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Influence of Age on Sleep Deprivation Induced Oxidative Stress in Different Brain Regions of Young and Old Rats

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Abstract **Original Research Article**

Sleep plays a crucial role in maintaining brain function and neuronal signalling and helps maintain overall health of the individuals. Sleep deprivation (SD) disturbs the circadian physiology and exerts a negative impact on brain and behavioural functions. The aim of the present study was to investigate the SD induced oxidative stress with advancing age in different brain regions. In the present study, young and old rats were taken as control and experimental rats (n=6) respectively. Lipid peroxidation and protein oxidation significantly increased ($p<0.05$) and reduces antioxidant levels in experimental young and old rats. The most affected part of the brain was hippocampus followed by cerebral cortex. The biochemical changes observed in SD treated young rats were comparable to those of untreated old control rats. Therefore, SD may play a role in the premature aging of animals.

Key word: Sleep Deprivation; Aging; oxidative stress.

1. INTRODUCTION

Sleep can be defined as an active state characterized by reduced alertness and responsiveness that is rapidly reversible (Arias-Carrion *et al*., 2011; Wu *et al*., 2011; Gaine *et al*., 2018). Serious physiological changes may occure due to prolonged sleep deprivation of animals (Siegel 2005). Sleep deprivation could be responsible for recruiting neurobiological mechanism related with stress as well as oxidative processes. In today's modern era of society, the population is greatly affected by sleep deprivation or insufficient rest each night (Bishir *et al*., 2020). Consistent sleep deprivation over time could be a leading factor of neuronal damage and Continuous lack of sleep can also be caused death (Hudson *et al*., 2020). The eye function may begin to generate even less coherent images possibly resulting in sleep deprivation (Frau *et al*., 2020).

Sleep deprivation may also cause reduced weight, loss of memory, sleep paralysis, altered immune system and elevated blood pressure (Havekes *et al*., 2015). Sleep deprivation caused deterioration in alertness, reasoning and response (Ben *et al*., 2020) and high order of cognitive impairment (Atrooz and Salim 2019). In the present study, we hypothesized that sleep may have several neurophysiological functions; that is, when impaired, sleep deprivation determines the subject to activate neurophysiological mechanisms to induce significant change in several systems. The exact mechanism of sleep deprivation induced alterations in neurophysiology still not clear but antioxidative mechanism proposed and accepted by the researchers (Rechtschaffen and Bergmann, 2002).

Various studies has been documented that SD potentiate oxidative stress and changes in lipid peroxidation and antioxidant defence system (Villafuerte *et al*., 2015). Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen derived oxidants commonly known as reactive oxygen species (ROS). ROS are reported to damage almost all macromolecules of the cell, including polyunsaturated fatty acids of membranes, thus causing impairment of cellular functions (Cobley *et al*., 2018).

It is hypothesized that, sleep architecture changes with age and lack of adequate sleep is common in older adults, but sleep loss may also induce oxidative stress in the brain. To the best of our knowledge, no study has directly tested the effects of SD on oxidative stress in the different part of the brain and no comparison was made between young and old animals. Therefore, in the present study attempt has been made to compare the effect of sleep on young and adult.

2. MATERIAL AND METHODS

2.1. Animals

Healthy young (n=12, age- 6 months, weight- 140 ± 5) and old (n=12, age- 24 months, weight- 480±5) male albino rats, Charles Foster strain, obtained from the Institutional animal house were used in the study. The animals were separately housed in polypropylene cages in a room, which was maintained at a temperature of 22±2 ^{0}C, relative humidity of 50±10 % and 12h light dark cycles and allowed access to water ad libitum. The Institutional Animal Ethics Committee approved the study prior to the initiation of the experiment and also approved all experimental protocols. Animals were divided in to two subgroups namely control and SD treated group.

