A study on SMF and immombilization induced stress modulates the antioxidant activities in wistar rats

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Abstract

Stress is a vulnerable homeostasis state that modifies physiologic and behavioural reactions. Numerous organs, including the plasma, brain, liver, kidney, and heart, endure oxidative damage as a result of acute stress. The method static magnetic fields (SMFs) work, however, is not fully understood. We focused on changes that occur in various markers of oxidative damage to demonstrate the effects of static magnetic fields (128mT) and immobilized stressed animal in these studies. In immobilized stress, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), non-enzymatic antioxidants, Plasma corticosterone levels and vitamins C and E were also observed to be more significant changes. Revelation to SMFs over 21 days (1 hr/day) diminished the levels some of SOD, CAT, GPx, and GSH while increasing GPx. The response to SMFs varied over time. So, we suggested that sub-chronic exposure to SMFs might result in increased stress. Our findings introduced a new ideology on health studies, especially in the context of severe stress.

Keywords Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), SMF Static Magnetic field.

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INTRODUCTION

Stress is termed as acute or chronic, depending on the period of exposure to the stressor and the reaction. Acute stress is transient and activates the "fight or flight" response, increasing alertness and physical activity readiness while suppressing functions like feeding or reproduction[38]. While the body can quickly adapt to acute stress, repeated exposure to stress brought on by chronic stressors can result in an inappropriate basal activity or hyper-responsiveness, which may cause long-term damage to tissues like the hippocampus and prefrontal cortex in the brain[9,20].

Stress changes the physiological and behavioural reactions since it creates a vulnerable homeostasis state. The amendments on the level of stress, the nature and length of the stressful events. The central nervous system (CNS) and other systems become dysfunctional as an outcome of efforts to restore the body's homeostasis[47,17]. Cognitive changes are connected to psychiatric disorders like depression, which may develop as a result of CNS changes[5]. Furthermore, significant alterations in hemodynamic variables like heart rate and arterial blood pressure have also been measured[13,21].

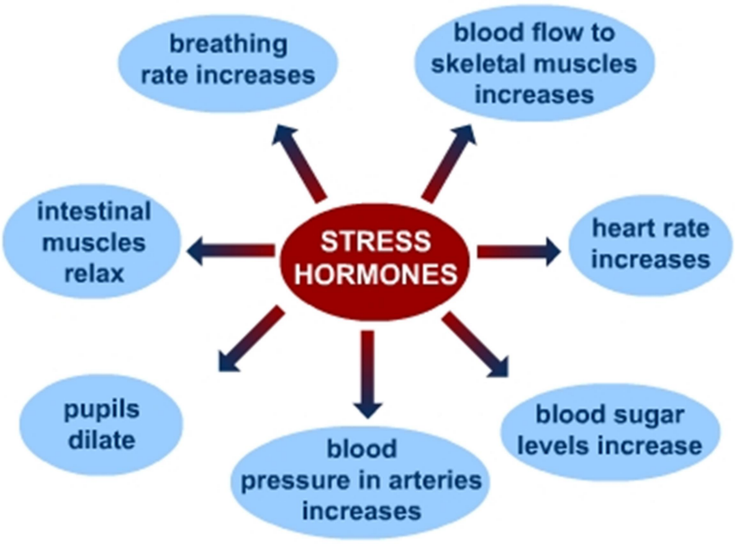
These variations can be viewed as a consequence of general adaptation syndrome as postulated by Hans Selye[6] and frequently return to their normal status after the stimulus has extinct from the section. Strong and prolonged exposure to stress[22,3,43] can cause an upregulation in energy negative balance, which can then lead to a reduction in adaptation mechanisms, an elevation in susceptibility to pathogen infection, a decline in productivity, and ultimately a significant economic loss[22,3].

Stress can be categorized as acute (such as from surgery, physical activity, or a cold environment) or chronic (such as from social isolation, work stress, or a protracted cancer treatment), depending on its length and intensity. The term "acute stress response" was first coined by Walter Cannon stated that generally animals respond to pressures by discharging their sympathetic nervous system[10]. The reaction was later identified as the initial phase of a general adaptation syndrome that controls stress reactions in vertebrates and other living things.

