SCREENING OF ANTISTRESS PROPERTIES OF *CHLOROPHYTUM BORIVILIANUM* TUBER

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Summary

Chlorophytum borivilianum belonging to family liliaceae is a very famous for its adaptogenic, immunomodulatory properties in traditional Indian system of ayurveda. In recent era there is a great thrust on screening of herbal extracts and formulations for Adaptogenic action as stress is a daily phenomenon faced by every human. The aim of the present study is to investigate the antistress activity of Aqueous and alcoholic tuber extracts of *Chlorophytum borivilianum*. This property is assessed by swim endurance stress, anorexic test in rats and despair swim test. Cold stress induced Gastric ulceration model was also selected to evaluate antiulcer activity. The effect of single oral dose of the extracts was evaluated at 30, 100 and 300 mg/ kg. It was found that alcoholic extract significantly increases swimming time and reduces the ulcer index compared to that of control group. A significant effect ($p < 0.001$) from 200 mg/ kg dose for both the extracts was observed in all four models.

Key Words: Antistress, anorexic test, despair swim test, swimming endurance test, Cold stress

Introduction

Chlorophytum borivilianum belonging to family liliaceae is a well known plant for its aphrodisiac as well as adaptogenic activity in India.[1] Roots are claimed to be useful to treat oligospermia, pre and post natal symptoms, arthritis, diabetes and dysuria [2.] Saponin, polysaccharides and mucilage are major constituents of *Chlorophytum borivilianum3,4.* Recent pharmacological studies on tubers of *Chlorophytum borivilianum* has indicated antiviral[5,] anticancer[6,] anti-oxidant[7,] antidiabetic[8,] antistress[9,10,] aphrodisiac[11], antimicrobial[12,] antidiabetic[13,] hypolipidemic[14,] anti-inflammatory[15], immunomodulatory[16,] properties. Recently four new steroidal saponins have been isolated from plant tuber[17.] This multifunctional nature of *Chlorophytum borivilianum* tubers made to select the present study to evaluate its anti-stress (adaptogenic) properties. Previously two models of this property have been already studied by Kenjale R. D. et.al 2007 and Patil et al. 2006 but present study is evaluating more models to confirm plant,s adaptogenic property..

Materials and Methods

Plant material collection and authentication:
Chlorophytum borivilianum roots were purchased from local vendor. The roots were dried, powdered to coarse size and stored in airtight container for further use. Plant species is authenticated by Botanist Dr.Prabha Bhogaonkar, Vidarbh institute of science and humanities, (V.M.V), Amravati. A specimen sample is deposited at Dept. of Botany, Vidarbh institute of science and humilities, (V.M.V), Amravati.

Extraction:
The *C. borivilianum* tubers were powdered and defatted by petroleum ether. Marc then extracted with ethanol for 3 hour with mild heating. The Aqueous extract was prepared by maceration process by treating 100g of fresh powder with 500ml of distilled water along with 10ml of chloroform as a preservative .The maceration process was carried for 7 days with intermittent stirring. Both the extracts were filtered and evaporated to dryness under vacuum. This Alcoholic (AL) and Aqueous (AQ) extracts were used for further study.

Preliminary Phytochemical Screening[18]
Both extracts were screened for the presence of various secondary metabolites by adopting standard procedures.

Experimental Animals
Adult Swiss albino mice (20- 25g) and Wistar Rats (150 -200g) of either sex were used for the study. The mice and rats were fed with standard pellet and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by IAEC. (No.CPCSEA/IAEC/PC-01/04-2K8)

The toxicity study:

Acute toxicity[19]
The study was performed according to the acute toxic classic method (as per OECD guidelines). Swiss albino mice were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the test drug extract dissolved in water was administered orally at the dose of 2000 mg/kg and observed for 14 days. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion). The toxicity study carried out as per the guidelines of AOT- 421 using albino mice. The extracts were found to be safe till 2000mg/kg. Hence the three doses (30, 100, 300 mg/kg) have been used.

Swim endurance test[20]

Swiss albino mice were randomly divided into 4 groups of 6 animals each consisting Group II, II III and IV were subjected to the oral administration of extract at the dose of 30, 100 and 300 mg/kg, p.o respectively whereas group I received dose of 30, 100 and 300 mg/kg, p.o. for 21 days. This duration was selected from our pilot study.

