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Protective effect of *Ocimum sanctum* and *Camellia sinensis* on stress-induced oxidative damage in the central nervous system of *Rattus norvegicus*.

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ABSTRACT

Effect of aqueous extracts of *Ocimum sanctum* and *Camellia sinensis* on restraint stress-induced oxidative damage in the central nervous system (CNS) of *R. norvegicus* were evaluated in terms of lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Restraint stress was used for 3h/day for 6 days consecutively, caused a significant elevation in LPO and reduction in GSH, SOD and CAT in cerebrum, cerebellum and brain stem. Post-treatment of *O. sanctum* and *C. sinensis* (100 mg/kg/day for 6 days) resulted in alteration of these parameters towards their control values. In conclusion, *O. sanctum* and *C. sinensis* post-treatment protected the CNS in different manner against restraint stress-induced oxidative damage in all three regions of brain.

Keywords: Restraint stress, *Ocimum sanctum, Camellia sinensis,* oxidative stress, antioxidants, *R. norvegicus.*

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INTRODUCTION

Stress is one of the major contributory factors that stimulate numerous intracellular pathways leading to the increased free radical generation causing oxidative damage [1-2]. Free radicals are highly reactive moieties playing an important role in health and disease. The brain is especially vulnerable to free radical-induced damage because of its high oxygen consumption, abundant lipid content and low levels of enzymatic and nonenzymatic antioxidants [3].

Restraint stress is an easy and convenient method of inducing both psychological and physical stress resulting in restricted mobility and aggression [4]. Numerous reports have shown that restraint stress results in the imbalance of antioxidant status which ultimately leads to increased oxidative stress thereby resulting in oxidative damage [5-6].

Exogenous supplementation of antioxidants has been proposed beneficial in preventing the stress-induced oxidative damage in brain [3]. *O. sanctum* and *C. sinensis* are well known medicinal plants having antioxidant property. *O. sanctum* is a well-known medicinal plant widely distributed throughout India, and its role as a healing herb is well identified in traditional Indian medicine system 'ayurveda' where it is considered to be panacea for many diseases [7]. The aqueous and alcoholic extracts from the leaves of this plant have been shown to possess anti-stress, antioxidant, hepatoprotective, anti-inflammatory, antibacterial and radioprotective properties [8-13]. Various aspects of the stress alleviating potential of the crude extracts of *O. sanctum* have been established [14]. The restraint stress alleviating effect of *O. sanctum* has also been documented earlier [15-16]. However, there is a lack of information regarding the effect of *O. sanctum* on oxidative damage in CNS induced by restraint stress.

Green tea is a widely consumed beverage brewed from the plant species '*Camellia sinensis*' originated in China. *C. sinensis* has many beneficial effects to our body, such as antimutagenic, antiproliferative and anticarcinogenic properties, as well as neuroprotective activity in degenerative disorder models [17-20]. It contains an abundance of naturally occurring polyphenols called catechins of which epigallocatechin gallate (EGCG) is the most prevalent. *C. sinensis* polyphenols (CSPs) have aroused considerable attention in recent years for preventing oxidative stress-related disease [21]. There are active hydroxyl hydrogens in the molecular structure of CSPs that can end the chain reaction of excessive free radicals that result in oxidative stress. CSPs possess strong antioxidant [22] and antilipidperoxidative properties [23]. Aqueous extract of *C. sinensis* has been shown to reduce reactive oxygen species resulting in oxidative stress [24]. But there is no study on the protective effects of aqueous extract of *C. sinensis* on oxidative damage induced by restraint stress in the CNS of *R. norvegicus.*

Hence, the goal of the present study was to investigate the efficacy of *O. sanctum* and *C. sinensis* as a source of antioxidants, on restraint stress-induced oxidative damage in different parts of brain (cerebrum, cerebellum and brain stem) of *R. norvegicus,* evaluated in terms of measurement of free radical scavenging enzymes like SOD, CAT, GSH and LPO.

