammation refers to a response with a rapid onset and relatively short duration and characterised by particular vascular phenomenon. Albezzia lebbeck, Cyperus rotandus and Solanum xanthocarpum used in the present research trial are use from era to treat asthma in Ayurveda. In order to search the probable mechanism of their action and their pharmacological activity the present research trial was conducted with the aim to show the anti-inflammatory and analgesic activity of drug in animal model.

MATERIALS AND METHODS

Plant collection

The plants Albizzia lebbeck, Cyprus rotandus and Solanum xanthocarpum were collected from local market of Varanasi. The identification of the drugs was done by Prof.A.K. Singh, Department of Dravyaguna, S.S.U., Varanasi.

Extraction of the plant material and sample preparation

Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug was carried out by Hot percolation method through soxhlet appratus. Thereafter extract was dried using rotary evaporator and dried extract was put to the process of standradization.The extract was dissolved in distilled water and three different concentration namely 50mg/ml, 200mg/ml & 500mg/ml was prepared.

Drugs and Chemicals

Aminopyrine, Carrageenan, Pentazocin, Endomethcin & Acetic acid were purchused from Sigma-Aldrich, Germany.

Experimental animal

Adult Charles Foster Albino rats (150± 30g) of either sex were obtained from the Animal Research Branch of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in polyvinyl cages and. were fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed under standard conditions of temperature (22 ± 2^0C), relative humidity ($60 \pm 5\%$) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). The saline fed group served as control and one group was treated with a standard drug in each protocol. Before experimentation, the animals were kept on fast for 24 h but water was given *ad libitum* except during experimental test period. During experiments, animals were also observed for any alteration in their general behavior.

All the experiments and the care of the laboratory animals were according to current ethical guidelines by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi.

Anti-inflammatory study

Carrageenin,a sulphated polysaccharide,extracted from sea weed, has been extensivelyused to induce inflammatory reaction in a number of animal species. Winter et al. (1962) introduce the carrageenin edema of rat hind paw for assay of anti-inflammatory drugs. The reproducibility of the fact that inflammation entirely depends upon local inflammatory reaction devoid of anigenic properties, has made carrageenin the most widely employed phlogistic agent. For the present experiment, carrageenin suspension was prepared as a homogenous suspension of powder in 0.9% sodium chloride solution (sterile normal saline) with the help of mortar & pestel. A volume of 0.1ml of suspension was injected through a 26 gauge needle into the plantar surface of the right hind paw below the plantar aponeurosis 1h after the oral administration of test materials. The volume of hind paw of the rats upto the ankle joint was measured plethysmographically, by the mercury displacement method. The volume was measured 1h, 2h ,3h, 4h & 24 after the administeration of drug. The extract was administered at 50, 200 and 500 mg/kg body weight. Endomethacin 25 mg/kg body weight was used as standard anti-inflammatory agent.

Acetic acid induced writhing test

The peripheral analgesic activity of hydroalcholic extract of Shishadi Ayurvedic compound was measured by the acetic acid induced writhing test as described earlier (Saha *et al*., 2007). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Aminopyrine at oral dose of 50 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 10 min after the application of acetic acid.

Radiant heat tail-flick method

The central analgesic activity of the plant material was studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails by using a Medicraft Analgesiometer Mask-N (D'Amour and Smith,1941) and described previously (Saha *et al*., 2007). Briefly, the current intensity passing through the naked nicrome wire was maintained at 5 ampere. The distance between the heat source and the tail skin was 1.5 cm and cut-off reaction time was fixed at 10 second to avoid any tissue damage. Morphine was used to compare the analgesic effect of the plant extract.

Table 1: Anti-inflammatory activity of crude extract of *Shirishadi* compound by carrageenan induced rat paw edema.

GROUP	% Increase in Paw Volumes (ml × 1000) ± SEM (% inhibition)				
	1h	2h	3h	4h	24h
Control	1.78 ± 0.77	3.0 ±0.15	3.61 ± 0.20	4.0± 1.2	1.93 ± 0.11
Standard (Indomethacin 25mg/kg)	0.72 ± 3.7 (59%)	0.69 ± 0.60 (77%)	0.67 ± 0.66 (81%)	0.65 ±0.37 (92%)	0.66 ± 0.52 (66%)
Shirishadi 50mg/ Kg bwt	1.06 ± 0.68 (40%)	0.99 ± 0.66 (67%)	0.93 ± 0.33 (74%)	0.89 ±0.33 (77%)	0.78 ± 0.41 (59%)
Shirishadi 200mg/Kg bwt	1.0 ± 1.0 (43%)	0.97 ± 0.14 (67.66%)	0.92 ± 0.24 (74.28%)	0.88 ±0.57 (79%)	0.77 ± 0.24 (60%)
Shirishadi 500mg/Kg bwt	0.95 ± 0.33 (46.6%)	0.95± 0.37 (68%)	0.83 ± 0.66 (77%)	0.76 ± 0.63 (81%)	0.66 ± 0.33 (65%)

Values are mean±SEM (N=3).

Table :2 Effect of Shishadi Extract on acetic acid induced writhing response in rodents.

