# ATTENUATION OF OXIDATIVE STRESS BY ETHANOLIC EXTRACT OF NETTLE (*URTICA DIOICA*) IN MICE

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***Abstract***

*This study was undertaken to evaluate the antioxidative potential of ethylic fraction of nettle (Urtica dioica) in stressed mice. In this present study mice were divided into the four groups and each group containing five mice. Group I mice were orally administered with 1 ml of 0.9% NaCl with is the positiv control group. Group II mice were administered with ethylic fraction of Urtica dioica. Group III stressed mice were orally administered with 1 ml of 0.9% NaCl with is the negativ control group. Group IV stressed mice were administered with ethylic fraction of Urtica dioica.*

*The activity of enzymic antioxidants (U/ mg of protein) such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were found to be significantly high in ethylic fraction treated stressed mice when compared to the negative control mice. The levels of nonenzymic antioxidant such as reduced glutathione in the ethylic fraction treated stressed mice was found to be significantly higher than that found in control mice.*

*These results suggest that nettle (Urtica dioica) has very good antioxidant and hepatic protective effect of oxidative stress in mice. Currently, the importance of oxidative stress in the pathophysiology of many disorders has been highlighted, thus use of this plant as an herbal medicine is highly recommended.*

***Key words***: antioxidative enzymes, glutathione, polyphenols, thiobarbituric acid reactive substances (TBARS).

## INTRODUCTION

In this modern era, stress has become an integral part of human life (Ravindran R. *et al.*, 2005). It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress (Verma N. and Khosa R.L., 2009). Stress is considered to be any condition which results in perturbation of the body's homeostasis (Emeny R.T. and Lawrence A. D., 2007). If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. (Lakshmi B.V.S. and Sudhakar M., 2009). Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous

system (CNS), endocrine system, and metabolic system (Rai D. *et al.*, 2003). Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Herbal formulations have been globally in use for many for human well-being. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body (Lakshmi B.V.S. and Sudhakar M., 2009).

## MATERIALS AND METHODS

### *Preparation of ethanolic extracts*

In this study, there were used dried aerial parts of nettle (*Urtica dioica*). The interest parts of plant were powdered and extracted with ethanol 60 % (1:10 ratio, w:v) for 3 hours at 60ºC. The homogenates obtained were filtered using filter paper Watman no. 1 and the filtrates were then centrifuged for 20 min at 5000 rpm and 5ºC. After the ethanol was evaporated, the aqueous residues were utilized.

### *Animals*

The animals used in this study were purchased from Cantacuzino Institute. In this study, twenty adult female albino mice (25-30 g weight, nine weeks old) were used as experimental animals. They were kept in polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark, 22 ± 2 °C temperature, fed with a normal rodent diet one week before the experiment. Starvation was used prior to all assays because polyphenols extracts were always administered orally (by gavage) using distillated water as vehicle. Oxidative stress was induced in experimental albino mice by keeping them in special lighting conditions (six hours of daylight and 18 hours of darkness). The administration of plant extracts to mice began seven days before inducing oxidative stress. All the pharmacological experimental protocols respected European legislation for experimental animals.

### *Experimental protocol*

The mice were randomly divided into 4 groups of 5 animals each, as follows:

- Group 1: Normal control mice treated with 0.9 % NaCl: mice were orally administered 1 mL 0.9 % NaCl, for 21 days.
- Group 2: Normal control mice treated with nettle (*Urtica dioica*) extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 3: Control mice group was stressed and treated with 0.9 % NaCl:

mice were orally administered 1 mL 0.9 % NaCl, for 21 days.

- Group 4: Stresed mice were treated with nettle (*Urtica dioica*) extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.

Twenty-four hours following last administration, the animals were sacrificed by cervical dislocation. The abdomen was excised and the liver was removed immediately by dissection, washed in ice-cold isotonic saline and blotted between two filter papers. The liver was transferred into preweighed vials to determine the wet weight. A 10% (w/v) liver homogenates was prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5.

***Determination of lipid peroxidation***

The measurement of liver lipid peroxide by a colorimetric reaction with thiobarbituric acid was done as described by Ohkawa et al. (Ohkawa *et al*., 1979), and the determined lipid peroxide is referred to as malondialdehyde. Briefly, in a test tube, 20% trichloroacetic acid solution and 0.67% thiobarbituric acid solution were added to the homogenate. The color of thiobarbituric acid pigment was developed in a water bath at 100°C for 20 min. After cooling with tap water to room temperature, 2mL *n*-butanol was added and shaken vigorously. After centrifugation, the color of butanol layer was measured at $\lambda_{max}$ 532 nm. The TBARS concentration of the sample was calculated using the extinction coefficient of MDA ($1.56 \times 10^5$ $M^{-1}cm^{-1}$) and the values were expressed as nmol/mg protein.

