

Protective effect of *Ocimum sanctum* on lipid peroxidation, nucleic acids and protein against restraint stress in male albino rats

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Abstract

Effect of restraint stress on brain oxidative stress parameters and their modulation by *Ocimum sanctum* Linn (OS) were evaluated in male albino rats. Rats were subjected to restraint / immobilization stress 3h/day for 6 consecutive days. Post administration of aqueous extract of OS (100 mg/kg for 6 consecutive days) was given following restraint stress. MDA a marker of lipid peroxidation, nucleic acids and proteins were estimated in cerebrum, cerebellum and brain stem. Exposure to restraint stress caused a significant elevation in the rate of lipid peroxidation, reduction in nucleic acids and proteins as compared to control in all three regions of brain of male albino rats. Post treatment of aqueous extract of OS prevented the stress induced changes in these biochemical parameters. The results of the study indicate the protective nature of OS on different regions of brain against the detrimental effect of restraint stress.

Keywords: *Ocimum sanctum*, restraint stress, nucleic acids, lipid peroxidation, brain.

Introduction

Stress is defined as "non specific result of any demand upon the body" (Selye, 1980). Stress can be either physical or psychological. It can be induced in experimental animals in various forms e.g. Immobilization, forced swim, exposure to cold environment, starvation etc. The mechanism underlying stress-induced tissue damages are not yet fully understood, however, accumulating evidence has implied that the production of free radicals plays a critical role in these processes (Liu et al., 1996; Olivenza et al., 2000; Zaidi et al., 2003). Previous studies have indicated that stress stimulated numerous pathways leading to increased levels of free radicals (Liu et al., 1996; Olivenza et al., 2000). Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. Brain is the target for different stressors because of its high sensitivity to stress induced degenerative conditions. Restraint stress resulted in elevated levels of malondialdehyde (MDA) an index of free radical generation and lipid peroxidation in brain (Pal et al., 2006; Chakraborti et al., 2007). Restraint stress is an easy and convenient method of inducing both psychological and physical stress resulting

in restricted mobility and aggression (Singh et al., 1993)). Less well understood is the contribution of stress to oxidant production, especially in the brain. This is important because of considerable evidence that the formation of oxidants, damaging cellular molecules such as DNA, is a major contributor to ageing and the degenerative diseases of ageing such as brain dysfunction, cancer, cardiovascular diseases, and immune system decline. Stress-induced DNA damage has been studied by Adachi et al., (1993). This DNA damage has been implicated in cellular ageing and in malignant transformation of cells (Robbins pathologic basis of disease). The study of RNA is very helpful in knowing the rate of protein synthesis, and also to understand the functional status of the nervous tissue (Bergen et. al., 1974). Protein is one of the important biochemical components of the brain in vertebrates. Cells generally contain thousands of different proteins each with a biological activity. These functions include enzymatic catalysis (superoxide dismutase), molecular transport (hemoglobin), nutrition (casein), cellular defense (immunoglobulin), movement (tubulin), regulation (insulin) etc. The specific neuronal functions such as transmission are extensively mediated by

proteins (Bock, 1978). This has wide implications as restraint stress damages biomolecules-DNA (nucleolar and mitochondrial), RNA and protein.

