



Research Article

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ANTI-INFLAMMATORY POTENTIAL OF CHLOROPHYTUM BORIVILIANUM SANT. & FERN. ROOT TUBERS

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ABSTRACT

The methanolic extract of *Chlorophytum borivilianum* Sant.& Fern. root tubers (MECB) (200 & 400mg / Kg b.w., p.o.) were tested in Carrageenan induced rat paw edema and cotton pellet induced granuloma in rats to assess anti-inflammatory potential of MECB. The paw volumes in rats were reduced significantly ($P<0.05$) as compared to that of control and standard drug. Inhibition of granuloma formation in rats were significant ($P<0.05$) as compared to that of control and standard drug. This result indicates that the extract is possessing anti-inflammatory potential.

Key words: Safed Musli, *Chlorophytum borivilianum*, Anti-inflammatory, Carrageenan, edema, granuloma.

INTRODUCTION:

Anti-inflammatory drugs are counter acting or suppressing inflammation. One of the characteristic of living tissues is its ability to react to injury. The reaction of living tissues to injury, which comprises a

series of changes of the terminal vascular bed, blood & connective tissues, which tend to eliminate the injurious agent to repair the damaged tissues, may be called as 'inflammation'. There are two types of

inflammation i.e. Acute and Chronic inflammation. The classical sign of acute inflammatory reaction are like warmth, redness, pain, swelling & loss of function.

The intensity and the localization of the reaction is determined by both severity of the injurious agent and the reactive capacity of the host. Chronic inflammation is also characterised by pain, redness and swelling but it does not subside in a period of days, but may instead have a relentless, damaging course of several weeks, months or years. Various in- vivo & in -vitro models have been proposed as being able to detect anti - inflammatory effect.

The most common screening model of acute inflammation has been the prevention of carrageenan induced edema in rats. This is believed to be biphasic. The 1st phase is due to release of histamine and serotonin, the 2nd phase is caused by the release of bradykinin, protease, prostaglandin and lysosome. It has been reported that the 2nd phase of edema is sensitive to most clinically effective anti-inflammatory agents ¹(Mukherjee PK, 2002)

Cotton pellet granuloma is used as an experimental animal model of chronic inflammation test. Increment in the dry weight of the pellets is taken as a measure for granuloma formation² (Winter and Porter, 1975). The test drug should

significantly inhibit the granuloma formation in rats. Multiplications of small blood vessels as well as proliferation of fibroblasts are the characteristic feature at the repair phase of inflammation. Such proliferating cells penetrate the exudate, producing a highly vascularized reddened mass known as granulation tissue ³ (Swingle, 1974). The test drug extract when effectively and significantly reduced cotton pellet granuloma suggests its activity in the proliferative phase of the inflammation process.

Indomethacin is the choice of standard drug for both the model and used in both acute as well as chronic inflammation.

Chlorophytum borivilianum Sant. & Fern. (Safed musli) is a short, rhizomatous herb, distributed in tropical & sub-tropical regions at an altitude of 1500 mts of the world and belongs to family liliaceae ⁴ (Satija and Singh,2005). It is a native Indian plant with versatile therapeutic uses. An age-old and popular constituent in Indian folklore medicine, it is also a key ingredient in several Ayurvedic, Unani, Siddha formulations. It is used as tonic for complete rejuvenation of human body. It acts as aphrodisiac, galactagogue and useful in bleeding piles, increasing sperms and arthritis. In Rajasthan it is used as a folklore medicine in the form of Battisha is given to the

women during post pregnancy 5 (Gurav and Singh, 2004).

Present authors are working extensively on this subjected plant and already reported on botanical identification characters, Pharmacognostical & phytochemical studies for proper identification of this plant along with different Pharmacological activities like, acute oral toxicity & psychopharmacological efficacy, analgesic, anti-diabetic activities, etc. of this wonder plant known in market as 'Safed Musli'.

No researcher has yet reported on anti-inflammatory activity of root tubers of this plant. Therefore, it is worth conducting an investigation on the anti-inflammatory activity of methanolic extract of *C. borivilianum* root tubers.