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MATERIALS AND METHODS

Ocimum sanctum Linn

Leaves of *O. sanctum* were collected from University campus and identified by a pharmacognist, Department of Botany, Aligarh Muslim University, Aligarh. Its I.D. No. is Husain1375 and deposited in A. M.U, Herbarium.

Camellia sinensis

Leaves of *C. sinensis* were purchased from an authorized dealer and it has also been identified by a pharmacognist, Department of Botany, Aligarh Muslim University, Aligarh. Its I.D. No. is Husain 395 and deposited in A.M.U, Herbarium.

Extraction of O. sanctum and C. sinensis

Leaves of *O. sanctum* and *C. sinensis* were dried, washed and powdered. The powdered leaves of *O. sanctum* and *C. sinensis* were refluxed for 5 hour in double distilled water (DDW) at 100^{o}C. Thereafter it was cooled and filtered. The water was removed under reduced pressure to get product [25]. The yield of the extracts of *O. sanctum* and *C. sinensis* was 9% and 7% (w/w) in terms of dried starting material. The residue was stored in the refrigerator until further use.

Animals

Adult male albino rats (200 ±50gm) were obtained from Central Animal House facility of J.N Medical College, A.M.U, Aligarh. The animals kept in polypropylene cages, were housed in air conditioned room and maintained on standard pellet diet and water *ad libitum*.

Experimental protocol

A total of 36 rats were used in this study and they were divided into six groups. First group treated as control and second was of restraint stress (3h/day for 6 consecutive days). Third and fourth group were treated with the post-treatment of *O. sanctum* and *C. sinensis* aqueous leave extracts following restraint stress. Fifth and sixth group were treated with *O. sanctum* and *C. sinensis* alone, respectively. The study was approved by Institutional Animals Ethics Committee.

For this study, the animals were killed by cervical dislocation. Cerebrum, cerebellum, and brain stem were separated and cleaned with ice cold saline. Brain parts were homogenized in a proportion of 1:10 (w/v) ice cold phosphate buffer (0.1M, pH 7.4). Homogenate was centrifuged at 10,000 × g at 4^{o}C for 15 min to obtain post mitochondrial supernatant (PMS)

which was used for the study of superoxide dismutase and catalase. Protein concentration was determined according to the method of Lowry et al. [26].

Lipid peroxidation assay

LPO was determined as described by Okhawa *et al* (1979) [27]. Briefly, the reaction mixture consisted of 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 95^0C 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 in Beckman DU-640 spectrophotometer and MDA content was expressed as nmol/mg protein using molar extinction coefficient.

Reduced glutathione assay

GSH was measured according to the method of Ellman, (1959) [28]. An equal quantity of PMS was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) and 0.4 ml of double distilled water were added. The mixture was vortexed and absorbance was read at 412 nm with in 15 min. The concentration of reduced glutathione was expressed as µ mol/g tissue using molar extinction coefficient.

Superoxide dismutase assay

The activity of superoxide dismutase in the PMS was determined by the method of Nandi and Chatterjee (1988) [29]. The ability of superoxide dismutase to inhibit the autooxidation of pyrogallol makes this reaction a basis for a simple assay of this dismutase. The activity of superoxide dismutase was expressed as unit/mg protein using molar extinction coefficient.

Catalase assay

CAT activity was measured according to the method of Aebi, (1984) [30]. Briefly, 0.1 ml of PMS was added to a cuvette containing 1.9 ml of 50 mmol phosphate buffer (pH 7.0). The reaction was started by addition of 1 ml of freshly prepared 30 mmol H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically by changes in absorbance at 240 nm. The activity of CAT was expressed as unit/mg protein using molar extinction coefficient.

Statistical analysis

All results were expressed as the Mean ± S.E. The results were analyzed for statistical significance by Student-*t*-test using SPSS package program (version 10.0).

