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Anti-stress and anti-oxidant effects of roots of *Chlorophytum borivilianum* (Santa Pau & Fernandes)

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The aqueous extract of *C. borivilianum* (250 mg/kg for 7 days) significantly reverted the elevated levels of plasma glucose, triglycerides, cholesterol and serum corticosterone and also reduced the ulcer index, adrenal gland weight more as effectively as the standard drug (diazepam) in rats. At 125 mg/kg po, it showed a mild anti-stress activity. Under *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical scavenging assay and lipid peroxidation assay the extract considerably inhibited, in a dose-dependent manner, the levels of DPPH• free radicals and thiobarbituric acid reactive substances, respectively thus showing significant antioxidant property. The results suggested that it could be used for the treatment of oxidative stress-induced disorders.

Keywords: Adaptogen, Anti-oxidant, *Chlorophytum borivilianum*, Immobilistation stressor, Lipid peroxidation

During stressful situations, the energy requirement of the organism is increased, resulting in enhanced generation of free radicals[1-3]. Free radicals cause oxidation of nucleic acids and proteins. Free radicals also damage biomembranes, reflected by increased lipid peroxidation, thereby compromising cell integrity and function. During this process, the ability of the body's defense system to combat the oxidative stress may diminish due to reduced anti-oxidants. Supplementation with various macro- and micronutrient and herbal preparations has been evaluated for their adaptogenic activity during exposure to a stressful environment[4–7]. It is possible to support the body's adaptation by using food supplements, dietary elements, herbs and minerals for increasing physical and mental performance, described in various oriental systems of medicine including the ancient Indian medical system, Ayurveda. Such substances have been described as 'adaptogens'[8].

An adaptogen (i) produces a non-specific response in an organism; i.e., an increase in power of resistance against multiple stressors including physical, chemical or biological agents; (ii) has a normalizing influence on physiology, irrespective of the direction of change from physiological norms caused by the stressor, and (iii) is incapable of influencing normal body functions more than required to gain non-specific resistance[9].

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Roots of *Chlorophytum borivilianum* have been traditionally used as an aphrodisiac, adaptogenic and a general health promotive tonic. It is also said to be endowed with *Rasayana* properties i.e. it delays the ageing process by rejuvenating the entire system. It consists of alkaloids and steroidal saponins (stigmasterol) which are considered to be the active constituents[10]. In India, according to Ayurvedic system of medicine, several plants are claimed to possess adaptogenic potential. However, in most cases the validity of these claims has not been scientifically tested. Roots of *Chlorophytum borivilianum* belonging to family Liliaceae, is one such example. Hence, in the present study the adaptogenic and anti-oxidant activities of *Chlorophytum borivilianum* (Santa Pau & Fernandes) have been evaluated.

Materials and Methods

The crude root powder of the plant *Chlorophytum borivilianum* was procured from Dr. K.S. Laddha, Pharmacognosy Lab, Mumbai University Institute of Chemical Technology. The crude root powder was authenticated botanically as *Chlorophytum borivilianum* belonging to family Liliaceae.

The crude powder was subjected to standard chemical tests to determine qualitatively the presence or absence of alkaloids, flavonoids, phenols, steroids and triterpenoids, oils, saponins, amino acids and peptides. Preliminary chemical testing of the aqueous solution showed the presence of carbohydrates,

proteins, and saponin glycosides in the aqueous extract of the root powder. The aqueous extract gave almost 50% yield while a methanolic extract gave only about 4% yield. Hence the aqueous extract was selected for further study.

Preparation of the water extract: Exhaustive extraction — Aqueous extract of the root powder was prepared by adding the powder to distilled water in a ratio of 1g:6mL in a beaker heating it in water bath for 2 hr with constant stirring. The resulting aqueous solution was filtered through muslin, while the residue was subjected to the same procedure again for 2 hr; the solution obtained was added to the earlier solution to give the final extract that was dried in hot air oven. The dry aqueous extract (CB) so obtained was stored in a dry container at room temperature for further use.

The extract was solubilised in distilled water 2-3 hr prior to experimental use to obtain the desired concentrations (125 and 250 mg/kg body weight) in 1 mL.

Animals — Study was conducted on healthy, male Wistar albino rats weighing 180-200 g obtained from Nicholas Research Centre, Mumbai and were housed in the registered animal house (CPSCEA No 87/1999) at MUICT in group of 6 in polyethylene cages under standard housing conditions with 12:12 hr light and dark cycle. The animal feed in the form of dry pellets, obtained from M/s D. S. Trading Ltd., Mumbai, and tap water was given *ad libitum*. Standard hygiene conditions were maintained. All the procedures were performed in accordance with the Institutional Ethical Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi, India. Approval No. of Institutional Animal Ethical Committee for this study was: UICT/PH/IAEC/0206/23.

