Antioxidant Property of Triphala on Cold Stress Induced Oxidative Stress in Experimental Rats

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Stress is one of the basic factors in the etiology of number of diseases. The present study was aimed to investigate the antioxidant properties of Triphala (*Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*) during cold-stress. Four groups of albino rats were employed namely control, Triphala, cold-stress and Triphala with cold-stress. The oxidative stress was assessed by measuring the lipid peroxidation (LPO), enzymatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and non-enzymatic (Vitamin C) antioxidant status in adrenal tissue and plasma corticosterone level. Following cold-exposure (8°C for 16 hr/d/15 days), enzymatic and non-enzymatic antioxidants were significantly reduced with concomitant increase in LPO and corticosterone levels were observed. Administration of Triphala (1 g/kg/body weight/48 days) significantly prevents the cold-stress-induced oxidative stress and elevation in LPO and corticosterone levels. This study concludes that Triphala supplementation significantly prevents the cold-stress-induced oxidative stress may due to its antioxidant properties.

Key words —— triphala, cold-stress, antioxidant

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 the 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°C from normal could impair both physical and mental task.[3] When the surrounding temperature drops below 18°C, the body may not be able to warm itself, and hence serious cold-related illnesses, permanent tissue damage and death may result.[4]

Antioxidants play an important protective role against the reactive oxygen species (ROS).[5] Reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases.[6,7] 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.[8] It has been found that some compounds in their natural formulations are more active than their isolated form.[9]

Triphala is a traditional Ayurvedic herbal formulation, consisting equal parts of three medicinal plant fruits namely *T. chebula*, *T. bellerica* and *E. officinalis*. Triphala has been used extensively as a drug against number of diseases.[10] Gallic acid found to be a major ingredient of Triphala.[11] *Emblica officinalis* has been reported as a rich source of vitamin C, which plays an important role in scavenging free radicals.[12] This study was designed to evaluate the antioxidant properties of Triphala by measuring the lipid peroxidation (LPO), enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and non-enzymatic (Vitamin C) antioxidant status and corticosterone level against cold-stress.

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MATERIALS AND METHODS

Experimental Groups —— The study was approved by the Institute's Animal Ethical Committee of the University of Madras (IAEC. No. 08/006/02) 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. The following four groups were used in this study and each group had six animals (n = 8). Group I (saline control), saline (1 ml) was administered intragastrically for 48 days. Group II animals were administered Triphala (1 g/kg/b.w) intragastrically for 48 days. Group III (cold-stress) animals were subjected to cold-stress (8°C for 16 hr/d) for 15 days. 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. Ether was used to anaesthetize the animals to collect the blood samples by using the technique of Feldman and Conforti[13] to avoid stress.

Drug and Dosage —— *T. chebula*, *T. bellerica* and *E. officinalis* 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. Equal proportion (1 : 1 : 1) of powder from each fruits were mixed with saline (1 ml) at the dose of 1 g/kg of animal body weight and administered intragastrically for 48 days.

Cold-Stress Procedure —— Rats were exposed to cold-stress by maintaining them at 8°C for 16 hr in a refrigerated compartment in wire mesh cages.[14]

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.*[15] Protein estimation was done according to the method of Lowry *et al.*[16]

SOD —— The superoxide dismutase activity was measured as the degree of inhibition of autooxidation of pyrogallol at alkaline pH by the method of Marklund and Marklund.[17]

CAT —— The activity of catalase was measured as the amount of hydrogen peroxide consumed per minute per milligram of protein by the method of Sinha.[18]

GPx —— Glutathione peroxidase level was estimated by measuring the amount of reduced glutathione consumed in the reaction mixture according to the method of Rotruck *et al.*[19]

Vitamin C Estimation —— Vitamin C was estimated by the method of Omaye *et al.*[20] Vitamin C was oxidized by copper to form dehydroascorbic acid which reacts with 2, 4-dinitrophenyl hydrazine to form bis-2, 4-dinitrophenyl hydrazone. This undergoes further rearrangement to form a product with absorption maximum at 520 nm. Thiourea provides the reducing medium to prevent interference from non ascorbic acid chromogens.

Corticosterone Estimation —— Corticosterone was estimated with the spectrofluorometric method as described earlier.[21] To 1ml of plasma, purified dichloromethane (7.5 ml) was added and gently shaken for 5 min. The supernatant was discarded and 2.5 ml of a fluorescence reagent (ethanol and concentrated H_2SO_4 in the ratio 3 : 7) was added to the sediment and shaken vigorously for 20 sec. The resulting fluorescence of the acid layer was read at excitation 470 nm and emission 530 nm in spectrofluorometry.

Statistical Analysis —— All data's 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, U.S.A.). When there was a significant difference, Tukey's multiple comparisons were performed by fixing the significance level at $p < 0.05$.

RESULTS

The result of the LPO analysis is given in Table 1. Both plasma and adrenal malondialdehyde levels were significantly increased by cold-stress. The cold stress-induced increase in both plasma and adrenal malondialdehyde levels were significantly reduced by Triphala supplementation.

