**Interaction of exercise training and alcohol treatment on brain antioxidant defense system in two different age groups of male albino rats**

**Abstract**

**Background** : The biochemical mechanisms by which exercise significantly benefits health and well being, including antioxidant enzymes status, are not well understood in alcohol treated aged rats.

**Aim** : The present study was carried out to know the impact of exercise training on alcohol-induced oxidative damage and antioxidant status in the brain tissue of young and old albino rats.

**Methods** : The age-matched wistar rats (3 months young, n=24; 18 months old, n=24) were randomly divided into four groups: normal control (NC), exercise trained (Ex), alcohol treatment (At), and exercise plus alcohol treatment (Ex + At). The oxidative stress parameters specifically catalase (CAT), glutathione s transferase (GST), xanthine oxidase, (XOD), uric acid, (UA), ascorbic acid (AA), and malondialdehyde (MDA) were estimated in the brain tissue.

**Findings** : In alcohol treated rats CAT, AA levels are decreased and UA, XOD, GST, MDA contents were increased. These changes were found to be greater in the aged rats than that of the young rats. In exercise training rats, the levels of these parameters were increased than normal rats. Where as in both age group rats, exercise plus alcohol treatment upregulated these antioxidant enzymes status. This study showed that alcohol intoxication increased the lipid peroxidation and decreased the antioxidant status in the brain tissue of old aged rats. However, the levels of these antioxidant enzymes and antioxidant status were augmented with exercise training in both age groups of alcohol treated rats.

**Conclusion** : The present study suggests that exercise can be considered as a therapeutic means of countering the effects of alcohol intoxication in aged rats and that it may provide a useful strategy for enhancing the brain antioxidant enzymes status in rats.

**Keywords**: Alcohol, exercise, age, antioxidant enzymes, brain

**Introduction:**

The beneficial effects of regular exercise have been consistently reported in a series of human situations and diseases and in studies with experimental animals. A plethora of studies have provided evidence that exercise enhances antioxidant enzymes status in both humans and animals (Kramer *et al*., 1999). There is evidence indicating that physical activity may reduce antioxidant status in age rats and it is recommended as a therapeutic strategy to prevent, or recover from, neurodegenerative disease (Radak *et al*.,1995). There is a paradox regarding the effect of exercise on health and well being because while it can induce free radical formation which may be detrimental for cellular functions, it also reduces a variety of age-related diseases.

Studies have shown that alcohol administration presumably exert one of its toxic effect in the brain. Brain alcohol exposure can be associated with oxidative perturbation of cellular oxidant/antioxidant balance (Calabrese *et al*., 2000). Alcohol induces the formation of reactive oxygen species (ROS), such as hydrogen peroxide (H2O2), hydroxyl radical (HO.) and superoxide anion radical (O2· −) in brain tissue. The main damage to cells after alcohol results from the reactive oxygen species (ROS).

Aging is a complex process involving morphologic and biochemical changes in single cells and in the whole organism. Aging is an inevitable biological process, leading to loss of function and of resistance to stress. Oxidative stress, an unavoidable consequence of oxygen metabolism in aerobic cells, is postulated to be one of the most important causes of age-related changes (Kokoszka *et al*., 2001). Aging brings about impairment of the functions of the central nervous system, which is manifested by changes in memory and cognitive performance, concomitant with the aging process is the neuronal loss in the brain and decrement of antioxidant enzymes status.

Exercise, alcohol and aging induces free radical formation, which may be detrimental for cellular function. Reactive oxygen species (ROS) are generated by a variety of physiological and pathological conditions and despite their vital importance to normal cell function including proliferation, growth, signaling and apoptosis, they cause continuous damage to lipids, proteins and DNA (Goto and Nakamura, 1997). Lipid peroxidations, especially in membranes, play a crucial role in tissue injury. (Renis *et al*., 1996). The physiological ROS concentration might be dependent on cell types, age, and even the history of oxidative stress exposure could be a modulating factor (Radak *et al*., 1998). The damaging effects of reactive oxygen species on cellular biomolecules are well documented and the consequences of such have been implicated in the aetiology of a number of human disorders. In order to neutralize ROS, the body uses enzymatic antioxidants and non enzymatic antioxidant such as superoxide dismutase, catalase, and ascorbic acid, uric aicd etc.

So far, various age related changes have been investigated the lipid peroxidation and antioxidant enzyme capacity in rat tissues. However it must be stressed that these experimental results are inconsistent (Semsei *et al*., 1991). It is also currently unaware that exercise training could provide defensive action against alcohol induced oxidative damage in aged rats. Hence in this study, we made an attempt to know the effect of exercise training on antioxidant enzymes and antioxidant status in alcohol treated groups by comparing young and old rats.

