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Protective Effect of Triphala on Cold Stress-induced Behavioral and Biochemical Abnormalities in Rats

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Stress is one of the basic factors in the etiology of number of diseases. Cold-stress occurs when the surrounding temperature drops below 18℃, the body may not be able to warm itself, and hence serious cold-related illnesses, permanent tissue damage and death may results. The present study was aimed to investigate the effect of Triphala (*Terminalia chebula, Terminalia belerica* and *Emblica officinalis*) against the cold stress-induced alterations in the behavioral and biochemical abnormalities in four different groups (saline control, Triphala, cold-stress and Triphala with cold-stress) of Wistar strain albino rats. In this study cold-stress (8℃ for 16 h/d/15 days) was applied and the oxidative stress was assessed by measuring the extent of lipid peroxidation (LPO) and the changes in corticosterone levels. Upon exposure to the cold-stress, a significant ($P<0.05$) increase in immobilization with decrease in rearing, grooming, and ambulation behavior was seen in open field. Following cold-exposure, significant increase in the LPO and corticosterone levels was observed. Oral administration of Triphala (1 g/kg/animal body weight) for 48 days significantly prevented these cold stress-induced behavioral and biochemical abnormalities in albino rats. The results of this study suggest that Triphala supplementation can be regarded as a protective drug against stress.

Key words——triphala; cold-stress; lipid peroxidation; open field behavior

INTRODUCTION

Stress is one of the basic factors in the etiology of number of diseases. Stressful condition leads to the formation of excessive free radicals which are a major internal threat to cellular homeostasis of aerobic organisms.[1] Environmental stress has been demonstrated to cause an increase in the oxidative stress, an imbalance in the antioxidant status.[2] Exposure to cold is a direct threat to the body. It was observed that the specific changes in body temperature of ±4℃ from normal could impair both physical and mental task.[3] When the surrounding temperature drops below 18℃, the body may not be able to warm itself, and hence serious cold-related illnesses, permanent tissue damage and death may result.[4]

During experimental cold condition, plasma concentrations of few vitamins, minerals and insulin were reported to decrease together with an increase in plasma corticosterone level.[5,6] Environmental stress has been reported to accelerate the lipid peroxidation (LPO) level due to the production of free radicals.[7,8] In animals, experimental stress had been shown to induce various behavioral changes, including decreases in general locomotor activity and exploratory behavior.[9,10]

Antioxidants play an important protective role against the reactive oxygen species.[11] Reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases.[12,13] Hence search for natural antioxidants is essentially important. Although initial research on antioxidants was mostly on isolated pure compounds, but much focus is on natural formulations.[14] It has been found that some compounds in their natural formulations are more active than their isolated form.[15] Herbal medicines are in use for over hundreds of years before the development and spread of modern medicine and are still in use.

Ayurvedic rasayana are widely used to enhance the natural resistance to various diseases.[16] Triphala is a traditional Ayurvedic herbal formulation, consisting equal parts of three medicinal plant fruits namely *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. Triphala has been used extensively as a

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drug against number of diseases.[17] It is prescribed for various symptoms like fatigue, assimilation and infectious diseases such as tuberculosis, pneumonia and AIDS.[18] Gallic acid found to be a major ingredient of Triphala.[19] *Emblica officinalis* has been reported as a rich source of vitamin C and flavonoids which plays an important role in scavenging free radicals.[20]

This study was designed to evaluate the effect of Triphala supplementation against cold stress-induced behavioral and biochemical abnormalities in albino rats.

MATERIALS AND METHODS

Experimental Animals The study was approved by the Institute's Animal Ethical Committee of the University of Madras (IAEC) and confirmed to National Guidelines on the Care and Use of Laboratory Animals. Male albino rats (Wistar strain), weighing 180–200 g were used for this study. Rats were housed individually in polypropylene cages (25 cm×13 cm×12 cm) under 12 : 12 h light/dark (light during 7.00 A.M.–19.00 P.M.) cycle at 24±1℃ with food and water *ad libitum*.

Experimental Groups The following 4 groups were used in this study and each group had eight animals ($n=8$). Group I (saline control), saline (1 ml) was administered orally for 48 days. Group II animals were administered Triphala (1 g/k.g/b.w) orally for 48 days. Group III (cold-stress) animals were subjected to cold-stress (8℃ for 16 h/d) for 15 days. The cold-stress induced changes were studied in this group. Animals in group IV were treated with Triphala for 48 days and were further subjected to cold-stress from day 33 of the experiment onward till day 48. All the experiments were done on the 49th day. Behavioral activities were assessed on day 1, 8 and 15 day of the experiment.

Heparnized syringe was used to collect the blood samples from the jugular vein. This procedure was conducted during 08.00–10.00 A.M to avoid circadian rhythm influences. Ether was used to anaesthetize the animals to collect the blood samples by using the technique of Feldman and Conforti to avoid stress.[21]

Drug *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* (Table 1) were collected and authenticated by the Chief Botanist, Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation (TAMPCOL) Ltd, Chennai, India. The seedless fruits were dried under shade and made into fine powder.