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RESULTS

Lipid peroxidation

MDA is a marker of LPO, the rate of MDA increased from 3.24 ± 0.17 (control) to 4.85 ± 0.05 (stress) in cerebrum, 3.61 ± 0.03 to 5.32 ± 0.10 in cerebellum and from 3.16 ± 0.08 to 4.68 ± 0.05 in brain stem after 3h/day for 6 days of restraint stress in comparison to control rats (P<0.001). A significant depletion in the lipid peroxide level was observed after post treatment of *O. sanctum* and *C. sinensis* aqueous leave extracts as compared to restraint stress group. *O. sanctum* attenuated LPO levels as 3.34 ± 0.02 in cerebrum, 3.58 ± 0.04 in cerebellum and 3.28 ± 0.03 in brain stem whereas *C. sinensis* 3.28 ± 0.03 in cerebrum, 3.36 ± 0.04 in cerebellum and 3.13 ± 0.03 in brain stem respectively (P<0.01; Table-1, Fig.1).

Reduced glutathione

GSH level decreased from 4.16 ± 0.07 (control) to 2.83 ± 0.03 (stress)in cerebrum, from 5.10 ± 0.07 to 3.52 ± 0.05 in cerebellum and from 3.95 ± 0.05 to 2.82 ± 0.06 in brain stem after restraint stress as compared to control (P<0.001). Post administration of *O. sanctum* and *C. sinensis* significantly increased the level of GSH as compared to stress group. *O. sanctum* recovered the GSH level as 4.13 ± 0.04 in cerebrum, 5.16 ± 0.04 in cerebellum and 4.06 ± 0.04 in brain stem whereas *C. sinensis* 4.28 ± 0.03 in cerebrum, 5.28 ± 0.02 in cerebellum and 4.11 ± 0.03 in brain stem respectively (P<0.01; Table-2, Fig.2).

Superoxide dismutase

SOD activity inhibited from 4.42 ± 0.04 (control) to 3.44 ± 0.02 (stress) in cerebrum, from 3.76 ± 0.03 to 2.85 ± 0.05 in cerebellum and from 4.38 ± 0.02 to 3.46 ± 0.05 in brain stem by restraint stress (P<0.001). Post administration of *O. sanctum* and *C. sinensis* significantly protect the level of SOD in comparison to control group. *O. sanctum* enhanced the level of SOD as 4.62 ± 0.02 in cerebrum, 3.88 ± 0.02 in cerebellum and 4.47 ± 0.02 in brain stem whereas *C. sinensis* 4.48 ± 0.03 in cerebrum, 3.66 ± 0.04 in cerebellum and 4.32 ± 0.04 in brain stem respectively (P<0.01; Table-3, Fig.3).

Catalase

Restraint stress-induced CAT depletion from 2.22 ± 0.02 (control) to 1.58 ± 0.04 (stress) in cerebrum, from 1.69± 0.02 to 1.23±0.06 in cerebellum and from 1.34 ± 0.02 to 1.01 ± 0.02 in brain stem as compared to their respective control group (P<0.001). A significant elevation was observed after the post-treatment of *O. sanctum* and *C. sinensis* as compared to restraint stress group. *O. sanctum* increased CAT level as 2.35 ± 0.03 in cerebrum, 1.73 ± 0.02 in cerebellum and 1.38 ± 0.01 in brain stem whereas *C. sinensis* 2.25 ± 0.02 in cerebrum, 1.67 ± 0.02 in cerebellum and 1.33 ± 0.02 in brain stem respectively (P<0.05; Table-4, Fig.4). There was no

significant change observed when *O. sanctum* and *C. sinensis* given alone as compared to control in all of the above parameters.

DISCUSSION

This is the first study in which *O. sanctum* and *C. sinensis* were evaluated on restraint stress-induced oxidative damage in different parts of brain (cerebrum, cerebellum and brain stem) and determines which one is more protective according to antioxidant status and LPO markers in different regions of brain. The activities of GSH, SOD and CAT were decreased while the level of LPO was increased in the stressed rats than control. The increase was observed in LPO, is in agreement with earlier studies [1, 2] Further, our results showing that GSH, SOD and CAT became decreased in different parts of brain following restraint stress. Therefore, it could be suggested that the elevated LPO in stress could be resulted from the depletion of antioxidant enzymes [31, 32]. GSH an endogenous thiol related antioxidant is involved in protection of brain cells against oxidative damage. GSH depletion may lead to an increased LPO, possibly due to the lowering of the cellular defense system against endogenous toxic intermediates [33]. Our results are in agreement with Madrigal and co-workers [34] who have reported that stress induces GSH depletion, LPO and mitochondrial dysfunction in rat brain.
Exogenous supplementation of antioxidants has been reported to exert protective effect in various pathological states in which free radicals are involved [35]. In the present study, *O. sanctum* and *C. sanctum* were used since they were recently gained considerable attention as an antioxidant but not evaluated, simultaneously in a single investigation.

