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Effect of *Solanum nigrum* on Immobilization Stress Induced Antioxidant Defense Changes in Rat

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Abstract: In the present study, the antioxidant potential of *Solanum nigrum* leaves extract was evaluated on the modulation of restraint induced oxidative stress. Rats were treated with crude extract of *S. nigrum* alone and both before (pre-extract stress treated) and after (post-extract stress treated) 6 h of stress exposure. Pro-oxidant effect of rat plasma was evaluated by determining the activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione-S-Transferase (GST) and the levels of glucose, uric acid and lipid peroxidation (MDA). About 6 h of restraint stress caused a significant decrease in the activities of SOD, CAT and GST and the level of glucose, while increase in the levels of MDA and uric acid. The post treatment of crude extract was found more effective in restoring restraint stress induced changes in rat plasma than pre treatment. In order to reduce oxidative stress, observed in many pathological conditions, the *S. nigrum* leaves extract can be given both as a prophylactic and therapeutic supplement for scavenging free radicals.

Key words: Immobilization stress, antioxidant effect, *Solanum nigrum* extract

INTRODUCTION

In the recent years, there has been an upsurge in the clinical use of indigenous drugs, such as herbal plants, originally used in traditional system of medicine, are now being effectively tried in a variety of pathophysiological states.

Solanum nigrum (Mako) is a weed of wasteland, old fields and ditches. It is an annual branched herb up to 90 cm high with dull dark green leaves. Flowers are small and white with a short pedicellate and 5 widely spread petals, found in most parts of India and Southern Europe. Some of the beneficial uses of *Solanum nigrum* extract include, its action against microbial infections, cure of skin diseases and as a hypoglycemic and antiulcerogenic agent (Satyavati *et al*., 1976; Chopra *et al*., 1951; Rao *et al*., 1969).

Immobilization/restraint stress is an easy and convenient method to induce both psychological (escape reaction) and physical stress (muscle work) resulting in restricted mobility and aggression (Ramanova, 1994; Singh *et al*., 1999). Recently, various stresses have been associated with enhanced free radical generation causing oxidative stress. One of the most important consequences of the generation of free radical is the peroxidation of membrane lipids. Moreover, stress has been suggested to decrease the level of glutathione (GSH) and vitamin C, which play an important role in protection of tissues from oxidative damage (Liu *et al*., 1994; Levi and Basuaj, 2000).

In the present study we have evaluated the antioxidant/pro-oxidant effect of *Solanum nigrum* on antioxidant enzymes like Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione-S-Transferase (GST) and the levels of thiobarbituric acid reactive substances (TBARS), glucose and uric acid in both normal and stressed rats with a view to elucidate the probable biological mechanism involved in the effects of this indigenous drug on oxidative metabolism of rats.

MATERIALS AND METHODS

Inbred male Wistar rats weighing (180-200 g) were selected. Animals were housed in-group cages; Purina diets and tap water were supplied to them ad libitum. Prior to commencement and throughout the experiment the rats were housed at 24±2°C room temperature and 12 h light/dark cycles. All the chemicals and reagents were purchased from commercial sources. All the experimental protocols adhered to the guidelines of the animal welfare committee of the University. The numbers of experimental rats were kept only 6 in each group according to the latest guidelines for reduction of animals in experiments.

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Table 1: Effect of single dose of crude extract of *Solanum nigrum* leaves on circulating levels of SOD, Catalase, GST, MDA, Glucose and Uric acid

	SOD Units mg^{-1} protein	Catalase Units mg^{-1} protein	GST nmoles mg^{-1} protein	MDA nmoles mg^{-1} protein	Glucose mg dL^{-1}	Uric acid mg dL^{-1}
Control (6)	2.96±0.63	0.75±0.06	0.25±0.03	5.65±0.36	174.50±19.97	6.99±1.55
Stresses rats (6)	1.63^{b}±0.36	0.37^{c}±0.04	0.11^{b}±0.01	10.05^{c}±0.10	136.36^{c}±0.52	8.88^{b}±0.72
Solanum nigrum alone (6)	2.52^{a}±0.15	0.76^{a}±0.02	0.36^{a}±0.03	4.86^{a}±0.28	175.91^{b}±6.04	5.60^{a}±0.59
Pre-extract stress treated (6)	1.99^{b}±0.12	0.41^{b}±0.02	0.17^{b}±0.05	8.99^{c}±0.18	186.50^{a}±2.66	7.99^{a}±0.05
Post-extract stress treated (6)	2.52^{c}±0.23	0.69^{b}±0.01	0.22^{b}±0.01	6.69^{c}±0.09	193.00^{a}±3.34	6.35^{c}±0.70