2.2. Mode of treatment

Chronic sleep deprivation was induced using columns-in-water (modified multiple platform) model as described (Alhaider *et al*., 2011; Alzoubi *et al*., 2011). Briefly, experimental animals were placed on platforms; 20 cm high and 5 cm diameter, 7 cm apart edge-to-edge) surrounded by water (24 ± 1 ◦C) in an aquarium where water and food were accessible to animals. The water level in the aquarium was about 4 cm below the edge of the platform. Same were applied in the control without water. The sleep SD treatments were administered between 8:00 and 9:00 am with 8 h/day for 45 days.

2.3. Tissue Homogenate Preparation

After 45 days of experimental period, rats were sacrificed by anesthetic overdose and the brain was removed immediately and dissected into different parts (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) for biochemical estimations. Ten percent (w/v) homogenate of different brain regions was prepared with the aid of York's homogenizer fitted with Teflon plunger in KCl (0.15M) or 0.1 M phosphate buffer (pH 7.1), as per requirement.

2.4. Biochemical investigations

Protein carbonyl group (the oxidized protein products) levels were estimated as described by Liu *et al*., (2003**)**. The optical density of which was read in UV range at 280 nm. Carbonyl contents were calculated by using a molar extinction coefficient (*e*) of 22,000 M ¯ 1 cm ¯1. Data were expressed as nmoles carbonyl /mg. Lipid peroxide levels were estimated by the method of Ohkawa *et al*., (1979). A clear butanol fraction obtained after centrifugation of the tissue homogenate was used for measuring the absorbance at 532 nm. An appropriate standard made up of malondialdehyde (MDA) 2.5 nmol was run simultaneously. Super oxide dismutase was estimated in the tissue homogenate by the method of McCord & Fridovich, (1969). The unit of enzyme activity was defined as the amount of enzyme required to inhibit the reduction in optical density of Nitroblu tetrazolium up to 50%, in one min at 560 nm under the assay condition. Results were expressed as unit/mg protein. Catalase was estimate by the method of Aebi, (1974). Pipetted 3.0 ml of H_2O_2 phosphate buffer into the cuvette, added the required amount of tissue supernatent (cytosolic fraction) as enzyme source, and the contents were mixed thoroughly. The decrease in absorbance at 240 nm was recorded after every 30 seconds for 3 minutes. Glutathione peroxidase was estimated in the different part of the brain (Paglia & Valentine, 1967). The activity was calculated on the basis of molar extinction coefficient for NADPH ($6.22X10^3$ $M^{-1}cm^{-1}$) and result was expressed as n moles NADPH oxidized/ min/ mg protein. Reduced glutathione (Ellman *et al*., 1959) in the sample were calculated using the standard curve and the results were expressed as µmoles/ g tissue.

3. RESULTS

In the present study, male Charles Foster rats of two age groups were taken for the study. The young rats (6 months, 120 ± 5.5-gram weight) and old rats (24 months, 520 ± 5.5-gram weight) were sleep deprived treated the terminal body weight and brain weight were found to be significantly reduced of SD treated old rats when compared with the old control rats, while, insignificant difference was observed in SD treated young and old control rats (Table-1). The piloerection and hair loss were observed in SD treated young and old rats as physical sign of toxicity.

The concentration of protein carbonyl content in the various regions of the brain of young and old rats were investigated (Figure-1). The protein carbonyl content was found to be significantly ($p>0.05$) changed between groups. A maximum increment of protein carbonyl content was observed in the hippocampus (97%) of SD treated young rats and cerebrum (122%) of SD treated old rats, when compared with their respective controls.

The lipid peroxide levels in various regions of the brain of young and old rats were investigated and presented in Figure-2. The highest increment of LPO was observed in hippocampus (97%) of Al treated young rats and cerebrum (122%) of SD treated old rats, when compared with their respective controls. While, there was minimum increment in cerebellum (43%) of SD treated young rats and hypothalamus (67%) of SD treated old rats, as compared with their respective controls. The maximum increment was observed in cerebrum (64%) of SD treated young rats and hippocampus (44%) of SD treated old rats, when, compared with their controls.