Swimming test is carried out on 21st day one hour after the drug administration with upper cut off of 600 sec. on day 14 and 21 mice were allowed to swim in cylindrical container filled with water maintained at 25 ± 2°C till they got exhausted and the moment they drowned was considered as the endpoint ("Swimming Time"). The time was noted and the data obtained were subjected to statistical analysis.

Anoxic stress tolerance test[20]

Swiss albino mice (25 ± 2 g) of either sex were used for evaluating anoxic stress tolerance test. The normal animals were treated with normal saline (10 mL/kg, p.o.) and other five groups were treated with test drug extract in three different doses (30, 100 and 300 mg/kg, p.o.). Conical flasks of 250 mL capacity were used for the study. These flasks were made airtight using rubber cork before beginning the experiment. On day 14th and 21st, 1 hr after the treatment, each animal was placed in the airtight vessel and time was observed using a stopwatch. The moment animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of "Anoxic stress tolerance". The data obtained were subjected to statistical analysis.

Chronic stress induced behavioral despair test [20]

Wistar albino rats (180-200 g) of either sex were used for the study. Four groups treated as per schedule along with daily foot shock of 5mA for the period of 06 second with the interval of 90 seconds for 15 minutes. One hour after the drug administration on 14th and 21st day rats were forced to swim individually in a cylindrical container (60 x 40 cm, h x d) containing water (maintained at 25°C ± 1°C) upto 30 cm height, which ensured that Rat's feet did not touch the floor of the vessel for a period of 05 minutes and total duration of immobility was noted down by complete cessation of swimming with the head floating.

Cold restraint ulcers (Stress Ulcers) [21, 22, 23]

Rats were divided in to 04 groups treated with respective extracts for a period of 07 days. These rats were deprived of food and water for 24 hrs prior to test. On the 8th day rats were immolised by strapping the fore paw and hind limb on a wooden plank and kept for 2hrs at a temp of 4 ^{o} C in an environmental chamber. Thereafter rats were sacrificed by decapitation, stomach was cut open at greater curvature and ulcers were observed and ulcer index was calculated by the method of Alphin and Ward (1969).

Statistical Analysis

The data were analyzed by ANOVA followed by Student's't' test P values <0.01 were considered significant.

Result and Discussion

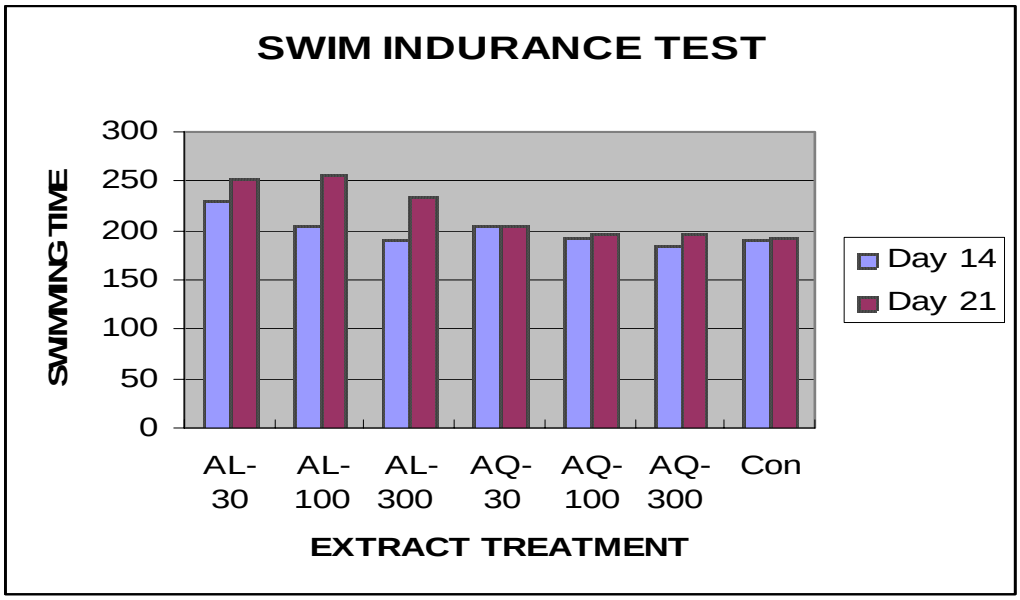
Stress is the body's physical, mental or chemical reaction observed usually during excitation, confusion or in unsafe condition. A large proportion of all illness is believed to occur due to stress closely associated with modernization of life. Fortunately Mother Nature has an answer to this challenge - a unique class of herbal products called "adaptogens". Adaptogens have the broad-spectrum healing properties of any herbal medicines, but their unique value is that they specifically relieve stress. According to modern science adaptogens are natural plant products that increase the body's ability to cope with internal and external stress factors, and normalize the functions of the organism.