Numbers of experimental rats are indicated in the parenthesis, a = $p<0.02$, b = $p<0.05$, c = $p<0.001$ as compared with stress alone treatments

Preparation of aqueous extract of *S. nigrum* leaves: Fresh leaves of *S. nigrum* were collected locally, shade dried and powdered. Aqueous extract was prepared by refluxing with distilled water at 80°C and concentrated under vacuum. The weight/volume of the extract to solvent after complete dissolution was fixed at 100 mg mL^{-1} for oral administration with the help of catheter.

Immobilization stress was accomplished by placing individual animals in wire mesh cages of their size attached to a wooden board. The rats were deprived of food and water during stress exposure (Singh *et al*., 1999). The animals were subjected to 6 h of stress and then sacrificed by injecting sodium pentobarbital (i.p. 50 mg kg^{-1} of body weight). Control rats were handled at the same time as the stressed and were placed in individual cages during the corresponding time.

To elucidate the effect of *S. nigrum* extract on immobilization stress induced pro-oxidant changes (Zaidi and Banu, 2004), 24 rats were selected and divided into 4 groups of 6 rats each. The first group received normal-saline orally and served as control. The second group received *S. nigrum* aqueous extract (100 mg kg^{-1} body weight) orally, while the third and fourth group received the extract 1 h prior to (pre-extract stress treatment) and 1 h after (post-extract stress treatment) the 6 h stress session.

After the termination of experiment the rats were sacrificed by injecting sodium pentobarbital (i.p. 50 mg body $weight^{-1}$) and immediately exsanguinated. Blood was collected and centrifuged at 5000 rpm for 15 min; plasma was separated and quick-frozen at -40°C until assay.

The plasma was subjected for the assay of superoxide dismutase (Marklund and Marklund, 1974), catalase (Beers and Sizer, 1952), glutathione-S-transferase (Habig *et al*., 1974), thiobarbituric acid reactive substances (TBARS) (Halliwell and Chirico, 1993), glucose (Rabbo and Terkildsen, 1960) and uric acid by standard methods. The protein content was determined by the method (Lowry *et al*., 1951).

Statistical analysis: One-way ANOVA was used followed by pair wise comparison (Tukey's honestly significant Post hoc analysis) for significant differences between control and stress treatments. Statistical significance was defined at $p<0.05$. The statistical procedure was performed with SPSS analytical software USA. Data were expressed as mean±SEM. Similar statistical treatments were also given to the treatments with respect to the stress alone or non-stressed controls. The results are summarized in Table 1.

RESULTS AND DISCUSSION

Treatments with crude extract of *S. nigrum* (100 mg kg^{-1} body weight) did not show any significant change in general behavior, food intake or body weight in rats. Post treatment with crude extract of *S. nigrum*, markedly neutralized restraint stress induced changes in above parameters.

Effect of aqueous extract of *S. nigrum* leaves on stress induced oxidative changes: The present study revealed that 6 h of immobilization stress caused a significant decrease in the circulating activities of superoxide dismutase ($F_{1\text{-}9}$ = 21.256, $p<0.05$), glutathione-S-transferase ($F_{1\text{-}9}$ = 19.256, $p<0.05$), catalase ($F_{1\text{-}9}$ = 23.215, $p<0.001$) and the levels of glucose ($F_{1\text{-}9}$ = 22.325, $p<0.001$) with a significant increase in the level of TBARS ($F_{1\text{-}9}$ = 26.235, $p<0.001$) and uric acid ($F_{1\text{-}9}$ = 17.256, $p<0.05$) in comparison to non stressed controls. A single oral dose (100 mg kg^{-1} of body weight) of the extract did not cause significant change in the above-mentioned biochemical parameters.