***Determination of superoxide dismutase activity (SOD)***

The activity of superoxide dismutase in liver was measured using a commercial kit (Fluka analytical). This method uses xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4- iodophenyl)- 3- (4- nitrophenol)- 5- (2, 4- disulfophenyl)-2H-tetrazolium, monosodium salt to form a water soluble formazan dye. The values are expressed as Units/mg of protein in liver tissue.

***Determination of catalase activity (CAT)***

Catalase activity was measured by the method described by Aebi (Aebi, 1984). Supernatant was added to cuvette containing 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of freshly prepared 30 mM $H_2O_2$. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as Units/mg of protein.

***Determination of glutathione peroxidase activity (GPx)***

The method is based on the oxidation of gluthatione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate, Reduced) (Gupta and Baquer, 1998). Final reaction mixture (3 ml) was 65 mM $KH_2PO_4/K_2HPO_4$, pH 7.5, 2 mM GSH, 1 U glutathione reductase, 0.12 mM NADPH and 8 mM tert buthyl hydroperoxide. Activity of glutathione peroxidase was expressed as Units/mg of protein.

***Determination of liver reduced glutathione (GSH)***

Reduced glutathione in liver was determined by the method of Jollow *et al.* (Jollow *et al.*, 1974). An aliquot of liver homogenate (10% in 0.1M phosphate buffer) was precipitated with sulfosalicylic acid (4%). The samples were kept at 4°C for 1h and then subjected to centrifugation at 4000 rpm for 15 min at 4°C. The assay mixture contained 0.1 mL aliquot from the supernatant, 0.1M phosphate buffer (pH 7.4) and dithionitrobenzene (DTNB) in a total volume of 3.0 ml. The optical density of the yellow color developed was read immediately at 412 nm in a spectrophotometer. GSH was expressed as mg / 100 g tissue using a GSH standard curve.

***Determination of total proteins***

Total proteins were determined according to the method of Lowry, using bovin seric albumin (BSA) as a standard (Lowry *et al.*, 1951).

***Statistical data interpretation***

Statistical data interpretations were calculated with EXCEL program from Microsoft Office package. All the data are shown as mean value ± standard deviation (SD). Number of mice per group n = 5. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p>0.05$ statistically insignificant; $*p<0.05$ statistically significant; $**p<0.01$ strong statistical significance; $***p<0.001$ very strong statistical significance.

## RESULTS AND DISCUSSION

***Determination of lipid peroxidation***

When the antioxidant capacity is insufficient against ROS, lipid peroxidation occurs and TBARS is formed. In the present study, stress induced oxidative damages in liver characterized by increased MDA concentration. TBARS contents of the liver tissue in group 2 was found to significantly decreased as compared to group 1 ($P < 0.01$). Pretreatment of normal mice with nettle (*Urtica dioica*) alcoholic extracts decreased

TBARS level in the liver; however the polyphenolic treatment in stressed mice (G4) have the same effects on TBARS in this tissue (Fig. 1).

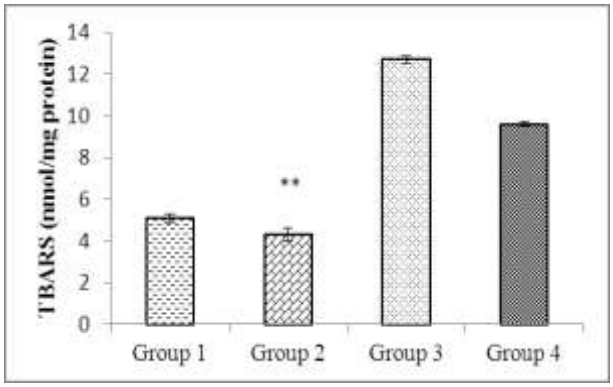


Fig. 1. The influence of oral administration of plant polyphenols (100 mg/kg) on lipid peroxidation in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. ** $p < 0.01$ vs 1st mice group.

***Determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities***

SOD, GPx, CAT, GST and G6DH activities of liver mice in the experimental groups are given in Figure 2, 3 and 4. When stressed mice were treated with nettle (*Urtica dioica*) extracts, SOD, CAT and GPx activities were found to be increased from those of the group 3 (G3).

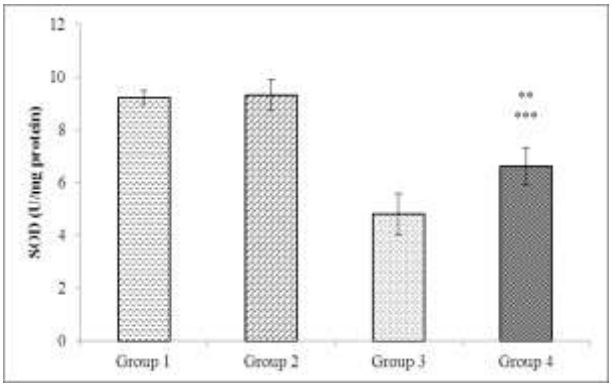


Fig. 2. The influence of oral administration of plant polyphenols on superoxide dismutase (SOD) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 2nd mice group and ** $p < 0.01$ vs 3rd mice group.