Adaptogenic (anti-stress) activity — The experimental model used was Chronic Cold Restraint Stress model.

Purpose and rationale— During stress induction various biochemical enzymes and immunological system are affected. Some of the stress-induced alterations have been attributed to an imbalance in neuro-endocrine system. Hence, measurement of the some of the endocrinal parameters will serve as important basis for the evaluation of anti-stress activity. The distinct advantage of using immobilization as a stressor lies in the fact that it produces both physical as well as an in-escapable psychological stress[11,12].

Procedure—The rats were divided into following 5 groups of 6 each: control (non-stress group), chronic stress (CS) group, drug treated groups CB125 and CB250 (125 mg/kg and 250 mg/kg, po respectively), and standard drug (diazepam 1 mg/kg) group.

The drugs were administered orally 45 min prior to the stress regimen up to seven consecutive days except that the rats were kept fasted overnight on the 6th day after drug feeding and stress exposure. The stress was produced by restraining the naïve animals inside an adjustable cylindrical plastic tube (6.2 cm diameter, 20 cm long). The rats were confined individually and exposed continuously to cold stress at 4°C for 50 min once only for 7 consecutive days. On day 7 the rats were sacrificed immediately after stress by decapitation, and the blood was collected in tubes containing 4% EDTA, kept on ice and in polypropylene tubes to collect blood plasma and blood serum, respectively. The blood was centrifuged (3000 rpm for 20 min at 4°C) and plasma and serum were separated out and stored at -20°C for biochemical and hormonal assays. The plasma levels of glucose, triglycerides, and cholesterol were estimated using the respective assay kit (Ecoline®) procured from Merck Ltd., USA, whereas serum corticosterone levels were estimated using flourimetry. The adrenal glands were dissected out and weighed. The stomach was dissected out and cut open along the greater curvature for scoring the incidence of ulcer. Ulcer index (UI) was scored according to the method of Gupta *et al.*[13]. Briefly, immediately after the animals were sacrificed the stomach was isolated and cut along the greater curvature. It was cleared of its contents by washing in ice cold phosphate buffered saline. The inner gastric mucosa was then observed for any ulceratic patches. These were given a score depending on the degree of ulceration from 0 to 3, where a score of 0 indicated no ulcer, 1 indicated moderate ulcer, 2 indicated severe and 3 indicated very severe ulcer.

In vitro anti-oxidant activity

Assay of DPPH• radical-scavenging activity — To measure antioxidant activity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical-scavenging assay was carried out by U.V. spectrophotometric method[14].

This method is based on the principle that DPPH• being a stable free radical can accept an electron or hydrogen radical to become a stable diamagnetic

molecule. Due to its odd electron, the methanolic solution of DPPH• shows a strong absorption band at 517 nm. DPPH• radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavenger. Reduction of the DPPH• radicals can be observed by the decrease in absorbance at 517 nm.

The DPPH• radical scavenging activity was measured in a reaction mixture containing 0.1 ml of 1m M DPPH• radical solution, 0.8 ml of 99% methanol, and 0.1 ml of sample solution. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. The antioxidant activity of test drug was expressed as IC_{50}, which was defined as the concentrations of test compounds required for inhibition of the formation of DPPH• radicals by 50%.

Thiobarbituric acid reactive substances (TBARS) assay: In this method, the rat was sacrificed by decapitation and liver was quickly removed and washed with ice-cold normal saline and, homogenized in glass teflon homogenizer with 10 volumes of ice-cold 5 m*M* potassium phosphate buffer (*p*H 7.4) Different concentrations of the test drug in distilled water were prepared and added to rat liver homogenate.

Lipid peroxidation in the rat liver homogenate was initiated by adding 100 µl of 15 m*M* $FeSO_4$ solution to 3 ml of liver homogenate (final concentration was 0.5 m*M*). After 30 min of incubation at room temperature, 0.1 ml (10%w/v) of liver homogenate was taken in a tube containing 0.1 ml of 8.1% w/v sodium dodecyl sulphate (SDS), 0.75 ml of 20% acetic acid and 0.75 ml of 0.8% thiobarbituric acid (TBA) aqueous solution. The volume in each tube was made to 2 ml with distilled water and then heated on water bath at 95°C for 60 min. After 60 min, the volume in each tube was made up to 2.5 ml and then 2.5 ml of n Butanol:Pyridine (5:1) was added in each tube. The reaction mixture was vortexed and centrifuged at 4000 rpm for 10 min. The organic layer was then removed and absorbance was read at 532 nm in a UV spectrophotometer.

