

LETTER TO THE EDITOR

EFFECT OF OCIMUM SANCTUM LINN ON CHANGES IN LEUCOCYTES OF ALBINO RATS INDUCED BY ACUTE NOISE STRESS

Sir,

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Ocimum sanctum linn (OS), commonly called Tulsi or Thulasi belongs to the family Labiatae (Lamiacea). Many reports are available regarding the antistressor activity of this plant material on the changes induced by different stress agents (1-4). However, the antistressor action of OS on the changes produced by noise had not been explored so far.

In our earlier study, we have noticed the antistressor effects of ethanol extract of OS leaves on noise induced changes in plasma corticosterone level (5). Hence, in this study, it was decided to evaluate the effect of ethanol extract of OS leaves on the changes in total count (TC) and differential count (DC) of leucocytes in albino rats subjected to noise stress, because, the hematological parameters too are acclaimed to be the important stress indices.

Exposure of rats to noise stress

Exposure of rats to noise stress was done as in our previous study (6) by placing them in a noise chamber. Pure tone noise of sine waves with a frequency of 10 KHz was produced by a function generator. These sound waves were amplified and fed into a loud speaker fixed at the roof of the chamber. The intensity of noise reaching the

floor of the chamber was set at 100 dB by using decibel meter.

Extract of ocimum sanctum leaves

The leaves of ocimum sanctum plants were dried in shade and powdered. Ethanol extract of this powder was prepared as in the previous study (5). The final yield of the extract in terms of dried leaves was 20% (w/w). The final product was used for experimental purpose by dissolving in propylene glycol (10%, w/v).

Experimental animals

36 adult male albino rats of Wistar strain with same age group weighing 150-170 g were selected for the study. Standard conditions with free food and water were provided for the animals. The rats were randomly divided into 6 equal group viz.

- Group 1 : Control animals.
- Group 2 : *Noise stress*- This group of animals were exposed to noise (100 dB) for 30 minutes and then sacrificed after 30 minutes.
- Group 3 : *Noise with OS* - These rats

were pretreated daily with ethanol extract of OS (100 mg/kg) orally for 7 days. On the 8th day, these rats were exposed to 30 minutes noise stress and sacrificed after 30 minutes.

Group 4 : OS 7 days - OS extract (100 mg/kg) was administered to these rats for 7 days. And these rats were sacrificed on the 8th day.

Group 5 : Stressed vehicle control - Animals of this group were given propylene glycol (1 ml/kg) orally for 7 days. On 8th day, these rats were sacrificed after 30 minutes.

Group 6 : Vehicle control - These rats received propylene glycol (1 ml/kg) orally daily for 7 days and were sacrificed on 8th day.

In order to avoid the influences of circadian fluctuations, all the experimental procedures were done in the forenoon between 0600 h and 0800 h. The animals were sacrificed by quick decapitation. The trunk blood was collected in a heparinized container to enumerate the TC and DC. Among the different white blood cells, only the neutrophils, eosinophils and lymphocytes were considered since the basophils and monocytes were in negligible numbers. All the experiments concerned with cell counts were done in duplicate and the mean of these values were taken into account.

Statistical analysis

The values of TC and DC of leucocytes from different groups of animals were subjected to one-way Analysis of Variance - ANOVA. This was followed by Tukey's multiple comparison test to determine the significant difference between the groups. Statistical significance level was fixed at $P < 0.05$.

RESULTS

In the rats subjected to acute noise stress, there was a significant reduction in T.C ($F = 14.376$; d.f = 5,30; $P < 0.001$), lymphocyte count ($F = 34.029$; d.f = 5,30; $P < 0.001$) and eosinophil count ($F = 12.287$; d.f = 5,30; $P < 0.001$). However, there was an increase in neutrophil count ($F = 21.890$; d.f = 5,30; $P < 0.001$).

