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A STUDY TO EVALUATE THE EFFECT OF RESTRAINT STRESS ON ANTIOXIDANTS STATUS IN RATS

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ABSTRACT: Stress exerts detrimental effects on several cellular functions through impairment of antioxidant defences, leading to oxidative damage and onset of many cardiovascular and neurological diseases. The restraint stress or immobilization stress is widely accepted model to induce emotional stress and the deleterious effect of restraint stress on the biological system is well documented. The precise molecular and cellular events induced by restraint stress needs to be evaluated.

Aim: To determine the effect of sub-acute restraint stress on antioxidant status in rats.

Methodology: Adult male Wistar albino rats weighing about 180-200g were taken for the study and was divided into two groups - Control group (n=6) and Sub-acute restraint stress group (n=6). Restraint stress were given in wire mesh restrainers for 15 days (6 hrs/ day), the blood from the jugular vein was collected for estimation of antioxidant status (SOD, GPx, CAT, Vit C and Vit E) in rats.

Results: After 15 days of restraint stress, there was a significant ($P<0.05$) increase in LPO in restraint stress when compared with their control group. In enzymatic and non-enzymatic antioxidants like SOD, CAT, GPx, Vit C and Vit E was significantly ($P<0.05$) decrease in restraint stress compared to control group.

Conclusion: The exposure to repeated stress induces oxidative stress. These alterations may contribute to the deleterious effects observed after restraint stress.

INTRODUCTION: The adaptation response to stress would be different according to the type, duration, intensity and history of the stress [11, 9, 25, 27]. Repeated stress causes a disturbed homeostasis due to internal or external environment like physical or psychological stimuli known as stressors.

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The mechanisms underlying stress-induced tissue damage are not yet fully understood. But, increasing evidence has implied that the production of free radicals plays a critical role in these processes [14, 32].

Earlier studies have reported that exposure to repeated stress could stimulate many mechanisms leading to an increase in the production of oxygen free radicals. One of the reasons for the stress-induced development of free radicals may be the elevation of hydroxyl radical (OH^-), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and nitric oxide (NO) production.

These species may interact with oxygen generating reactive oxygen species (ROS). ROS are believed to be involved in oxidative damages and these substances can directly damage the cellular proteins, DNA and lipids and may cause the onset of many cardiovascular and neurological diseases.[12,15]

Restraint Stress is also known to alter antioxidant status and cause tissue damage. Immobilization for 6 hr or 8 hr causes a significant increase in lipid peroxidation, oxidative damage and resulted in reduction in the level of reduced glutathione and also decreased the activities of catalase and superoxide dismutase [23]. Cells are able to defend themselves from destructive potential of O_2 radicals in normal physiologic condition by means of their own antioxidants mechanism. In these studies alterations in the antioxidant status or oxidative damage have been studied after exposure to a repeated restraint stress. Exposure to a stressor after a stressful condition alters antioxidant status and thus increases oxidative damage or system gets habituated to stress exposure. Earlier studies have reported the effects of acute and chronic exposure of restraint stress in rats and hence the present study aims at investigating the effect of 15 days exposure of restraint stress on antioxidant status in rats.

METHODOLOGY: Adult male Wistar rats (12) weighing 150-180g were taken for this study. The institutional animal ethical committee approval was obtained. The rats were maintained (3rats/cage) under 12h: 12h light and dark cycle and were provided with food and water *ad libitum*. The rats were divided into 2 groups (a) Control group (6), and (b) restraint stress group (6). The control group were kept in home cage without any disturbance, whereas rats in stress group were restrained for 6hours/day for 15 days by placing each rat in a wire mesh cage [32]. After 15 days of restraint stress the blood samples were collected from jugular vein for estimating antioxidant status of animals. The enzymatic and non-enzymatic activity like Lipid peroxidase (LPO), (OhKawa *et al*., 1979), Superoxide dismutase (SOD) (Marklund & Marklund - 1974), Glutathione peroxidase (GPx), (Rotruck *et al*., 1973), Catalase (Sinha 1972), Vitamin C (Omaye *et al*.,1979), Vitamin E (Desai 1984).

