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IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *CISSUS PALLIDA*

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ABSTRACT

The present investigation deals with the *in-vivo* acute toxicity studies and *in-vivo* anti-inflammatory activity by Carrageenan induced rat paw edema model of methanolic extract in the stems of *Cissus pallida*. The Literature review reveals the presence of steroids, flavonoids, triterpenoids, and glycosides in *Cissus pallida*. Some plants belonging to Cissus genus have been reported to posses anti-inflammatory activity hence methanolic extract of stems of *C. pallida* were screened for anti-inflammatory activity. Carrageenan induced significant inflammation when compared with the animals treated with the extracts. Standard drug, indomethacin at 10 mg/kg inhibited the inflammation significantly at all time intervals. *C.pallida* exhibited significant anti-inflammatory activity in dose dependant manner. C.*pallida* inhibited the carrageenan induced inflammation significantly at doses of 500 and 1000 mg/kg at 2, 3, 4 and 5 hours.

KEY WORDS: *Cissus pallida*, Carrageenan induced Paw edema, Anti-Inflammatory, Acute toxicity.

1. INTRODUCTION

Plants have been used for medicinal purposes long before recorded history in many countries such as India, China and Africa. Since then, thousands of indigenous plants have been used for the treatment of chronic ailments. Many other herbs and minerals were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants (Agarwal,2007). In 20th century, as part of industrial revolution, the practice of allopathic medicines gained popularity. Eventually, the spirit of herbal medicine declined from conventional medicinal use as safety and effectiveness of herbal medicines were not scientifically corroborated and remain largely unknown. Due to the fact that the safety of medicine has always been around for us, it becomes a common theme to rely on safe treatments. Usage of herbal traditional medicine is rising steadily, because of lesser side effects, affordability and in certain diseases where no suitable allopathic medicines are available. Based on the therapeutics significant of herbs and by proper monitoring of quality, dosage of the drug by the ayurvedic physician is essential. Recent advances in the methodologies used to extract, purify and evaluate plant extracts for biological activity have enable the miniaturization and automation of extremely specific biochemical tests. As a result, in number of patients a resurgence of interest on plants and plant derived products as a source of medicine is increasing. There is an urgent need to re-emphasize and enhance research in natural products in many therapeutic areas (Bhujbal,2008).

Inflammation term is derived from Latin word *inflammare*, which means - to set on fire. Inflammation refers to a vital response of a tissue against injury elicited by physical trauma, noxious chemical or microbiological agents. It is considered to be body's defensive reaction either to inactivate or destroy the injurious foreign agent or organism. It is triggered by the release of chemical mediators which includes amines such as histamine, serotonin and lipids such as prostaglandins and small peptides such as kinins from the injured tissues and migrating cells. Mainly, inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms: Accordingly, pharmacological methods for anti-inflammatory evaluation have been developed by artificially inducing inflammation by using phlogistic agent (irritants) such as: Brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, aerosol, sulfated polysaccharides like carrageenan. The effects can be measured by several methods such as UV-erythema in guinea pigs, Vascular permeability, Croton-oil ear edema in rats and mice, Paw edema in rats and Granuloma pouch technique (Brooks,1991).

2. METHODOLOGY

2.1 Toxicity Studies on stems of methanolic extract of *C. pallida* in rats

2.1.1 Acute toxicity study in rats with test drugs: The animals were used with the approval of Institutional Animal Ethics Committee (Reg .No. 627/02/A/CPCSEA). Two groups, each of three female rats, were

treated with methanolic extract of stems of *C. pallida* by oral administration at a dosage of 2000 mg/kg body weight. The test drugs were formulated in vehicle (distilled water) at a concentration of 2000 mg/kg and administered at the dose of 1ml/kg.

2.1.2 Treatment: The animals received a single dose of the test item by oral administration at 2000 mg/kg body weight for groups I and II, after being fasted for approximately 18.0 hours but with free access to water. Food was provided again at approximately 3.0 hours after dosing for both the groups. The administration volume was 10 ml/kg body weight. The animals were dosed using 18 G oral Stainless steel feeding tubes. The animals were observed daily during the acclimatization period and mortality/viability and clinical signs were recorded. All animals were observed for clinical signs during first 30 minutes and at approximately 1, 2, 3 and 4 hours after administration on test day 0 and once daily during test days 1-14. Mortality/viability was recorded twice daily during days 1-14 (at least once on day of sacrifice). Body weights were recorded on test day 0 (prior to administration), test days 7 and 14. All animals were necrosed and examined macroscopically(Dorni,2006).

2.1.3 Necroscopy: All animals were sacrificed at the end of the observation period by carbon dioxide in euthanasia chamber and discarded after the gross/macroscopic pathological changes were visually observed and recorded. No organs or tissues were retained.

2.2 Screening for anti-inflammatory activities of methanolic extract of stems of *C. pallida* in rats

2.2.1 Carrageenan induced rat paw edema model: The model is based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation. All these events occur as a result of metabolism of arachidonic acid. The pharmacological screening of the *C. pallida* was carried out using standard protocols[1]. The crude extract was suspended in 1% carboxy methyl cellulose (CMC) for administration to albino rats. Albino rats of 150-200 g were used for present investigation and were used with the approval of the Institutional Animal Ethics Committee (Regd. No. 627/02/A/CPSCSEA). They were kept in polypropylene cages in an air-conditioned area at 25 $\pm$ 2^{o}C in 10-14 hours light dark cycle. They were provided with Amruth brand balanced feed and tap water *ad libitum* (Eshrat MH, 2003).

