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Full Length Research Paper

Effects of sub-chronic exposure to moderate intensity static magnetic field (SMF) on oxidative stress parameters of male rats

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Increasing exposure to electromagnetic fields has become inevitable for people living in industrialized environments. Magnetic fields can increase the production of free radicals; reports also suggest that extremely low frequency magnetic fields (ELF-MF) can increase free radical life-span. This study evaluated the effects of moderate intensity static magnetic field (SMF) (56 Hz, 41 mT, 6 h/day) on the oxidative status of male rats. Plasma and tissue (liver, heart, kidney, brain and testes) superoxide dismutase (SOD) and catalase (CAT) activities, as well as malondialdehyde (MDA) concentrations were measured after 35 consecutive days of exposure to the SMF. SMF exposure caused significant reductions in the body weight of rats but did not alter relative organ weight. Plasma and tissue MDA concentration were statistically insignificantly reduced, tissue SOD activities were slightly increased, while plasma and liver CAT activities were significantly ($p<0.05$) increased in SMF exposed rats. The conditions of our study show that while an oxidative challenge was experienced by the rats exposed to SMF, oxidative stress did not occur. Higher intensity SMF and/or longer duration of exposure may however lead to oxidative stress.

Key words: Static magnetic field, oxidative stress, superoxide dismutase, catalase, malondialdehyde.

INTRODUCTION

All life forms are continually exposed to the magnetic field of the earth, this field is weak and unobtrusive and people are generally unaware of its existence (Mottelay, 1922). The wide spread use of gadgets that produce non-ionizing radiations (such as static magnetic fields) with exponentially higher strength, have continued to be a source of concern with respect to health safety. The inconsistences in reports on the effects of these fields on biological systems have further compounded the issue. What is clear however is that the adverse effects of these fields worsen with increase in strength/intensity and duration of exposure (Canseven et al., 2008; Goraca et al., 2010). Static magnetic fields have virtually the same strength inside the body as outside. Very intense static magnetic fields can alter blood flow or change normal nerve impulses. However, such high field strengths are not found in everyday life (WHO, 1998). Extremely low frequency magnetic fields (ELF-MF) have been considered to be therapeutic and they are frequently used in medicine. ELF-MF can initiate a number of biochemical and physiological alterations in biological systems of different species (Kula et al., 2000; Jelenkovic et al., 2006; Hashish et al., 2008). Magnetic fields can increase the production of free radicals (Rollwitz et al., 2004). Reports also suggest that

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ELF-MF can increase free radical life-span (Lee et al., 2004; Yokus et al., 2005). Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are causative factors in the oxidative damage of cellular structures and molecules such as lipids, proteins and nucleic acids.

This damage to structure ultimately affects the functions of these biomolecules. Biological membranes that are rich in unsaturated fatty acids are particularly susceptible to free radical attack (Riley, 1994). Free radicals react with unsaturated fatty acids in cell membranes promoting a process called lipid peroxidation. Malondialdehyde (MDA), one of the end products of lipid peroxidation, is a highly toxic molecule implicated in a range of pathologies by producing oxidative damage in tissues (Goraca et al., 2010).

The objective of this study, therefore, was to assess the sub-chronic effects of exposure to moderate strength static magnetic fields on the oxidative status of various tissues of male rats.

MATERIALS AND METHODS

Animals

Twelve post-weaned rats of the Wistar strain weighing an average of 100 g, obtained from the Animal Unit of the University of Ibadan Teaching Hospital (UCH), Ibadan, Nigeria, were used for this experiment. The rats were allowed to acclimatize for two weeks before the commencement of the experiment and then they were randomly divided into two groups. Since ROS detoxification may differ depending on gender (Julicher et al., 1984), only male animals were used. The rats were housed in groups of six per cage and maintained in a room with a 12-h light/dark cycle and allowed free access to food and water. The rats were weighed weekly. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-23, revised 1985).

Experimental protocol

Animals were randomly divided into two groups as follows: Group I (n = 6)- the control group, animals were housed in the same conditions as the experimental group. Group II (n = 6)- animals were exposed to SMF in a rectangular shape solenoid of frequency 56 Hz, generating 41 mT SMF. The Group II rats were exposed to magnetic field for 35 days at the same time of the day from 9:00 am to 3:00 p.m. (6 h/day).

