

## EFFECT OF OCIMUM SANCTUM LINN ON NOISE INDUCED CHANGES IN PLASMA CORTICOSTERONE LEVEL

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**Abstract:** Ethanol extract of leaves of *ocimum sanctum* was screened for its antistressor actions against acute and chronic noise stress in albino rats by investigating the plasma corticosterone level in these animals. There was a significant elevation of the corticosterone level in plasma of rats subjected to 30 min noise (100 dB) stress. Chronic exposure (4 hr daily for 30 days) to noise with same intensity reduced the hormonal level significantly.

Treatment of animals with ethanol extract of *Ocimum sanctum* prevented the changes in plasma level of corticosterone induced by exposure to both acute and chronic noise stress, indicating the antistressor property of the plant against noise.

**Key words :** noise stress  
corticosterone

*ocimum sanctum*

### INTRODUCTION

*Ocimum sanctum* Linn (OS) is considered as a sacred plant by Indians. It is commonly called Tulsi (Hindi), Thulasi (Tamil) or Holibasil (English). The plant belongs to the family Labiatae (Lamiaceae). In India, this plant is widely used in various traditional systems of Medicine, particularly Ayurveda and Unani (1, 2).

The antistressor effect of OS on the changes caused by different types of stress had been studied in animals. The ethanol extract of the plant was found to increase the physical endurance and survival time in swimming mice (2). The severity of convulsions in mice subjected to electroshock was reported to be reduced by the ethanol extract of OS (3). The ethanol extract of the leaves from OS was found to prevent the changes in the

concentration of adrenergic hormones in brain of rats exposed to swimming stress and gravitational stress (4). Sen et al (5), had reported that the essential oil from OS leaves and seeds reduced the psychobiological changes induced by restraint stress.

However, the antistressor effect of OS on the noise induced changes has not been reported so far. Hence, the present study is undertaken to evaluate the antistressor effect of OS on albino rats subjected to acute and chronic noise stress. The plasma corticosterone level is used as a tool.

### METHODS

#### Plant extract

Fresh leaves of *Ocimum sanctum* were collected from the garden of Sri Ramachandra Medical College and Research Institute,

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Porur, Madras. The plant was identified and authenticated by Professor Dr. K. Thorthathri, Madras University Botany Field Research Laboratory, Madras. Powder of the shade dried leaves was extracted by percolation at room temperature with 70% ethyl alcohol as described by Bhargava and Singh (2). The extract was concentrated in vacuo below 50°C till the residue was obtained. The yield of the extract was 20% (w/w) in terms of dried starting material. For the experimental use, the product was dissolved in propylene glycol (10 gm/100 ml) as done by Bhargava and Singh (2).

#### Stress procedure

Exposure of animals to noise stress was carried out as done in the previous study (6). The animals were placed in a noise stress chamber. Sine waves were obtained by a function generator and amplified by an amplifier which was connected to a loud speaker fixed at the roof of the chamber at a height of about 40 cm above the animal cage. A column speaker was used in order to obtain uniform intensity of noise in whole area of the chamber. The instruments were set to produce a pure tone noise with an intensity of 100 dB and a frequency of 10 KHz. The intensity of the sound was measured by a sound level (decibel) meter (Kamlex SLM3). The ambient noise in the room where the chamber was placed was <45 dB, which was due to the ventilation system of the room.

#### Animals

Adult male albino rats of Wistar strain were used for the study. All the rats were of same age group weighing 150-170 gms. The rats were maintained under standard conditions and received food and water *ad libitum*.

Six groups of animals were selected for acute studies and another six groups for chronic studies. A common control group was used for both the studies. All the groups had equal number of (6) rats.

#### Animals of acute studies

- Group I : *Control animals*
- Group II : *Acute stress* - These animals were exposed to noise (100 db) stress for 30 min and sacrificed after 30 min.
- Group III : *Acute stress with OS* - This group of rats were pretreated daily with ethanol extract of OS (100 mg/kg) orally for 7 days. On 8th day, these were subjected to 30 min noise (100 dB) stress and sacrificed after 30 min.
- Group IV : *OS 7 days* - These rats received OS extract (100 mg/kg) orally, daily for 7 days and were sacrificed on the 8th day.
- Group V : *Stressed vehicle control* - This group of animals were treated with propylene glycol (1 ml/kg) orally for 7 days. On 8th day, these were exposed to 30 min noise (100 dB) stress and sacrificed after 30 min.
- Group VI : *Vehicle control* - These were given propylene glycol (1 ml/kg) daily orally for 7 days and sacrificed on 8th day.

#### Animals of chronic studies

- Group I : *Control animals*
- Group II : *Chronic stress* - The rats of this group were subjected to 4 hr continuous noise (100 dB) for 30 days and killed on 31st day.

- Group III : *Chronic stress with OS* - These animals were treated with extract of OS (100 mg/kg) daily for 7 days. On the 8th day, onwards, the treatment with OS extract was continued with 4 hr noise (100 dB) stress daily for 30 days and were sacrificed on the 38th day.
- Group IV : *OS 37 days* - These rats received OS extract (100 mg/kg) orally, daily for 37 days and were sacrificed on the 38th day.
- Group V : *Stressed vehicle control* - This group of animals were treated with propylene glycol (1 ml/kg) orally for 7 days. From 8th day, propylene glycol was given followed by 4 hr noise (100 dB) stress and sacrificed on 38th day.
- Group VI : *Vehicle control* - The rats belonging to this group were given propylene glycol (1 ml/kg) daily for 37 days and sacrificed on 38th day.