Materials and Methods

Plant material:

Authenticated planting materials were collected through NBPGR (ICAR) from NRC for M & AP, Anand, Gujrat bearing DS no. 413 dated 5th July 2004 and planted by following standard method of cultivation in herbal garden. *C. borivilianum* root tubers were harvested in time and voucher specimen preserved for future references.

Root tubers were made free from aerial parts & wiry rootlets and thoroughly washed, peeled & shade dried. They were

powdered to 40 mesh and stored in airtight glass container for further work.

Preparation of Extract

The powder root tubes were subjected to successive extraction with Pet. Ether (40^o – 60^oC), chloroform, ethyl acetate and methanol in a soxhlet apparatus. Solvent from the methanolic extract was removed by vacuum distillation and a brown colour viscous residue was obtained (Yield- 9.78%w/w with regard to the dry weight basis of plant material) and stored in a desicator. The preliminary phytochemical analysis, of the extract showed positive test and for the presence of alkaloid, glycoside, steroids, saponin and flavonoids. The methanolic extract of *Cholorophytum borivilianum* Sant. & Fern. Root tubers (MECB) used in preparation of test drug and subjected for evaluation of anti-inflammatory potential.

Animals:

Adult Wister albino rats weighing between 150-200g of both sexes in equal numbers were used for the study. The animals were housed in standard polypropylene cages at room temperature and provided with standard diet with water *ad libitum*. The study was permitted by the Institutional Animal Ethics committee and all efforts were made to minimize animal suffering and to reduce the number of animals.

Preparation of anti-inflammatory agent:

1% w/v solution of Carrageenan was prepared with normal saline.

Preparation of standard and test drugs:-

Indomethacine at dose of 10 mg / kg body weight suspended in 2 % gum acacia in distilled water was used as standard drug and methanolic extract (MECB) at 200 & 400 mg/kg body weight suspended in 2 % w/v gum acacia in distilled water were used as test drugs.

Evaluation of anti-inflammatory activity**(a) Carrageenan-induced rat hind paw edema (acute test model)**

Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-oedematous effect of natural products. The anti-inflammatory activity of MECB was evaluated by using Carrageenan induced rat paw edema method^{6,7} (Winter *et al.* 1962. Bodakhe *et al.* 2008). Rats were divided into four groups of six animals in each and fasted over night with free access to water prior to the study. Group-I was served as control group and received only vehicle 2% w/v gum acacia in distilled water in dose of 2ml / kg of body weight orally. Group-II served as standard group & received standard drug Indomethacine in dose of 10mg/kg body weight orally and Group-III & IV served as test groups were treated with methanolic extract of C.

borivilianum root tubers (MECB) as test drug at doses of 200 & 400mg/ kg body weight orally. The doses of extract were chosen, based on those in an earlier study. Half an hour after the respective treatment, 0.1ml of 1% w/v freshly prepared Carrageenan in normal saline was injected in sub-planter region of left hind paw of rats to produce the acute inflammation. The paw volume was measured at 0 hr i.e. immediately after Carrageenan injection and then at 1,2,3 & 4 hr by the volume displacement method using Plethysmometer. The average paw swelling in the group of extract treated rat was compared with control & standard group and percent change in edema was calculated by the formula:

% of edema inhibition (anti-inflammatory activity) = $[1-(V_t/V_c)] \times 100$

Where, V_t is the volume of treated group and V_c is the volume of control group.

Cotton pellet Granuloma (Chronic test model)

Chronic Inflammation in rats were produced by sub-cutaneous implantation of pellet of cotton in rats method^{8,9} (Winter & Porter, 1975, Bodakhe *et al.*,2009). In this method, some Giant cells and connective tissues can be observed beside the fluid infiltration after seven days of sub-cutaneous implantation of pellet of cotton in rats. Animals were grouped as described in acute test model to study the

anti-inflammatory activity. The groups were fasted and treated with drugs / doses similar to that of Carrageenan induced hind paw edema. Sterile cotton pellets each weighing 10 ± 0.7 mg were prepared and sterilized in a hot air oven at 123°C for 3 hrs. Each animal was placed under light ether anesthesia and an incision was made on the lumber region by blunted forceps. A sub-cutaneous tunnel was made and a sterilized cotton pellet was inserted in the groin area. All the animals received either MECB or indomethacin or vehicle (2% w/v gum acacia) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion. On the 8th day, animals were anesthetized again and cotton pellets were removed & dried to constant mass.

