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Antiinflammatory and Antioxidant Activity of *Chlorophytum borivilianum* Root Extracts

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The aqueous, hydro alcoholic and methanolic extract of *Chlorophytum borivilianum* (liliaceae) roots were investigated for antiinflammatory and antioxidant activity. Antiinflammatory activity was studied by carragenan induced rat paw oedema model. Methanolic and aqueous extract shows most significant antiinflammatory activity as compared to hydro alcohloic extract. Same extracts were studied for antioxidant activity. The assay methods used were DPPH and nitric oxide method. In both free radicals induced assay methods, methanolic extract shows maximum activity as compared to aqueous and hydro-alcoholic extracts.

Key Words: *Chlorophytum borivilianum*, Antiinflammatory, Antioxidant activities.

INTRODUCTION

Chlorophytum borivilianum belonging to family Liliaceae is found in natural forest right from East Assam to Gujarat[1]. It holds very important position in Ayurveda and Unani system where it is mostly used to treat oligospermia, pre and post natal symptoms, arthritis, diabetes and dysuria[2-4]. Present study was done to evaluate its antiinflammatory and antioxidant activities.

EXPERIMENTAL

Chlorophytum borivilianum roots were purchased from local nursery. All the samples were authenticated from Dr. Bhogaonkar, Botany Department, Vidharbha Institute of Humanities and Science, Amravati. The roots were dried, powdered to coarse size and stored in airtight container for further use.

Preparation of the extracts: The powdered root material was extracted using water, methanol and 70 % alcohol in a soxhelet apparatus. The solvent was completely removed by using rotary flash evaporator and to obtain semi solid mass, except water extract which was obtained as dried powder.

Animals: Albino wistar rats of either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory condition for 7 d before commencement of experiment. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. The study was duly approved by IAEC.

Acute toxicity studies: Acute toxicity study was performed according to method of Litchfield and Wilcoxon[5] by observing the number of deaths of mice in different groups (n = 10) treated with the extract in dose range of 200-1000 mg/kg. After an overnight fast, the extracts were administered orally in graded doses (100-1000 mg/kg). They were observed continuously for the first 2 h upto 24 h for toxic symptoms.

Carragenan induced Paw Oedema: Oedema was produced in rats by subplantar injection of 0.1 mL of 1 % (w/v) freshly prepared suspension of carragennan to each animal of 5 groups. All doses were given orally 0.5 h prior to the injection of caragennan. The paw volume was measured at 0, 1, 2 and 3 h after carrageenan injection using a plethysmometer by method of Winter and Poster[6,7].

Antiinflammatory activity: The oedema induced animals were divided into 5 groups (n = 6). The therapeutic treatments were as follows. Doses were calculated as per acute toxicity studies: • Group I- carragenan + 2 % v/v aqueous Tween 80; • Group II- carragenan + diclofenac 5 mg/ kg body weight; • Group III- carragenan + aqueous extract in dose 700mg/kg body weight, p.o; • Group IV- carragenan + methanol-extract in dose 500mg/kg body weight, p.o; • Group V- carragenan + hydro alcoholic extract in dose 500mg/kg body weight, p.o. The % inhibition of paw oedema in the various treated groups was then calculated by using the formula:

$$\text{Percentage inhibition} = (1 - Vt/Vc) \times 100$$

where Vt = is oedema volume in drug treated; Vc = is oedema volume in the control group.

Antioxidant activity

DPPH method: 1 mL of different concentration of extract solutions (100, 250, 500, 1000 µg/mL) and standard were taken in different vials. To this 5 mL of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37 ºC for 20 min. Control experiment without test sample but with equivalent amounts of DPPH was conducted in an identical manner. The absorbance of chromophore formed was measured[8] at 516 nm. Experiment was repeated in triplicate. The decline in radical concentration indicated the radical scavenging activity of the sample. BHA was used as a standard.

Nitric oxide method: 4 mL of sodium nitroprusside (5 Mm) in standard phosphate buffer solution was incubated with different concentration of 1 mL of test solution dissolved in standard phosphate buffer and tubes were incubated at 25 ºC for 2.5 h. Control experiment without test sample but sample with equivalent amount of buffer was conducted in identical manner. After 2.5 h, 1 mL of incubated solution was removed and diluted with 1.5 mL of Griess reagent (10 % sulphanimide 2 % H_3PO_4) and 1.0 % naphthalene diamine dihydrochloride). The absorbance of pink chromophore formed during diazotization of nitrite with subsequent coupling with naphthalene diamine was read[9] at 546 nm. Experiment was repeated in triplicate. Results were calculated by same formula as per DPPH method. BHA was used as standard.

Statistical analysis: The experimental results were expressed as the mean ± SEM data were assessed by the method of analysis of Anova followed by Dunnet's test.

RESULTS AND DISCUSSION

Aqueous and methanolic extracts exhibited significant antiinflammatory activity as compared to hydroalcoholic extract. The results are summarized in Table-1. From the results it can be concluded that aqueous and methanolic extracts after 3 h of treatment in carragenan induced paw oedema, shown maximum activity *i.e.* 83.33 and 80.55 %, respectively. Whereas hydroalcoholic extract at 500 mg/mL shown 50.55 % activity. Standard drug diclofenac showed 91.11 % activity.

TABLE-1
MEAN INCREASE IN PAW VOLUME AND INHIBITION (%)
AFTER TREATMENT

	Dose	Mean increase in Paw volume ± SD			
	(mg/kg, p.o.)	0 h	1 h	2 h	3 h
Carrageenan	0.1 mL/kg subcutaneously	-	0.25 ± 0.06	0.29 ± 0.12	0.36 ± 0.2
Diclofenac	0.5	-	0.06† ± 0.02	0.03† ± 0.04	0.032†±0.05
Inhibition (%)			**76.00**	**89.65**	**91.11**
Aqueous extract	700	-	0.17 ± 0.09	0.18 ± 0.04	0.06† ± 0.09
% Inhibition			**32.00**	**37.93**	**83.33**
Hydroalcholic extract	500	-	0.19 ± 0.03	0.2 ± 0.2	0.16† ± 0.7
Inhibition (%)			**24.00**	**31.03**	**55.55**
Methanol extract	500	-	0.1* ±0.09	0.06† ± 0.07	0.07† ± 0.05
Inhibition (%)			**60.00**	**79.31**	**80.55**

n = 6, * = $p < 0.05$, † = $p < 0.01$, ‡ = $p < 0.001$.

The methanolic extract showed promising antioxidant activity in both type of method while hydroalcoholic extract showed moderate activity and aqueous extract showed poor activity (Table-2).

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY OF BHA BY DPPH METHOD

Concentration of butylated hydroxyl anisole (BHA)	Radical (%) scavenging activity (DPPH method)	IC_{50} (µg/mL) (DPPH method)	Radical (%) scavenging activity (nitric oxide method)	IC_{50} (µg/mL) (nitric oxide method)
10	62.19 ± 0.16	8.03	56.159 ± 0.11	8.90
20	73.82 ± 1.38		67.130 ± 0.12	
40	80.32 ± 0.17		75.790 ± 1.23	
60	88.52 ± 0.05		89.540 ± 0.62	

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