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ROLE OF *OCIMUM SANCTUM* ON NOISE STRESS INDUCED ALTERATIONS IN GLUCOCORTICOID AND CARBOHYDRATE METABOLISM

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ABSTRACT

Noise is a common environmental stress factor that leads to alterations in plasma catecholamine, adrenocorticotropic hormone (ACTH) and cortisol concentrations. The countries are now a day's interested in natural remedies, phytomedicines. In our present study we examined the effects of noise stress on glucocorticoid and glucose metabolism and the efficacy of ethanolic extract of *Ocimum sanctum* (*OS*) in preventing these alterations. Wistar albino male rats were divided into four groups-Groups I (Control), Group II (*OS* treated), Group III (stressed) and Group IV (*OS* pre treated and stressed). Noise stress for 15 days (100dB for 4hrs/ day) was given to the group III and IV and *OS* was injected to Group II and Group IV (100 mg/ kg bw. i.p). On the 16th day, animals were sacrificed and blood samples were analyzed for various biochemical parameters. In our study, corticosterone, glucose, insulin, glycogen phosphorylase levels and adrenal/body weight ratio were found to be significantly increased in the stressed group compared to the control and the levels of liver glycogen, muscle glycogen, glycogen synthase and thymus/body weight ratio were found to significantly decrease in the stressed group compared to the control. Our study showed that these alterations could be significantly reversed by *OS*.

KEY WORDS: **Noise-stress, Glucose metabolism, Corticosterone, *Ocimum sanctum.***

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INTRODUCTION

Stress is a daily phenomenon faced by every living being and it is essential for learning. It is the non-specific response of the body to any demand made upon it[1]. Stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). GAS involves three stages; Alarm stage, Resistance stage and Exhaustion stage[2]. GAS involves two major systems of our body, the nervous system and the endocrine system. The autonomic nervous system (ANS) affects many bodily functions instantly and directly, while hormones have slower yet wider effect on the body [3]. Both hormones and neurons communicate with cells and create the delicate dynamic balance between the body and its surroundings, through paired systems and feedback mechanisms [4]. The productive stress is called eustress while the other harmful stress is called distress[5]. Loud noise is considered as an environmental stress factor. Cortisol a glucocorticoid (a naturally occurring steroid), which triggers the fight-or-flight response is intended to save human beings when they are confronted by danger. Stimulation of this system results in increased glucose availability, increased blood flow and increased behavioral responsiveness during stressful situations. Although the short-term effects of glucocorticoids are essential, the long-term effects are damaging [6]. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases [7]. These effects have been attributed to antioxidant components such as plant phenolics, including flavanoids and phenyl propanoids [8]. A number of reports are available for such herbal antidotes. Both the ethyl acetate as well as the methanolic extract of *Acorus calamus* protected most of the changes in the rat brain due to antioxidant property of alpha asarone [9]. It is found that white noise (100 dB) exposure to male Wistar albino rats significantly increased the levels of plasma corticosterone and nor epinephrine in all three durations (acute, 1 day; sub-acute, 15 days; chronic, 30 days) of noise exposure [10]. Earlier studies in the department showed that *Ocimum sanctum* when administered alone did not induce any change in all the parameters studied and it was able to attenuate the noise induced oxidative stress indicating its adaptogenic property [11]. Adaptogens are such herbal agents, which help the body to overcome excess stress even in chronic cases without affecting the normal levels. The chief components present in *OS* are eugenol, methylchavicol, and alpha and beta bisabolen. Additional constituents are the flavonaglyca luteolin, epigenin and their 7-O-glucuronides as well as the C-glycosides orientin and molludistin and the triterpene acid ursolic acid. Of these components eugenol is responsible for the therapeutic activities if *OS* [12]. Hence, this present study was undertaken to evaluate the efficacy of *Ocimum sanctum* against 15 days noise stress induced changes in the glucose metabolism.

MATERIALS AND METHODS

(i) Animals

Adult male Wistar albino rats were selected for the study. Animal experiments were carried out after getting proper IACE and well as CPCSEA permission (IAEC no. 8/014/08).

(ii) Chemicals

All the biochemicals used in this experiment were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

(iii) Experimental protocol

Animals were divided into four equal (six animals each) groups. The different groups and their experimental schedule are given in Table1:

Table 1
Groups, experimental schedule and significance of group division

Groups	Experimental schedule	Significance
Group I	Control rats	To understand the normal levels.
Group II	*OS* treated control animals (100 mg/ kg bw. i.p)	To understand whether administration of *OS* could alter the normal levels or behave as adaptogens
Group III	15 days noise stress exposed animals (100dB for 4hrs / day)	To understand the influence of stress on the parameters studied
Group IV	15 days noise stress to 15 days *OS* pre treated animals (100 mg/ kg bw. i.p)	To understand the effect of *OS* during stress exposure.