Acute stress can typically mobilize an adaptive response as a stimulant to keep homeostasis, which is advantageous to the body. The endocrine system and behavioural

responses are affected by stressors when they continue for a long time and can contribute to a sum of diseases. The hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) make up the neuroendocrine system, the most thoroughly researched mediator linking stress and magnetic field. Three main hormones, including glucocorticoid, epinephrine and norepinephrine, are released in response to stress[58].

Static magnetic fields (SMFs) are widely used in contemporary science and knowledge, industrial and agricultural production, medicine, healthcare, and other fields. The effects of SMFs on biological systems are receiving increasing scientific and public attention[57]. Owing to the innate magnetism of living things, exposure to SMFs causes a change of physical and chemical effects. By creating electric fields and currents, SMFs have been known to cause biological effects since the mid of the 19th century. With an increase in SMF intensity, magnetic resonance imaging's (MRI) resolution and imaging capabilities get better.



Stress hormones and diseases

It is possible for either humans or animals to be exposed to a variety of stressors throughout the course of a single day, including exposure to SMF found in electronics and home appliances. It is unclear whether this changes antioxidant status and thereby increases oxidative damage or whether the system becomes accustomed to stress exposure. Due to their capacity to detoxify free radicals like reactive oxygen species (ROS), major antioxidants like SOD, CAT, GSH, GPx, Vitamin E, and Vitamin C are crucial for cellular protection[26]. In my research work alterations in the antioxidant status or oxidative damage have been studied after exposure to acute stress and SMF.

METHODOLOGY

Experimental animals

Healthy adult albino Wistar rats, bred and nurtured in Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University were used for the experiment. We stick to males to avoid complications due to the oestrous cycle. Weight matched animals (150-180 g) were selected and housed in polypropylene cages lined as bed with husk and kept in a semi-natural light/dark condition (12 h light/dark). The animals were allowed free access to water *ad libitum* and standard pellet diet (Hindustan Liver Ltd., Bangalore, India) consisting of protein (22.21%), fat (8.0%), fibre (3.11%), balanced with carbohydrates (>67%), vitamins and minerals. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University and animals were cared in accordance with the "Committee for the purpose of control and supervision on experimental animals" (Reg. No: 160/1999/CPCSEA), [CPCSEA guidelines for laboratory animal facility, 2004].

Animals were randomized and separated into 4 groups of 9 animals each and housed in individual well-ventilated cages. Feed and water were provided *ad libitum* to the animals. Control rats, stress-treated rats, SMF-exposed rats and co-exposed rats.

Experimental design

Group	Exposure
I	Untreated control rats
II	Stress induced rats
III	Static Magnetic Field (SMF) exposed rats
IV	Stress and SMF co-exposed rats

After 21 days of exposure, the rats were abstained overnight anesthesia by injecting ketamine hydrochloride (30mg/kg body weight), sacrificed by decapitation and the blood samples were obtained in separate tubes containing heparin as the anticoagulant for the estimate of various parameters. The liver, kidney, heart and brain tissues were dissected out and collected in ice cold saline for numerous estimations.

Immobilization stress procedure

Animals were immobilized daily for 21 days by being placed in 20 cm × 7 cm plastic tubes[36]. The furthest end of the tube had 3mm holes for breathing. They tolerable ample air but animal was unable to move.

Static magnetic field exposure

Static magnetic field (SMF) were measured by gauss meter and standardized in the total floor area of the Plexiglas cage (20, 10, 20 cm) at 128mT. Male rats were exposed to the SMF, 1 h/day during 21 consecutive days[1]. Without smearing to SMF control rats were placed under the same conditions.

Analysis of behaviour:

Open field test:

Open field behaviour is a simple test to evaluate the status of the animal by placing the animal in a brightly lit box considerably larger than home cage. This elicits a series of behaviour like exploratory behaviour, immobilization, motor activity like grooming and rearing relating to emotional status of the animal[19,50].

Passive avoidance test:

On 21st day passive avoidance behaviour (PAB) was studied in a one trial learning, step-through situation, which utilizes the natural preference of rats for a dark environment[39]. After 2 min habituation to the dark compartment the rat was placed on the illuminated platform and allowed to enter the dark compartment. Two more approach trials were given on the following day with a 2 min interval. At the end of the second trial unavoidable scrambled electric foot shock (0.25 mA, AC, 2s) was delivered through the grid floor of the dark compartment (learning trial). 24 h later was tested recall of the passive avoidance responses by placing the animal on the platform and measuring the latency to re-enter the dark compartment to a maximum of 300s.

