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Effect of *Ocimum sanctum* Linn. on cardiac changes in rats subjected to chronic restraint stress

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Abstract

Male Wistar rats were subjected to chronic restraint stress (CRS; 6 h/day for 21 days) alone or along with either hydroalcoholic extract of *Ocimum sanctum* (Os; 100 mg/kg; orally) or MK-801, an NMDA receptor antagonist (0.3 mg/kg; i.p.). In the rats subjected to only CRS, plasma cAMP level was significantly raised on day 21, with no significant change in plasma corticosterone level. There was a significant ($p < 0.05$) fall in myocardial glutathione level, along with a significant ($p < 0.05$) rise in myocardial superoxide dismutase (SOD) and catalase activities, while light microscopy showed evidence of myocardial edema. Both Os and MK-801 significantly prevented the CRS-induced rise in plasma cAMP level, myocardial SOD and catalase activities as well as the light microscopic changes in the myocardium. This study revealed that *Ocimum sanctum* protects rat heart from chronic restraint stress induced changes, through its central effect.

Keywords: Chronic restraint stress; Myocardium; Oxidative stress; *Ocimum sanctum* (Labiatae); MK-801

1. Introduction

Psychological stress has recently been identified as a major cardiovascular risk factor (Wittstein et al., 2005). Stress leads to the increased release of catecholamines and glucocorticoids by the activation of sympathoadrenal and hypothalamic pituitary adrenal (HPA) axis, (Pettersson et al., 1990; Aguilera et al., 1995) as well as increased release of excitatory amino acid (EAA) in brain (Moghaddam, 1993). Chronic exposure to all these mediators has deleterious biological effects, in which oxidative stress plays a major etiopathological role (Simmons et al., 1991). In this regard, a number of medicinal plants with antioxidant properties have been shown to modify or alter the course of stress-associated diseases. These plants have been identified as a class of metabolic regulators (of natural origin), which increase the ability of the organism to adapt to environmental stressors (Pannossian et al., 1999).

Ocimum sanctum Linn. (Labiatae) commonly known as Tulsi in India has extensively been used in Indian traditional systems of medicine for various human ailments. The salutary effects of *Ocimum sanctum* have earlier been reported in cardiovascular diseases, where oxidative stress plays a major role (Sharma et al., 2001; Sood et al., 2005). The psychological stress alleviating effect of *Ocimum sanctum* has also been documented earlier (Bhargava and Singh, 1981; Sen et al., 1992). However, there is a lack of information regarding the effect of *Ocimum sanctum* on the cardiac changes associated with psychological stress. Therefore, the present study was designed to investigate whether *Ocimum sanctum* has any effect on the myocardial oxidative stress markers and the associated histopathological changes in rats, subjected to chronic restraint stress.

2. Material and methods

The study conforms with the guide for the care and use of laboratory animals US, NIH (Publication no. 85-23, 1985) and all procedures were approved by the institute animal ethics committee (no. 119/IAEC/01).

2.1. Experimental animals

Male Wistar rats (200–250 g body weight; 10-week old) were randomly divided in five groups ($n = 8$ in each group) with food and water ad libitum except during the stress period. Animals

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were maintained in a 12 h light/dark cycle, temperature of 22 ± 2 °C before and throughout the experiment. Body weight was measured every week and expressed as body weight gain per 100 g body weight of rat.

All chemicals were of analytical grade and were obtained from Sigma chemicals (St. Louis, USA) except otherwise mentioned. Double distilled water was used for all biochemical assays.

2.2. Experimental treatment groups

2.2.1. Chronic restraint stress (CRS) (Madrigal et al., 2001b)

Rats were subjected to restraint stress of 6 h/day (10–16 h) in adjustable Plexi glass restrainers (INCO, Ambala, India) for 21 days. During the restraint sessions, the rats had no access to food and water. The sham control animal group were not subjected to restraint but were devoid of food and water for 6 h daily (10–16 h) for 21 days.

Groups studied:

- Group C—control.
- Group sham C—sham control with no food and water.
- Group CRS—chronic restraint stress for 6 h/day for 21 days.
- Group CRS + Os 100—chronic restraint stress along with *Ocimum sanctum* (100 mg/kg; orally) for 21 days.
- Group CRS + MK-801—chronic restraint stress along with MK-801 (0.3 mg/kg; i.p.) for 21 days.

2.3. Preparation of plant extract

The leaves were purchased from the local market and the pharmacognostic authentication was carried out by the Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India (voucher no. 60). The method for extraction was same as described by Bhargava and Singh (1981). Briefly the sun dried powder of the *Ocimum sanctum* leaves was extracted by percolation at room temperature with 70% ethyl alcohol. The extract was concentrated in vacuo below 50 °C till a residue was obtained. The yield of the extract was 13% (w/w) in terms of dried starting material. The residue of *Ocimum sanctum* extract was suspended in 1% carboxymethyl cellulose to make a solution of 50 mg/ml and fed orally at the dose of 100 mg/kg once daily to Group IV. The extract was found to be rich with flavonoids, glycosides, alkaloids and ascorbic acid.