The use of natural resources e.g. medicinal plants may prove to be useful approach towards the management of stress-linked mental health problems (Yoydim and Joseph, 2001). *Ocimum sanctum* (OS) (Family Lamiaceae) is commonly known as Tulsi or holy basil in India. The plant grows wild in India but is also widely cultivated in home and temple gardens. Traditionally, fresh juice or decoction of OS leaves is used to promote health and in treatment of various disorders as advocated in Ayurveda, the Indian system of Medicine. In Ayurveda, OS is described as 'rasayana' (plants having adaptogen like properties). It has been reported to exhibit several medicinal properties. This reputed medicinal plant has recently been shown to possess very interesting pharmacological properties relevant to the present study. Recent investigations has shown that different extracts of OS possess significant anti-inflammatory (Singh et al., 1996), antioxidant (Uma Devi and Ganasoundari, 1999), and anti stress properties (Sood et al., 2006). Flavonoids which were isolated from the aqueous extract of OS have been shown significant anti oxidant activity, both *in vivo* and *in vitro* (Uma Devi et al., 2000). OS leaf extract showed strong protective effects against radiation injury (Uma Devi and Ganasoundari, 1995). The ethanolic extract of OS leaves was found to prevent noise induced oxidative stress in discrete regions of the brain (Samson et al., 2007)). The anti-stressor effect of essential oil from leaves and seeds of OS in rats exposed to restrained stress has been reported (Sen et al., 1992). However, no study was done to evaluate the protective effect of OS on restraint stress-induced damage of LPO, DNA, RNA and protein in CNS of male albino rats. Therefore, the present study was designed to investigate the protective effect of aqueous extract of OS on stress-induced damage of these biochemical parameters in cerebrum, cerebellum and brain stem of rats.

Materials and Methods

Ocimum sanctum extract preparation

Leaves of OS were collected from University campus and identified by Prof. Wajahat Husain, taxonomist, Department of Botany, Aligarh Muslim University, Aligarh. Its I.D. No. is Husain1375 and deposited in A.M.U, Herbarium. The leaves were dried under shade, and powdered. The aqueous extract was prepared following the method of Ganasoundari et al., (1998). The shed dried powder of OS was refluxed for 24 hour with double distilled water (DDW) at 100°C, cooled and filtered. The solvent was removed under reduced pressure to get the product. The final yield of the product was 9% (w/w) of the starting material and this was stored in refrigerator until further use.

Animals

Adult male albino rats (200 ± 50 gm) were obtained from Central Animal House facility of JN Medical College, A.M.U., Aligarh. The study was approved by institutional ethics committee. The animals were kept in air conditioned room and had free access to pellet diet (Hindustan Lever Ltd. Mumbai, India) and water *ad libitum*.

Experimental design

All the animals were randomly divided into four groups with six animals in each.

Group I: This group of rats served as control.

Group II: Rats of this group were subjected to restraint stress 3h/day for 6 consecutive days.

Group III: This group received aqueous extract of OS (100 mg/kg/day, orally for 6 consecutive days) after restraint stress.

Group IV: Aqueous extract of OS alone (100mg/kg) was given for 6 days consecutively.

Isolation of brain areas

The animals were sacrificed by cervical dislocation. Dissection for separating the cerebrum, cerebellum and brain stem was carried out. Proper care was taken to avoid damage of any brain part tissues while departing from the skulls. The tissues of the brain parts were used for the assay of lipid peroxidation (LPO), nucleic acids (DNA, RNA) and protein.

Extraction and estimation of lipid peroxidation

Briefly the reaction mixture consisted 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid and 0.2 ml of brain homogenate. The mixture was made up to 4 ml with distilled water and heated at 95°C for 60 min. After cooling with tap water, 5 ml of n-butanol and pyridine (15: 1, v/v) and 1 ml of distilled water were added and centrifuged. The organic layer was separated out and its absorbance was measured at 532 nm using UV-visible spectrophotometer and MDA content was expressed as nmol/mg protein (Okhawa et al., 1979).

Extraction and estimation of nucleic acids and protein

Nucleic acids were isolated by using method of Searchy and Macinnis (1970). Different brain regions were weighed and homogenized in 5.0 ml of 0.5 N perchloric acid. The homogenates were heated at 90°C in boiling water bath for 10 min, cooled and centrifuged at 3,000 rpm for 10 min. Supernatants were taken in graduated test tubes and the volume was maintained up to 5.0 ml with 0.5 N perchloric acid. This extract was used in the estimation of DNA (Burton, 1956) and RNA (Dische, 1995). Protein level was also estimated in homogenate by Lowry et al., (1951).

Statistical analysis

The statistical software package SPSS 10.0 for windows was used to analyze the data. Statistical analysis was undertaken by using student-t-test. $P < 0.1$ was considered statistically significant.