Rats were treated with *O. sanctum* and *C. sinensis* showed an increase in the activity of GSH with a decrease in LPO. The protective activity of *O. sanctum* and *C. sinensis* aqueous leaves extract (ALE) against stress-induced oxidative damage, may to some extent, be mediated through the release of intracellular antioxidants which, in turn, will scavenge the free radicals and also may help in repair of oxidative damage. Experimental studies have shown that phenolic compounds, particularly flavonoids and catechins are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenol and the number and location of the hydroxyl groups [36].

The *O. sanctum* flavonoids, Orientin and Vicenin have strong antioxidant activity *in vitro* and *in vivo,* which strongly suggest free radical scavenging as a major mechanism by which *Ocimum* products protect the cellular damage [25, 37]. In accordance with the earlier studies [16, 38], *O. sanctum* has been found to be highly effective to protecting stress-induced LPO is responsible for its free radical scavenging action [12]. So, our results are strongly supported by above these studies.

Protective effect of the *C. sinensis* extract expressed in decreased level of LPO has also been reported on brain and liver [39]. plasma and erythrocytes [40]. Structure of polyphenols occurring in the *C. sinensis* suggest that o-dihydroxy or o-trihydroxyphenyl B-ring (catechol structure) is responsible for the most effective property in the inhibition of LPO [41, 42]

Catechins react with peroxyl radicals in phospholipid bilayers via a single electron transfer followed by deprotonation [41]. Recent studies propose that the B ring in green tea catechins is finally the principal site of antioxidant reactivity [42]. Data of present study is in agreement with the studies of Lin *et al.* [43] who reported antioxidative property of *C. sinensis* against iron-induced oxidative stress in rat brain homogenates *in vitro.* Further, it has been reported that *O. sanctum* and *C. sinensis* ALE significantly enhances the GSH content [16, 39] which reduces the free radical generated cellular damage.

This study indicated that stress induced a significant inhibition in SOD and CAT. They are scavenger enzymes that are reported to work together to eliminate toxic free radicals [44]. So, the possible reason for inhibition of SOD and CAT could be the decreased of these enzymes caused by enhanced free radicals in the stress condition [45, 32]. On the other hand, declined SOD and CAT activities were brought back to a normal level by the administration of *O. sanctum* and *C. sinensis*. This is possible due to the constituents of *O. sanctum* and *C. sinensis* that are characterized by their ability to scavenge free radicals that produced during the restraint stress.

The increase in the activity of SOD was observed with *O. sanctum* and *C. sinensis* can be explained by increasing effect of *O. sanctum* and *C. sinensis* on nerve growth factor (NGF), NGF provides expression of superoxide dismutase gene which is a factor leading to increment of SOD [46]. Catalase requires NADPH for its regeneration from its inactive form [47]. NADPH bringing up by enhanced glucose uptake by cells which stimulate both the pentose phosphate shunt and oxidative phosphorylation and thereby also enhances the activity of CAT in restraint stress. Lipoic acid is an antioxidant, able to increase glucose uptake *in vitro* [48]. Therefore, it can be assumed that *O. sanctum* and *C. sinensis* may also be acting on similar trends. Further increase in SOD and CAT activities after administration of *O. sanctum* and *C. sinensis* were accompanied by a concomitant decrease in the level of LPO and the present study is supported by some recent studies [49, 38, 50, 40].