Estimation of plasma corticosterone

The fluorometric method was used to estimation the plasma corticosterone as an index of HPA function. Plasma stored at -70°C overnight was used for the estimation. A reaction mixture consisting of 1 ml of plasma and 7.5 ml of dichloromethane was shaken for 2 minutes, centrifuged (in order to separate the phases), and then the plasma layer was removed. At zero-time, 2.5 ml of fluorescent reagent (7:3 v/v concentrated sulfuric acid and ethanol) was added and shaken for 2s. The supernatant was discarded and exactly after 12 minutes, the acid extracts were read at 530 nm emission with 470 nm excitation[22].

Enzymic Antioxidants

Assay of superoxide dismutase (SOD, EC. 1.15.1.1)

The activity of superoxide dismutase was assayed by the method of Kakkar et al., [27].

The assay is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90 s adding glacial acetic acid stopped the reaction. The colour developed at the end of the reaction was extracted into n-butanol layer and measured.

Estimation of catalase (CAT, EC 1.11.1.6)

The activity of catalase in the plasma and tissues was determined by the method of Sinha[54]. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H_2O_2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H_2O_2 as chromic acetate was determined calorimetrically.

Estimation of glutathione peroxidase (GPx, EC. 1.11.1.19)

Glutathione peroxidase activity was estimated by the method of Rotruck et al[51]. A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH. GPx utilize GSH for the decomposition of H_2O_2. After a specific time period, the remaining GSH content was measured.

Estimation of reduced glutathione

Reduced glutathione in the plasma and tissues was estimated by the method of Ellman[14]. This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added to compounds containing sulphydryl groups.

Non-enzymic antioxidant

Estimation of ascorbic acid (vitamin C)

Ascorbic acid in the plasma and tissues was estimated by the method of[49]. The ascorbic acid was converted to dehydroascorbic acid by mixing with norit and then coupled with 2, 4 dinitrophenylhydrazine (DNPH) in the presence of thiourea, a mild reducing agent. The coupled dinitrophenylhydrazine was converted into a red colored compound when treated with sulphuric acid.

Estimation of α-tocopherol (vitamin E)

α–Tocopherol in the plasma and tissues was estimated by the method of[28]. The method involves the reduction of ferric ions to ferrous ions by α–tocopherol and the formation of a red colored complex with 2, 2' dipyridyl. Absorbance of the chromophore was measured at 520 nm.

Statistical analysis

Data were checked by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially accessible statistics software package (SPSS for Windows, V. 11.0, Chicago, USA). Outcomes were accessible as means ± SD. *p* values <0.05 were observed as statistically significant.

RESULTS AND DISCUSSION

Behavioural Changes

Fig.NO.1. shows the behavioural changes in the immobilization stress induced rats. Our data indicates that 21 days of immobilization stress rats show a significant difference between groups in outer sector crossings more than controls. The total number of outer sector crossing increased over time for both immobilization stress and SMF than control. The immobilization stressed group shows a significant learning, in Fig.NO.2. rats did not enter the darker compartment in control whereas co-exposure of immobilization stress and SMF have much more memory loss compared to control animals.

Physical and mental stress are both caused by many different stressors. Numerous studies using stress models have demonstrated that physiological and psychological stress stimuli activate the sympathetic nervous system activation and the hypothalamic-pituitary-adrenal (HPA) axis. As a result, the adrenocortical cells55 release the catecholamines and glucocorticoids37. More reactive oxygen species (ROS) are produced in the plasma, the liver, the kidney, the heart, and the brain have too many glucocorticoids and catecholamines present. As a result, oxidants from stress may contribute to hypertension, ulcers, impaired fertility, immunosuppression, and degenerative aging diseases like brain dysfunction.