Results

Lipid peroxidation

The level of the rate of MDA, a marker of LPO, increased significantly in cerebrum, cerebellum and brain stem after 3h/day for 6 days restraint stress in comparison to non stressed control rats ($P < 0.001$). Maximum elevation was in cerebrum (33%) and minimum was in cerebellum and brain stem

(32%). A significant depletion in the lipid peroxide level was observed in cerebrum (31%), cerebellum (32%), and brain stem (30%) after post treatment of OS (100 mg/kg for 6 days) as compared to RS group ($p < 0.01$) while OS was given alone there was no change as compared to control (Table 1, Fig. 1).

Deoxyribonucleic acid

Restraint stress significantly inhibited the level of DNA in all the three regions of brain as compared to control group ($p < 0.001$). The maximum inhibition of DNA was in cerebrum, cerebellum (30%, 31%) and minimum in brain stem 26%. Oral administration of OS significantly recovered DNA level 29% in cerebrum, 32%, in cerebellum and 25% in brain stem as compared to restraint stress group. ($p < 0.001$, $p < 0.01$). When control animals were compared with OS per se group, there was no difference (Table 1, Fig. 2).

Ribonucleic acid

RNA level was decreased significantly in various regions of brain after restraint stress as compared to control group ($p < 0.001$). The inhibition of RNA was 27% in brain stem, 22% in cerebrum and 25% in cerebellum. This decreased level was significantly brought back to normal level after post treatment of OS as compared to stress group ($p < 0.001$, $p < 0.05$). OS increased the level of RNA 21% in cerebrum 26% in cerebellum and 27% in brain stem. Here too OS per se group did not show any change (Table 1, Fig. 3).

Protein

Protein levels were also significantly inhibited by restraint stress in all three regions of brain in comparison to their respective control group ($p < 0.05$). The maximum inhibition of protein was in cerebellum (24%) and minimum in brain stem (17%). OS administration was found to induce significant increment of protein level in different regions of brain ($p < 0.001$, $p < 0.01$). OS increased the level of protein 26% in cerebrum, 29% in cerebellum and 15% in brain stem. OS alone showed no change (Table 1, Fig. 4).

Table 1: Protective effect of *O. sanctum* post-treatment (100 mg/kg/day for 6 days) on lipid peroxidation, nucleic acids and proteins of different parts of brain subjected to restraint stress (3h/day for 6 days).

BRAIN PARTS	GROUPS	LPO (nmol/mg Protein) [Mean \pm S.E.]	DNA (mg/g tissue) [Mean \pm S.E.]
Cerebrum	Control	3.24 \pm 0.176	5.91 \pm 0.127
	Restraint stress	4.85 \pm 0.052*	4.16 \pm 0.108*
	Restraint stress + OS	3.34 \pm 0.029**	5.84 \pm 0.02**
	OS	3.01 \pm 0.033	6.13 \pm 0.033
Cerebellum	Control	3.61 \pm 0.035	7.51 \pm 0.169
	Restraint stress	5.32 \pm 0.104*	5.13 \pm 0.122*
	Restraint stress + OS	3.58 \pm 0.040**	7.56 \pm 0.019 ^a
	OS	3.41 \pm 0.042	7.64 \pm 0.023
Brain stem	Control	3.16 \pm 0.087	5.18 \pm 0.153
	Restraint stress	4.68 \pm 0.051*	3.82 \pm 0.092*
	Restraint stress + OS	3.28 \pm 0.033**	5.13 \pm 0.014**
	OS	3.19 \pm 0.030	5.31 \pm 0.018

(Table 1 is continued on next page...)