CONCLUSION

Present study concluded that both of the plants *O. sanctum* and *C. sinensis* aqueous leave extracts administration were able to attenuate the restraint stress-induced oxidative damage in cerebrum, cerebellum and brain stem indicating their adaptogenic property. Therefore, it is clear by studying different biochemical parameters that both of these plants are affected but in different manner. Thus the study indicates that *O. sanctum and C. sinensis* are potential candidates for further evaluation as an antioxidant to attenuate restraint stress induced oxidative damage in the CNS of *R. norvegicus*.

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	Control	RS	RS + O.S	O.S	RS + C.S	C.S
Brain parts	(n mol/mg protein) Mean ± S.E					
Cerebrum	3.24 ± 0.17	4.85 ± 0.05*	3.34 ± 0.02**	3.01 ± 0.03	3.28 ± 0.03**	2.95 ± 0.04
Cerebellum	3.61 ± 0.03	5.32 ± 0.10*	3.58 ± 0.04**	3.41 ± 0.04	3.36 ± 0.04**	3.26 ± 0.03
Brain stem	3.16 ±0.08	4.68 ± 0.05*	3.28 ± 0.03**	3.19 ± 0.03	3.13 ± 0.03**	3.05 ± 0.02

Table.1: Effect of restraint stress, *O. sanctum* and *C. sinensis* on lipid peroxidation (LPO) at 7th day in *R. norvegicus*.
* $P < 0.001$. Statistically significant as compared to control group.
** $P < 0.01$. Statistically significant as compared to restraint stress group.

	Control	RS	RS + O.S	O.S	RS + C.S	C.S
Brain parts	(μ mol/gm tissue) Mean ± S.E					
Cerebrum	4.16 ± 0.07	2.83 ± 0.03*	4.13 ± 0.04**	4.42 ± 0.05	4.28 ± 0.03**	4.46± 0.02
Cerebellum	5.10 ± 0.07	3.52 ± 0.05*	5.16 ± 0.04**	5.46 ± 0.03	5.28 ± 0.02**	5.74 ± 0.04
Brain stem	3.95 ± 0.05	2.82 ± 0.06*	4.06 ± 0.04**	4.25 ± 0.03	4.11 ± 0.03**	4.27 ± 0.03

Table.2: Effect of restraint stress, *O. sanctum* and *C. sinensis* on reduced glutathione (GSH) at 7th day in *R. norvegicus*.
* $P < 0.001$. Statistically significant as compared to control group.
** $P < 0.01$. Statistically significant as compared to restraint stress group.

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	Control	RS	RS + O.S	O.S	RS + C.S	C.S
Brain parts	(Unit/mg protein) Mean ± S.E					
Cerebrum	4.42 ± 0.04	3.44 ± 0.02*	4.62 ± 0.02**	4.88 ± 0.02	4.48 ± 0.03**	4.52 ± 0.05
Cerebellum	3.76 ± 0.03	2.85 ± 0.05*	3.88 ± 0.02**	3.92 ± 0.03	3.66 ± 0.04**	4.11 ± 0.03
Brain stem	4.38 ± 0.02	3.46 ± 0.05*	4.47 ± 0.02**	4.56 ± 0.04	4.32 ± 0.04**	4.86 ± 0.04

Table.3: Effect of restraint stress, *O. sanctum* and *C. sinensis* on superoxide dismutase (SOD) at 7th day in *R. norvegicus*.
*$P < 0.001$. Statistically significant as compared to control group.
**$P < 0.01$. Statistically significant as compared to restraint stress group.

	Control	RS	RS + O.S	O.S	RS + C.S	C.S
Brain parts	(Unit/mg protein) Mean ± S.E					
Cerebrum	2.22 ± 0.02	1.58 ± 0.04*	2.35 ± 0.03**	2.76 ± 0.02	2.25 ± 0.02**	2.33 ± 0.03
Cerebellum	1.69± 0.02	1.23 ± 0.06*	1.73 ± 0.02**	1.84 ± 0.07	1.67 ± 0.02**	2.04 ± 0.03
Brain stem	1.34 ± 0.02	1.01 ± 0.02*	1.38 ± 0.01**	1.76 ± 0.02	1.33 ± 0.02**	2.13 ± 0.03

Table.4: Effect of restraint stress, *O. sanctum* and *C. sinensis* on catalase (CAT) at 7th day in *R. norvegicus*.
*$P < 0.001$. Statistically significant as compared to control group.
**$P < 0.05$. Statistically significant as compared to restraint stress group.