2.4. Preparation of MK-801

MK-801 was dissolved in isotonic saline and administered intraperitoneally daily to stressed rats (0.3 mg/kg; i.p.; once daily) for 21 days.

2.5. Blood sampling

Rats from all the groups were subjected to blood sampling a day before beginning the restraint stress (S_0), immediately after the sixth hour of stress on the first day (S_1) and sixth hour of stress on the 21st day of stress (S_{21}), from tail vein. Blood was quickly collected (∼15 s per rat) in heparinised tubes and immediately centrifuged at 1000 × *g* for 10 min at 4 °C. The plasma obtained was frozen at −20 °C for cAMP and corticosterone assay.

2.6. Plasma cAMP and corticosterone assay

Plasma cAMP and corticosterone were measured by using a RIA kit (Immunotech, France) and ELISA kit (Assay Designs, USA), respectively. The plasma obtained after centrifugation was diluted (1:15 for cAMP and 1:100 for corticosterone assay) and then evaluated for the same using the kits. The coefficients of variation within and between assays were 5–10%, respectively, for both the kits. Assay sensitivity was 2 nM for cAMP assay and 26.9 pg/ml for corticosterone assay.

2.7. Biochemical studies

At the end of 21 days of restraint stress, rats were sacrificed by cervical dislocation in all the groups and heart was removed, washed with ice cold normal saline, and dipped in liquid nitrogen and stored at −70 °C, for estimation of oxidative stress markers.

2.8. Tissue processing

Myocardial tissue samples were thawed once and homogenized in 10% (w/v) ice-cold 0.05 M potassium phosphate buffer (pH 7.4). Aliquot (0.2 ml) from the homogenate was used to estimate myocardial thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979) and an aliquot of the homogenate was mixed with 10% trichloroacetic acid and centrifuged at 5000 × *g* for myocardial reduced glutathione (GSH) estimation (Ellman, 1959). The remaining homogenate was centrifuged at 15,000 × *g* for 60 min at 4 °C. The supernatant was then used for enzyme assays like superoxide dismutase (SOD) (Kakkar et al., 1984), Catalase (CAT) (Aebi, 1974) and glutathione peroxidase (GPx) (Wendel, 1981). Protein concentration was determined according to Bradford (1976).

2.9. Histopathological studies

Histopathological studies were done by whole body perfusion with 4% buffered formalin (pH 7.4) in two rats from each group. After perfusion fixation, heart was isolated and processed for paraffin sectioning. The sections (7 μm) were stained with hematoxylin and eosin (H&E), and were analyzed for histopathological studies.

2.10. Statistical analysis

Results are expressed as mean ± S.E.M. Biochemical data and body weight changes were analyzed using one-way ANOVA followed by post hoc Bonferroni multiple range test. Plasma cAMP and corticosterone levels were analyzed by two-way ANOVA followed by post hoc Dunnett's test using SPSS (10.0)

Table 1
Changes in body weight and endogenous myocardial antioxidants in groups CRS, CRS + Os 100 and CRS + MK-801

Groups	C	Sham C	CRS	CRS + Os 100	CRS + MK-801
Body weight gain (g/100 g rat after 21 days)	23.1 ± 3.7	12.5 ± 1.4**	3.4 ± 0.4##	25.3 ± 1.8+++	10.8 ± 1.7++
TBARS (nmol/g wet weight tissue)	95.9 ± 3.6	93.6 ± 7.7	100.7 ± 2.9	74.9 ± 9.4	99.5 ± 14.6
GSH (μg/g wet weight tissue)	345.5 ± 13.1	362.2 ± 29.3	275.2 ± 7.8#	302.3 ± 57.3	303.1 ± 9.5+
SOD (units/mg protein)	3.4 ± 0.2	3.2 ± 0.5	5.2 ± 0.5#	2.6 ± 0.3+++	3.4 ± 0.3+
Catalase (units/mg protein)	16.5 ± 0.4	15.7 ± 0.7	26.3 ± 0.8#	14.6 ± 2.5+	18.2 ± 1.0+
GPx (munits/mg protein)	0.192 ± 0.018	0.222 ± 0.017	0.196 ± 0.007	0.177 ± 0.015	0.221 ± 0.026

All values are expressed as mean ± S.E. ($n = 8$). C, control rats; sham C, control rats with no food and water, but not subjected to chronic restraint stress; CRS, control rats subjected to chronic restraint stress 6 h/day for 21 days; Os 100, 100 mg/kg *Ocimum sanctum* orally for 21 days; MK-801, 0.3 mg/kg MK-801 i.p. for 21 days.
** $p < 0.01$ vs. C.
# $p < 0.05$.
$p < 0.01$ vs. sham C.
+ $p < 0.05$.
++ $p < 0.01$.
+++ $p < 0.001$ vs. CRS.

statistical software. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Body weight change (Table 1)

There was a significant ($p < 0.01$) fall in body weight gain in group sham C as compared to group C. A significant ($p < 0.01$) fall in body weight gain in group CRS was observed when compared to group sham C. Fall in body weight gain in group CRS was significantly prevented in group CRS + Os 100 ($p < 0.001$) and group CRS + MK-801 ($p < 0.01$).