BRAIN PARTS	GROUPS	RNA (mg/g tissue) [Mean \pm S.E.]	PROTEIN (mg/g tissue) [Mean \pm S.E.]
Cerebrum	Control	6.35 \pm 0.064	106.61 \pm 1.18
	Restraint stress	4.93 \pm 0.080*	81.58 \pm 1.32***
	Restraint stress + OS	6.27 \pm 0.025**	110.21 \pm 0.217**
	OS	6.66 \pm 0.066	103.03 \pm 0.159
Cerebellum	Control	7.22 \pm 0.077	111.31 \pm 2.38
	Restraint stress	5.41 \pm 0.071*	83.67 \pm 1.15***
	Restraint stress + OS	7.28 \pm 0.079 ^a	117.53 \pm 0.197**
	OS	7.15 \pm 0.079	108.81 \pm 0.239
Brain stem	Control	5.78 \pm 0.092	92.98 \pm 1.30
	Restraint stress	4.23 \pm 0.079*	76.48 \pm 1.15***
	Restraint stress + OS	5.81 \pm 0.036**	90.06 \pm 0.224 ^a
	OS	5.44 \pm 0.079	91.61 \pm 0.208

*P<0.001, *** p<0.05 statistically significant as compared to control.

^aP<0.001, ** p<0.01 statistically significant as compared to restraint stress group.

Fig. 1: Ameliorative action of *O. sanctum* (100 mg/kg/day for 6 days) on restraint stress induced alteration (3h/day for 6 days) on the rate of lipid peroxidation (LPO) in different brain parts of rats.