STATISTICAL ANALYSIS

The observation values are reported as mean \pm SEM of six observations. The significance of difference among the various treated groups and control group were analysed by means of one way ANOVA followed by Dunnet's t-test. The value of less than 5% ($p < 0.05$) was considered statistically significant¹⁰ (Kulkarni SK,1993).

RESULTS AND DISCUSSION:

Effect on carrageenan induced rat paw edema:

The results of carrageenan induced rat paw edema are summarized in Table-I.

The result obtained indicates that the extract possesses significant ($P < 0.05$) anti-inflammatory activity in rats. The MECB at the test doses 200 and 400 mg/kg body weight reduced the edema by carrageenan induced method to 35.06% & 42.8% and 34.6% & 42.3% respectively at 2h & 3h, whereas the standard drug showed 58.4% & 55.1% of inhibition respectively at 2h & 3h as compared to the control group (Figure -I).

The pre-treatment with MECB resulted in a significant and dose-dependent reduction in carrageenan induced paw edema in rats. The percent inhibition was comparatively less at 1 hr after treatment with MECB at all the doses when compared to the effect of indomethacin. The maximum inhibition of paw volume was observed at 2 and 3 hr after treatment with MECB at each dose but the activity was almost consistent from 2 to 3 hr and it decreased after 3 h.

Effect on cotton pellet granuloma in rats:

The results of the effect of MECB on granuloma formation in the cotton pellet method are presented in Table II. MECB treatment at the test doses of 200 and 400 mg/kg body weight caused 33.9%, 39.8% inhibition respectively. Significant effect was observed at 200 & 400 mg/kg body weight as shown in figure -II. The standard

drug indomethacin caused 51.8% inhibition as compared to the control.

From the result, the test drugs show effectively and significantly reduced cotton pellet granuloma, which suggests its activity in the proliferative phase of the inflammation process. Such proliferative cells penetrate the exudate, producing a highly vascularized reddened mass known as granulation tissue.

The test drugs significantly inhibit the granuloma formation in rats. Multiplication of small blood vessels as well as proliferation of fibroblasts are characteristic features at the repair phase of inflammation.

DISCUSSION:

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3-4 h after carrageenan injection) kinin and prostaglandins are involved¹¹ (Hernandez and Rabanal, 2002). Our results revealed that administration of MECB inhibited the edema starting from the first hour and during all phases of inflammation, which is probably due to inhibition of different aspects and chemical mediators of inflammation.

To further verify the anti-inflammatory activity of MECB on the proliferative phase of inflammation, the cotton pellet granuloma bioassay model was used. At the doses used in the model, MECB, showed a significant inhibitory effect on granuloma formation. This study revealed that MECB was active against the inflammation induced by a foreign body as well. The cotton pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transudate, the dry weight of the pellet correlates with the amount of granulomatous & tissue^{12,13} (Lowry *et al.*, 1951; Castro *et al.*; 1968). In cotton pellet granuloma the efficacy of extract to inhibit the inflammation might be due to an increase in number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation¹⁴ (Arragonime, 1977). The MECB showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

CONCLUSION:

This study substantiated the reason for using the plant for arthritis in the traditional system of medicine. Since the extract contains a mixture of compound, studies are planned to work with the

extract for further isolation and characterization of compound and find which compound / compounds are responsible for such activity.