Procedure for estimating the Antioxidant levels:

Parameter studied:

- **Biochemical estimation:**
  - **Lipid peroxidase (LPO) (OhKawa et al., 1979):** Melondialdehyde (MDA) a secondary product of LPO reacts with Thiobarbituric acid to form a pink chromogen which was measured spectrophotometrically at 532nm and expressed as nmoles of MDA formed/min/mg/protein in tissue samples.

- **Antioxidants status:**

Enzymatic:

- **Superoxide dismutase (SOD) (Marklund & Marklund - 1974):** Pyrogallol auto oxidizes rapidly in aqueous solution at a faster rate with higher PH (8.0) to produce several intermediate products.The inhibition of auto oxidation brought about by addition of enzyme is evaluated at early stage as an increase in absorbance at 420nm and expressed as units/min/mg of tissue protein.

- **Glutathione peroxidase (GPx) (Rotruck *et al*., 1973):** GBH is converted to GSSG in presence of GPx and the colour developed was read at 412nm using spec and expressed as glutathione oxidized/min/ mg/protein**.**

- **Catalase (Sinha 1972):** Dichromate in acetic acid is reduced to chromic acetate when heated in presence of H_2O_2 resulting in formation of per chromic acid as an unstable intermediate. The Chromic acetate measured using spec at 570nm and expressed as µmoles of H_2O_2 utilised/min/mg/protein.

Non-Enzymatic:

- **Vit C (Omaye *et al*., 1979):** Ascorbic acid is oxidized by Cu to form dehydroascorbic acid and diketogluconic acid when with DNPH forms a derivative, bis 2, 4 dinitrophenyl hydrazone when combined with H_2SO_4 forms a yellowish orange produced which was measured at 520nm and expressed as µg/mg protein.

- **Vit E (Desai 1984):** Tocopherol in liver tissue extracted in to Xylene and its content was measured using Emmeric-Eegel reaction. This reaction is based on reduction of Fe^{3+} to Fe^{2+} in the presence of α, α1 dipyridyl forming red colour and absorbance read at 530nm and expressed as µg/mg/protein.

RESULTS: All data were analyzed and expressed as the mean ± SE and by using students 't' test were used to test the significant difference between mean values of different groups (**fig. 1-3, table 1**).

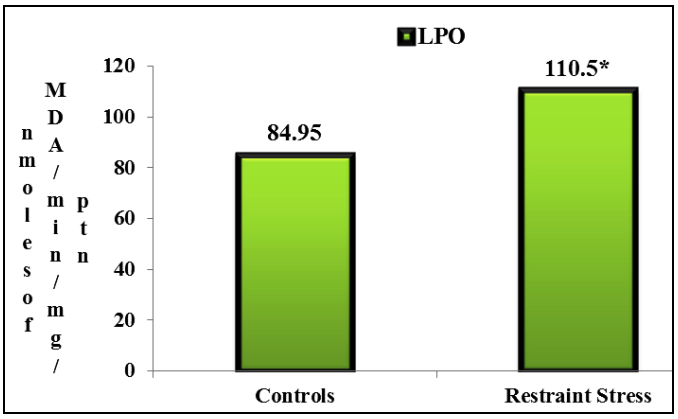

FIGURE 1: COMPARISON OF LPO LEVELS IN CONTROL AND RESTRAINT STRESS GROUP. There was a significant increase in LPO activity after restraint stress (P<0.05) compared to controls.

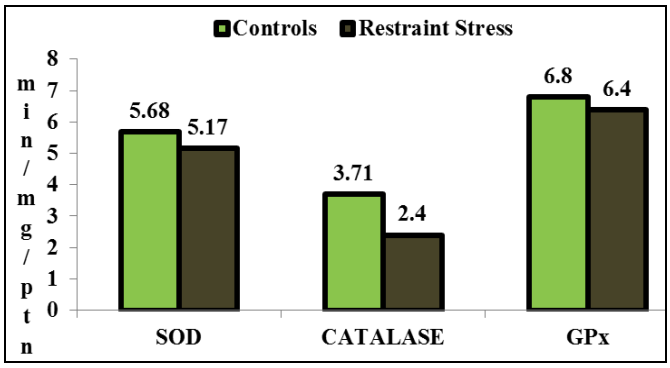

FIGURE 2: COMPARISON OF ENZYMATIC ANTI-OXIDANT LEVELS IN CONTROL AND RESTRAINT STRESS GROUP. The enzymatic activity of the SOD, CAT and GPx were significantly reduced (P<0.05) when compared to controls.