Percentage inhibition for both the assays (DPPH• and TBARS) was calculated based on the following formula:

$$\text{Inhibition (\%)} = \frac{\text{absorbance of control} - \text{absorbance of drug sample}}{\text{absorbance of control}} \times 100$$

Statistical analysis — Statistical analysis was done using one-way ANOVA followed by Dunnett's test. *P* values <0.05 were considered significant.

Results

Adaptogenic (anti-stress) activity —CS resulted in a significant increase in plasma glucose level as well as plasma cholesterol and triglycerides levels compared to the control. Serum corticosterone levels were also significantly elevated in the CS group as compared to control. CS also increased the ulcer index and adrenal gland weight (hypertrophy) considerably as compared to the control.

Effect on plasma glucose levels: Pretreatment with *Chlorophytum borivilianum* (CB) aqueous extract at both dose levels (125 and 250 mg/kg) reverted the rise in plasma glucose levels indicating its adaptogenic potential (Table 1). CS did not considerably alter the body weight in seven days.

Table 1 — Changes in plasma glucose, plasma cholesterol, plasma triglyceride, ulcer index, serum corticosterone and adrenal gland weight in control, chronic stress, drug-treated groups (CB125 and CB250) and standard drug group.

[Values are mean ± SE from 6 animals in each group]

	Control	CS	CS + CB125	CS + CB250	CS + Std.
Glucose (mg/dL)	96.9 ± 0.04	121.2 ± 0.11*	108.8 ± 0.08	77.3 ± 0. 16*	75.7 ± 0. 07**
Cholesterol (mg/dL)	86.2 ± 5.3	99.7 ± 3.8*	87.6 ± 12.2	83.3 ± 5.6*	71.4 ± 6.1**
Triglycerides (mg/dL)	58.55± 7.36	93.95 ± 4.29*	75.31 ± 0.79	71.21± 7.1	63.06 ± 06*
Serum corticosterone (mg/dL)	18.56 ± 5.231	48.91 ± 6.512**	39.21 ± 4.213	32.29 ± 4.19*	23.23 ± 5.0*
Ulcer index	0	2.83 ± 0.31**	1 ± 0.22*	0.5 ± 0.06**	0.66 ± 0.03**
Adrenal gland weight (mg/100gm body wt.)	9.49 ± 0.93	14.08 ± 1.48**	12.75 ± 1.13	10.81 ± 1.54	10.96 ± 1.45

P values: * <0.05, ** <0.01, (compared with control, one-way ANOVA followed by Dunnett's test)

Effect on plasma cholesterol levels: In animals pretreated with CB at both dose levels, lower plasma cholesterol levels were found compared to control group. CB250 showed statistically significant reduction in cholesterol levels similar to the standard drug used (Table 1).

Effect on triglyceride levels: Lower triglyceride levels were obtained with CB125 (75 mg/dL), CB250 (971mg/dL) and standard drug diazepam, (63 mg/dL), as compared to control (94 mg/dL) (Table 1).

Effect on corticosterone levels: Dose-dependent decrease in serum corticosterone was observed after treatment of CB. Infact CB250 mg/kg was found to significantly reverse the elevation of plasma corticosterone levels during experimental stress (Table 1).

Effect on cold restraint stress induced ulcer formation: CB125 and CB250 significantly reduced the ulcer formation a dose dependant manner in animals compared to control group. CB250 showed a better protection to ulcers than even the standard drug Diazepam (Table 1).

Effect on organ weights: Pretreatment with CB125 and CB250 reverted the increase in adrenal weight caused due to the stress, thus inhibiting the basic signs of stress response. From the above observations, it can stated that *Chlorophytum borivilianum* possesses significant adaptogenic (antistress) activity (Table 1).

Anti-oxidant activity —In the DPPH• radical-scavenging assay the drug at various concentrations of the methanolic solution of the drug produced inhibition of the DPPH• free radical in a dose dependent manner. The IC_{50} value was found to be around 1200 mcg. (Table 2).

In the TBARS (lipid peroxidation) assay it is observed that the different concentrations of the drug showed significant % inhibition of *Thiobarbituric Acid Reactive Substances*. The IC_{50} value was found to be around 300 mcg. (Table 3).