The results of the Tukey's Multiple Comparison Tests for all the parameters are given in Table I :

Pretreatment of rats with OS extract before the exposure to noise stress (Group 3) prevented the leucopenia and the changes in DC. Pretreatment of animals with propylene glycol before exposure to noise (Group 5) did not affect the stress induced changes in both TC and DC.

The values of TC and DC were not altered from control levels in rats which received OS extract (Group 4) or propylene glycol (Group 6) for 7 days before sacrificing.

TABLE I : Tukey's Multiple Comparison Test for total leucocyte count and differential leucocyte count.

Parameter	No.	Sample name	Mean \pm SEM n = 6	C.I. Overlap
Total leucocyte count (per cu. mm)	1.	Control	13,383 \pm 488	3,4,6
	2.	Noise stress	10,258 \pm 348	5
	3.	Stress with OS	12,975 \pm 437	1,4,6
	4.	OS 7 days	13,742 \pm 395	1,3,6
	5.	Stressed vehicle control	10,133 \pm 352	2
	6.	Vehicle control	13,208 \pm 536	1,3,4
Neutrophil count (per cu. mm)	1.	Control	3,288 \pm 152	3,4,6
	2.	Noise stress	4,185 \pm 120	5
	3.	Stress with OS	3,158 \pm 147	1,4,6
	4.	OS 7 days	3,458 \pm 154	1,3,6
	5.	Stressed vehicle control	4,128 \pm 140	2
	6.	Vehicle control	3,201 \pm 146	1,3,4
Eosinophil count (per cu. mm)	1.	Control	421 \pm 41	3,4,6
	2.	Noise stress	120 \pm 32	5
	3.	Stress with OS	411 \pm 51	1,4,6
	4.	OS 7 days	457 \pm 58	1,3,6
	5.	Stressed vehicle control	111 \pm 37	2
	6.	Vehicle control	406 \pm 48	1,3,4
Lymphocytes count (per cu. mm)	1.	Control	9,670 \pm 189	3,4,6
	2.	Noise stress	5,949 \pm 127	5
	3.	Stress with OS	9404 \pm 152	1,4,6
	4.	OS 7 days	9,792 \pm 160	1,3,6
	5.	Stressed vehicle control	5,891 \pm 116	2
	6.	Vehicle control	9,598 \pm 158	1,3,4

C.I Overlaps confirm Null hypothesis.

No. Overlap indicates significant difference at the level of $P < 0.05$.

DISCUSSION

Since the changes in most of the stress indices start appearing 30 minutes after exposure to acute noise stress (6), the animals were sacrificed in this study 30 minutes after the end of stress period. Few reports are available in support of the results obtained in this study regarding noise induced changes in TC and DC. The leucopenia was observed in mice subjected to 90 minutes noise stress (7), and one hour confinement stress (8). It could be due to the excessive release of corticosteroids in stress conditions which was known to cause leucopenia (7). The reduction in lymphocyte count was also noticed in rats exposed to

restrain stress (10). The stress induced reduction in lymphocyte count was attributed to redistribution and trapping of lymphocytes in lymph nodes (9). Under normal conditions in rats, the neutrophil count is less than that of lymphocyte (10). And, there is an inverse relation between these two cells both in basal conditions and stressed conditions. Thus, the noise induced increase in neutrophil count could be secondary to the reduction in lymphocyte count.

The results of the present study revealed the normalizing activity of OS extract on noise induced changes in TC and DC in rats. In our earlier study, the normalizing

activity of OS extract was noticed on the increased corticosterone level induced by acute noise stress (5).

Thus, OS is an 'adaptogen' that acts as an antistressor (1, 2, 11). However, the exact mechanism by which it acts as an adaptogen is not known. According to Brekhman and Dardymov an adaptogen acts by inducing a "State of Non-specific Increased Resistance (SNIR) in animals and man (11). However, in order to include OS in the group of

"Adaptogens" against noise, it is necessary to explore its normalizing actions on many other physiological changes induced by noise stress. It is also worthwhile to find out whether OS acts by inducing SNIR.

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