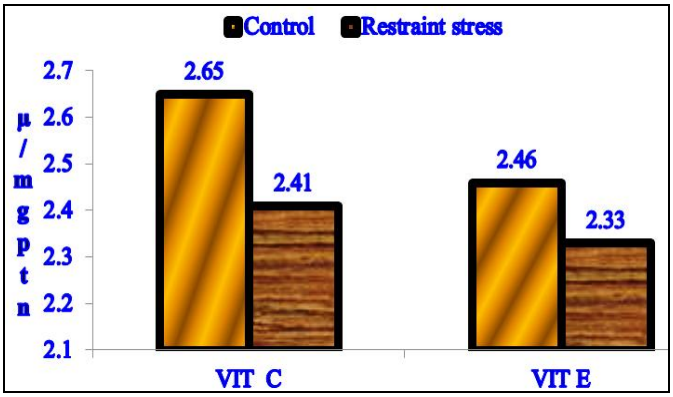

FIGURE 3: COMPARISON OF NON-ENZYMATIC ANTI-OXIDANT LEVELS IN CONTROL AND RESTRAINT STRESS GROUP. The non-enzymatic activity of the Vit C and Vit E were significantly reduced in restraint stress condition as (P<0.05) compared to controls.

TABLE 1: EFFECT OF RESTRAINT STRESS ON ANTIOXIDANTS

Groups	LPO (nmoles of MDA/min/mg/ptn)	SOD (min/mg/ptn)	CAT (min/mg/ptn)	GPx (min/mg/ptn)	VIT C (µ/mg ptn)	VIT E (µ/mg ptn)
Controls	84.98±1.33	5.68±0.09	3.71±0.08	6.801±0.11	2.65±0.02	2.46±0.02
Restraint stress	110.5±1.72*	5.17±0.02*	2.40±0.04*	6.405±0.06*	2.41±0.02*	2.33±0.02*

Values are means ±standard error. Significance fixed at P<0.05*

DISCUSSION: Stress may also impair the antioxidant defence system, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors. There is a change in lipid metabolism in restraint stress resulting in increase in **LPO** levels. Malondialdehyde is a susceptible indicator of lipid peroxidation and these generate a cascade producing LPO that causes a major damage in the mechanism of cell and even cell death [1, 18]. The fact that restraint stress enhanced MDA levels are probably associated with increased free radical generation. Oxygen free radicals are extremely reactive and unstable, thus easily reacts with lipids, proteins, carbohydrates and DNA in the body [28]. The repeated stress is capable of inducing reactive oxygen species.

The production of ROS is seen in variety of stress situation and the consequences of ROS formation depends upon the strength of the stress which causes the increasing level of antioxidant. A significant inhibition of SOD is induced by restraint stress that causes increasing the level of LPO under this stress condition [5].

Superoxide is dramatically increased during stressful condition. This superoxide is one of the main reactive oxygen species in the cell which are chemically reactive molecules containing oxygen also increased. This significant decrease of SOD may result in damage to cell structures and thus causes oxidative stress.

The function of the SOD is converting superoxide radicals into hydrogen peroxide which is effective in ROS scavenging by the reaction of CAT and GPx. This combined form of enzymes produced hydrogen peroxide

The function of the SOD is converting superoxide radicals into hydrogen peroxide which is effective in ROS scavenging by the reaction of CAT and GPx. The combined form of CAT and GPx is produced hydrogen peroxide A significant inhibition of SOD is induced by restraint stress that causes increasing the level of LPO under this stress condition [5].

The decline in **Catalase** and **GPx** can be attributed to ineffective scavenging of H_2O_2 resulting in increasing H_2O_2 levels, which can react with O2 to give OH radical and thus increased LPO. Catalase requires NADPH for its inactive form [23]. **Vit. E** and **Vit. C** have been shown to be an effective antioxidant inhibiting NO induced LPO [29].

CONCULSION: The repeated restraint stress induced oxidative stress. These alterations which may contribute to the deleterious effects on tissues were observed after restraint stress. The biochemical data were clearly showed that restraint stress induces free radical generation which may leads to oxidative damage and onset of many cardiovascular and neurological diseases.

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