Dosage Equal proportion (1 : 1 : 1) of powder from each fruits were dissolved with saline (1 ml) at the dose of 1 g/kg of animal body weight and administered orally for 48days.

Cold-stress Procedure Rats were exposed to cold-stress by maintaining them at 8℃ for 16 h in a refrigerated compartment in wire mesh cages.[22]

Biochemical Parameters

Lipid Peroxidation (LPO) Thiobarbituric acid reactive substances are indirectly indicating the LPO level and was estimated spectrophotometrically (532 nm) according to the method of Ohkawa *et al*.[23]

Corticosterone Estimation Corticosterone was estimated with the spectrofluorometric method as described earlier by Mattingly.[24] To 1 ml of plasma, purified dichloromethane (7.5 ml) was added and gently shaken for 5 minutes. The supernatant was discarded and 2.5 ml of a fluorescence reagent (ethanol and concentrated H_2S0_4 in the ratio 3 : 7) was added to the sediment and shaken vigorously for 20 s. The resulting fluorescence of the acid layer was read at excitation 470 nm and emission 530 nm in spectrofluorometric.

Behavioral Assessment In the present study, the changes in behavior following cold-stress in rats were evaluated by open-field method.

Open Field Behavior (OFB) The open field test is widely used to measure general locomotor and explorative activity.[25] The apparatus is made of wood covered with impermeable formica and has a black floor of 100×100 cm (divided by white lines into 25 squares of 20×20 cm) and white walls, 40 cm high.

Table 1. Studied Plants (Triphala)

Scientific Name	Family	Harvested period	Part used
Terminalia chebula	Combretaceae	January	seedless dry fruits
Terminalia belerica	Combretaceae	December	seedless dry fruits
Emblica officinalis	Euphorbiaceae	December	seedless dry fruits

The 100-W white bulb was placed 1 m above the field. Rat used in this study was placed in the center of the open field, which was novel to the animal, and the following variables were scored for 5 min: (i) *Immobilization*: Rats had eyes open, holding its head against the gravity but without any head, body or limb movements. (ii) *Grooming*: Rhythmic paw movements over the face and/or head for face washing might include episodes of biting and cleaning of paws. (iii) *Rearing*: Standing still on upright on its hind limb only. (iv) *Ambulation*: When all the four limbs were in one particular square (central or peripheral) of the field.

Statistical Analysis All data were expressed as mean±S.D. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 10.0 (SPSS, Cary, NC, USA). When there was a significant difference, Tukey's multiple comparisons were performed by fixing the significance level at $p<0.05$. Food and water intake, animal body weight were statistically analyzed by Student's t test $p<0.05$.

RESULTS

Lipid Peroxidation (LPO) The results of the LPO analysis are given in Table 2. The plasma malondialdehyde levels were significantly increased in cold-stress group. The cold stress-induced increase in plasma malondialdehyde levels were significantly reduced by Triphala supplementation.

Corticosterone The results of the corticosterone estimation are also given in Table 2. Cold-stress exposed rats showed a significant ($p<0.05$) elevation in the corticosterone level. Prior treatment with Triphala for 48 days, cold-stress induced elevation in the corticosterone level was significantly ($p<0.05$) reduced when compared to cold-stress group.

Open-field Behavior (OFB) The results of the open field tests are given in Table 3. Results show that a significant ($p<0.05$) increase in immobilization with concomitant decrease in ambulation in peripheral and total squares, rearing and grooming behavior was observed on day one of cold-stress exposed rats. On day 8, same result was observed as day one. However, on fifteenth day of exposure to cold-stress, the changes in behavior in OFB were the significant ($p<0.05$) reduction in ambulation in central squares with significant ($p<0.05$) increase of immobilization behavior was observed when compared to normal controls. This cold-stress induced change in the peripheral, central, immobilization and rearing were significantly ($p<0.05$) prevented in Triphala treated cold-stress exposed group.

DISCUSSION

The present study reveals the protective effect of Triphala against cold-stress induced behavioral and biochemical abnormalities in Wistar strain albino rats. Preliminary study with different dosages of Triphala (0.5 g, 0.75 g and 1 g/kg body weight), 1 g/kg body weight dosage significantly ($p<0.05$) prevents the cold stress-induced decline in corticosterone level (data not shown).

Open field test detects motor deficits which will be manifested by decreased line crossing, rearing and grooming activities.[26] Excessive shivering due to cold-stress contributes to fatigue and makes performance of motor skills more difficult. In our study, cold exposure induced and increased immobilization in OFB was observed. Along with the changes, decrease in rearing, grooming and ambulation also revealed that the cold exposure may cause fatigue and altered excitability of the nervous system. The cold stress-induced behavioral changes in OFB were prevented in Triphala treated group (Table 3). However, the results of behavioral analysis on day 15 indicated that adaptations of the animals to the stressful environment may be the reason for very less or

Table 2. Protective Effect of Triphala (1 g/kg animal body weight) on Vitamin C, LPO and Corticosterone Level in Albino Rats Exposed to Cold-stress.

