Antioxidant Property of *Emblica officinalis* during Experimentally Induced Restrain Stress in Rats

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The present study was aimed to investigate the antioxidant property of *Emblica officinalis* (*E. officinalis*) during restrain-stress in albino rat. Three groups of albino rat were employed namely control, restrain-stress (4 hr/day for 15 days) and *E. officinalis* + restrain-stress. The oxidative stress was assessed by measuring the lipid peroxidation (LPO), enzymatic antioxidant status superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the lymphoid organs of thymus and spleen and plasma corticosterone level. Following restrain-stress, enzymatic antioxidants status was significantly reduced with concomitant increase in LPO and corticosterone levels were observed. Administration of *E. officinalis* (500 mg/kg body weight for 30 days) significantly prevents the restrain-stress-induced oxidative stress and elevation in LPO and corticosterone levels. This study concludes that administration of *E. officinalis* significantly prevents the restrain-stress-induced oxidative stress and this may due to its strong antioxidant property.

Key words —— *Emblica officinalis*, restrain-stress, antioxidant

INTRODUCTION

Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stressors.[1)] A stressful condition leads to the excessive production of free radicals, which results in oxidative stress, an imbalance in the oxidant/antioxidant system. Generation of free radicals is an integral feature of normal cellular functions, in contrast, excessive generation and/or inadequate removal of free radical results in destructive and irreversible damage to the cell.[2)] Corticotropin-releasing hormone is released during stress and stimulates the release of corticosterone from the adrenal cortex. Elevation in the corticosterone level accelerates the generation of free radicals[3)] and suppresses the immune function.[4)] Present study reveals the adverse effects of restraint stress on the lymphoid organ (thymus and spleen) antioxidant status. Restraint stress has been commonly used by other workers as stress inducers in rats.[5, 6)]

Antioxidants play an important protective role against the reactive oxygen species (ROS).[7)] Reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases.[8, 9)] 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 forms.[10)] It has been found that some compounds in their natural formulations are more active than their isolated form.[11)]

Emblica officinalis (*E. officinalis*; Family: Euphorbiaceae) are used in Ayurveda as a potent rasayanas, a class of plant-derived drugs reputed to promote health and longevity by increasing defence against disease.[12)] *E. officinalis* has been reported as a rich source of vitamin C, which plays an important role in scavenging free radicals.[13)] This study was designed to evaluate the antioxidant property of *E. officinalis* by measuring the lipid peroxidation (LPO), enzymatic antioxidant status superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and corticosterone level against restraint stress.

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MATERIALS AND METHODS

Animals and Treatments —— Healthy adult Wistar strain male albino rats, weighing between 180–200 g (3 months old), were used in this study. The rats were kept in controlled ambient temperature (24 ± 2°C), humidity and light (14 hr; 10 hr light/dark cycle, lights on 07:00 AM) with food and water *ad libitum*. The animal procedures were approved by the Institutional Animal Ethical Committee. All efforts were made to minimize both the number of animals used and unwanted stress or discomfort to the animals throughout experimental procedures. Rats were divided in to three groups (Control, Restrain stress, *E. officinalis* + Restrain stress) and each group had six animals. Seedless fruit *E. officinalis* was powdered, dissolved in Phosphate buffered saline (PBS, 1 ml) and administered orally at the dose of 500 mg/kg body weight for 30 days. Rats were immobilized by keeping them in transparent plastic jars with holes, for ventilation, for 4 hr a day for 15 days.[14]

Sample Preparation —— Thymus and spleen were removed and 10 % homogenate was prepared using ice cold Tris–HCl (100 mM, pH 7.4) buffer by a motor driven Teflon-glass tissue homogenizer. The homogenate was centrifuged at 2000 (runs per minute) for 15 minutes at 4°C and the supernatant was used for the biochemical study.