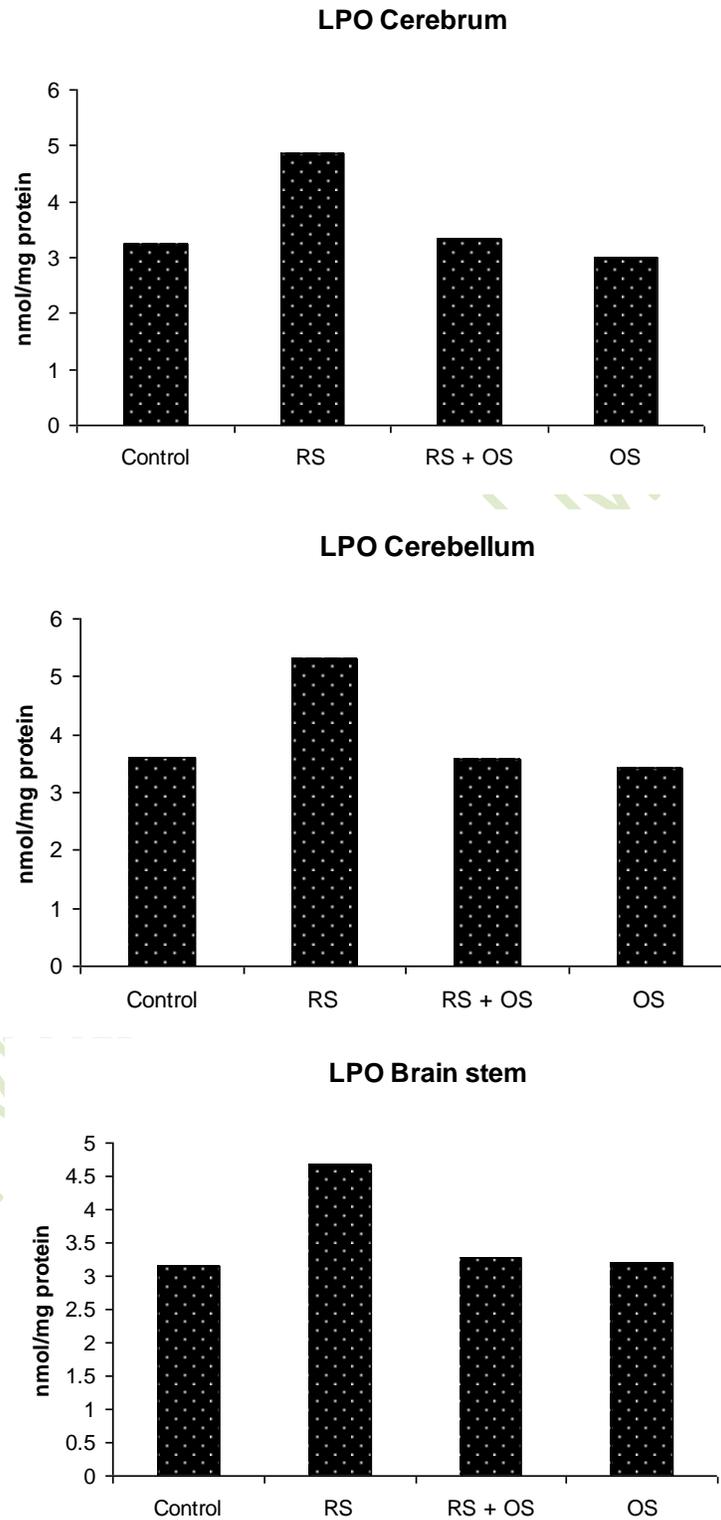


Fig. 2: Ameliorative action of *O. sanctum* (100 mg/kg/day for 6 days) on restraint stress induced alteration (3h/day for 6 days) of deoxyribose nucleic acid (DNA) on different brain parts of rats.