**Materials and methods**

**Animals**

Wistar strain male albino rats of two different age groups, that is, young (3 months n=24) weighing 170±10 g and old (18 months n=24) weighing 240±10 g, were used in the present investigation. This study was approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CP CSEA/ dt.17.07.2001; resolution number 9/IAEC/SVU/ 2001/dt. 04.03.2002). The rats in the 3-month age group were considered as ‘‘younger’’ and the 18-month age group were considered as ‘‘older’’ or adult rats as per the life span of Wistar strain. The rats were housed in clean polypropylene cages, six rats per cage and maintained under hygienic conditions, temperature controlled room (27±2˚C) with a photoperiod of 12-h light and 12-h dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum.*

**Groups:**

Age-matched wistar albino rats were randomly divided into four groups and six rats in each group and treatment was given as follows.

**Group I – Normal Control (NC) :** Six rats were put on a six-channel, motor driven treadmill for 5 days / week for a period of 2 months and given 2 m / mm exercise for 5 mm for equivalent handling, and also the rats received normal (0.9%) saline orally via orogastric tube.

**Group II - Exercise Training (Ex) :** Six rats were given exercise training on a six-channel, motor driven treadmill for 5 days / week for a period of 2 months at the running speed of 23m / mm 30 mim at a constant gradient of 7.5%. Treadmill was custom-built at University Scientific Instrumentation Center (USIC) - Sri Venkateswara University Campus.

**Group III – Alcohol Treatment (At) :** Six rats were received the 20% of alcohol with a dose of 2.0 grams / kg bodyweight via orogastric tube for a period of 2 months.

**Group IV - Exercise Training + Alcohol Treatment (Combination treatment) (Ex+At) :** Six rats were exercised on treadmill for 30 minutes as described in group II and the same rats were received 20% of alcohol as described in group III after exercise training.

After completion of 2 months treatment the animals were sacrificed by cervical dislocation and the brain tissue are excised at 40C. The tissues are washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80˚C for further biochemical analysis.

**Chemicals**

All the chemicals used in the present study were of Analar grade and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburgh, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India).

**Analytical procedures**

After completion of 2 months exercise training and alcohol treatment the animals were sacrificed by cervical dislocation and the brain tissue was excised at 4˚C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored in deep freezer at -80˚C for further biochemical analysis. The activities of catalase, (CAT), glutathione s transferase (GST), and xanthine oxidase (XOD) were measured by the methods of Aebi (1984), Habig *et al*., (1974), Srikanthan and Krishnamurthy (1954). Where as ascorbic acid (AA), uric acid (UA) and malondialdehyde (MDA) levels by the methods of Omaye *et al*. (1971), Martinek (1970) and Ohkawa *et al*. (1979) repectively.

**Statistical analysis**

The data were analyzed by using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects, age and treatments along with their interactions. The data were compared using two way ANOVA with Dunnett's multiple comparison test and differences were considered significant at *P* < 0.001.

**Results**

CAT activity in the brain of exercise trained rats increased significantly in young and old rats. In the current study, a significant (P<0.001) decrease in brain CAT activity was observed with alcohol treatment and also in age rats. However exercise alone and combined with alcohol significantly elevated the CAT activity in both young and old age groups than that of their respective controls. (Fig. 1).

The activity of GST was significantly increased in alcohol treated rats than control group. Alcohol significantly (P<0.001) increased the GST activity when compared to other groups in both age groups. Where as with exercise training along with alcohol significantly (P<0.001) increased the GST activity when compared to alcohol treated group. GST activity was more in the young alcohol group indicate that young animals may be less susceptible to alcohol-induced oxidative damage. (Fig. 2).

Figure. 3 depicts that the brain XOD activity. XOD activity was increased in old rats than young rats and this was further augmented with alcohol treatment. The activity of XOD was increased with exercise training and combination of alcohol when compared to control. The increased XOD activity was greater in alcohol treated rats than that of exercise training alone. However, in the combination treatment exercise plus alcohol XOD activity was significantly (P<0.001) decreased than that of exercise and alcohol treated groups.

The effect of exercise, alcohol and the combination of both (Ex + At) on brain uric acid level is depicted in the figure 4. UA level was significantly increased in exercise, alcohol and exercise+alcohol groups. Uric acid content was increased with advancement of age in the brain tissue. Exercise-induced elevation of uric acid in old rats is indicative of exercise’s beneficial role in countering the high amount of ROS.