After completion of the stress procedure the animals were left in the cages for 24hrs. On the 16th day animals were sacrificed along with control rats.

(iv) Noise stress procedure

Broad band (White) noise was produced by a white noise generator and amplified and connected to a loudspeaker. The intensity of the sound was measured by a sound level meter (Cygnet systems-D 2023 Serial No. F02199, India) and maintained at 100 dB intensity [13].

(v) Preparation of OS extract

Fresh OS plants were collected at IMPCOPS farms, Chennai, dried under shade and the leaves were powdered and the ethanol extract was prepared [14]. This method involves percolation at room temperature using 5 liters of 70% ethanol to which 500gm of powder was mixed and kept for 7 days. The supernatant was decanted, filtered and 2 liters of ethanol was added to the residue and kept for 2 days. The preparation was filtered and filtrate was added to the first filtrate. The residue was percolated once more with 2 liters of alcohol for 2 days. The filtrate collected in all the three stages was pooled, concentrated below 50°C in a vacuum centrifuge till the final residue was obtained. This was stored in deep freezer at -20°C.

(vi) Biochemical analysis

Plasma corticosterone level was estimated by the method proposed by Mattingly[15]. Glucose level in plasma was estimated by Merckotest Glucose kit (GOD POD, No 11862900011730) from Merck, India. Plasma insulin levels were assayed using standard Mercodia Rat Insulin ELISA enzyme immunoassay kit (no: 10-1124-04) by Mercodia, Sweden. Glycogen was estimated by the method of Hassid and Abraham [16]. Glycogen synthase was assayed by the method of Leior and Goldenberg [17]. In the presence of glycogen primer, glycogen synthase forms the bond between the glucose of uridine diphosphoglucose (UDPG) and C_4 of the terminal glucose residue of glycogen liberating uridine diphosphophosphate (UDP). The assay is based on the measurement of the amount of UDP formed from UDPG. Glycogen phosphorylase enzyme was estimated by method of Cornblath [18]. Glycogen phosphorylase cleaves the phosphoric bond of α-1, 4 linkages of glycogen molecule, to yield glucose-1-phosphate. The property of synthesizing glucose from glucose-6-phosphate by liberating inorganic phosphorous is made use of in this procedure. The phosphorous content was analysed by the method of Fiske and Subbarow [19].

(vii) Statistical analysis

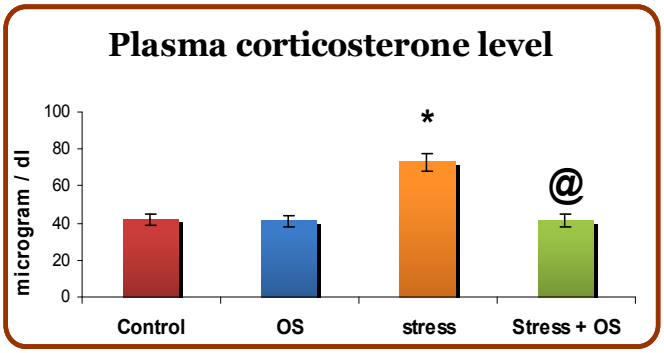
The data are represented with Mean ± SD in the form of bar diagram. The statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version 10.0 (SPSS, Cary, NC, USA). When there was significant difference, Tukey's multiple comparisons were performed by fixing the significance at $P<0.05$.

RESULTS

The data is represented as bar diagram with Mean ± SD.
* represents Control Vs Stress and @ represents Stress Vs *OS* treated stressed animals.
Control (Group I), Rx/OS (Group II), Stress (Group III) and Stress Rx/OS (Group IV)

1. Plasma corticosterone

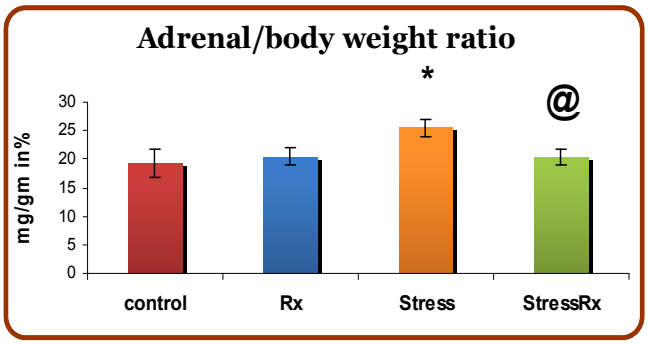
Graph 1
Plasma corticosterone levels in various group animals



There was a significant increase (df =3, F=109.4) in the corticosterone level in the stressed group animals when compared to the control animals. In the *OS* treated control animals corticosterone level was similar to control groups. The *OS* pretreated animals showed marked suppression in plasma corticosterone level when compared to the stress group similar to controls.