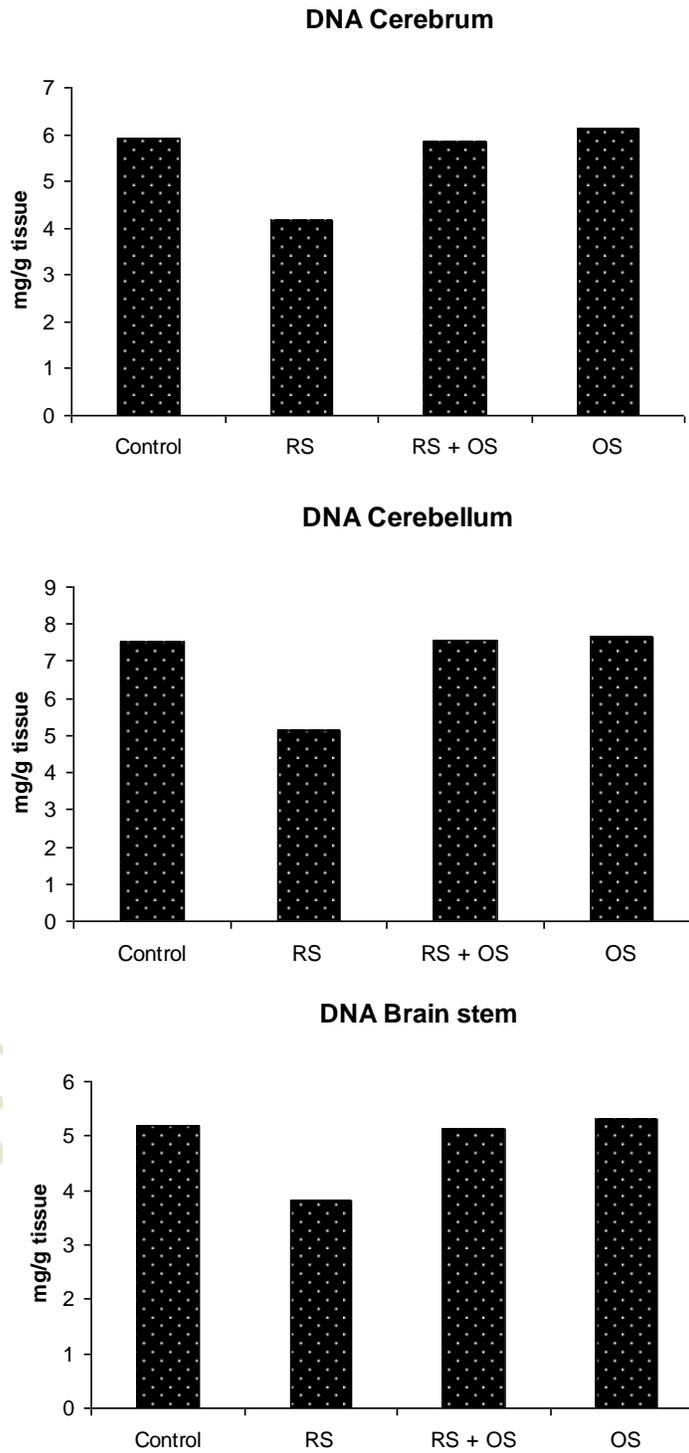


Fig. 3: Ameliorative action of *O. sanctum* (100 mg/kg/day for 6 days) on restraint stress induced alteration (3h/day for 6 days) of ribose nucleic acid (RNA) on different brain parts of rats.