The activity of superoxide dismutase (SOD) in various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of SD treated young and old rats are presented in figure-3. The activity of SOD was found to be decreased following 90 days of SD treatment. The maximum reduction was observed in cerebellum (- 19 %) of SD

treated young rats and hippocampus (- 45 %) of SD treated old rats when compared with their respective controls. While the least reduction of SOD was observed in cerebrum (-14%) of SD treated young rats and cerebellum (-17%) of SD treated old rats as compared with controls. The activity of SOD was found to be reduced with the passage of time. The maximum reduction was observed in hippocampus (-40 %) and minimum in brain stem (-12%) of old control rats as compared with young control rats respectively. An age dependent effect of SD was also found in (fig 5.14) the activity of SOD in different regions of the rat brain between SD treated old rats as compared with the SD treated young rats. The maximum decrement of SOD was found in hippocampus (-61%) and least in brain stem (-13%) of SD treated old rats when compared with the SD treated young rats.

The activity of catalase (CAT) in various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of SD treated young and old rats are presented in figure-4. The CAT was found to be decreased following 90 days of SD administration to rats. A maximum reduction was observed in hippocampus (- 36 %) of SD treated young rats (-52 %). While the least reduction of CAT was observed in hypothalamus (-29 %) of SD treated young rats and brain stem (-38 %) of SD treated old rats as compared with their respective controls.

The activity of glutathione peroxidase (GSHPx) in various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of SD treated young and old rats are presented in figure- 5. The activity of selenium containing glutathione peroxidase (GSHPx) was found to be decreased following 90 days of SD treatment to rats. The maximum reduction was observed in hippocampus (-33 %) of SD treated young rats and hypothalamus (-44 %) of SD treated old rats, when, compared with their age matched control rats. While the least reduction of GSHPx was observed in hypothalamus (-12 %) of SD treated young rats and brain stem (-14 %) of SD treated old rats as compared with their respective controls. The age dependent reduction in the activity of GSHPx was found in old rats as compared with young controls. Highest reduction of GSHPx was observed in hippocampus (-39 %) and minimum in hypothalamus (-16 %) in old control rats as compared with young control rats. The age dependent decrease in GSHPx activity was also found in SD treated old rats as compared with the SD treated young rats (fig 5.16). A maximum decrement in GSHPx activity was found in hypothalamus (-44%) and minimum in cerebellum (-20 %) of SD treated old rats, when, compared with the SD treated young rats.

The concentration of reduced glutathione (GSH) in various regions (namely, hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain of young and old rats following 90 days of SD administration is depicted in figure-6. The maximum reduction of GSH was observed in brain stem (-33 %) of SD treated young rats and hippocampus (-38 %) of SD treated old rats when compared with their respective controls. While, there was least reduction in GSH levels of cerebellum (-15 %) of SD treated young rats and brain stem (-22 %) of Al treated old rats, as compared with their respective controls. It may be seen from fig 4.10 that the concentration of GSH was decreased in aged rats' brain in all the regions but their levels varied significantly. The age dependent reduction of GSH were found to be maximum in hippocampus (-57%) and minimum in brain stem (-19 %) of old control rats as compared with the young controls. An age dependent effect of SD is also seen in figure (5.18). In SD treated rats, there was highest reduction of GSH in hippocampus (-64 %) and least in cerebellum (-45%) of SD treated old rats, when, compared with the SD treated young rats respectively.

Table-1: Terminal body weight and brain weight

Groups	Terminal body Wt (g)	Brain weight (g/kg)
Young Control	255.8 ± 8.3	4.64 ± 0.07
Young treated	237.5 ± 9.2[a]	3.89 ± 0.15[a]
Old Control	569.2 ± 11	3.92 ± 0.17
Old treated	505.8 ± 8.7[c]	3.12 ± 0.21[c]

The value of the terminal body weight and brain weight are presented as mean ± SEM of six animals per group. Superscripts relate significant ($p < 0.05$) comparison with young control (a), young Al treated (b), aged control (c) and aged treated rats.