Biochemical analyses

At the end of the exposure period, the rats were stunned and in this unconscious state, the thoracic and abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture and placed in heparinized blood bottles. The liver, kidney, heart, brain and testes were also collected. The blood samples were centrifuged at 5,000 rpm for 5 min; the plasma was then separated, tissues were homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant was stored in the freezer until analyses.

Lipid peroxidation

The malondialdehyde (MDA) concentration was used to estimate the level of lipid peroxidation. MDA levels were determined in the plasma, liver, kidney, heart, brain and testes by the thiobarbituric acid reactive substances (TBARS) method (Varshney and Kale, 1990).

Catalase

Catalase activity was determined in the plasma, liver, kidney, heart, brain and testes by the method of Sinha (1972).

Superoxide dismutase (SOD)

Superoxide dismutase activity was determined in the plasma, liver, kidney, heart, brain and testes by the method of Misra and Fridovich, (1972).

Statistical analysis

The data are expressed as means of 6 determinations ± S.E.M. The differences between groups were analyzed by the Independent Sample T-Test. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. P value lower than 0.05 was considered significant.

RESULTS

Weekly assessment of body weight revealed that the body weight of the control rats rose steadily from week 1 to 5 (Figure 1). However, the body weight of the SMF exposed rats decreased gradually from week 1 to week 5. As compared to the control rats, the decrease in the body weight of the SMF exposed group in week 5, was statistically significant ($p<0.05$).

After 35 days of SMF exposure, no statistically significant ($p<0.05$) differences were observed in organ-body weight ratio of the SMF exposed rats as compared to control (Figure 2). However, while liver and testes relative weights of SMF exposed rats were numerically lower; relative brain weight was numerically higher than that of control rats.

The plasma, liver, kidney, brain and heart malondialdehyde (MDA) concentrations of the SMF exposed rats were insignificantly lower than that of control rats (Figure 3). However, the decrease observed in testes MDA concentration of the SMF exposed rats was statistically significant ($P<0.05$) as compared to control rats.

Plasma and tissue superoxide dismutase (SOD) activeties evaluated after 35 days exposure to SMF revealed a slight decrease in plasma SOD activities (Figure 4) of the SMF exposed rats as compared to the control. Tissue SOD activities of SMF exposed rats were generally higher than control values, with the heart recording signifycantly ($p<0.05$) higher SOD activity.

After 35 days of continuous exposure to 41 mT SMF, the plasma and liver catalase (CAT) activities of SMF exposed rats were significantly ($p<0.05$) higher than control values (Figure 5). For all other tissues, numerically

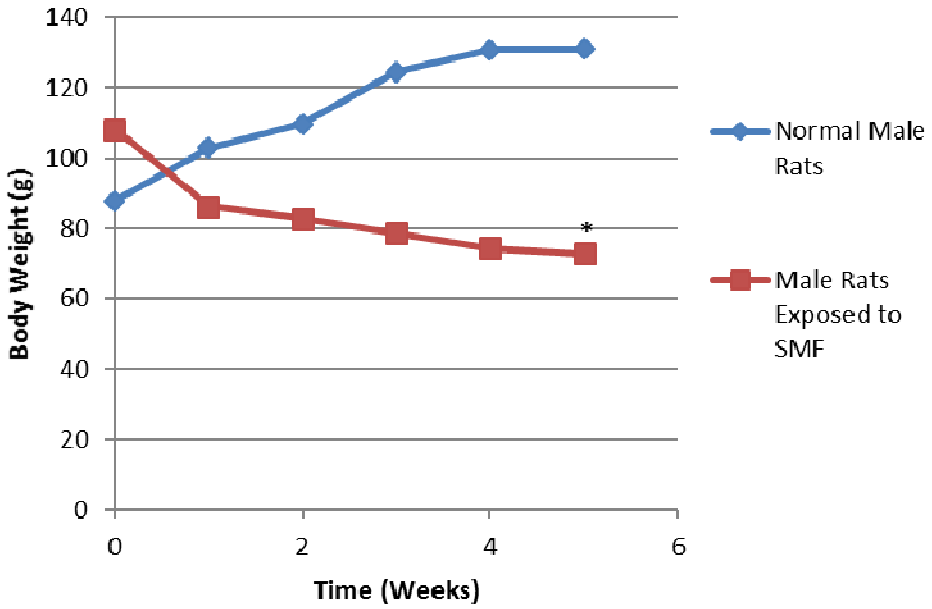

Figure 1. Effect of static magnetic field (56 Hz, 41 mT, 6 h/day) on the body weight gain of normal male rats. Data were obtained weekly, values carrying notations are statistically different from control at $p<0.05$.