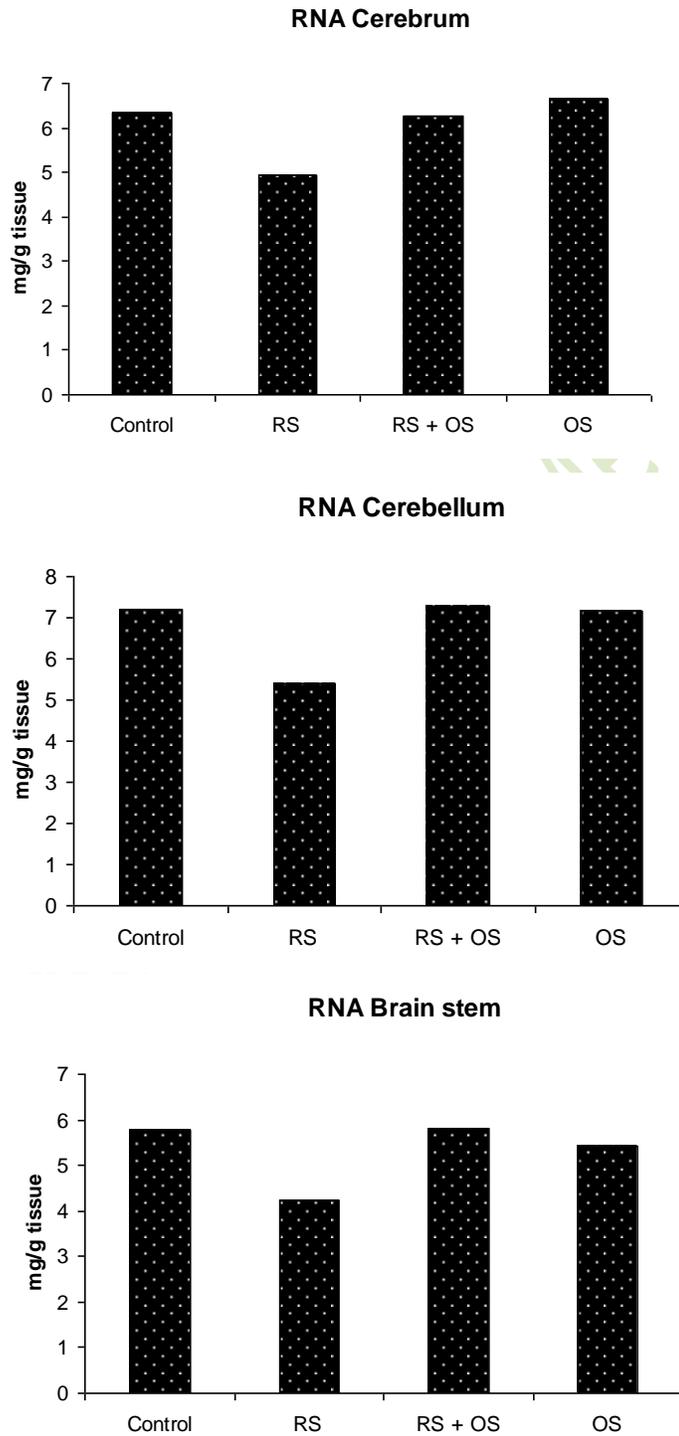
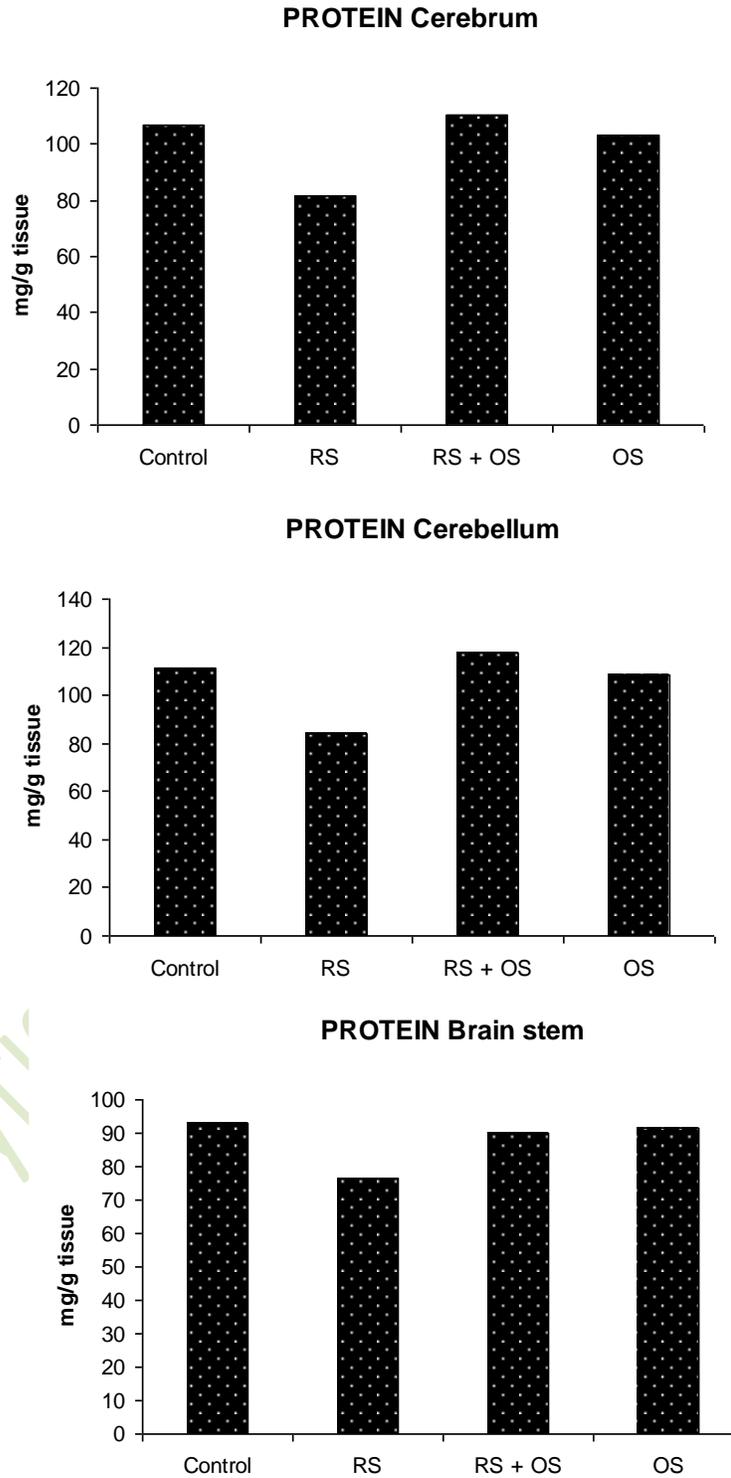


Fig. 4: Ameliorative action of *O. sanctum* (100 mg/kg/day for 6 days) on restraint stress induced alteration (3h/day for 6 days) of protein on different brain parts of rats.