Groups	Dose of Drug	Writhing[b]	% Inhibition
Control	--	15.6 ± 0.50	--
Standard	Pentazocin 10mg/Kg, i.p.	4.3 ± 0.66 **	71.2%
	Aspirin 25 mg/Kg, i.p.	8.0 ± 1.15 **	63.5%
Shirishadi	200mg/ Kg bwt, p.o.	7.66 ± 0.88 **	65.6%
	500 mg/ Kg bwt, p.o.	6.33 ± 0.88 **	70.9%
One way ANNOVA	F	46.94	--
	df	4,12	--
	P	<0.001	--

[a] 1hr after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 minutes after the injection, the number writhing was counted for 10 min. [b] Values are mean ± SEM (n = 5, for control & 3in drug treated groups); One-way ANOVA; ***P*<0.001, compared to control.

Table 3: Effects of crude extract[a] on radiant heat tail-flick response in rodents.

Groups	Dose of Drug	Reaction Time (sec) 30 (mins) (% elongation)	60 (mins) (% elongation)	120 (mins) (% elongation)
Control	--	4.65 ± 0.15	4.82 ± 0.16	4.98 ± 0.20
Standard	Pentazocin 10mg/ kg bwt, i.p.	8.65 ± 0.71**	6.45 ± 0.45**	5.89 ± 0.37**
Shirishadi Compound	200 mg/ Kg bwt, p.o.	7.41 ± 1.17** (58.1%)	7.10 ± 0.30** (56.3%)	6.08 ± 0.21** (49%)
	500 mg/ Kg bwt, p.o.	7.98 ± 0.12** (61.1%)	7. 57 ± 0.45** (59%)	6.75 ± 0.15** (54%)
One way ANOVA	F	68.5	27	5.34
	P	< 0.001	< 0.001	< 0.001
	df	7,40	7, 40	7,40

a .per oral administration of vehicle and crude extract, radiant heat intensity was 6 amp. b morphine was administered sub-cutaneously. [c] Values are mean ± SEM (n =5); One-way ANOVA; df = 7, 40; ***P*<0.01, **P*<0.05 compared to control.

Table 4 Intergroup comparision of shirishadi treated and Endomethacin treated group with control by using One Way ANNOVA followed by Post- Hoc test.

Groups	Change in Paw Volume expessed as Mean ± SE (per hour) 1h	2h	3h	4h	24h
Shirishadi 50mg/ 100g bwt	1.06 ± 0.068**	0.99± 0.006**	0.93 ± 0.03**	0.89± 0.003**	0.78 ± 0.04**
Shirishadi 200mg/100g bwt	1.10 ± 0.05**	0.97 ± 0.01**	0.93 ± 0.02**	0.89± 0.05**	0.77 ± 0.02**
Shirishadi 500mg/100g bwt	0.95 ± 0.03**	0.95 ± 0.03**	0.83 ± 0.06**	0.76± 0.06**	0.66 ± 0.03**
Endomethacin	0.72 ± 0.03**	0.69 ± 0.06**	0.67 ± 0.06**	0.65 ± 0.03**	0.66 ± 0.03**

* Values are mean ± SEM (n =3); One-way ANOVA; df = 4, 10; 14,***P*<0.001, **P*<0.05 compared to control.

STATISTICAL ANALYSIS

Data were analyzed by one-way ANOVA followed by Dunnet's test and *P* values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In the carrageenan-induced rat paw edema test (table 1)for acute inflammation, the extract of *Shirishadi* compound in doses of 50mg, 200 mg and 500 mg/kg body weight showed 77% and 79% and 81% inhibition of edema, respectively, at the end of 4h which is comparable to that of standard (endomethacin) i.e. 92%. In the acetic acid induced writhing test the extract of *Shirishadi* compound (200 and 500 mg/kg body weight) showed a significant (p<0.001) reduction in the number of writhes with 65.6% and 70.9% of inhibition, respectively (table2). In radiant heat tail-flick test the crude extract produced 40.74% (p<0.001) and 61.48% (p<0.001) elongation of tail flicking time 30 minutes after oral doses of 200 and 500 mg/kg body weight respectively (table 3). After 60 minutes the extract showed 31.29% (p<0.001) and 41.37% (p<0.001) elongation of tail flicking time. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways (Ronaldo *et al*., 2000). The extract of *Shirishadi* compound produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. In the radiant heat tail-flick test, the polyhderbal extract prolonged the stress tolerance capacity of the rat, indicating the possible involvement of a higher center (Whittle, 1964). Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of nonsteroidal anti-inflammatory drugs and selective COX[1]-2 inhibitors (Winter CA,et.al 1962).. Several lines of evidence indicate that the COX-2-mediated increase in prostaglandin (PG) E_2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. In the paw, the early phase was associated with increases in PGE_2 and thromboxane (TX)B_2 levels and with a peak of COX-2 (Vinegar R,et.al.1969). Therefore, the inhibition of carrageenan induced inflammation by the extract of *Shirishadi* compound could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. The present study on extract of *Shirishadi* compound has demonstrated that this compound has significant analgesic and anti-inflammatory properties, and thus can be use in Bronchial asthma and other inflammatory & painful conditions.

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