2. Adrenal/body weight ratio

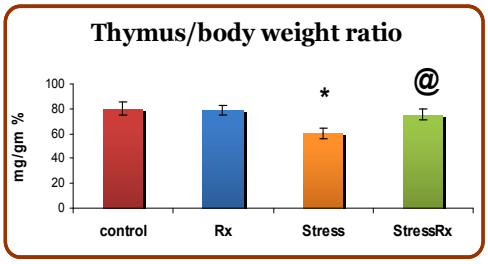
Graph 2
Adrenal/body weight ratio in various group animals



Adrenal / body weight ratio was significantly increased (df =3, F=14.1) in stressed animals when compared to control animals. *OS* treated control animals did not vary from the control groups. The *OS* pretreatment prevented the alteration in the adrenal / body weight ratio and the ratio remained similar to controls.

3. Thymus/body weight ratio

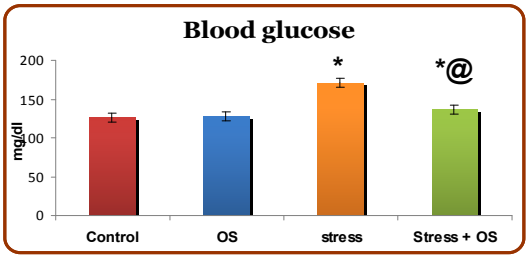
Graph 3
Thymus/body weight ratio in various group animals



Thymus /body weight ratio was significantly decreased (df =3, F=24.8) in stressed animals when compared to control animals. *OS* treated control animals did not vary from the control groups. The *OS* pretreatment prevented the alteration in the thymus weight ratio and the ratio remained similar to controls.

4. Glucose estimation

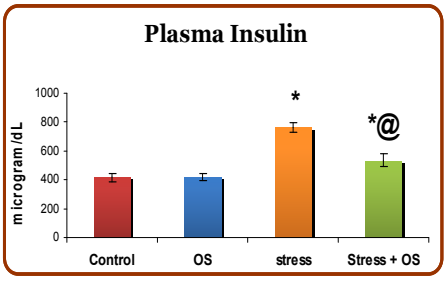
Graph 4
Blood glucose levels in various group animals



There was a significant increase in the glucose level in the stress group animals (df =3, F= 80) when compared with the control animals. *OS* treated control animals did not vary from the control groups. The *OS* pretreated animals showed marked decreased the glucose level from the stressed group. However; it was showing still a marked increase from control levels.

5. Insulin estimation

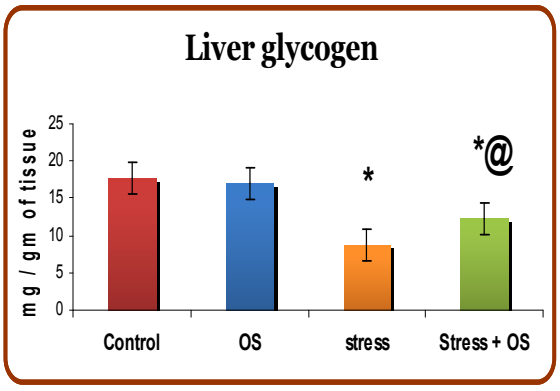
Graph 5
Plasma Insulin levels in various group animals



There was a significant increase (df =3, F= 147.9) in the insulin level in the stressed group animals when compared with the control animals. In *OS* treated control animals, no marked change was observed. The *OS* pretreatment markedly suppressed the elevation of plasma insulin from the stressed group though it showed a marked increase from control animals.