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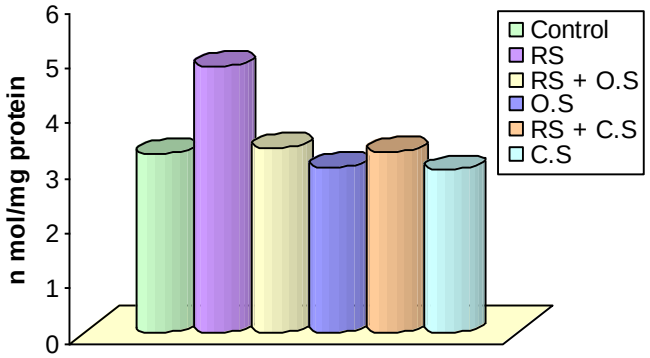
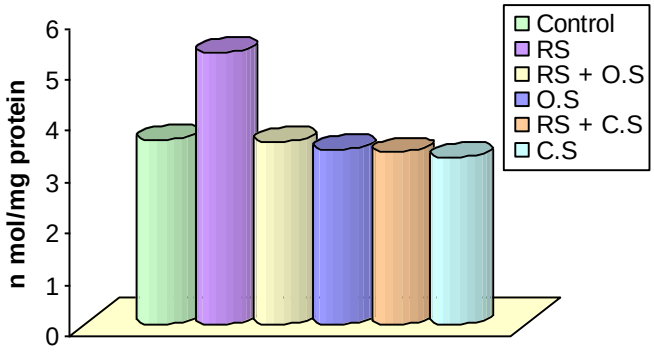
Fig.1