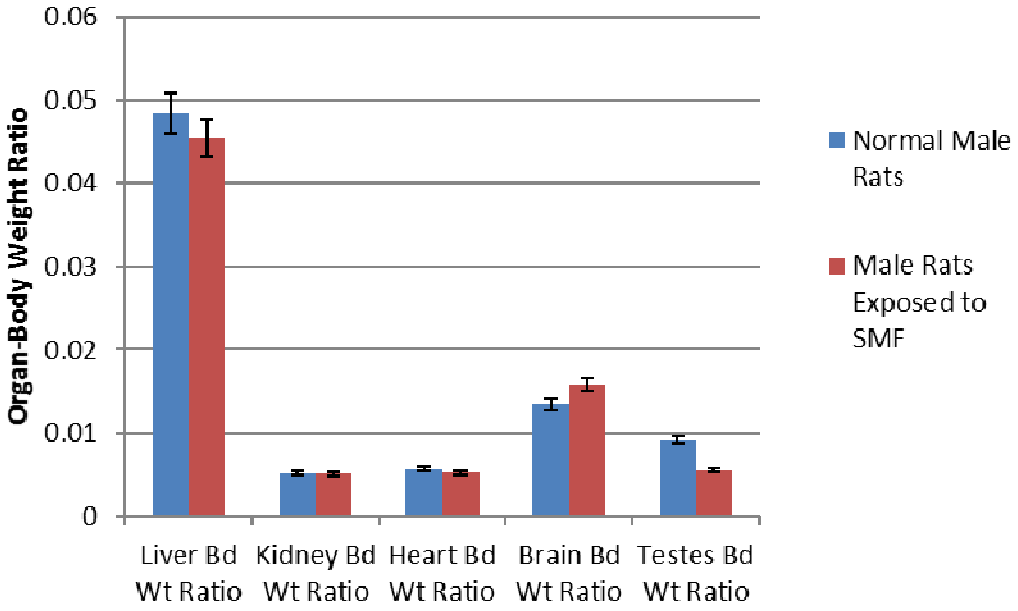

Figure 2. Effect of static magnetic field (56 Hz, 41 mT, 6 h/day) on the organ-body weight ratio of normal male rats. Data were obtained from excised tissues at the end of 35 days of exposure.

higher CAT activities were observed in the SMF exposed rats as compared to the control values.

DISCUSSION

Increasing exposure to electromagnetic fields has become inevitable for people living in industrialized environments. In particular, people living near power lines are continuously exposed to SMF of intensities greater than 20 mT (Moghaddam et al., 2005), which far exceeds naturally occurring static magnetic fields. Biological systems can respond to a wide range of SMF, some of these responses seem to be mediated partly through free radical reactions (Amara et al., 2009).