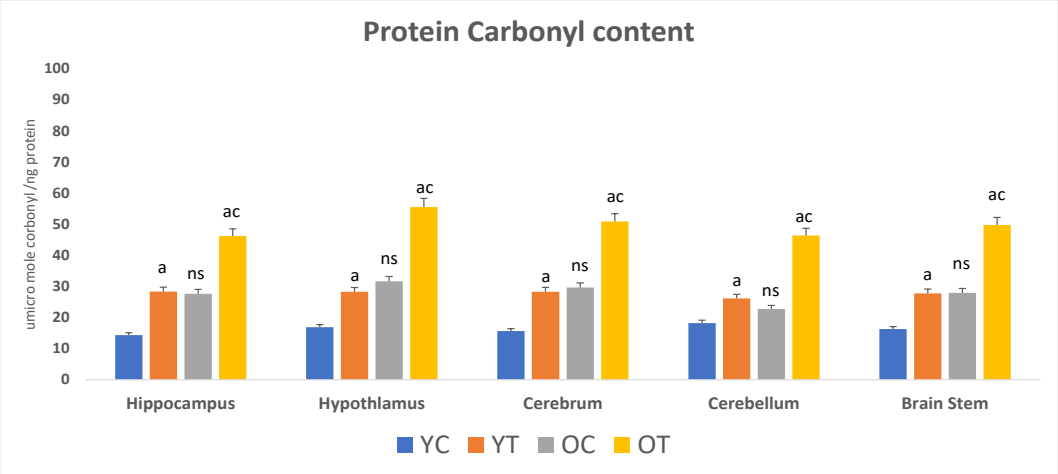

Fig-1: The protein carbonyl content (nmole carbonyl / mg protein) evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c)

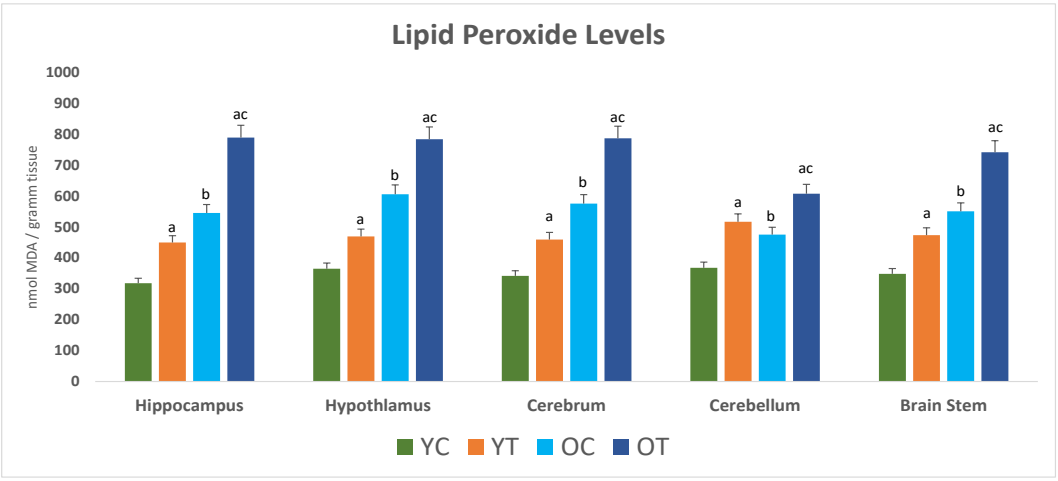

Fig-2: Lipid peroxide levels (nmole MDA / g tissue) evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c)