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of $O_2$•– (Crivineanu M., *et al.* 2010). In the present study, nettle (*Urtica dioica*) extract administration caused a very strong significant decreased ($p<0.001$) of SOD activities in liver, when it is compared with G2, and a strong significant increase ($p<0.01$) of liver SOD activity when it is compared with G3.

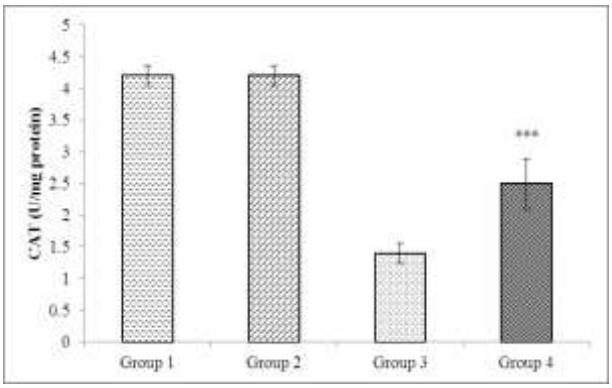


Fig. 3. The influence of oral administration of plant polyphenols on catalase (CAT) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 3rd mice group.

In the present study, the very strong significant increase ($p<0.01$) of CAT activity was observed in liver stressed mice treated with nettle polyphenolic extract. Also, strong significant decrease of liver GPx activity was observed in stressed mice treated with nettle polyphenolic extracts (G4).

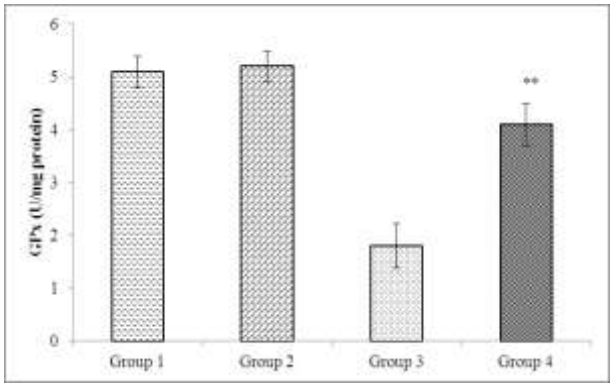


Fig. 4. The influence of oral administration of plant polyphenols on glutathione peroxidase (GPx) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. ** $p < 0.01$ vs 2nd mice group.

***Determination of liver reduced glutathione (GSH)***

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx) (Verma N. and Khosa R.L., 2009). Decreased level of GSH is associated with an enhanced lipid peroxidation (Papuc C. et al., 2010). Administration of *Urtica dioica* very strong significantly ($P<0.001$) increased the level of GPx in group 4 as compared with group 3. The significant ($p<0.01$) reduction in the liver non enzymatic antioxidant system (GSH) in stressed mice (G4) as compared to the control unstressed treated group (G2) could be responsible for increased lipid peroxidation levels observed during oxidative stress induced. Olso, GSH concentration for stressed mice group (G3) was significantly ($p < 0.001$) decreased when it is compared with G1 (Fig. 5).

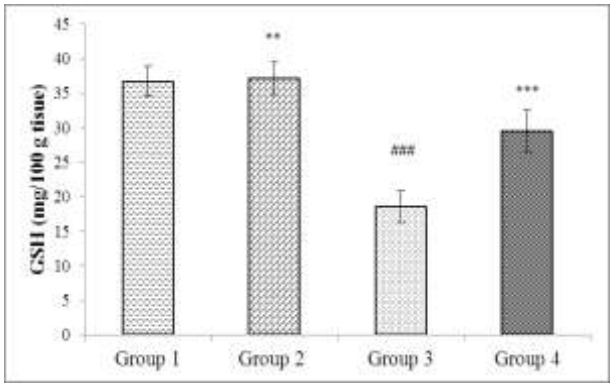


Fig. 5. The influence of oral administration of plant polyphenols on GSH levels in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 3rd mice group; ### $p < 0.001$ vs 1st mice group; ** $p < 0.01$ vs 4th mice group.

## CONCLUSIONS

Polyphenols supplementation to stressed mice decreased the level of TBARS, compared to control group.

The treatment of stressed mice with plant polyphenols in quantity of 100 mg/kg significantly improved the levels of SOD, CAT and GPx activities in liver homogenates.

Polyphenols extracted from *Urtica dioica* improved the levels of GSH in

stressed mice liver homogenates when compared with stressed untreated mice.

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