6. Liver glycogen

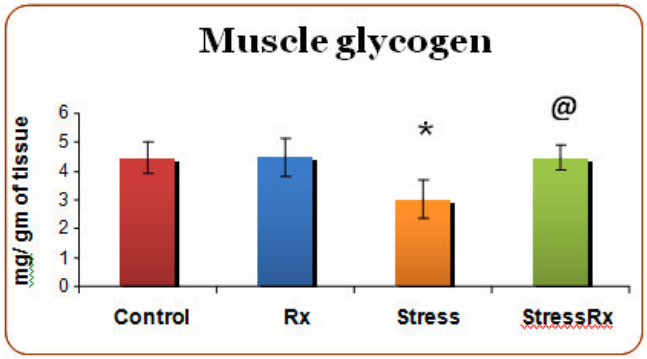
Graph 6
Liver glycogen levels in various group animals



There was a significant decrease in the liver glycogen level (df =3, F=29.4) in the stressed animals when compared to the control animals as well as *OS* treated stressed animals. *OS* treated control animals showed no changes in its glycogen level from the control groups. The pretreatment of animals with the extract of *OS* elevated the glycogen content markedly from stressed animals. However, still it showed marked decrease for the control animals.

7. Muscle glycogen

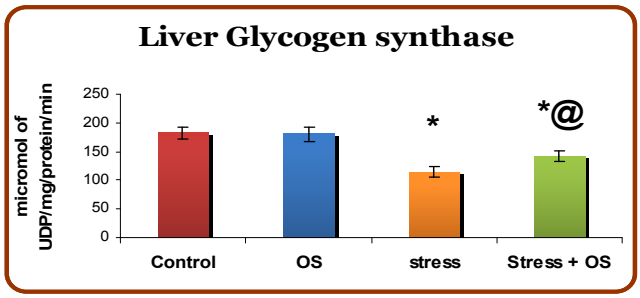
Graph 7
Muscle glycogen levels in various group animals



There was a significant decrease (df =3, F=.618) in muscle glycogen level in the stressed group animals when compared to the control animals as well as *OS* treated stressed animals. *OS* treated control animals showed no significant changes from control groups. The pretreatment of animals with the extract of *OS* markedly elevated the level from stressed animals and level of muscle glycogen was similar to the controls.

8. *Glycogen synthase*

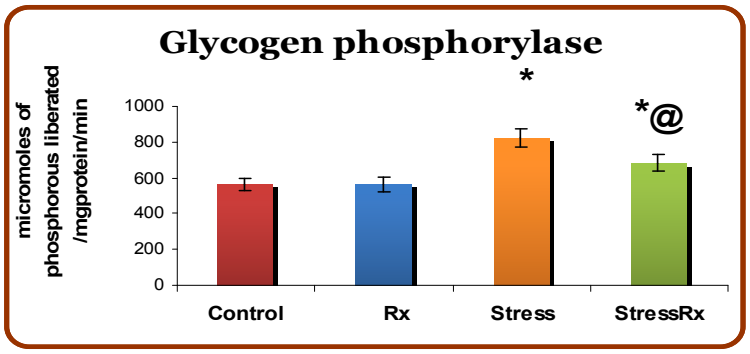
Graph 8
Liver glycogen synthase levels in various group animals



There was a significant decrease (df =3, F=59.6) in the enzyme activity in the stressed animals when compared to the control animals. In *OS* treated control animals, the glycogen synthase activity also remained similar to the control groups. The pretreatment of animals with the extract of *OS* decreased of glycogen synthase activity markedly from stressed group animals; though it still showed an increase in its activity from controls.

9. *Glycogen phosphorylase*

Graph 9
Glycogen phosphorylase levels in various group animals



There was a significant increase (df =3, F=47.6) in the enzyme activity in the stressed animals when compared with the control animals as well as from *OS* treated stressed animals. *OS* treated control animals showed no significant changes from the control groups. The pretreatment of animals with the extract of *OS* suppressed the elevation of glycogen phosphorylase markedly from stressed animals. However, it showed a marked increase from control animals.

DISCUSSION

In this study most of the parameters studied revealed that the stress is not yet adapted and there was an increase in the corticosteroid level even after fifteen days of repeated noise stress exposure. Activation of adrenal glands in stress was due to ACTH, the enhanced level of which always preceded the elevation of glucocorticoids [20]. This persistent increase in steroid level can be attributed to be due to the changes in the glucocorticoid receptor levels,

which were associated with the altered gluocorticoid negative feedback sensitivity in rats [21]. The *OS* treatment during stress could prevent this increase in the corticosteroid level indicated its anti stressor activity. In this study, the adrenal gland also showed a marked increase in its weight ratio whereas thymus weight ratio showed a marked decrease indicating the hypertrophy of the adrenal gland and atrophy of the thymus gland. ACTH apart from increasing the secretory activity of adrenal cortex could also cause hypertrophy and proliferation of adrenocortical cells [22]. This explanation gives the possible reason for the increase in the weight ratio of the adrenal gland. Similar results have been observed in rats subjected to noise, which showed a significant increase in adrenal gland weight ratio with decrease in thymus and spleen weight ratio [23]. In this study, the treatment with *OS* appears to be more beneficial as the adrenal as well as thymus weight ratio was not altered during stress exposure. *OS* extract is found to prevent the increase in adrenal weight in mice subjected to swimming stress [14].