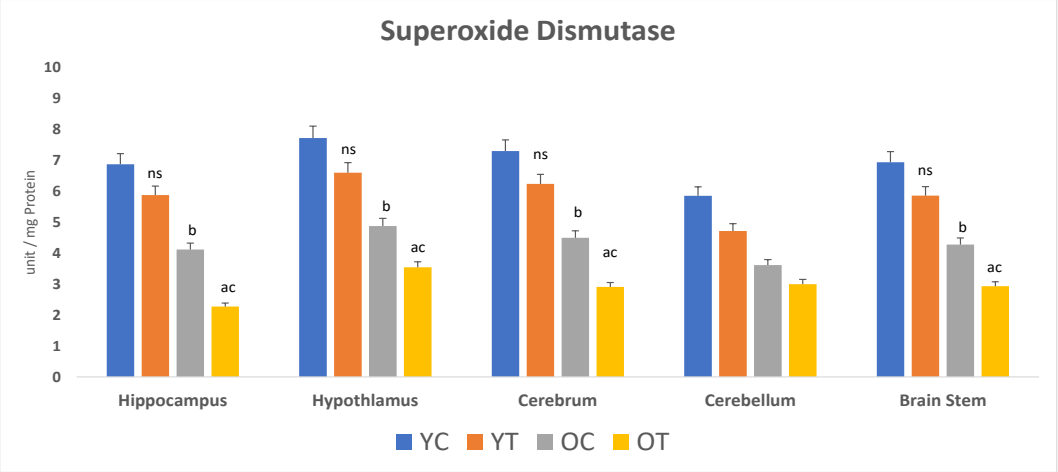

Fig-3: Superoxide dismutase activity was evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c)

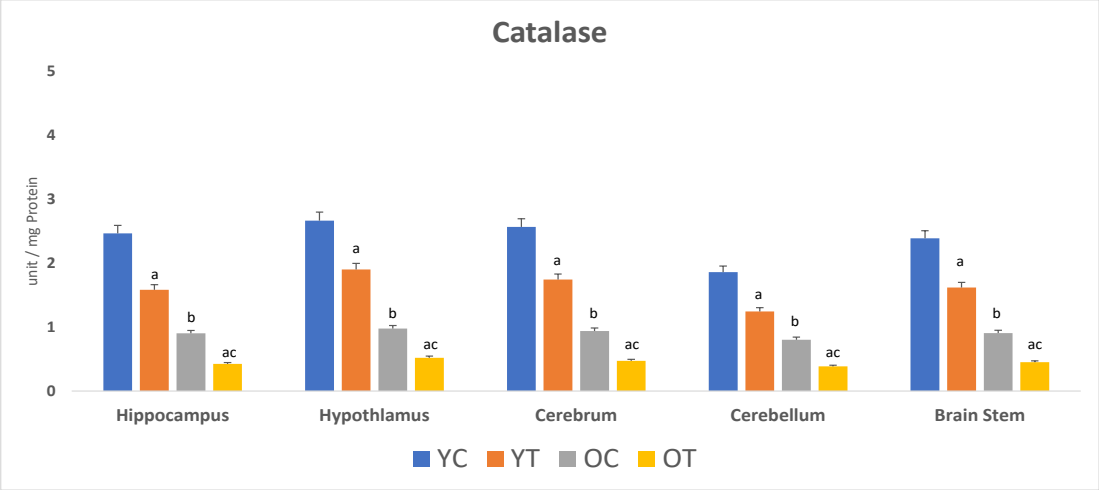

Fig-4: Catalase activity was evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c).