2.2.2 Experimental procedure: Seventy two rats were divided into five groups (n=6) starved overnight with water adlibitum prior to the day of experiment. The group I kept as carrageenan control, groups II to IV received test drugs at different doses and group V kept as standard drug control, respectively. Left paw was marked with ink at the level of lateral malleolus; basal paw volume was measured plethysmographically by volume displacement method using Plethysmometer (UGO Basile 7140) by immersing the paw till the level of lateral malleolus. In the experiment, animals from the control group were given vehicle control, Carboxy Methy Cellulose (CMC) and animals from standard drug were treated with Indomethacin 10 mg/kg b.w orally as given in table 2. Other groups were treated with different doses of test drugs orally as given in table 2. After 30 min. of drug treatment the rats are charged by a subcutaneous injection of 0.1ml of 1% solution of carrageenan into the sub-plantar side of the left hind paw (Gupta R, Bajpai KG, 2008). The paw volume is measured again at 1, 2, 3, 4 and 5 hours after charging. The increase in paw volume is calculated as percentage compared with the basal volume. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percent increase in paw volume was calculated using formula as follows.

$$\% \text{ increase in paw volume} = \frac{(V_t - V_c)}{V_c} X\, 100$$

Where Vt = paw volume at a time t, Vc = paw volume at a time 0

3. RESULTS

All animals survived in group I and group II until the end of the experimental period. All the animals dosed at 2000 mg/kg body weight did not show evident toxicity throughout the experimental period. The animals showed increase in their body weight by day 14 as compared to day 0. No abnormalities were detected for all the animals at necropsy. Based on the results, the median lethal doses (LD_{50}) of, *C. pallida*

was greater than 2000 mg/kg b.w and were classified as category 4 (it indicates that no death was observed at 2000mg/kg b.w.) as per OECD guidelines (Hoareau and DaSilva,1999).

4. CONCLUSION

In the control group, carrageenan induced significant inflammation when compared with the animals treated with the extracts. Standard drug, indomethacin at 10 mg/kg inhibited the inflammation significantly at all time intervals. *C. pallida* exhibited significant anti-inflammatory activity in dose dependant manner. C. *pallida* inhibited the carrageenan induced inflammation significantly at doses of 500 and 1000 mg/kg at 2, 3, 4 and 5 hours.

Table.1 Body weight analysis of test drug treated rats

Test drug	Group	Dose(mg/kg bodyweight)	Animal Numbers	Sex	Test day 0 (treatment)(g)	Testday7 (g)	Testday14 (g)
C. pallida	I	2000	002007	Female	211.83	222.95	254.06
			002008	Female	206.12	231.41	261.16
			002009	Female	205.04	231.78	260.85
	II	2000	002010	Female	212.12	240.54	255.72
			002011	Female	211.35	231.35	253.24
			002012	Female	201.54	231.54	252.63

mg/kg = miligram/kilogram, g = gram

Table.2 Macroscopic findings of animals from test drug treated groups

Test drug	Group	Dose (mg/kgbw)	Animal Numbers	Sex	Mode of death	Macroscopic findings
C. pallida	I	2000	002007	Female	T.S.	N.A.D.
			002008	Female	T.S.	N.A.D.
			002009	Female	T.S.	N.A.D.
	II	2000	002010	Female	T.S.	N.A.D.
			002011	Female	T.S.	N.A.D.
			002012	Female	T.S.	N.A.D.

bw = body weight,T.S.= Terminal Sacrifice, N.A.D.= No abnormalities Detected

Table 3: Showing percentage of inflammation indicating Anti-inflammatory activity of test drugs on carrageenan induced rat paw oedema

Groups	Drug Doses	Percentage of inflammation at time (hours)				
		1	**2**	**3**	**4**	**5**
I	Carrageenan Control	38.83± 1.03	81.83± 1.16	113.3± 2.39	131.0± 4.98	146.72± 5.46
II	*C.pallida* 250mg/kg	36.60± 1.15	79.17± 2.18*	102.62± 6.05**	111.52± 4.45***	119.80± 5.37 ***
III	*C.pallida* 500mg/kg	35.83± 1.97**	62.57± 1.74***	80.63± 3.33***	93.37± 2.84***	108.56± 3.46 ***
IV	*C.pallida* 1000mg/kg	29.52± 1.63***	57.22± 1.62***	71.35± 1.91***	80.55± 1.42***	98.64± 2.68 ***
V	Indomethacin 10 mg/kg	12.55± 0.86***	19.1± 0.48 ***	27.17± 1.07***	32.65± 1.52***	30.15± 2.50 ***

* P<0.05, ** P<0.01, *** P<0.001 significant from control

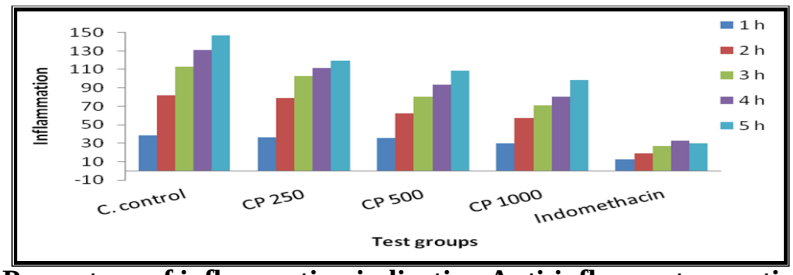

Figure.1 Percentage of inflammation indicating Anti-inflammatory activity of *C. pallida* in carrageenan induced rat paw oedema

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