The food and water intake and the behaviour of all the animals were observed throughout the study. All the experimental procedures were carried out in the forenoon between 6 a.m. and 10 a.m. to avoid the influences of circadian rhythm. The administration of OS extract or propylene glycol was done in the morning at 6 a.m. for all the groups of treated animals. The rats were sacrificed by quick decapitation without any initial disturbances. The trunk blood was collected in a heparinized container.

#### Estimation of plasma corticosterone

This was done by the method of Mattingly (7). The extraction of free and protein bound corticosterone from plasma was done by using

dichloromethane. This extract was shaken with a sulphuric- ethanol reagent. The resulting fluorescence was read in fluorimeter at excitation of 470 nm and emission of 530 nm.

#### Statistical analysis

The data of all results from different groups belonging to Acute and Chronic studies were analysed by the application of one-way Analysis of Variance - ANOVA. The multiple comparisons to elicit the significant difference between the groups were performed by means of Tukey's test and  $P < 0.05$  level was fixed for statistical significance.

## RESULTS

*Acute studies (Table I):* There was a significant increase in plasma level of corticosterone in rats subjected to 30 min noise stress ( $F = 14.38$ ;  $df = 5,30$ ;  $P < 0.001$ ). Tukey's test also showed a significant difference between control and all stressed animals (with or without propylene glycol). The elevation of corticosterone level induced by stress was prevented in animals pretreated with extract of OS. The hormonal level in rats administered with OS extract or propylene glycol for 7 days (without exposure to stress) was similar to that of controls.

*Chronic studies (Table II):* Corticosterone level was significantly reduced after chronic exposure to noise in both Chronic stress group and Stressed vehicle control group ( $F = 23.84$ ;  $df = 5,30$ ;  $P < 0.001$ ). Tukey's test did not reveal any change in the corticosterone level from basal value in all the other groups.

## DISCUSSION

The increased corticosterone level in animals exposed to 30 min noise (100 dB) has

TABLE I : Plasma corticosterone level in acute studies.

Group		Corticosterone (mg%)	Tukey's test C.I. overlap
1. Control	(6)	89.70 ± 3.65	3, 4, 6
2. Acute stress	(6)	158.52 ± 3.93	5
3. Stress with OS	(6)	99.72 ± 3.02	1, 4, 6
4. OS 7 days	(6)	91.02 ± 3.77	1, 3, 6
5. Stressed vehicle control	(6)	156.20 ± 2.07	2
6. Vehicle control	(6)	90.07 ± 4.08	1, 3, 4

Values are expressed as Mean ± SEM.

Number in parenthesis indicates the number of animals used.

C. I overlaps confirm Null Hypothesis.

No overlap indicates Significant Difference (P<0.05).

TABLE II : Plasma corticosterone level in chronic studies.

Group		Corticosterone (µg%)	Tukey's test C.I. overlap
1. Control	(6)	89.70 ± 3.65	3,4,6
2. Chronic stress	(6)	58.62 ± 2.96	5
3. Stress with OS	(6)	86.55 ± 2.85	1,4,6
4. OS 37 days	(6)	91.88 ± 3.55	1,3,6
5. Stressed vehicle control	(6)	59.52 ± 2.58	2
6. Vehicle control	(6)	90.90 ± 3.82	1,3,4

Values are expressed as Mean ± SEM.

Number in parenthesis indicates the number of animals used.

C.I Overlaps confirm Null Hypothesis.

No overlap indicates Significant Difference (P < 0.05).

been already reported from this laboratory (6). Similar observation was reported by De Boer et al also (8).

The elevation of corticosterone at 30 min after exposure to noise was prevented by pretreatment of animals with OS extract for 7 days.

In the present study, the corticosterone level was significantly reduced in rats exposed to 30 days noise stress. Similar result were noticed by Smoker and Buckley (9) in rats after 5 weeks exposure to noise.

The mechanism involved in reduction of corticosterone level by chronic exposure to stress was not understood well. The probable

cause suggested by Henkin and Knigge (10) was the blockade in release of ACTH through hypothalamo - hypophyseal axis. Role of opioid receptors in the reduction of corticosterone level was also suggested by Wilson et al (11).

The results of this study revealed that the reduction in corticosterone level caused by chronic exposure to noise stress was prevented by the treatment of animals with OS extract.

Thus, the observations of the present study indicated the normalizing activity of OS extract on the increased corticosterone level induced by acute noise stress and on the decreased corticosterone level caused by chronic exposure to noise.

This could fulfill the criteria suggested by Brekhman and Dardymov (12) that the adaptogenic agents could possess the normalizing action irrespective of direction of forgoing pathological changes. The concepts of Brekhman and Dardymov (12) regarding the adaptogenic properties of plant materials had been accepted by recent investigators also (2, 3).

According to Brekhman and Dardymov (12), another important criteria for naming a plant material as an adaptogen, is that the material should be innocuous and cause minimum disorders in physiological functions of an organism. The LD<sub>50</sub> of OS extract was found to be 4508 ± mg/kg by oral route (1), indicating the low toxicity of the plant material. In this study, the long term treatment of rats with OS extract did not

cause any change in the body weight food intake and the behaviour of the animals indicating the absence of adverse effect of the plant material itself.

According to Lazarev, who coined the name "Adaptogens" for the substances showing antistressor actions, these substances showed their activity by inducing a "State of Nonspecific Increased Resistance" (SNIR) in animals and man (12).

This is only a preliminary study on the antistressor activity of OS against noise stress. To name this plant material as an "Adaptogen", it is worthwhile (i) to explore its actions on many other changes induced by noise stress and (ii) to confirm that this plant material acts by inducing SNIR.

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