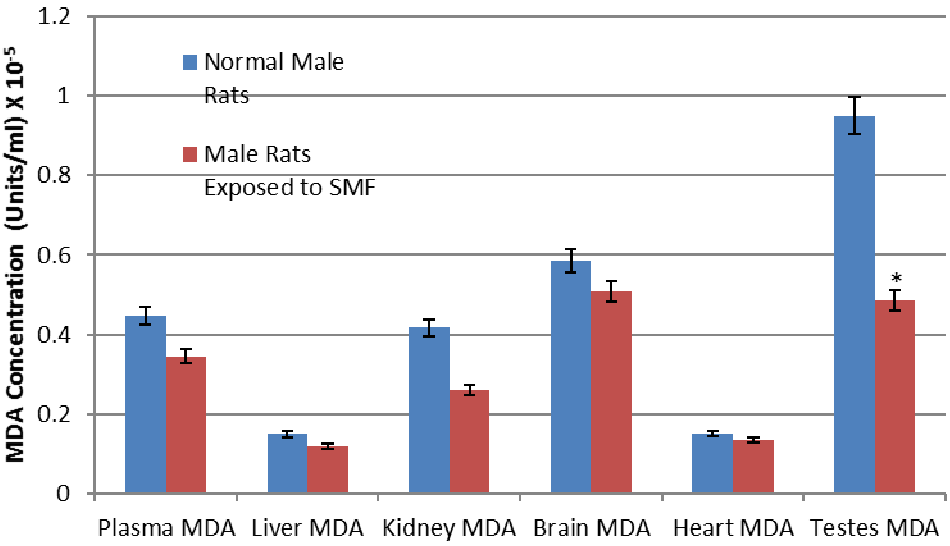

Figure 3. Effect of static magnetic field (56 Hz, 41 mT, 6 h/day) on the plasma and tissue MDA concentration (units/ml homogenate x 10^{-5}) of male rats. Data were obtained from plasma and tissue homogenates at the end of 35 days of exposure. Values carrying notations are statistically different from control at $p<0.05$.

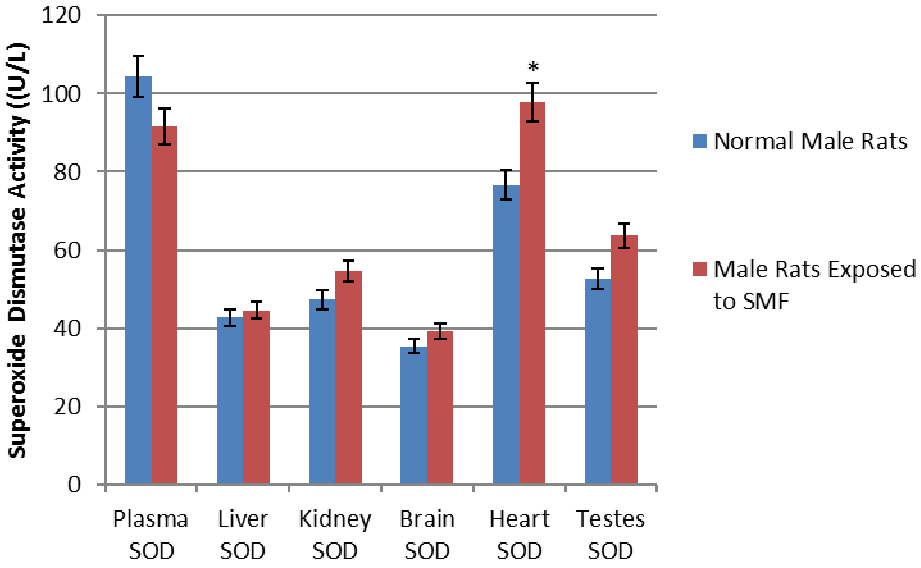

Figure 4. Effect of static magnetic field (56 Hz, 41 mT, 6 h/day) on the plasma and tissue superoxide dismutase (U/L) activities of male rats. Data were obtained from plasma and tissue homogenates at the end of 35 days of exposure. Values carrying notations are statistically different from control at $p<0.05$.

To simulate the continuous exposure to moderate intensity SMF; this study examined the effects of exposure to SMF (6 h/day at 41 mT, 56 Hz), for 5 weeks, on the oxidative status of male rats. There are several systems developed by living organisms to protect themselves from oxidative stress, antioxidant enzymes are major elements of such systems (Politański et al., 2010). This study demonstrated that exposure of male rats to 41 mT static magnetic field for 35 days reduced body weight gain; this reduction was statistically significant at week 5. Amara et al. (2006), reported that SMF exposure of rats for 30 consecutive days (1 h/day at 128 mT) significantly decreased body weight by the second week, Lahbib et al. (2010), also reported a decrease in body weight of rats exposed