In this study, ascorbic acid (vitamin C) content was depleted in alcohol treated and also in old age rats. The significant decrease in ascorbic acid due to alcohol treatment was more in old rats than that of young rats. Where as exercise training upregulated AA level more in young alcohol treated rats than old rats. This indicates that alcohol toxicity was more in old age individuals. Exercise training along with alcohol significantly (P<0.001) increased the ascorbic acid content and restored the contents in both young and old age rats, than that of alcohol treated rats. (Fig. 5).

The analysis of oxidative stress marker MDA in brain of control and experimental animals are showed in figure 6. MDA levels is significantly (P<0.001) increased in the groups given exercise, alcohol and in combination groups (Exercise+Alcohol). Alcohol significantly increased the MDA values when compared to other groups in both ages. The higher levels of MDA in the old alcohol group indicate that old animals may bemore vulnerable to alcohol-induced oxidative damage.

**Discussion :**

Aging is a process characterized by several changes that include a reduced capacity to use oxygen along with impaired cardio circulatory capacity and respiratory adaptation, deterioration of the nervous system, degeneration in muscle mass characterized by a reduction in muscle fiber diameters. It has been reported that alcohol-induced oxidative stress and decreased antioxidant status could be reversed by exercise training in young rats. Moreover, brain antioxidant enzymes status decreased and MDA levels increased with advancement of aging in alcohol treated rats (Fukui *et al.,* 2001). In this study, we reported that the alcohol treatment decreased antioxidant status and increased the lipid peroxidation in aged rat brain was reversed by exercise training.

Catalase, localized in peroxisomes, is one of the enzymes responsible for converting H2O2 to water and oxygen. In this study we observed decreased activity in alcohol treated group. The decrease of catalase activity could consequently induce a high sensitivity to oxidative stress in rats treated with alcohol. Dinu and Zamfir (1991) also reported the decreased activity CAT in the brain of alcohol treated rats. It has been revealed that during alcohol metabolism free radicals are generated (Lieber, 1997). It plays a major role in alcohol induced oxidative stress, which may be additionally enhanced by depletion in antioxidant defense system and in consequence by an imbalance between oxidants and antioxidants. This suggests the increased damage to this tissue as a result of uncontrolled generation of partially reduced oxygen species (Mahendran and Shyamala Devi, 2001). The increased catalase activity indicates its active involvement in the decomposition of hydrogen peroxide during exercise. Increased CAT activity in response to exercise in the brain of all age groups although to a lesser extent in the old rats, suggests the production of excess amount of oxygen from H2O2. Thus, it is likely that the decrease in the CAT activity in the brain of the old rats may predispose the area to oxidative attack. Decreased CAT activity in the old age suggests increased enzyme protein oxidation due to H2O2 accumulation. Here, we demonstrated that the age dependent deterioration against alcohol induced ROS insult was reversed by exercise training. However, the activity of this enzyme is upregulated in the exercise plus alcohol treated group. This implies that an adaptative response occurred to counterbalance the diminished catalase activity in alcohol treatment rats in response to exercise training. To emphasize, young age group rats which received combination treatment exhibited a pronounced elevation of CAT activity than the old age group rats. The combination of exercise training and alcohol ingestion augmented CAT activity in the brain suggests that exercise training may help to develop a resistance in the brain to cope with alcohol induced oxidative injury and maintain the normal levels of antioxidant enzymes.

The specific activity of GST was significantly elevated with exercise training in both the age groups of young and old rats. Increased GST activity is may be due to the sufficient availability of glutathione content in the brain, which is also increased with exercise training already reported in the present study. Vani *et al.* (1990) reported a significant increase in brain GST activity subsequent to swimming training. To support our data, several scientists reported that hepatic GST activity increased with response to exercise training (Mallikarjuna, 2005). An upregulation of GST activity due to endurance exercise training indicate its active role in detoxification of the toxic free radical compounds in the brain tissue. The elevation of GST activity in the brain tissue with treadmill exercise trained animals elucidates the possible elevation of enzyme activity or its synthesis in response to exercise training. Sen *et al.* (1993) reported that endurance exercise training significantly upregulated the hepatic GST activity in rats. The activity of GST was significantly increased with alcohol consumption in both age groups of young and old rats. Sekhar et al., (2024) reported the increased GST activity with alcohol treatment in liver. Das and Vasudevan (2005b) in their dose dependent alcohol studies reported increased GST activity which is in consistence with these findings. Increased GST activity suggests its activation due to oxidative stress (Aniya and Daido, 1994). Enhancement of GST activity in brain of alcohol fed rats could be considered an adaptive response protecting the tissue against alcohol induced oxidative stress. The specific activity of GST was drastically decreased in old age rats over the young rats. During aging process oxidative stress increases in the tissue and cause damage to the tissues. (Navarro *et al.*, 2004). Ji *et al.* (1990) reported a significant reduction in the brain tissue GST activity in older animals. In senescence a reduction of brain protein synthesis, an increase in protein degradation or both are possible. The estimated GST activity in the combination of exercise plus alcohol treatment showed a significant elevation in the brain tissue. This elevation was noticed in both the age groups of rats. However exercise training provides possible adaptation by increasing GST activity, which is involved in the removal of toxic peroxides (Anuradha and Balakrishnan, 1998). Increased GST activity with exercise training indicate the ability of tissue to cope against alcohol- induced toxicity in the body.