Fig.NO.1. Open Field test in experimental rats

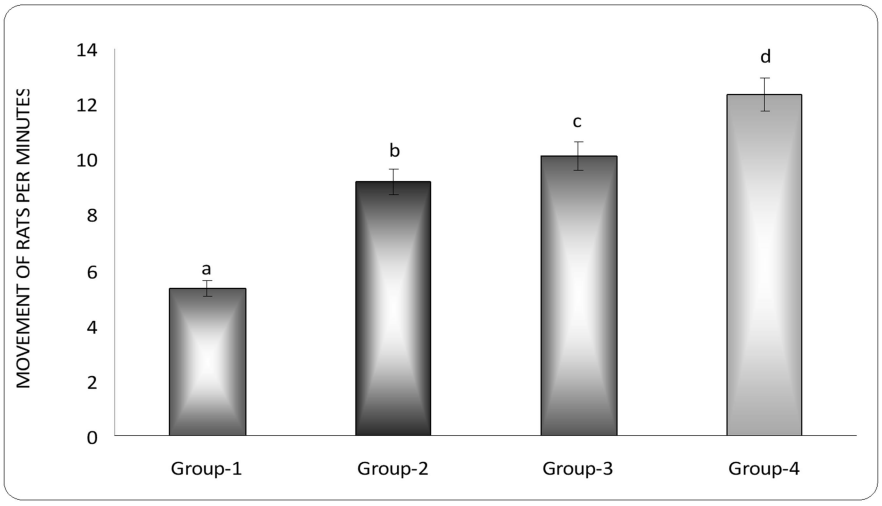
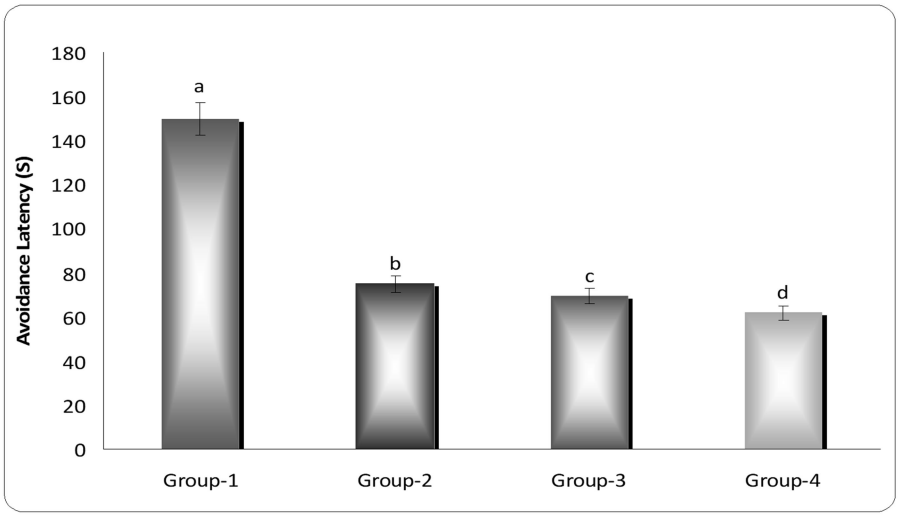


Fig.NO.2. Passive Avoidance Behaviour change in experimental rats



Group -1 Control; Group-2 Stress; Group-3 SMF; Group -4 Stress + SMF

Data represent the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT). SMF: Static Magnetic Field

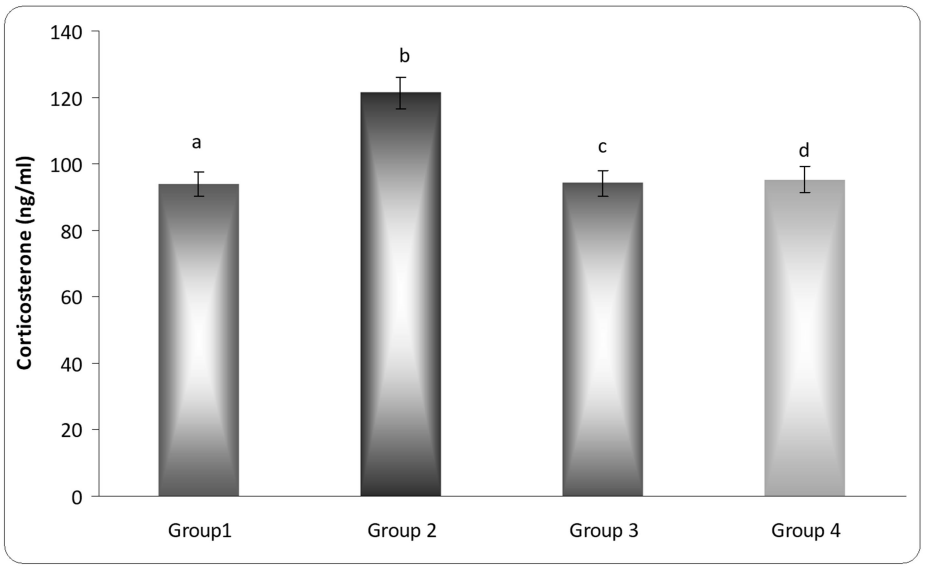
Values not sharing common alphabets as superscripts significantly different from each other.