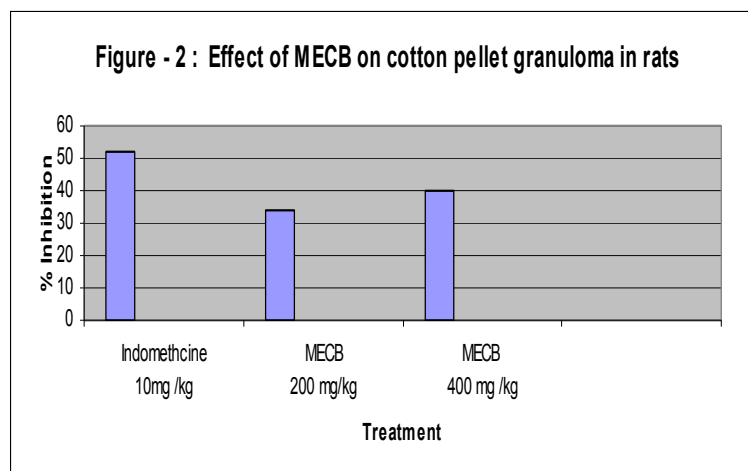
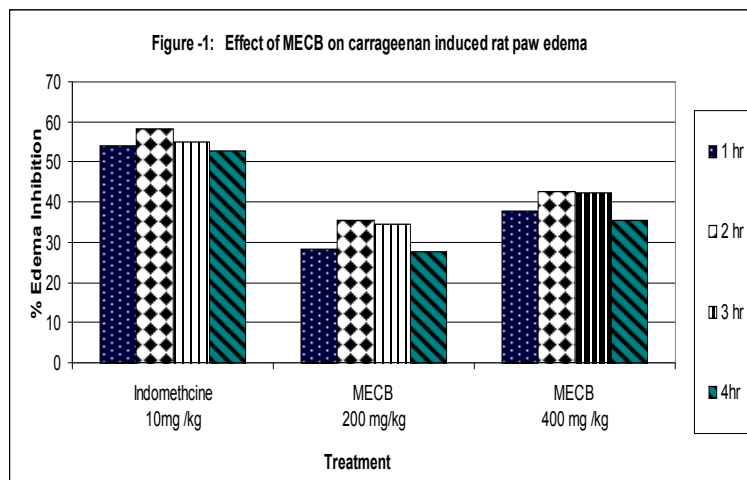


Table-I: Effect of MECB on carrageenan induced rat paw edema

Group (n=6)	Treatment	Dose	0 hr	Paw volume (ml) and percentage inhibition (%)							
				1.00 hr		2.00 hr		3.00 hr		4.00 hr	
				(ml)	(%)	(ml)	(%)	(ml)	%	(ml)	(%)
Gr- I Control	2 % w/v Gum acacia in distilled water	2ml/kg	0.30±0.05	0.74±0.03	-	0.77±0.06	-	0.78±0.02	-	0.76±0.06	-
Gr- II Standard drug	Indomethacin	10 mg /kg	0.26±0.02	0.34±0.02*	54.1	0.32±0.06*	58.4	0.35±0.04*	55.1	0.36±0.03*	52.6
Gr-III Test drug-1	MECB	200 mg/kg	0.28±0.02	0.53±0.02	28.3	0.5±0.1*	35.0	0.51±0.03*	34.6	0.55±0.02	27.6
Gr- IV Test drug -2	MECB	400 mg/kg	0.29±0.04	0.46±0.05*#	37.8	0.44±0.03*#	42.8	0.45±0.03*#	42.3	0.49±0.01*	35.5

Values are expressed as mean ± SEM of six animals in each group

*P<0.05 as compared to control

#P<0.05 as compared to Indomethacin treated group

Table-II: Effect of MECB on cotton pellet granuloma in rats

Group (n=6)	Treatment	Dose	Weight of dry cotton pellet granuloma (mg)	Percentage inhibition (%)
Gr- I (Control)	2 % w/v Gum acacia in distilled water	2ml/kg	35.6±1.29	-
Gr- II (Standard drug)	Indomethacin	10 mg /kg	17.15±1.35*	51.8
Gr-III (Test drug-1)	MECB	200 mg/kg	23.52±0.422*	33.9
Gr- IV (Test drug -2)	MECB	400 mg/kg	21.42±1.3*#	39.8

Values are expressed as mean ± SEM of six animals in each group

*P<0.05 as compared to control

#P<0.05 as compared to Indomethacin treated group

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