The results of the SOD, CAT and GPx analysis are given in Table 1. Cold stress exposed rats showed a significant decrease in SOD, CAT and GPx in adrenal tissue. When Triphala pretreated rats exposed to cold-stress, the cold-stress induced decline SOD, CAT and GPX were significantly prevented.

The vitamin C (Table 1) in both plasma and adrenal tissue were significantly decreased by cold-stress. Cold-stress induced decline in the vitamin C level was significantly prevented with pretreatment of Triphala.

The results of the corticosterone estimation are also given in Table 1. Cold-stress exposed rats showed a significant elevation in the corticosterone level. Prior treatment with Triphala for 48 days, cold-

Table 1. Effect of Triphala (1 g/kg Animal Body Weight) on SOD, GPx, CAT, Vitamin C, LPO and Corticosterone Level in Albino Rats Exposed to Cold-Stress

Parameters	Control	Triphala	Cold-stress	Triphala + cold-stress
Adrenal SOD (Units/min/mg Protein)	2.55 ± 0.20	2.63 ± 0.30#	1.25 ± 0.22*	2.37 ± 0.21#
Adrenal CAT (μmol of H_2O_2 consumed /min/mg protein)	38.27 ± 3.67	39.53 ± 3.25#	24.50 ± 2.47*	37.62 ± 2.50#
Adrenal GPx (μmole/mg Protein)	8.28 ± 0.61	8.15 ± 0.67#	4.32 ± 0.42*	7.93 ± 0.51#
Adrenal vitamin C (mg/100 g of tissue)	263.63 ± 17.50	266.50 ± 13.34#	169.88 ± 9.02*	250.25 ± 16.85#
Adrenal LPO (MDA nmol/mg protein)	1.34 ± 0.06	1.28 ± 0.12#	2.28 ± 0.09*	1.55 ± 0.04#
Plasma vitamin C (mg/dl)	1.55 ± 0.24	1.60 ± 0.20#	0.91 ± 0.06*	1.53 ± 0.23#
Plasma LPO (MDA nmol/mg protein)	3.37 ± 0.06	3.30 ± 0.14#	5.30 ± 0.09*	3.66 ± 0.16#
Plasma Corticosterone (μg/dl of plasma)	39.41 ± 3.30	38.33 ± 2.28#	88.05 ± 4.62*	44.66 ± 3.47#

Values are expressed as mean ± S.D. of six 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°C/16 hr/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. *$p < 0.05$, compared with saline control; #$p < 0.05$, compared with cold-stress.

stress induced elevation in the corticosterone level was significantly reduced when compared to cold-stress group.

DISCUSSION

The present study reveals the antioxidant property of Triphala against cold-stress. During forty eight days of Triphala administration the studied biochemical parameters were not altered significantly (Group II). Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals.[22] In the present study, rats exposed to cold-stress shown to increase LPO level, which may be lead by the uncompromised production of free radicals. Nevertheless, by administration of Triphala in cold stress exposed animals the level of LPO was decreased. LPO can be prevented at the initiation stage by free radical scavengers and antioxidants.[23] Triphala reported to rich in antioxidant, vitamin C and gallic acid contents might be an effective inhibitor in reducing LPO formation. This scrutiny reveals that Triphala is able to quench the LPO chain and is capable to shield the membrane from free radicals caused injuries.

The endogenous antioxidant system may counteract the ROS and reduce the oxidative stress with the enzymic antioxidants SOD, CAT and GPx. SOD accelerates the conversion of superoxide radical to hydrogen peroxide while CAT or GPx converts hydrogen peroxide to water. Vitamin C, well known as a potent water-soluble non-enzymic antioxidant effectively intercept oxidants in the aqueous phase before they attack and cause detectable oxidative damage.[24] Depletion in the activity of this enzymic and non-enzymic antioxidant can be owed to an enhanced radical production during stress conditions.

Addition to this physiological response to stress is the activation of hypothalamic-pituitary-adrenal axis and subsequently release of corticosterone from the adrenal cortex into the bloodstream.[25] The elevation of endogenous corticosterone due to stress response has been reported to accelerate the generation of free radicals.[26] In the present observation an increase in LPO was presumably associated with increased free radicals, confirming the fact that the free radicals inhibited the activities of SOD, CAT, GPx and vitamin C level. The observed reduction might be attributed to the utilization of these antioxidants to alleviate free radical induced oxidative stress. Activities of the enzymic and non-enzymic antioxidants, LPO and corticosterone level are significantly prevented in Triphala treated cold-stress exposed animals. This indicates the antioxidant potency of the drug.

The protective role Triphala may be due to the compound itself may scavenge the free radicals and/or prevent the antioxidants from ROS and additionally the compound can act by up regulating endogenous antioxidant defenses. The increase in the level of these antioxidants after the administration of Triphala in cold-stress exposed rats may be due to the direct reaction of Triphala with ROS. Since the Triphala reported to be a strong antioxidant and this antioxidant property may be due to the presence of gallic acid, vitamin C and flavonoid contents.[27)]

The present study underlines that administration of Triphala inhibits the level of LPO and corticosterone and significantly increases the enzymic and non-enzymic antioxidant defense mechanisms in cold-stress exposed animals.

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