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Discussion

The level of lipid peroxidation (LPO) increased while the levels of nucleic acids (DNA, RNA) and protein decreased after restraint stress in cerebrum, cerebellum and brain stem as compared to control group. The observed increase in LPO is in agreement with previous studies (Liu et al., 1996; Yargicoglu et al., 2003). Restraint stress resulted in the generation of oxidative stress / reactive oxygen species (ROS). These ROS may propagate the initial attack on lipid rich membranes of the brain to cause LPO (Das and Kanna, 1997). Decrease in DNA, RNA and protein are in accordance with earlier studies (Zahir et al., 2006; Ramtej and Devjani, 2008). Decline in nucleic acids and protein may be due to DNA damage caused by the free radicals and inhibition of RNA by direct interaction of ROS. Oxygen radicals can attack proteins, nucleic acids and lipid membranes, thereby disrupting cellular functions and integrity. Our results provide strong evidence that H_2O_2 and O_2 cause DNA damage because LPO products were increased with the passage of time as well as restraint stress. (Gupta et. al., 1991). The predominant radicals encountered in higher organisms are superoxide (O_2^-), peroxy (ROO^\cdot), nitroxy (NO^\cdot) and hydroxyl (HO^\cdot) radicals. Hydroxyl radical (HO^\cdot) is more reactive and is capable of causing damage to biomolecules such as lipids, proteins and DNA. It is generally recognized that in physiological system HO^\cdot is produced under aerobic condition by Fenton's reaction (Chen et. al., 1999) and its interaction with DNA causes oxidative damage. Oxidative RNA damage is also a feature in vulnerable neurons at the earliest stages of these diseases suggesting that RNA oxidation may actively contribute to the onset or to the development of disease (Nunomura et. al., 2006). There are only few studies about the causal effects of stress on protein oxidation in the brain (Liu et. al., 1996). In our study decrease in protein level of rats with restraint stress may be attributed to accumulations of constituents like phospholipids and cholesterol in the brain. Decrease in protein level also suggests high rate of utilization of protein in restraint stress.

Ocimum sanctum post treatment significantly prevented the rise in LPO levels suggesting that it attenuates the excessive

formation of ROS secondary to restraint stress. This is in agreement with the observation that OS possesses significant antioxidant activity (Devi, 2000). Protective effect of aqueous extract of OS against LPO has been reported (Geetha and Vasudevan, 2004). The antioxidants interrupt the free-radical chain of oxidation by donating hydrogen from phenol's hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids. Therefore, it can be assumed that OS may also be acting on similar lines. Post treatment of OS significantly increased the levels of DNA, RNA and protein in different parts of brain. Our results are strongly in favour of Ramtej and Devjani (2008) in which DNA, RNA and protein contents increased by *Embllica officinalis* aqueous extract induced by ochratoxin. Although the protective effect of OS on brain against stressors had been documented, the mechanism of action of OS extract has to be elucidated. Balanehru and Nagarajan (1992) had reported the reduction in free radical level by the component ursolic acid separated from OS extract. Thus it could be possible that the protective action of OS might be through the suppression of free radicals. The increased nucleic acids and protein is therefore, an indication that the brain's antioxidant machinery is activated to excessive generation of free radicals (Bannister et. al., 1987). Presence of flavonoids in OS may be held responsible for its attenuating activity because flavonoids have been reported as potentially useful exogenous agents in protecting the aging brain, other organs and tissues of the body against free radical induced damage (Blaylock, 1999). So it is evident now that OS prevents the stress-induced changes not only in the central cholinergic system, cardiac system (Sembulingam et al., 2005, Sood et al., 2006) but also in central dogma. To the best of our knowledge this is the first study reporting the protective effect of OS on stress induced damage of nucleic acids and proteins in different parts of brain.

Conclusion

Our study indicated that restraint stress significantly induced alterations in lipid peroxidation, nucleic acids and proteins. OS is an ideal antioxidant for the prevention of stress-induced elevation of LPO and

reduction of nucleic acids and protein in cerebrum, cerebellum and brain stem. Thus, OS could be used as a potentially effective therapeutic agent in clinical conditions associated with free radical damage in CNS.

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