The phytochemical studies of extracts revealed the presence of alkaloids, steroids, saponin glycosides in alcoholic extract and carbohydrates, saponin glycosides in Aqueous extract.

Swim endurance test

Rodent when forced to swim in restricted space become immobile after initial period of vigorous mobility. Hence Swimming endurance time was used as antistress parameters for preliminary adaptogenic activity screening of the extract. From results (Fig. 1) it is clear that mice pretreated with extract showed significant improvement in the swimming time and thereby showed the antistress activity. The alcoholic extract is more effective in this regard. However the dose of 100 mg/kg showed lesser effect at 21 days when compared with the effect of 14th days. This is seen with many CNS activities as part of saturation. Overall the AL extract at a dose of 300mg/kg is most effective dose and the peak action is seen at the end of two week which further showed sustained effect.

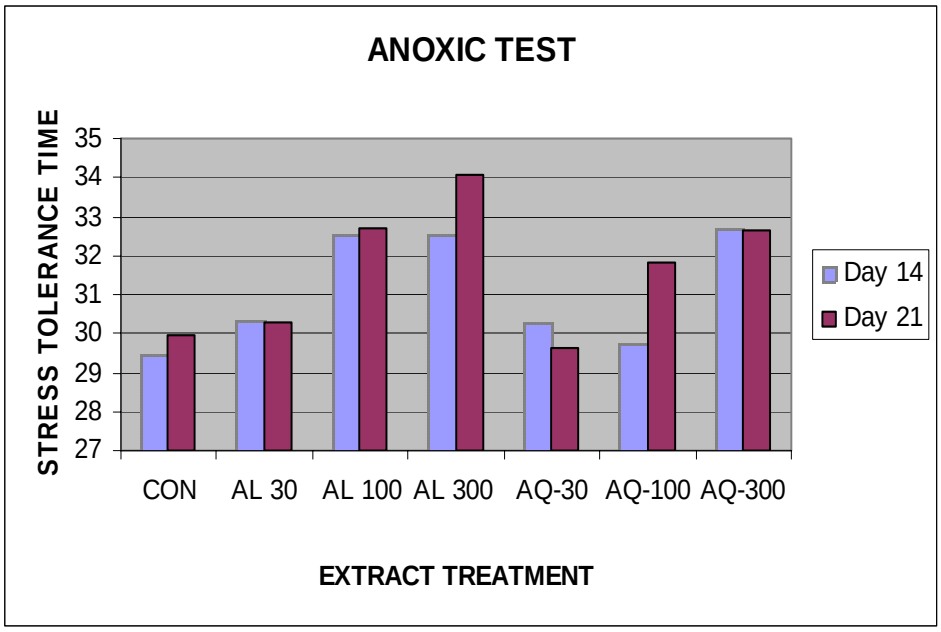
Figure no. 1: Effect of extracts on swimming time in swim endurance test



Anoxic stress

The exposure of hypobaric environment to mice for a specified period causes significant decrease in brain neurotransmitters, i.e. norepinephrine (NE), dopamine (DA), serotonin (5-HT) and acetylcholine (ACh) and hence the observed drug effective in this model may be effective by the modulation of above mentioned neurotransmitters. The significant increase in anoxia tolerance time is an indication of either resistance to it or reduction in cerebral oxygen consumption. Both these effect are quite useful to protect neuronal cell against oxidative stress, a clinical condition increasing with the modernisation of life. In this model of severe stress the alcoholic extract was found to be effective whereas Aqueous did not show any effect up to 200 mg/kg dose. Pretreatment with *C. borivilianum tuber AL extract* significantly *($p<0.01$)* increased (Fig. 2) the anoxic stress tolerance time (34.06 ± 0.67 minute) in mice in a dose related manner. Whereas AQ extract increased the anoxic stress tolerance only at 300mg/kg dose.