Plasma Corticosterone level

Fig.NO.3. shows the level of plasma corticosterone in the control and experimental animals. There was a substantial growth in the corticosterone levels in the plasma of stressed and SMF exposed group animals when related to control group. The increased plasma corticosterone level in immobilization stress group is in accord with earlier studies[59] shows that plasma corticosterone is a vital indicator of stress. It is stated that stress might central to oxidative injury in various tissues[18]. All of the stress manipulations produce an initiation of the HPA axis, as indicated by increased corticosterone levels in all cases. All the stress group levels of plasma corticosterone were found to be knowingly elevated with esteem to the

control group. Many researchers show amplified plasma corticosterone levels depending on stress[46]. Though there are substantial variances between the percentage of the amplified corticosterone levels of stress groups, the maximum corticosterone level was found in immobilization and co-exposed SMF group. Some reports suggest that tremendously low frequency magnetic fields may act on the HPA activity and alter the plasma corticosterone level. According to chater et al.,[8] in rats, sub-acute exposure to SMF stimulated biosynthesis of plasma corticosterone. In part, oxidate stress plays a major role in the mechanism stress response by SMF.

Fig.NO.4. Plasma Corticosterone levels in experimental rats



Group -1 Control; Group-2 Stress; Group-3 SMF; Group -4 Stress + SMF

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT). SMF: Static Magnetic Field

Values with superscripts that are significantly different from one another but do not share the same alphabets.

Enzymatic antioxidant changes

The activities of enzymatic antioxidants (SOD, CAT and GPx) in the plasma and tissues of control and acute stress along with SMF exposed rats are represented in tables 1, 2, 3. Immobilized stress alone shows a substantial reduction in the activity of enzymic antioxidants and co-exposure of immobilized stress and SMF is significantly amplified when

linked with control animals. Numerous antioxidant systems that function as a form of fortification against free radical. One of the main causes of oxidative damage is the imbalance of antioxidants that results from increased free radical production, antioxidant enzyme inactivation or excessive antioxidant consumption. As a result, a method for measuring the overall antioxidant capacity of biological samples has been created. Stress was found to lower the total antioxidant capacity[52]. The first cellular defences against oxidative injury are involved by free radical scavenging enzyme such as SOD, CAT and GPx are disposal of superoxide anions and H_2O_2[7].

Immobilization stress is an eminent technique for the creation of chronic stress[38] and acute stress. The original point of this study is examining the tissue from immobilization stress for the changes in antioxidant enzyme activities (SOD, CAT and GPx), GSH, protein oxidation and lipid peroxidation levels.

Superoxide dismutase (SOD) is a crucial component of the system that prevents the body from destructive free-radical activity. By oxidizing the superoxide radical to H_2O_2 and molecular oxygen SOD neutralizes it[29] (Kaviarasan, 2005). Its absence or diminished activity may have noxious metabolic outcome. A decline in the SOD activity that was found in steel workers exposed to electromagnetic field is due to accrual of superoxide anion radical in red blood cells[31]. According to Lee et al[33] stated that exposure to SMF (12 G, for 3 h) induced stress decreased SOD activity level in mouse tissues. Moreover, Boguslaw[42] specified that there is a link between the exposure to SMF and the oxidative stress because it disturbs the redox balance, which causes physiological disturbances. It is recorded that after exposure to magnetic field, mouse bone marrow derived macrophages and also in their precursor cells primarily produced superoxide anion radicals[23]. The current study specifies that acute stress and acute stress exposure to SMF declined the antioxidant activity in rat tissues.

