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Short-term Feeding Effects of *Origanum syriacum* Crude Extract on Immobilization Stress Induced Antioxidant Defense Changes in Rat

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Abstract: Ethnomedically genus *Origanum* L. is commonly used in many countries due to its stimulating, analgesic, antitussive, expectorant, sedative, anti-inflammatory and antihelminthic agent. The aim of this research was to study the antioxidant potential of the aerial parts of *origanum syriacum* extract and to evaluate its effect on the modulation of restraint induced oxidative stress. Rats were treated with crude extract of *origanum syriacum* 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 *O. syriacum* extract can be given both as a prophylactic and therapeutic supplement for scavenging free radicals.

Key words: Immobilization stress, antioxidant effect, *Origanum syriacum* extract, pro-oxidant, free radicals

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 (Ibrahim *et al.*, 2010).

Origanum syriacum 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 *Origanum syriacum* 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). Origanum essential oils were found to contain mainly thymol and carvacrol, Monoterpene glucosides, phenols including gallic acid, rosmarinic acid, caffeic acid, apigenin, naringenin and luteolin-7-O-glucoside (Zein *et al.*, 2011).

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 *et al.*, 1994; Singh *et al.*, 1993). 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). The present study have evaluated the antioxidant/pro-oxidant effect of *Origanum syriacum* on antioxidant enzymes like Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione-S-Transferase (GST) and the levels 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.

Preparation of aqueous extract of *O. syriacum* leaves:

Fresh leaves of *O. syriacum* 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.*, 1993). The animals were subjected to 6 h of stress and then sacrificed by injecting sodium pentobarbital (i.p., 50 mg kg⁻¹ of b.wt.). 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 *O. syriacum* extract on immobilization stress induced pro-oxidant changes (Kashif 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 *O. syriacum* aqueous extract (100 mg kg⁻¹ b.wt.) 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 kg⁻¹ b.wt.) 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), glucose (Raabo 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.

RESULTS AND DISCUSSION

Treatments with crude extract of *O. syriacum* (100 mg kg⁻¹ b.wt.) did not show any significant change in general behavior, food intake or body weight in rats. Post treatment with crude extract of *O. syriacum*, markedly neutralized restraint stress induced changes in above parameters.

Effect of aqueous extract of *O. syriacum* 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 (SOD) (1.63, p<0.05), glutathione-S-transferase (0.11, p<0.05), catalase (0.37, p<0.05) and the levels of glucose (136.36, p<0.001) with a significant increase in the level of uric acid (8.88, p<0.05) in comparison to non stressed controls.

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 b.wt.) was found to be more effective in restricting stress induced decrease of SOD (2.52, p<0.001), GST (0.22, p<0.05), catalase (0.69, p<0.001) and uric acid (6.35, p<0.001) and increase in the and glucose (193.0, p<0.02) than the pre extract treatment as compared to stress treatment alone as shown in the results in Table 1.

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 (Chaudiere and Ferrari-Iliou, 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

Table 1: Effect of single dose of crude extract of *Origanum syriacum* leaves on circulating levels of SOD, catalase, GST, MDA, glucose and uric acid

Groups	SOD (IU/mg protein)	Catalase (IU/mg protein)	GST (nM/mg protein)	MDA (nM/mg protein)	Glucose (mg dL ⁻¹)	Uric acid (mg dL ⁻¹)
Control	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	1.63±0.36 ^b	0.37±0.04 ^c	0.11±0.01 ^b	10.05±0.1 ^c	136.36±0.52 ^c	8.88±0.72 ^b
<i>Origanum syriacum</i> alone	2.52±0.15 ^a	0.76±0.02 ^b	0.36±0.03 ^a	4.86±0.28 ^a	175.91±6.04 ^b	5.60±0.59 ^a
Pre-extract stress treated	1.99±0.12 ^b	0.41±0.02 ^b	0.17±0.05 ^b	8.99±0.18 ^c	186.50±2.66 ^c	7.99±0.05 ^a
Post-extract stress treated	2.52±0.23 ^c	0.69±0.01 ^c	0.22±0.01 ^b	6.69±0.09 ^c	193.00±3.34 ^a	6.35±0.70 ^c

n = 6, a: p<0.02, b: p<0.05, c: p<0.001 as compared with stresses rats

(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, *Origanum syriacum* has been a subject of considerable contemporary research. However, the anti-stress profile of this has not been clearly outlined (Jainu and Shyamala Devi, 2004) and for the first time the present study shows that at least some biochemical changes by *Origanum syriacum* 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 *et al.*, 1985).

The decrease in glucose levels as observed here could be due to hypoglycemic effect of immobilization stress. Moreover, studies have shown that *O. syriacum* leaves have a hypoglycemic effect, either by enhancing peripheral glucose uptake or by interacting directly with β -cells of pancreas (Karunanayake *et al.*, 1984). This mechanism could be explained by the presence of bioactive compounds for this metabolic transformation process acting without indirectly through enhancing insulin secretion (Pari and Satheesh, 2004). Others had suggested that many plants may contain substances that would exert an effect on the insulin-secretion through either a stimulation of pancreatic insulin secretion or by up-regulation in proliferation of Langerhans β -cells (Holz, 2004). Like many other plant research, the mechanisms by which this plants constituents act to induce hypoglycemia was not elucidated. Whoever, the significance of this plant extracts to induce hypoglycemic in rats suggested that this plant contains ingredients with anti-diabetic properties (Iweala and Oludare, 2011). This action mechanism of the plant has been only hypothetically proposed where further studies needed to elucidate the mechanism of action at the molecular level. 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 Chirico, 1993; 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 *O. syriacum* resulted in reducing the oxidative stress by altering the activities of free radical metabolizing/scavenging enzyme system. *Origanum syriacum* 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, uric acid and glucose towards their normal values, as compared to either untreated controls or stress alone treated groups. Probably the extract of *O. syriacum* acted as a free radical scavenger through enhancing the activities of SOD, GST, catalase and uric acid levels. The rats that received *Origanum syriacum* 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). *Origanum syriacum* is reported to act as an effective antioxidant of major importance against diseases and degenerative process caused by oxidative stress (Sultana *et al.*, 1995; Lin *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 Mahotra, 1998) β -carotene and vitamin C.

CONCLUSION

This study suggest that the extract of leaves of *Origanum syriacum* is effective preventing the damage that can be caused by oxidative stress it also can be concluded that extract of leaves of *Origanum syriacum* can be used both as prophylactic or curative agent in preventing/combating oxidative stress generated due to various diseases. The data suggest an important role of *Origanum syriacum* in normalization of glucose elevation in rats where the levels of free radicals were decreased in

plasma. This could be an indicator of the important role as antioxidant protective role played concomitant with an important hypoglycemic effect. Further studies needed to elucidate the mechanism of action at the molecular level.

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