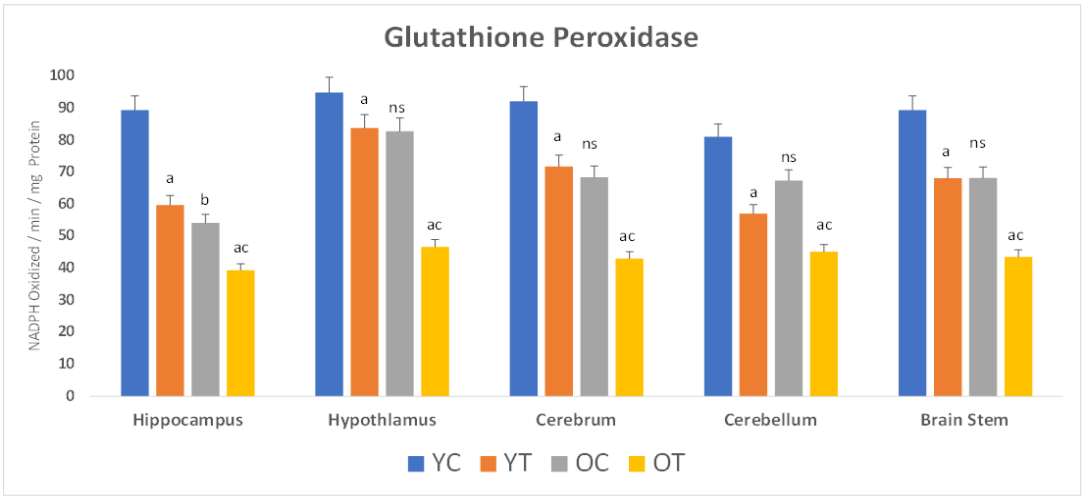

Fig-5: Glutathione peroxidase activity was evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c)

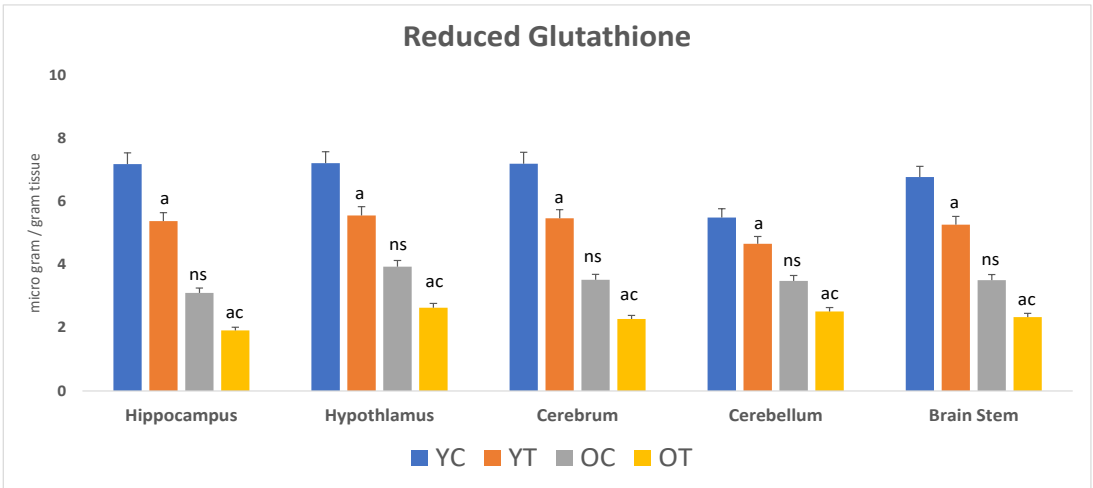

Fig-6: Reduced glutathione content evaluated in five different regions (hippocampus, hypothalamus, cerebrum, cerebellum and brain stem) of the brain following Al administration in young and aged rats. The data are expressed as Mean ± SEM in six rats of each group. The significant (p< 0.05) comparison using one-way ANOVA followed by Student Newman-Keuls post hoc test with young controls (a) and Al treated (b) and aged control (c)

DISCUSSION

Sleep plays a crucial role in maintaining brain function and maintaining neuronal signalling and helps maintain overall health. SD affects the immunological and redox system resulting in neuroinflammation and oxidative stress. Hence, it is important to understand the molecular and biochemical alterations that are the causative factors leading to these pathophysiological effects on the neurochemical function and associated aging may affect deleteriously.

In the present study, we found that the SD treatment to young and old rats had a detrimental effect on the body and brain weights of young and old rats when compared with their respective controls (Tables 1). Loss in body weight, following exposure to SD, has also been attributed to the increased mobilization of fat deposits, owing to enhanced synthesis of glucose from noncarbohydrate sources (Ganrot, 1986). The loss in brain weights after SD treatment could be credited to the retarded health. Moreover, these changes seem to be linked with SD and as they were more apparent in the old rats, it is likely that they may be due to the perturbation in cellular and molecular metabolism of aged rats.