Catalase (CAT), which acts as a preventive antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation. Hydrogen superoxide, a product of SOD activity is also a strong inhibitor of the enzyme. The elevation of SOD and CAT activity of immobilization stress decreased the reactive oxygen species (ROS) production on a large scale in tissues. That is the effective detoxication of active oxygen forms takes place with concordant SOD and CAT action. In our study SOD and CAT activity was decreased due to the effect of static electromagnetic field and acute stress exposure to SMF[2]. The result of decreased CAT activity in the plasma and other tissues is in accordance with the finding of Zaidi et al.,[60] found a decreased CAT activity in the plasma and other tissues of rats which were immobilized for 2 h/day. We may suggest that OH generated by

H_2O_2 decomposition *in vivo*, proteins that are rich in plasma and tissues, therefore may inactivate CAT, GPx, GSH activities and cause damage to tissues. The inactivation of antioxidant enzymes may lead to the increment of lipid peroxidation levels in the tissues[15].

Gultathione peroxidase (GPx) is a selenium containing metaloenzyme, partially located within cellular membrane, which can remove hydrogen peroxide by converting reduced glutathione into oxidized glutathione[12]. GPx also terminate the chain reaction of lipid peroxidation by removing lipid peroxides and H_2O_2 from the cell membrane[53]. Ozcan et al.,[42] reported declined activities of GPx in patients with affective disorders in compared with healthy controls in the study of depressive patients which was followed up on by[19, 56]. In the current study we have observed significantly reduced concentrations of GSH in acute stress compared to control. Both concurrent exposure to acute stress and SMF were noted to cause a down regulation of GSH. In a study, type 2 diabetic patients had lower levels of CAT, GPX, GSH and SOD[30]. While in another study[45] the activities of GPX, SOD and CAT in red blood cells were significantly reduced in diabetic subjects when compared with healthy controls. Some authors suggest reduced GPx activity as cardiovascular risk factor that was associated with amplified extent of atherosclerotic lesions[16]. A significant red blood cell GSH and GPx activity decrease was observed, such a tendency indicates abnormal function of the antioxidative system caused by the electromagnetic field and SMF[4].

Glutathione is a tripeptide normally present at high concentration intracellularly and constitutes the major reducing capacity of the cytoplasm[34]. The cellular system against toxic effects of lipid peroxidation is protected by Glutathione.

Non-Enzymatic antioxidant changes

The levels of non-enzymatic antioxidants such as GSH, vitamin C and vitamin E in the plasma and tissues of control, immobilized stress rats, both immobilized and SMF exposed rats are given in tables 4, 5 and 6. The levels of non-enzymatic antioxidants were significantly reduced in plasma and tissues of immobilized rats as related to control rats. Exposure of both immobilized and SMF exposed rats decreased the levels of non-enzymatic antioxidants.

The circulating activities of GSH were decreased while the level of TBARS was increased in stress treated rats as compared to control group. While in acute stress and SMF exposure showed more decreased levels of GPx and GSH levels were observed. The cells are protected by various antioxidants and free radical scavenging enzyme systems that exist the damaging effects of free radicals formed as a part of normal cell respiration and other cellular processes[24]. It has been demonstrated that Immobilization stress alters the antioxidant defense

mechanism in the plasma and tissues of rats[11]. According to[35] GSH play a major role in the detoxification of oxyradicals and their products. Stress has been linked to increased lipid peroxidation and free radical production[32]. The decreased activities of GSH as observed in the present study may be responsible for the elevated lipid peroxidation as represented by increased TBARS levels is stress. Thus, immobilization stress and SMF are found capable of generating severe oxidative stress in rats.

The non enzymatic antioxidant defence system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites. Vitamin C is a water-soluble antioxidant. It effectively scavenges and destroys free radicals in amalgamation with Vitamin E and glutathione[25].

The natural non enzymes like vitamins E and C are considered as a potent antioxidant defense system. Vitamin E and C are reported to act as an effective antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress[41]. Vitamin E is one of the major chains breaking lipophilic antoxidant with in all membrane everywhere it defends membrane fatty acids from lipid peroxidation. Vitamin E quenches the singlet oxygen and is changed to vitamin E radical. Vitamin E also responds with lipid peroxides to terminate the radical chain reaction in the membrane lipid[44]. The reductions in antioxidant enzyme systems induced by acute stress were normalized by Vitamin E that results were observed in our study. Kashif[28] also reported that predominant effect of vitamin E and C is decreased in acute stress and induced oxidative damage in rat tissues. Increased free radical scavenging/ oxidative damage activity under immobilization stress as well as SMF exposure might have caused the decrease in vitamin E and C levels[40].