In this study, XOD activity was increased in exercise trained rats. Exercise training significantly augmented the activity of xanthine oxidase in the brain tissue of both age groups of rats. Sekhar et al., (2024) reported increased xanthine oxidase activity with exercise training in liver tissue of old age rat. High intensity exercise may produce a cellular environment in favour of activation of xanthine oxidase pathway (Hellsten, 1994). During strenuous exercise brain effectively protect it self from circulating xanthine oxidase derived free radicals by having appropriate amounts of antioxidants (Radak *et al.*, 1995). The brain XOD activity was significantly elevated with alcohol intoxication and also with age. Acute alcohol exposure favours the conversion of xanthine dehydrogenase (XDH), which represents the main form of the enzyme, into the superoxide generating form xanthine oxidase. This conversion may be linked to hypoxia produced by alcohol in brain tissue and leading to activation of the calcium dependent proteases within such brain cells thereby converting XDH into XOD. Abbondanza *et al.* (1989) reported an increase in xanthine oxidase after alcohol administration and an increase in the intermediate XDH / XOD form after prolonged alcohol feeding. As age advances, the activity of XOD increases in the brain tissue. The higher values of XOD in old alcohol-treated rats indicate that alcohol toxicity occurs more in old individuals compared to young subjects. The age related increased XOD activity in liver and heart. The specific activity of brain xanthine oxidase was significantly increased with the combination treatment in both young and old age rats in the present study. In this investigation we observed the increased XOD activity in exercise plus alcohol combination, but this was definitely lower than alcohol and exercise alone treatment in both the age groups. These results conclude that exercise training could augment the alcohol metabolism, delayed free radical caused lipid peroxidation and may enhance the antioxidant enzymes activities.

Uric acid is the most abundant aqueous antioxidant, particularly effective in quenching the hydroxyl and superoxide anion radicals (Yu 1994). In this investigation, uric acid levels were significantly increased with alcohol, exercise, combination treatment, and also with age. Uric acid content was significantly elevated with exercise training in two age groups of rats. Increased uric acid level to exercise training was more in older rats which may be due to increased catabolism of purines during exercise. Sekhar et al., (2024) reported the increased uric acid level with endurance exercise training in old rats. Uric acid concentration was shown to increase in exercise trained rat muscle and in the plasma (Ji, 1999). The increased uric acid level with exercise may be attributed to the increased activity of xanthine oxidase as observed in the present study. Das and Vasudevan (2005a) reported significant increase in uric acid level in liver tissue of alcoholic group and high alcohol intake group showed higher uric acid value when compared with moderate alcohol intake. Serum uric acid level is known to be increased by alcohol via alcohol induced activation of adenine nucleotide turnover, which is triggered by the acetate formed from alcohol. Production of uric acid via adenosine nucleotide turnover may occur in every tissue in the body, and uric acid may be increased throughout the body, because the large amount of acetate formed from alcohol in the brain is probably released and utilized at brain tissues. The uric acid level was drastically increased with advancement of age in the brain tissue of rat. Increased xanthine oxidase may be responsible for the increased levels of uric acid in the tissue studied (Ji, 1999). However, in the combination treatment group we observed down regulation of uric acid level than exercise and alcohol treatment groups. Uric acid level was significantly augmented with the combination treatment in both the age groups of rats. These findings also attested with the previous reports of increase in uric acid levels in old age animals (Mallikarjuna, 2005). The increased uric acid level in brain tissue of old age exercised rat suggest that exercise and aging mediated free radical production was countered effectively by this aqueous antioxidant and there by preventing the tissue damage or injury.