Table 2— DPPH radical scavenging assay

Conc of CB (mcg/ml)	Absorbance at 517 nm	% Inhibition
0 (Control)	0.618	0
500	0.438	29
1000	0.354	42
1500	0.178	71
2000	0.121	80
2500	0.047	92

Table 3— Thiobarbituric acid reactive substances (lipid peroxidation assay)

Conc of CB (mcg/ml)	Absorbance at 532 nm	Inhibition (%)
0 (Control)	0.296	0
100	0.166	38.29
200	0.140	47.96
300	0.132	50.93
400	0.112	58.36
500	0.086	62.08
600	0.071	66.91

Discussion

During stress, (chronic cold restraint stress) release of various adrenal hormones such as catecholamines and glucocorticoids results in elevated plasma glucose levels because excess of cortisol causes insulin resistance leading to increased gluconeogenesis and eventually hyperglycemia. Release of corticosteroids may also induce hyperinsulinemia resulting in an increased synthesis of cholesterol. Stress has profound effect on metabolic functions of body[15].

Exposure to CS resulted in adrenal hypertrophy and gastric ulceration, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis, which is highly responsive to stress[16,17]. The hyper-activation of the para ventricular nucleus (PVN) of the hypothalamus during stress causes a decrease in mucosal blood flow and hyper-contractility through descending projections that induces pathogenesis of gastric ulcers[18]. The adrenal hypertrophy takes place in response to the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary for increased corticosterone from cortical cells to combat stress[19]. During CS, the increased level of glucose may be important for maintaining the ATP availability to muscles, CNS, and the organ of demand[20,21]. Hyperglycemic effect of corticosterone is reportedly due to increased glycogenolysis of glycogen in liver during stress[20,22]. The pretreatment with *C. borivilianum* decreased the CS induced UI, adrenal hypertrophy, plasma glucose, and circulating corticosterone. At 250 mg/kg *C. borivilianum* significantly reverted the elevated levels of corticosterone, glucose, trigyceride, cholesterol; it also produced significant decrease in the ulcer index and reverted the increase in adrenal gland weight, which is comparable with the standard diazepam. The decrease in the UI, adrenal gland weight, and corticosterone indicates its action on the HPA axis during the CS as these parameters are related with the markers of HPA axis regulation. The hyperglycemia

seems to be mediated through the circulating corticosterone level and the glucose.

Diazepam is reported to posses a non-specific anti-stress activity involving the mesocortical dopamine system and the norepinephrine and 5HT levels of whole brain and hypothalamus[23-25]. The mesocortical dopamine system is thought to play an important role in the etiology of the stress response. Dopamine (DA) has been shown to accumulate in the rat frontal cortex in response to a wide variety of stressors. Diazepam, an anxiolytic benzodiazepine, can reverse the effects of stress on cortical DA. It is proposed that this effect is produced through an enhancement of GABAergic neurotransmission by diazepam[23]. Exposure of animals to immobilization stress markedly and rapidly increases the concentration of NE in brain and hypothalamus[26]. Diazepam does not affect levels of NE of brain and hypothalamus in the control non-stressed animals. But pretreatment of rats with diazepam significantly attenuated stress-induced elevation of NE of brain and hypothalamus. As NE has been implicated in the activation of H-H-A axis (hypothalamo-hypophyseal-adrenocortical axis) during stress[26,27]. Simultaneous attenuation of stress-induced elevation of plasma corticosterone justifies the anti-stress action however definite involvement of NE in this anti-stress effect of diazepam cannot be confirmed at this stage. Ascending 5-HT neurons from raphe nuclei innervate and limbic sites and have an overall hypothalamic role in secretion of ACTH, during stress[28-30]. Restraint stress (RS) has been reported to enhance brain 5HT[31]. 5-HT may regulate ACTH secretion during stress by inhibiting negative feedback by corticosteroids on corticotrophin releasing hormone/ACTH axis[28]. The importance of 5-HT in the activation of the H-H-A axis in stress response has been reviewed[32-34]. Though diazepam does not affect the brain and hypothalamic 5-HT and plasma corticosterone in control rats, it attenuates stress-induced elevation of brain and hypothalamic 5-HT and also simultaneously diminishes the stress induced enhancement of plasma corticosterone levels.

Further studies involving selective estimation of brain and hypothalamic levels of NE and 5HT as well as cortical dopamine levels could help in elucidating the mechanism of action behind the significant anti-stress activity shown by *C. borivilianum.*

Although the IC_{50} value of 1200 mcg of the test drug may not be very potent antioxidant effect but it does implicate a correlation between the reduction of oxidative stress and the spermatogenic potential of the drug. Inhibition of TBARS indicates decrease in lipid peroxidase activity that indicates stability of cell membrane and arrest of cellular damage.

The above observations reveal that the drug has considerable antioxidant activity.

The crude aqueous extract of *C. borivilianum* produced significant % inhibition in the levels of DPPH• free radicals and TBARS.

Thus, it can be concluded that *Chlorophytum borivilianum* is a promising adaptogen or anti-stress agent as well as potential anti-oxidant.

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