Table 1. Effect of acute stress and SMF on the SOD levels in experimental animals

GROUPS	PLASMA (U/mg protein)	LIVER (U/mg protein)	KIDNEY (U/mg protein)	HEART (U/mg protein)	BRAIN (U/mg protein)
CONTROL	2.27 ± 0.22^{a}	3.49 ± 0.17^{a}	2.39 ± 0.21^{a}	2.09 ± 0.06^{a}	2.14 ± 0.12^{a}
STRESS	1.1 ± 0.06^{b}	1.37 ± 0.07^{b}	1.91 ± 0.05^{b}	1.31 ± 0.03^{b}	1.64 ± 0.07^{b}
SMF	0.9 ± 0.05^{c}	0.85 ± 0.07^{c}	0.85 ± 0.01^{c}	0.95 ± 0.024^{c}	0.87 ± 0.01^{c}
STRESS+SMF	0.62 ± 0.04^{d}	0.6 ± 0.015^{d}	0.64 ± 0.06^{d}	0.86 ± 0.022^{d}	0.63 ± 0.02^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field, SOD: Superoxide Dismutase. U-units, Enzyme required for 50% inhibition of NBT reduction/minute.

Values with superscripts that are significantly different from one another but do not share the same alphabets.

Table 2. Effect of acute stress and SMF on the CAT levels in experimental animals

GROUPS	PLASMA (U/mg protein)	LIVER (U/mg protein)	KIDNEY (U/mg protein)	HEART (U/mg protein)	BRAIN (U/mg protein)
CONTROL	3.41 ± 0.55^{a}	53.88 ± 1.05^{a}	40.78 ± 0.78^{a}	32.94 ± 1.56^{a}	39.53 ± 0.65^{a}
STRESS	2.22 ± 0.52^{b}	39.58 ± 1.05^{b}	34.17 ± 0.95^{b}	19.51 ± 0.47^{b}	31.25 ± 0.68^{b}
SMF	1.29 ± 0.48^{c}	34.13 ± 1.93^{c}	27.82 ± 0.61^{c}	14.15 ± 0.46^{c}	25.99 ± 0.94^{c}
STRESS+SMF	0.62 ± 0.04^{d}	26.29 ± 2.16^{d}	23.95 ± 1.03^{d}	11.21 ± 0.22^{d}	16.01 ± 0.41^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field, CAT: Catalase. U- µmoles of H_2O_2 utilised /minute. Values with superscripts that are significantly different from one another but do not share the same alphabets.

Table 3. Effect of acute stress and SMF on the GPx levels in experimental animals

GROUPS	PLASMA (U/mg protein)	LIVER (U/mg protein)	KIDNEY (U/mg protein)	HEART (U/mg protein)	BRAIN (U/mg protein)
CONTROL	12.08 ± 0.58^{a}	9.18 ± 0.71^{a}	9.41 ± 0.37^{a}	9.35 ± 0.35^{a}	9.26 ± 0.29^{a}
STRESS	9.93 ± 0.21^{b}	8.01 ± 0.65^{b}	8.5 ± 0.24^{b}	7.39 ± 0.28^{b}	8.1 ± 0.11^{b}
SMF	8.37 ± 0.39^{c}	6.12 ± 0.43^{c}	7.06 ± 0.13^{c}	6.78 ± 0.15^{c}	6.45 ± 0.09^{c}
STRESS+SMF	6.21 ± 0.13^{d}	5.04 ± 0.38^{d}	5.62 ± 0.18^{d}	4.96 ± 0.14^{d}	5.33 ± 0.07^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field, GPx: glutathione peroxide. U- µg of GSH utilised/minute. Values with superscripts that are significantly different from one another but do not share the same alphabets.