Cerebrum



Cerebellum



Brain stem

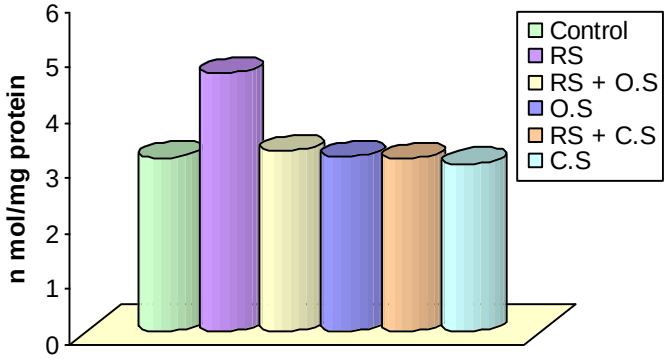


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Fig.2

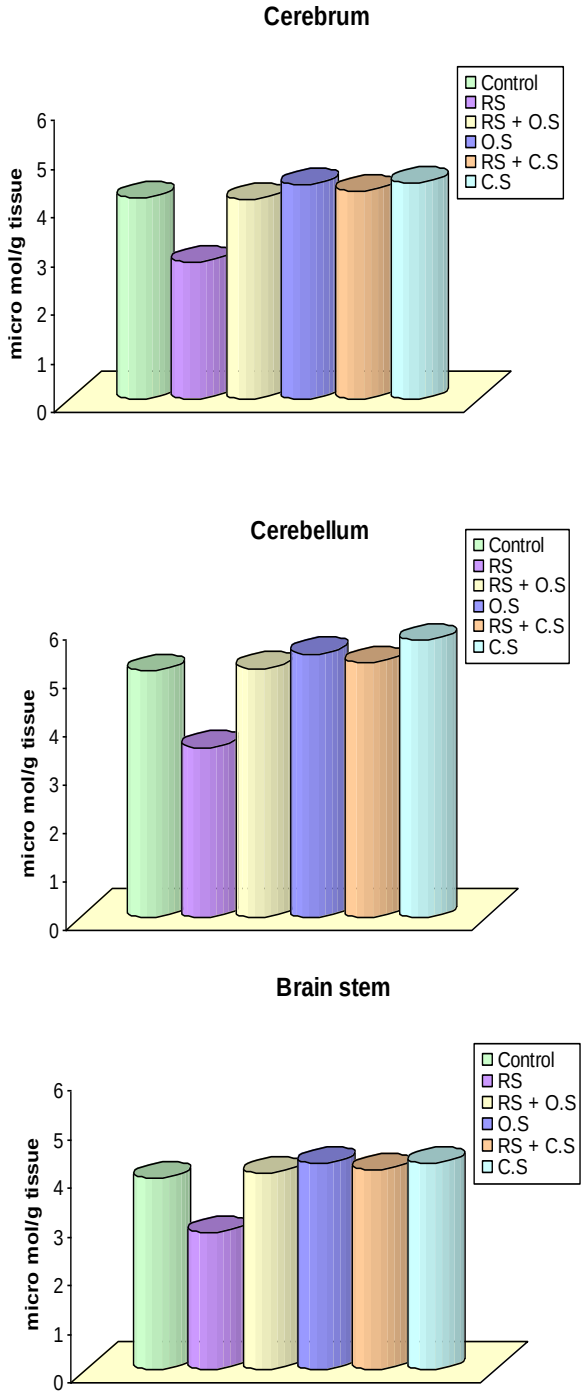

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Fig.3

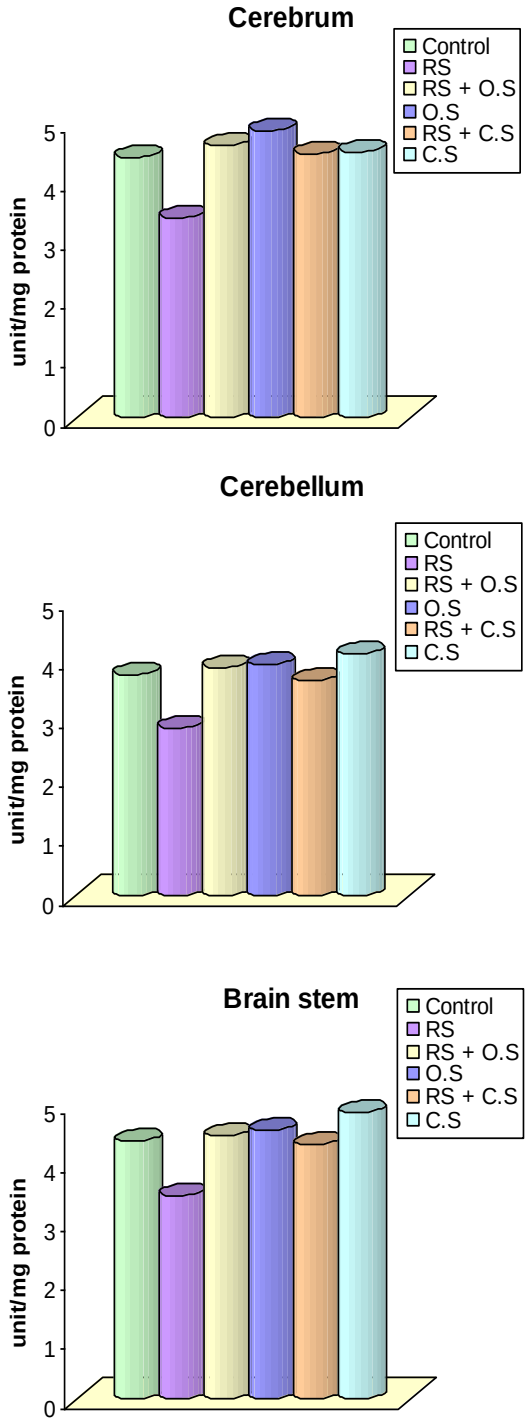

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Fig.4

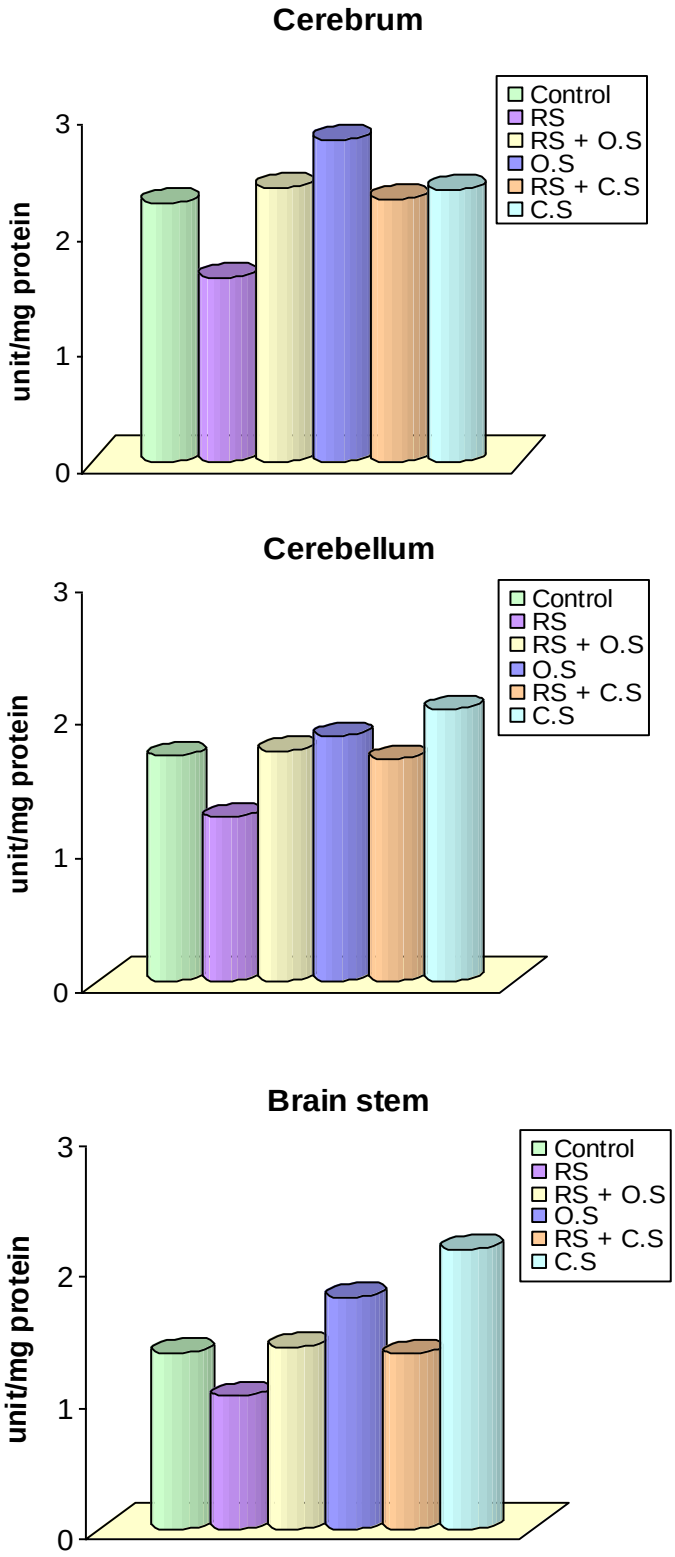

Figure caption:

Fig 1. LPO alteration in different regions of brain following restraint stress, *O. sanctum* and *C. sinensis* in *R. norvegicus.*

Fig 2. GSH alteration in different regions of brain following restraint stress, *O. sanctum* and *C. sinensis* in *R. norvegicus.*

Fig 3. SOD alteration in different regions of brain following restraint stress, *O. sanctum* and *C. sinensis* in *R. norvegicus.*

Fig 4. CAT alteration in different regions of brain following restraint stress, *O. sanctum* and *C. sinensis* in *R. norvegicus.*

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