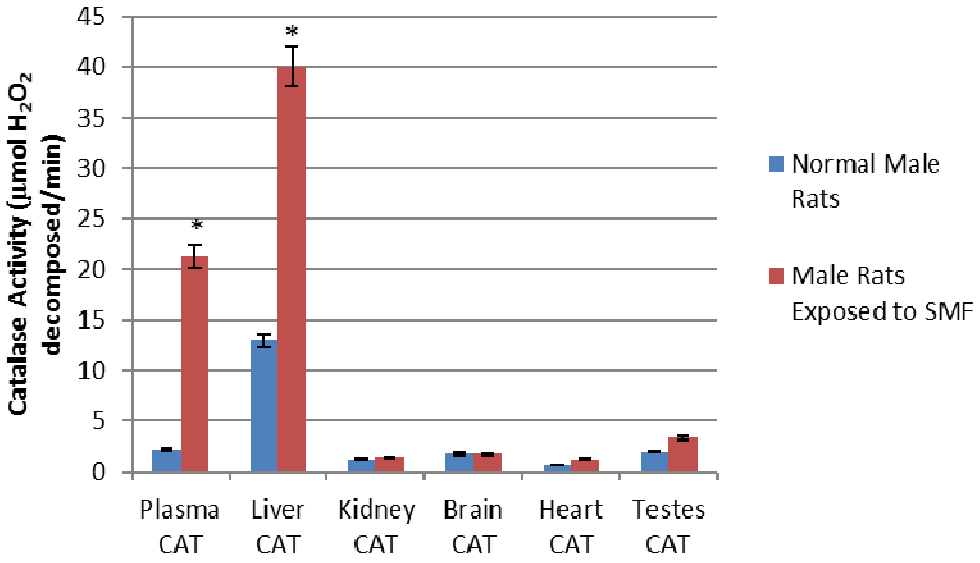

Figure 5. Effect of static magnetic field (56 Hz, 41 mT, 6 h/day) on the plasma and tissue catalase (µmol H_2O_2 decomposed/min) activities of male rats. Data were obtained from plasma and tissue homogenates at the end of 35 days of exposure. Values carrying notations are statistically different from control at $p<0.05$.

to SMF for 15 days (1 h/day at 128 mT). These reports are similar to our findings. Generally, under the conditions of our study, SMF exposure did not significantly alter relative tissue weights of male rats.

Peroxidation of unsaturated fatty acids leads to the formation of many products, like malondialdehyde (MDA) or 4-hydroxynonenal (4-HNE), which serve as markers of *in vivo* tissue peroxidation (Pompella, 1997). Tissues containing a high proportion of unsaturated fatty acids, such as brain, are particularly susceptible to lipid peroxidation (Bauer and Bauer, 1999). In this study, the concentration of MDA did not increase after 35 days of exposure of male rats to 41 mT SMF (6h/day), in fact most of the values recorded, especially testes MDA concentration, were lower than control. The implication of these results is that under the conditions of our study, lipid peroxidation did not occur. Studies which have evaluated lipid peroxidation levels in rats exposed to higher strength SMF (128 mT) have reported high levels of peroxidation products (Amara et al., 2006, 2009). The absence of peroxidation in our study may be a reflection of the lower intensity of SMF used in our study, several studies indicate that low frequency magnetic field has no effect on ROS generation in various tissues. For in-stance, Singh et al. (1999) reported that lipid peroxidation was inhibited in mice exposed to a 2 mT magnetic field. Akdag et al. (2006) showed that the exposure of male rats to 50-Hz magnetic field (2 h/day for 2 months) had no effect on MDA level in the liver, kidney and brain.

The slight increases observed in tissue SOD activities, as well as the significant increases seen in plasma and liver catalase activities of SMF exposed rats suggest that though the exposed rats experienced an oxidative challenge, hence the increase in the production of anti-oxidant enzymes, they did not experience oxidative stress. Politański et al. (2010) reported that catalase activity was significantly increased in rats exposed to SMF of 5 mT intensity, this phenomenon was attributed to explanation offered by the hypothesis presented by Blank and Goodman (2002), who suggested that magnetic fields may increase expression of some proteins (Blank and Goodman, 2002; Goodman and Blank, 2002). While it is possible that SMF may increase the production of anti-oxidant enzymes, this induction is in fact a reflection of the fact that the SMF may have first induced the production of free radicals. Again the intensity of the SMF used in this study could be responsible for this effect. Amara et al. (2009) reported decreases in SOD and catalase activities in the frontal cortex of rats exposed to SMF at 128 mT, 1 h/day during 30 consecutive days. Though we have used a longer period of exposure (6 h/day), it appears that intensity rather than duration of exposure is more pertinent in the generation of free radicals by SMF.

The oxidative challenge seen in this study as increases in SOD and catalase activities, is an indication of free radical production, it is possible that longer term exposure to these moderate strength SMF may eventually lead to oxidative stress.

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