Parameters	Saline control	Triphala	Cold-stress	Triphala+cold-stress
Plasma LPO (MDA nmol/mg protein)	3.37±0.06	3.30±0.14$^{\sharp}$	5.30±0.09*	3.66±0.16*$^{\sharp}$
Corticosterone (μg/dl of plasma)	39.41±3.30	38.33±2.28$^{\sharp}$	88.05±4.62*	44.66±3.47*$^{\sharp}$

Values are expressed as mean±SD of eight animals. Saline control-administration of saline for 48 days; Triphala- administration of Triphala for 48 days; Cold-stress-rats were exposed to cold-stress (8℃/16 h/day) for 15 days; Triphala+cold-stress-rats were treated with Triphala for 48 days and were further subjected to cold-stress from day 33 of the experiment onward till day 48. * compared with saline control; $^{\sharp}$ compared with cold-stress. The symbols represent statistical significance: *, $^{\sharp}$ $p<0.05$.

Table 3. Protective Effect of Triphala (1 g/kg animal body weight) on Ambulation (squares) in Open Field Behavior in Albino Rats Exposed to Cold-stress.

Parameters	Saline control	Triphala	Cold-stress	Triphala+cold-stress
Peripheral (day 1)	59.25±5.29	59.50±5.16#	44.63±4.22*	54.88±6.90#
Peripheral (day 8)	65.63±4.90	65.88±5.18#	55.88±4.15*	59.38±6.81
Peripheral (day 15)	59.88±5.10	60.38±5.91	57.63±3.26	56.63±4.35
Central (day 1)	1.75±0.45	1.88±0.34#	0.88±0.34*	1.63±0.72#
Central (day 8)	7.88±0.96	8.13±0.96#	3.00±1.46*	7.00±1.15#
Central (day 15)	8.00±1.03	8.25±1.00#	3.63±1.71*	7.25±1.13#
Immobilization (day 1)	27.25±4.37	26.63±4.67#	50.00±5.77*	31.00±3.10#
Immobilization (day 8)	25.38±4.47	24.75±3.04#	44.75±3.42*	26.25±4.43#
Immobilization (day 15)	22.50±2.37	22.13±2.16#	35.13±6.03*	20.50±2.37#
Rearing (day 1)	17.00±2.19	18.25±3.21#	7.00±1.93*	16.50±2.37#
Rearing (day 8)	16.25±3.00	16.63±3.10#	7.25±1.61*	13.00±2.42#
Rearing (day 15)	15.00±2.19	15.63±1.54#	10.63±2.42*	15.38±2.58#
Grooming (day 1)	10.00±0.78	10.29±0.91#	5.71±0.91*	7.86±1.17*#
Grooming (day 8)	8.57±0.94	8.86±1.17#	6.43±0.94*	7.29±1.64
Grooming (day 15)	7.00±1.24	7.14±1.17	6.43±1.74	7.29±1.54

Values are expressed as mean±SD of eight animals. Saline control-administration of saline for 48 days; Triphala-administration of Triphala for 48 days; Cold-stress-rats were exposed to cold-stress (8℃/16 h/day) for 15 days; Triphala+cold-stress-rats were treated with Triphala for 48 days and were further subjected to cold-stress from day 33 of the experiment onward till day 48. Peripheral and central squares are expressed in number of square entry. Immobilization was expressed in seconds. Rearing and grooming were expressed in number of attempts. * compared with saline control; # compared with cold-stress. The symbols represent statistical significance: *, # $p<0.05$.

rare changes in behavior in OFB was observed.

Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stressors. A stressful condition leads to the excessive production of free radicals, which results in oxidative stress.[27] In the present observation, increased LPO was presumably associated with increased free radicals, confirming the fact that the cold stress-induced generation of free radicals. Pretreatment with Triphala attenuated the cold stress-induced elevation in LPO and corticosterone levels.

A major physiological response to stress is activation of the hypothalamic-pituitary-adrenal axis and subsequently release of corticosterone from the adrenal cortex into the bloodstream.[28] The elevation of endogenous corticosterone due to stress response has been reported to accelerate the generation of free radicals.[27] These cold stress-induced elevations of LPO, and corticosterone level changes were significantly prevented in Triphala treated groups. Our early reports showed that the noise-stress induced oxidative stress and immune dysfunctions were significantly prevented by the administration of the herbal formulation Triphala.[29–31] The presence of vitamin C content in *Emblica officinalis* available in most stable form and an important antioxidant reported to reduce the corticotrophin-releasing hormone in the blood stream might be the reason for a significant protection in the studied behavioral and bio-chemical parameters.[32]

Cold stress-induced alteration in the behavioral and biochemical abnormalities was prevented in Triphala supplemented group. Therefore the present study suggests that Triphala supplementation may exert antioxidant effect and can be regarded as a protective drug against stress.

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