3.2. Plasma cAMP levels (Table 2)

There was a significant ($p < 0.05$) rise in plasma cAMP level in group CRS, group CRS + Os 100 and group CRS + MK-801 immediately after 6 h of stress on day 1 as compared to day 0. On day 21 the plasma cAMP levels were significantly high ($p < 0.05$) in group CRS with no significant change in group CRS + Os 100 and group CRS + MK-801 as compared to day 0.

Table 2
Changes in plasma cAMP level on day 0 (S_0), day 1 (S_1) and day 21 (S_{21}) in groups CRS, CRS + Os 100 and CRS + MK-801

Groups	Plasma cAMP levels (nM)		
	Day 0 (S_0)	Day 1 (S_1)	Day 21 (S_{21})
CRS	102.3 ± 14.5	333.1 ± 29.3*	258.3 ± 16.7*
CRS + Os 100	102.3 ± 14.5	140.4 ± 18.4*	80.4 ± 7.1
CRS + MK-801	102.3 ± 14.5	241.8 ± 18.6*	158.1 ± 23.2

All values are expressed as mean ± S.E. ($n = 8$). CRS, chronic restraint stress 6 h/day for 21 days; Os 100, *Ocimum sanctum* 100 mg/kg orally for 21 days; MK-801, MK-801 0.3 mg/kg i.p. for 21 days; S_0, day 0; S_1, immediately after 6 h of CRS on day 1; S_{21}, immediately after 6 h of CRS on day 21.
* $p < 0.05$ vs. S_0.

3.3. Plasma corticosterone levels

In group CRS, plasma corticosterone level was significantly ($p < 0.05$) high immediately after 6 h of stress on day 1 (42.3 ± 5.5 μg/dl) as compared to day 0 (19.3 ± 3.5 μg/dl) while no significant change on day 21 (10.9 ± 0.7 μg/dl) was observed as compared to day 0.

3.4. Biochemical changes (Table 1)

There was no significant change in myocardial TBARS in any of the group studied as compared to group C. There was no significant difference in myocardial GSH levels in group sham C as compared to group C, whereas a significant fall in ($p < 0.05$) in myocardial GSH level was observed in group CRS as compared to group sham C. In group CRS + Os 100 there was preservation of myocardial GSH levels though not significant, whereas MK-801 significantly ($p < 0.05$) prevented the fall in GSH levels as compared to group CRS. There was no significant difference in myocardial SOD activity in group sham C as compared to group C. However, a significant ($p < 0.05$) rise in myocardial SOD activity was observed in group CRS as compared to group sham C. The rise myocardial SOD activity was significantly prevented by group CRS + Os 100 ($p < 0.001$), and group CRS + MK-801 ($p < 0.05$) as compared to group CRS. There was no significant change in myocardial catalase activity in group sham C as compared to group C. However, a significant ($p < 0.05$) rise in myocardial catalase activity was observed in group CRS as compared to group sham C. The rise myocardial catalase activity was significantly prevented by group CRS + Os 100 ($p < 0.05$), and group CRS + MK-801 ($p < 0.05$) as compared to group CRS. There was no significant change in myocardial GPx activity in any of the groups studied.

3.5. Histopathological changes (Fig. 1a–d)

Fig. 1a shows a light micrograph of control rat heart showing normal architecture. In group CRS there was evidence of mild myocardial edema on light microscopy (Fig. 1b). Fig. 1c and d