OS helps the body to cope with stress in a better way. The changes in whole brain acetylcholine level after the exposure to noise stress was blocked by the *OS* extract indicating that *OS* constituents may act at the CNS level to reduce the stress. If *OS* could act on brain, level of ACTH may be reduced and where by the corticosteroid level was normalized. This may be reason for the normal organ weight ratios maintained. Corticosteroid over secretion causes the hypertrophy of adrenal and thymus weight reduction [24]. Blood glucose level depends on the liver glycogen, corticosteroid and insulin levels. Acute stress responses could increase the adrenaline, noradrenaline, glucagon and corticosterone levels within minutes [25]. This increase may be to meet certain energy demand in certain body areas. The primary function of cortisol is to promote gluconeogenesis, which is an essential component of our body's adaptation to stress, ensuring that the vital organs have enough energy to meet the increasing workload. This demand triggers mechanisms to compensate for the increased withdrawal of glucose from the blood to tissues. In this study, the corticosteroid elevation was accompanied by an increase in blood glucose, insulin and a decrease liver glycogen with the increase in the glycogenolytic enzyme phosphorylase with the decrease in the glycogen synthase's activity. Elevation of glucocorticoid causes an increase in glucose production and utilization [26]. The release of catecholamines and subsequently glucagon and corticosterone stimulate glycogenolysis and gluconeogenesis [27]. Hence the corticostreone and other stress hormones release may be responsible for the observed changes.

The increase in blood glucose might favor the insulin release. However, no increase in liver glycogen synthase activity or liver glycogen was observed. This may be due to the development of resistance to insulin. In fact, in vitro and in vivo studies also suggest that glucocorticoid excess causes insulin resistance by either altering the binding of insulin to its receptor or impairing the intra-cellular response to insulin [28]. Moreover in patients experiencing corticosteroid related hyperglycemia, persistent diabetes mellitus development was reported [29]. It has been suggested that basil leaves improve the beta cell function and enhance insulin secretion. But this may not be the mechanism as the insulin level in *OS* treated controls did not deviate from the controls in their insulin level [30]. Moreover in the *OS* treated stressed animals, though the level was reduced from noise stressed animals, there is a report that "tulsi" leaves inhibit absorption of glucose from the intestines, but the nature of active principle and exact mode of its action remains unclear [31]. OS possess anti-hyperglycemic activity [32]. The ability of saponin to inhibit both gastric emptying and intestinal glucose absorption was already reported [33]. Tannins via their anti oxidative actions increased the secretion of insulin and decreased the hyperglycemia in diabetic rats [34]. Eugenol decreases elevated blood sugar level, triglycerides, cholesterol and liver enzymes and is responsible for cardioprotective, hypolipidemic and hepatoprotective agent [12].

CONCLUSION

In conclusion, our study confirms that repeated noise stress exposure could affect the carbohydrate metabolism. 15 days repeated noise stress elevated the glucocorticoid (corticosterone) level indicating that the stress was not yet adapted, increased the blood glucose level which may be due to the release of stress hormones, decreased the glycogen level in liver and skeletal muscle as the phosphorylase and the glycogen synthase are modulated by stress hormones. Noise stress induced hormones increased the phosphorylase activity and decreased the glycogen synthase activity with the decrease in muscle and liver glycogen. Also blood insulin level was found to increase which may be due to the increase in the glucose in blood circulation. 15 days repeated noise stress increased the adrenal organ weight / body weight ratio whereas decreased the thymus weight ratio. Administration of *OS* to control animals did not alter any of the parameters studied, confirms the adaptogenic nature of OS. The changes induced due to 15 days noise stress were significantly reduced after OS administration. The dosage of OS used may not be adequate enough to prevent all the noise stress induced changes.One can reasonably hypothesize that *OS* may contain some substance that can block the afferent input so that the induction of alterations in the body is blocked. For a better understanding of this idea, it can be compared with the pain gate theory where the endogenous endorphins block the sensory input. Probably, the analgesic substance in *OS* blocks the sensory input. Further, more clinical studies are required to investigate the effectiveness of OS in humans,

CONFLICT OF INTEREST

Conflict of interest declared none

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