Assay of LPO, ROS-scavenging Systems and Corticosterone —— Thiobarbituric acid reactive substances which indirectly indicates the LPO level was estimated spectrophotometrically according to the method of Ohkawa *et al.*[15] Protein estimation was done according to the method of Lowry *et al.*[16] 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] The activity of catalase was measured as the amount of hydrogen peroxide consumed per minute per milligram of protein according to the method of Sinha.[18] GPx level was estimated by measuring the amount of reduced glutathione consumed in the reaction mixture fol-

Table 1. Effect of *E. officinalis* on Thymus LPO, SOD, CAT, GPx and Plasma Corticosterone Level in Male Albino Rats Exposed to Restrain-stress

Parameters	Control	Restrain-stress	*E. officinalis* + Restrain-stress
LPO (nmol of MDA formed/mg protein)	2.28 ± 0.29	5.83 ± 0.39*	2.43 ± 0.25#
SOD (units/mg protein)	2.64 ± 0.20	1.62 ± 0.13*	2.45 ± 0.16#
CAT (μ moles of H_2O_2 consumed /min/mg protein)	40.03 ± 3.37	27.17 ± 2.21*	39.17 ± 3.27#
GPx (μg of GSH consumed /min/mg protein)	8.87 ± 0.91	5.08 ± 0.47*	8.08 ± 0.76#
Corticosterone (μg/dl)	41.33 ± 3.34	55.67 ± 3.11*	45.33 ± 3.60#

Values are expressed as mean ± S.D. of six animals. *compared with control; #compared with restrain-stress. The symbols represent statistical significance: *,# $p < 0.05$.

Table 2. Effect of *E. officinalis* on Spleen LPO, SOD, CAT, GPx in Male Albino Rats Exposed to Restrain-stress

Parameters	Control	Restrain-stress	*E. officinalis* + Restrain-stress
LPO (nmol of MDA formed/mg protein)	2.07 ± 0.16	5.55 ± 0.51*	2.28 ± 0.32#
SOD (units/mg protein)	2.30 ± 0.24	1.12 ± 0.13*	2.25 ± 0.24#
CAT (μ moles of H_2O_2 consumed /min/mg protein)	35.17 ± 2.12	18.83 ± 0.94*	32.5 ± 3.12#
GPx (μg of GSH consumed /min/mg protein)	12.17 ± 0.94	5.17 ± 0.39*	11.33 ± 0.78#

Values are expressed as mean ± S.D. of six animals. *compared with control; #compared with restrain-stress. The symbols represent statistical significance: *,# $p < 0.05$.

lowed by the method of Rotruck *et al.*[19] Corticosterone was estimated spectrofluorometrically.[20] To the plasma, purified dichloromethane was added and gently mixed. The supernatant was discarded and fluorescence reagent (ethanol and concentrated H_2SO_4 in the ratio 3:7) was added to the sediment and shaken vigorously. The resulting fluorescence of the acid layer was read at excitation 470 nm and emission 530 nm.

Statistical Analysis —— All data were expressed as mean ± S.D. and statistical analysis was carried out using one-way analysis of variance (ANOVA). To evaluate the significance between the various groups studied, Tukey's multiple comparison tests were performed by fixing the significance level at $p < 0.05$.

RESULTS AND DISCUSSION

The lack of data on antioxidant property of *E. officinalis* during restrain-stress makes this study unique. Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals.[21] The enzymatic antioxidant system SOD, CAT and GPx counteract the free radical and reduce the oxidative stress. SOD accelerates the conversion of superoxide radical to hydrogen peroxide while CAT or GPx converts hydrogen peroxide to water.

The elevation of corticosterone in the present study (Table 1) due to stress response has been reported to accelerate the generation of free radicals.[22] An increase in LPO level was presumably associated with increased free radicals, confirming the fact that the free radicals reduced the antioxidant status in both thymus and spleen in restrain-stress rats. The observed reduction might be attributed to the utilization of these antioxidants to alleviate free radical induced oxidative stress. Decreased enzymatic antioxidant with associated increased LPO and corticosterone levels were significantly prevented in *E. officinalis* treated restrain-stress animals (Table 1 and 2) and indicates the antioxidant potency of the *E. officinalis*. The protective role *E. officinalis* may be due to the presence of compounds that regulates the enzymatic antioxidant defenses. The increase in the level of these antioxidants after the administration of *E. officinalis* in restrain-stress rats may be due to the direct reaction of *E. officinalis* with free radicals.

The present study underlines that administration of *E. officinalis* inhibits the levels of LPO and corticosterone and significantly protects the enzymatic antioxidant defense mechanisms in restrain-stress animals.

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