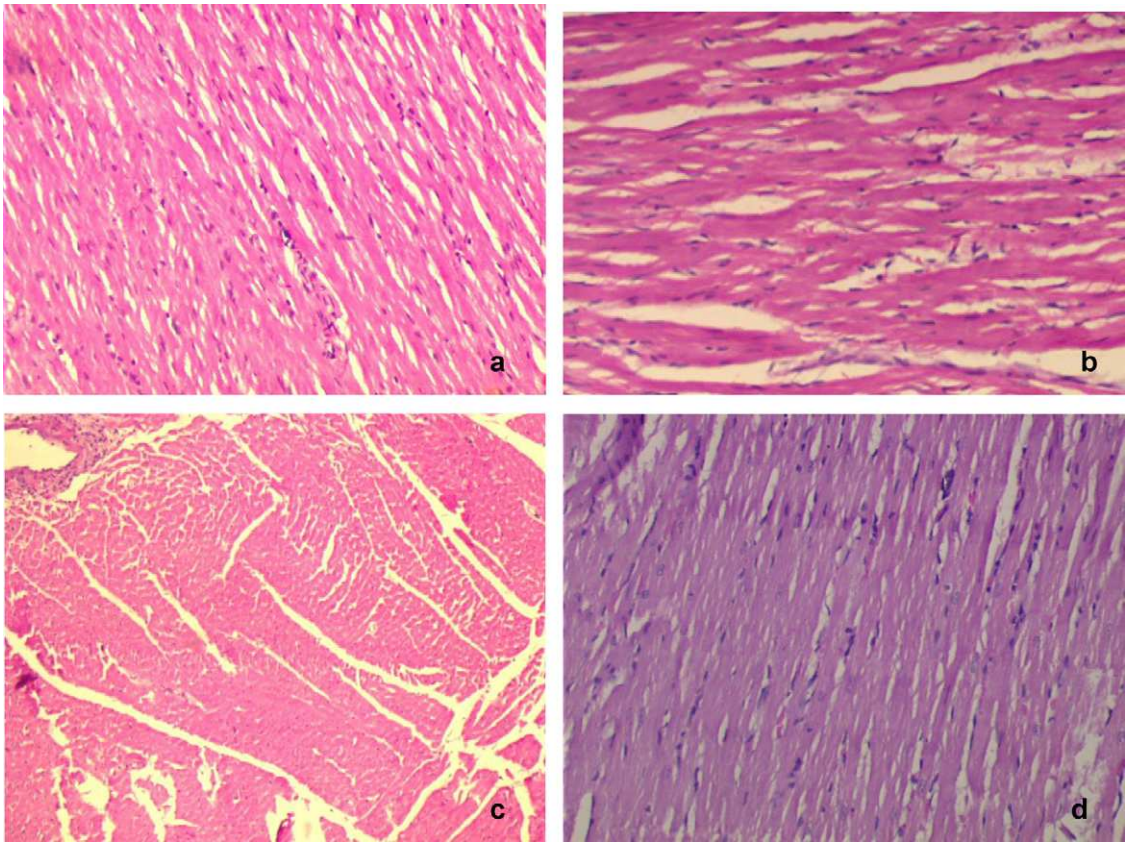

Fig. 1. Light micrograph of control rat heart showing (a) normal architecture in group C (10×, H&E), (b) myocardial edema (10×, H&E) in group CRS, (c) no evidence of myocardial edema in group CRS + Os 100 (10×, H&E) and (d) no evidence of myocardial edema in group CRS + MK-801 (10×, H&E).

shows normal morphology without any evidence of myocardial edema in group CRS + Os 100 and CRS + MK-801, respectively.

4. Discussion and conclusion

In the present study, a significant (46%) fall in body weight gain was observed in control rats devoid of food and water without CRS. In rats subjected to CRS, there was also a significant (85%) fall in body weight gain. CRS resulted in rise in plasma cAMP level (233% on day 1 and 152% on day 21 of CRS) while no significant change in plasma corticosterone levels was observed after day 21 of CRS. These were accompanied with increase in myocardial SOD (53%) and catalase activities (59%) along with fall in GSH level (20%) and mild myocardial edema. These changes were absent in the *Ocimum sanctum* and MK-801 treated rats, subjected to CRS.

To the best of our knowledge this is the first study of its kind, which has shown the effects of *Ocimum sanctum* on myocardial changes associated with CRS. In this regard, it was imperative to dissect the peripheral effect of *Ocimum sanctum* from its central action, especially in consideration of earlier reports about *Ocimum sanctum* having some direct cardioprotective effect (Sharma et al., 2001; Sood et al., 2005). This was attempted by comparing it with MK-801, an NMDA receptor antagonist. It is known to reduce brain oxidative damage caused by chronic stress by decreasing production of free radicals through inhibition of TNFα, NF-κB, iNOS (Madrigal et al., 2001a,b), in addition it its direct sympatholytic activity (Tsuda et al., 1994). But there are no studies indicating its benefit on cardiac changes associated with chronic stress. Our study shows that it exerts a beneficial effect on heart also, through these pathways vis-à-vis oxidative stress and decrease sympatholytic activity. The similar extent of protection for both *Ocimum sanctum* and MK-801 indicates the same mechanism of action for both. In conclusion, *Ocimum sanctum* has the potential to limit the harmful effects of stress on rat heart through its unique action on the damaging factors involved in chronic stress, which might be helpful in reducing the overall cardiovascular risk associated with psychological stress.

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