In the present study, we found that SD increases the rate of protein oxidation. There was maximum increment of the protein carbonyl concentration in hippocampus and cerebrum of SD treated old and young rats respectively, when compared with the controls. These results are concordance with the report of Ramanathan and Siegel (2011). The age dependent increased protein carbonyl content indicated that protein damage occurs under conditions of oxidative stress. It may represent direct oxidation of protein side-chains by ROS or RNS (Daiber *et al*., 2020). In addition to traditional ROS and RNS species such as peroxynitrite, oxidative damage to proteins can occur due to alternate oxidants (e.g., HOCl) and circulating oxidized amino acids such as tyrosine radical generated by metalloenzymes such as myeloperoxidase (Hazelton *et al*., 1985). The accumulation of oxidized protein is a complex function of the rates of ROS formation, antioxidant levels, and the ability to proteolytically eliminate oxidized forms of proteins. Thus, cells can generally remove oxidized proteins by proteolysis. However, certain oxidized proteins are poorly handled by cells, and together with possible alterations in the rate of production of oxidized proteins, this may contribute to the observed accumulation and damaging actions of oxidized proteins during aging and in pathologies such as diabetes, atherosclerosis and neurodegenerative diseases (Tönnies and Trushina, 2011).

Our results also demonstrated peroxidative damage of brain lipids, as measured by the increased levels of lipid peroxidation (MDA) in the different regions of the brain of young and old rats following SD treatment. This may be due to the excessive generation of ROS. Moreover, as the brain contains large amounts of lipids that are rich in polyunsaturated fatty acids, they can readily react with free radicals and undergo peroxidation. These free radicals attack lipids and proteins to produce lipofuscin pigment. SD administration is known to cause oxidative modification of proteins. The free radical theory of aging proposes that reactive oxygen species, mainly oxygen singlet and hydroxyl radicals, cause peroxidation of the phospholipid-bound polyunsaturated fatty acids (Saccà *et al*., 2018). Lipid peroxidation is a result of free radical damage to biomolecules, and, once initiated, the process of peroxidation can become autocatalytic as each lipid peroxides attacks a neighbouring fatty acid to yield additional lipid peroxide products. These lipid peroxides readily decompose to liberate highly reactive carbonyl fragments such as malondialdehyde (Khan *et al*., 2018). Malondialdehyde (MDA), a-3 carbon dialdehyde, is one of the final products of free radical chain reactions which take place during lipid peroxidation (Yang and Stockwell, 2016).

Our result demonstrating increased LPO in SD treated rats supported to the findings of Newairy *et al*., (2009), Sharma *et al*., 2009 and Sethi *et al*., (2008). These results show that biomembranes are rich in unsaturated fatty acids and these membrane lipids are susceptible to peroxidative attack. They cause extensive damage to enzymes and membrane fluidity and eventual loss of membrane integrity. Lipid peroxidation level can be easily correlated with the decrease of antioxidative defences observed with advancing age as well as with SD neurotoxicity. The decrease in the antioxidative defences (SOD, CAT, GSHPx and GSH) accompanied with the increased levels of lipid peroxides has also been reported earlier in rats by Villafuerte *et al*. (2015).

Among various antioxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic free radicals (McCord and Fridovich 1969). It is therefore possible that the decrease in SOD activity with age may be closely related to the aging and SD. The decrease in SOD activity with age may further accelerate the aging process (Bolduc *et al*., 2019). Furthermore, some authors even suggest a causal relationship between activities of antioxidant enzymes and the life span of animal species (Liguori *et al*., 2018). The effect of SD on the activities of SOD and catalase has been studied in a variety of organs and animals (Deloncle *et al*., 1999). Previous studies on the effects of age and SD on the antioxidant enzyme activity of the brain have yielded conflicting results and none of these studies have attempted to correlate the potential for oxidative metabolism with the enzyme activity. Kumar *et al*., (2012) report that superoxide radical formation increases with age; therefore, a decreased protection

against toxic radicals may have serious consequences for the aging brain. Ye *et al*., (2021) have found a direct correlation between the decrease in total activity of SOD and an increase in the level of lipid peroxidation in different regions of the aging rat brain.