Administration of crude extract of leaves both prior to and after immobilization stress resulted in a significant alteration in the circulating levels of antioxidant enzymes toward their control values. However, the post stress oral administration of extract (100 mg kg^{-1} of body weight) was found to be more effective in restricting stress induced decrease of SOD ($F_{1\text{-}9}$ = 13.265, $p<0.001$), GST ($F_{1\text{-}9}$ = 9.201, $p<0.05$), catalase ($F_{1\text{-}9}$ = 10.215, $p<0.05$) and uric acid ($F_{1\text{-}9}$ = 8.265, $p<0.001$) and increase in the levels of TBARS ($F_{1\text{-}9}$ = 15.325, $p<0.001$) and glucose ($F_{1\text{-}9}$ = 6.325, $p<0.02$) than the pre extract treatment as compared to stress treatment alone.

Immobilization stress has been shown to bring about antioxidant defense changes in rat plasma (Liu *et al*., 1994). SOD, GST, catalase play an important role in scavenging oxyradicals and their products (Mannervik and Danielson, 1988). In order to maintain the stability of a living organism it is necessary to reach a balance between the oxidative and anti oxidative defense, i.e., anti-FRS (free radical species). Enhanced free radical production with lipid peroxidation has been observed during stress (Clemens, 1991). The decreased activity of SOD, GST and catalase with decreased level of glucose, observed after 6 h of stress may be responsible for the elevation of free radical levels in stress (Claudiere and Ferrari-Illiou, 1999). The increase in the uric acid observed here could be body's natural response to scavenge excessive free radicals produced, as uric acid is one of the quencher of free radical/or because of enhanced xanthine oxidase activity, as observed during the oxidative stress (Davies *et al*., 1986). Thus, it seems that immobilization stress is capable of generating severe oxidative stress like situation in rats. In recent years, a number of drugs of plant origin have been investigated for their beneficial effects in man, *Solanum nigrum* has been a subject of considerable contemporary research. However, the anti-stress profile of this has not been clearly outlined (Mallika and Shayamla, 2004) and for the first time the present study shows that at least some biochemical changes by *Solanum nigrum* could help in explaining its adaptogenic role or property against the damaging effect of free radicals produced as a part of normal cell respiration and other cellular processes (Kaplowitz and Ookhtens, 1985).

The decrease in glucose levels as observed here could be due to hypoglycemic effect of immobilization stress. Moreover, studies have shown that *S. nigrum* leaves have a hypoglycemic effect, either by enhancing peripheral glucose uptake or by interacting directly with β-cells of pancreas (Karunanayake *et al*., 1984). Further, the decreased glucose concentration as observed here with decreased free radical scavenging enzyme activities might have contributed in aggravating the oxidative stress, because glucose is also a scavenger of OH^+ radicals, having a rate constant comparable with mannitol (Halliwell and Gutteridge, 1990). Other studies have also shown that immobilization stress significantly decreases circulating glucose level (Quirce and Maickel, 1981). According to the present study consumption of extract of *S. nigrum* resulted in reducing the oxidative stress by altering the activities of free radical metabolizing/ scavenging enzyme system.

Solanum nigrum extract was found to prevent and normalize/restore oxidative stress generated by immobilization stress, which was evident by return of the deranged activities of SOD, GST, catalase and levels of TBARS, uric acid and glucose towards their normal values, as compared to either untreated controls or stress alone treated groups. Probably the extract of *S. nigrum* acted as a free radical scavenger through enhancing the activities of SOD, GST, catalase and uric acid levels. The rats that received *Solanum nigrum* extract prior to stress exposure showed a resistance towards the derangement of their oxidative metabolism induced by immobilization stress, though post-extract treatment (curative) was found more effective in restoring the altered oxidative metabolism towards their control values than the pre-extract treatment (prophylactic).

Solanum nigrum is reported to act as an effective antioxidant of major importance against diseases and degenerative process caused by oxidative stress (Sarwat *et al*., 1995; Hui-Mei *et al*., 2008). The extract of this plant has been reported to contain many polyphenolic compounds, mainly flavonoids and steroids, some of the other chemical constituents reported in leaves are riboflavin, nicotinic acid, vitamin C, β-carotene, citric acid and oils. The antioxidant property of extract may, therefore be due to the presence of polyphenolic compounds (Rastogi and Mahrotra, 1998) β-carotene and vitamin C.

CONCLUSION

This study suggest that the extract of leaves of *Solanum nigrum* can be used both as prophylactic or curative agent in preventing/combating oxidative stress generated due to various diseases.

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