Figure no. 2: Effect of extracts on stress endurance time in anoxic test



Despair swim test

The despair swim test is a model of chronic stress. The chronic shock treatment cause significant increase in the immobility time, indicative of depression In this evaluation on 14th day, the alcoholic extract significantly *($p<0.01$)* reduced **(Table 1, 2)** the stress induced increase in the immobility period (39.63 ± 1.17 seconds) in the forced swimming test in rats at higher two dose showed significant anti stress activity whereas Aqueous extract only at 300mg/kg dose and upon long term administration have shown the effect **(Table 3, 4)**. This indicate that high dose of alcoholic extract can only be effective when there is role of multiple stressors.

*Table no1: Results of Alcoholic extract on 14*th day (**Despair Swim test**)

CON	AL 30	AL 100	AL 300 (14 days)
43.2	40.09	45.5	38.8
40.09	42.28	40.28	35.53
51.72	43	42.2	43.3
48.8	48.8	45.45	40.18
50.01	50.05	50.11	42.2
45.56	39.9	53.17	37.81
46.56	44.02 NS	46.11NS	39.63* (Mean)
1.8	1.78	1.96	1.17 (SEM)

*Table no.2: Results of Alcoholic extract on 21*st day (**Despair Swim test**)

CON	AL 30	AL 100	AL 300 (21 days)
47.7	42.29	38.17	35.55
42.2	44.4	39.9	37.18
43	48.81	41.27	40.09
44.19	39.95	39.73	42.29
39.98	49.18	37	36.02
46.6	43	40.07	38.87
43.94	44.60 NS	39.35*	38.33**(Mean)
1.16	1.5	0.62	1.05 (SEM)

*Table no.3: Results of Aqueous extract on 14*th day (**Despair Swim test**)

CON	AQ-30	AQ-100	AQ-300 (14 days)
40.03	37.77	39.9	43.3
38.89	38.26	41.25	44.01
37.16	43	43.3	40.56
36.66	39.04	38	39.82
43.02	38.27	44.17	42.16
42.2	41.1	42.2	38
39.66	39.57NS	41.47 NS	41.30 NS (Mean)
1.06	0.83	0.92	0.92 (SEM)

*Table no.4: Results of Aqueous extract on 21*st day (**Despair Swim test**)

CON	AQ-30	AQ-100	AQ-300 (21days)
35.55	36.6	35.45	33.3
34.4	37.28	38.89	35.55
39.02	39.91	39.9	38.78
41.16	40.01	40.02	31.11
37.73	41	41.19	30.09
40.02	38.87	42.02	32.28
37.98	38.94 NS	39.57 NS	33.51*(Mean)
1.06	0.69	0.93	1.30 (SEM)

Cold restraint ulcers (Stress Ulcers)

From the result (Table no. 5 and 6) of the anti ulcer activity showed that both Aqueous and alcoholic extract (from 200mg/kg) produced protection against gastric ulcer induced by cold stress and thereby pointed antiulcer activity. But alcoholic extract of *C. borivilianum* (300mg/kg) markedly reduced the ulcer index to 25.33± 1.58($p<0.01$) when compared with control (44.83±2.42) and standard (9.16 ± 0.6).The alcoholic extract showed significant anti ulcer activity when compared to Aqueous extract.

Table no.5: Results of Alcoholic extract on 7h day (**Cold Restraint Stress Ulcers)**

Con	AL-30	AL-100	AL-300 (14 days)	omez-20
41	41	45	20	9
39	40	34	28	7
50	43	40	26	8
42	40	38	24	11
53	50	41	31	10
44	46	33	23	10
44.83	43.33	38.5*	25.33**	9.16** (MEAN)
2.42	1.62	1.83	1.58	0.60 (SEM)

Table no.6: Results of Aqueous extract on 7h day (**Cold Restraint Stress Ulcers**)

Con	AQ-30	AQ-100	AQ-300 (14 days)	omez-20
41	43	36	36	9
39	35	33	30	8
50	39	39	29	7
42	31	31	27	10
53	42	30	28	10
44	40	29	33	9
44.83	38.33**	33**	30.5**	8.83** (MEAN)
2.42	1.85	1.57	1.38	0.47 (SEM)

Conclusion

C. borivilianum extract possess significant antistress and adaptogenic activity against different types of model indicating putative mechanism of action.

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