Increased ascorbic acid levels are indicative of improved availability of energy thorough efficient oxidoreductase activity and energy metabolism of tissue (Jhansi Lakshmi, 1998). Exercise training increases ascorbic acid level and it plays a major role in protecting tissue against oxidative stress (Rose, 1993). Liu *et al.* (2000) reported that exercise training increased rat brain ascorbic levels. This increased ascorbic acid levels may be attributed to the increased oxidative stress due to the increased oxygen consumption during the exercise training and due to increased HDL production and lipid protein lipase activity. These elevated ascorbate levels suggests their pivotal role in countering the age and exercise induced free radical toxicity. Recently Das and Vasudevan (2005a,b) reported the decreased levels of ascorbic acid with alcohol consumption in the hepatic tissue and also in plasma. These findings indicate that chronic alcohol subjected rats are encountered oxidative stress. Our results showing brain ascorbic acid levels with alcohol treatment in the present study are in agreement with the findings of Luczaj and Skrzydlewska (2004). The age related decrease in the brain, ascorbic acid may be due to the over utilization of ascorbic acid to nullify the oxygen derived free radicals which are produced during aging process (Youngman *et al.*, 1992). In particular, older rats exhibited greater depletion of ascorbic acid than younger rats. The rats that are exercised and received the alcohol for a period of 2 months have enhanced ascorbic acid levels indicate the activity of gluconolactone oxidase might have been increased with exercise training in the brain tissue. The high per cent elevation of ascorbic acid levels in the brain tissue with combination treatment suggest that glutathione and ascorbic acid system function together in protection against endogenously formed reactive oxygen species from alcohol and aging.

Malondialdehyde (MDA), a byproduct of lipid peroxides is the most frequently studied marker of oxidative stress and tissue damage during exercise. Depending on the exercise intensity and oxidative reaction, the antioxidant capacity may be perturbed and MDA may increase as a result of subsequent lipid peroxidation (Fadillioglu *et al.*, 2000). In the present study brain lipid peroxidation in terms of MDA formation was significantly elevated with exercise in both young and old rats. Several studies showed an increased lipid peroxidation with exercise training in various tissues like brain (Halliwell and Gutteridge 1984). It has been reported that exercise training increases lipid peroxidation in rat brain (Somani et al., 1995). Lipid peroxidation increased significantly in the brain tissue due to alcohol, suggesting that alcohol generates free radicals that could in turn increase brain lipid peroxidation. The activation of lipid peroxidation is one of the manifestations of alcohol neurotoxicity (Rouach et al., 1987). The increase in lipid peroxidation with age in many tissues has been reported to occur concomitantly with increased DNA and protein oxidation, and mitochondrial oxidative stress in animal as well as human models (Pansarasa et al., 2000). Fukui *et al.* (2001) reported that thiobarbituric acid (TBARs) contents in rat brain were significantly increased during aging. Lipid peroxidation levels were significantly elevated in the combination treatment of both young and old age rat. Sekhar et al., (2024) reported the increased lipid peroxidation levels in plasma of combination treatment i,e exercise plus alcohol group. However, the upregulation of lipid peroxidation that was observed with alcohol consumption was downregulated with the interactive effects of exercise and alcohol in the brain tissue. In this study, we reported that the exercise training modulated the alcohol induced lipid peroxidation in the aged brain. Our result indicates that alcohol increases ROS levels, but compensatory mechanism against ROS could be done by regular exercise training. It also suggests that a rise in metabolic rate during exercise may lead to an increase in the activity of brain antioxidant enzymes involved in metabolism of alcohol.

**Conclusion**: The data obtained in the present investigation reveal that there is a significant elevation of all major antioxidant enzyme activities in the brain tissue with response to treadmill exercise training in both the age group of rats. Age associated and alcohol intoxication decreased all the antioxidant enzymes. Here, we found that exercise training significantly reversed the alcohol induced oxidative damage in the aged rat brain. These decrement antioxidant enzymes activities were increased with exercise training up to certain levels indicating the beneficial role of exercise to cope with the free radical toxicity.

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Fig. 1. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on **Catalse (CAT)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).



Fig. 2. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on **Glutathione s transferase (GST)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).



Fig. 3. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on **Xanthine oxidase (XOD)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).



Fig. 4. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on **Uric acid (UA)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).



Fig. 5. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on **Ascorbic acid (AA)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).



Fig. 6. Effect of exercised trained (Ex), alcohol treatment (At), and combination of both (Ex + At) on M**alondialdehyde (MDA)** in the brain tissue of young and old male albino rats. The values are significant compared to the following: control (\*P<0.001), exercise (φ P<0.001), and alcohol (ψ P<0.001).