Table 4. Effect of acute stress and SMF on the GSH levels in experimental animals

GROUPS	PLASMA (mg/dL)	LIVER (mg/100g tissues)	KIDNEY (mg/100g tissues)	HEART (mg/100g tissues)	BRAIN (mg/100g tissues)
CONTROL	36.87 ± 1.33^{a}	35.65 ± 2.13^{a}	20.15 ± 2.67^{a}	17.99 ± 0.49^{a}	32.31 ± 2.02^{a}
STRESS	21.41 ± 0.96^{b}	28.59 ± 0.55^{b}	11.59 ± 0.39^{b}	12.03 ± 0.34^{b}	24.29 ± 0.66^{b}
SMF	17.15 ± 0.25^{c}	24.76 ± 0.44^{c}	9.28 ± 0.26^{c}	10.43 ± 0.23^{c}	21.32 ± 0.39^{c}

STRESS+SMF	15.59 ± 0.28^{d}	21.61 ± 0.34^{d}	7.64 ± 0.25^{d}	7.59 ± 0.19^{d}	18.23 ± 0.31^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field, GSH: glutathione reductase. Values with superscripts that are significantly different from one another but do not share the same alphabets.

Table 5. Effect of acute stress and SMF on the VITAMIN-C levels in experimental animals

GROUPS	PLASMA (mg/dl)	LIVER (mg/100g tissues)	KIDNEY (mg/100g tissues)	HEART (mg/100g tissues)	BRAIN (mg/100g tissues)
CONTROL	1.77 ± 0.11^{a}	1.38 ± 0.14^{a}	1.51 ± 0.11^{a}	1.32 ± 0.13^{a}	1.6 ± 0.19^{a}
STRESS	0.94 ± 0.04^{b}	1.03 ± 0.07^{b}	1.02 ± 0.014^{b}	0.99 ± 0.03^{b}	1.27 ± 0.03^{b}
SMF	0.81 ± 0.02^{c}	0.84 ± 0.07^{c}	0.83 ± 0.01^{c}	0.74 ± 0.01^{c}	0.93 ± 0.01^{c}
STRESS+SMF	0.72 ± 0.01^{d}	0.59 ± 0.04^{d}	0.51 ± 0.02^{d}	0.59 ± 0.01^{d}	0.76 ± 0.012^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field. Values with superscripts that are significantly different from one another but do not share the same alphabets.

Table 6. Effect of acute stress and SMF on the VITAMIN-E levels in experimental animals

GROUPS	PLASMA (mg/dl)	LIVER (mg/100g tissues)	KIDNEY (mg/100g tissues)	HEART (mg/100g tissues)	BRAIN (mg/100g tissues)
CONTROL	1.66 ± 0.11^{a}	1.67 ± 0.09^{a}	1.74 ± 0.04^{a}	1.60 ± 0.12^{a}	1.54 ± 0.07^{a}
STRESS	1.09 ± 0.04^{b}	1.32 ± 0.03^{b}	0.94 ± 0.02^{b}	1.38 ± 0.03^{b}	1.15 ± 0.03^{b}
SMF	0.83 ± 0.03^{c}	1.08 ± 0.25^{c}	0.81 ± 0.05^{c}	1.08 ± 0.26^{c}	0.88 ± 0.02^{c}

STRESS+SMF	0.71 ± 0.02^{d}	0.50 ± 0.02^{d}	0.70 ± 0.01^{d}	0.78 ± 0.01^{d}	0.78 ± 0.01^{d}

The data are the means ± S.D of six animals per group. $p < 0.05$, (ANOVA followed by DMRT), compared to control. SMF: static magnetic field. Values with superscripts that are significantly different from one another but do not share the same alphabets.

CONCLUSION

Immobilization stress is a good model for investigating the alterations occurring in oxidant–antioxidant balance in tissues of rats. According to our results, exposure to immobilization stress (2 h/day for 21 days) may lead to free radical generation which may have changed antioxidant enzyme activities of tissues. We conclude that immobilization stress targets brain for lipid peroxidation and liver for protein oxidation owing to their levels were initiate to be highest in these tissues. The increasing production of magnetic fields, due to the expanding use of electronic devices in normal life, is encouraging studies on the effects of magnetic field on living organisms, with a view to better protecting human health against their probable unfavourable effects. This finding may help the population who are all working in the stressed condition as well as in the magnetic field, to make awareness and to protect themselves from the destructive effects of SMF.

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