Hydrogen peroxide is considered as toxic for a wide range of living organisms. Oxidative stress occurs when there is an excess of pro-oxidants over antioxidants and it has been implicated in several neurological diseases. Catalase is involved in hydrogen peroxide catabolism and is important in defense against oxidative stress. The activity of catalase enzyme has also been shown to decrease following SD toxicosis in different regions of the rat brain. Results of the present study demonstrate that SD mediated and age dependent decrease in SOD and CAT activities. Numerous biochemical processes in aerobic cells lead to the production of peroxides by activated oxygen, which may cause oxidative damage in biological tissues (Nandi *et al*., 2019). Catalase deficiency could be associated with clinical features, pathologic laboratory test results, age and oxidative stress related disorders. Rather than considering it a benign condition, it should be considered as a complicating condition for aging and oxidative stress (Góth and Nagy 2013).

Glutathione peroxidase, a selenium dependent enzyme, metabolizes these peroxides and protects cell membrane from peroxidative damage and glutathioné reductase (GR) together act as components of a chain of reactions linking NADPH-generating systems to the detoxification of hydrogen peroxide and the maintenance of glutathione in the reduced state Flohé and Flohé (2020). The results obtained in the present study showed that the specific activity of GSHPx was depleted in SD treated old rats. The GSHPx activity was found reduces in catacholinergic rich regions (hypothamus and hippocampus) and these regions are highly susceptible to SD neurotoxicity and age-related changes. The inhibition of GSHPx activity due to the passage of time and neurotoxicity may predispose brain tissue to increased oxidative damage. Because brain tissue has a high rate of oxygen consumption, peroxides are formed by the amine oxidase enzymes (Cao and Dixon 2016).

In the present study, we observed a progressive depletion in the level of GSH in various regions of the brain of young and old rats following 45 days of SD treatment. The present evidence indicates that a low GSH content may be a general phenomenon of all ageing tissues and not restricted to a few specialized tissues. The age-related decrease in glutathione reduced levels has been reported by others as well (Wu *et al*., 2004). GSH may be the key factor in lowering reducing potential which occurs in senescent tissue. In support of this argument is the evidence that cellular GSH concentration may have a profound regulatory effect on the activity of pentose phosphate cycle enzymes (Wu *et al*., 2004). Owing to decreased GSH concentration, the NADPH coenzymes that are generated by the coupled enzyme systems of glutathione reductase and glutathione peroxidase may become linked and less available to the pentose phosphate-cycle enzymes. Consequently, NADPH reducing equivalents may decrease and in turn cause a lower biosynthetic activity. SD mediated decreased GSH levels could have a marked effect on the detoxification capacity of a senescent organism, since a major function of GSH is the detoxification of peroxides produced by normal metabolism and of xenobiotics via glutathione-S--transferases. Thus, this lower capacity may provide a toxicological basis for ageing and metal ion toxicosis. These results indicate a decrease in the do novo synthesis of GSH from amino acids and a concomitant increase in GSSG (oxidized glutathione). In sum, the decrement of GSH pool results in dysfunction of the oxidative defence mechanisms. Parallel to decrease in the GSH levels, we observed an increase in the level of lipid peroxides, lipid hydroperoxides in brain regions, suggesting extensive age-related oxidative stress associated with SD toxicosis in the brain tissue.

CONCLUSION

In the present study, an attempt has been made to delineate the SD induced neurochemical changes and associated with ageing, alters the central nervous system. It is evident that a close relationship exists between SD induced oxidative stress and aging. The biochemical changes observed in SD treated young rats were comparable to those of untreated old control rats. Therefore, SD may play a role in the premature aging of animals.

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