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## Genotoxicity and Molecular Changes of Hemoglobin Studies in Rats Exposed to 3 mT Static Magnetic Field

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**Abstract:** This investigation concerns with the potential adverse biological effects of subchronic exposure of male rats to low intensity Static Magnetic Field (SMF) on the biophysical properties of hemoglobin (Hb) molecule as well as possible genotoxic effects on DNA stability. Animals were equally divided into four groups: control group and three groups exposed to 3 mT SMF for different time intervals, the last group was used for delayed effect study. The viscometric and refractometry measurements for Hb (intrinsic viscosity, Huggins coefficient, slope and refractive index) as well as the DNA alteration in somatic and germ tissues using Random Amplified Polymorphic DNA (RAPD) method were carried out for all groups. The results revealed a remarkable change in viscometric and refractometry measurements in exposed animals indicating change in the molecular shape and dimensions of Hb and its protein conformation. Also, the data indicated changes in RAPD profiles in DNA isolated from germ tissue in exposed animals. In contrast the same treatment failed to induce DNA damage in somatic tissue. The results showed the dependence of SMF biological effect on the period of exposure. The data also revealed no sign of changes in all studies carried out on Hb and DNA for animals of delayed effect experiment indicating that the damage induced in Hb molecule and DNA isolated from testis were fully recovered or repaired and/or non cumulative effect of magnetic field.

**Key words:** Magnetic field, viscometry, refractometry, RAPD analysis, rat

### INTRODUCTION

Rapid technological development and the use of electricity exposes people constantly to low-intensity, extremely low frequency electromagnetic fields, particularly at the power frequencies of 50 and 60 Hz for both occupational and environmental exposure levels (Hill *et al.*, 2005; Hore, 2005). A part from the significant achievements that has created, has also contributed to the pollution of the environment, in which the modern society exists and develops. Such pollution affects the human health and the results that bring up on it are either long-term or short-term (Leszczynski, 2005; Feychting, 2005). It is notable that the National Institute of Environmental Health Science (NIEHS, 1998) has published major reviews and concluded that there is an association between exposure to magnetic fields and a variety of cancers including (brain, breast, thyroid testicular cancers in offspring of workers, lymphoma and many others). Magnetic fields induce changes in enzyme activity and gene expression, affect membrane structure and function and cause DNA damage (Simko *et al.*, 2001). *In vivo* studies Lai and Singh (2004) demonstrated that

exposure of rats to a 60 Hz magnetic field (0.01 mT for 24 h) caused an increase in DNA single- and double-strand breaks in brain. Prolonging the exposure to 48 h caused a larger increase. Also Chater *et al.* (2005) demonstrated that SMF induced apoptosis in thymic cells of rats. Some *in vitro* studies also showed positive mutagenic effects of SMF (Ahuja *et al.*, 1997; Hirose *et al.*, 2003). In contrast to the above results, negative effect of exposure *in vivo* and *in vitro* to SMF (Ikehata *et al.*, 1999; Zmyslony *et al.*, 2000; Heredia *et al.*, 2004) were reported.

DNA alterations can be detected by a number of laboratory techniques. For genotoxicity monitoring, recent advances in molecular technology facilitates understanding the mechanisms involved in genetic damage and allow evaluation of a wide range of effects upon DNA of exposed species (Haig, 1998). The randomly amplified polymorphic DNA (RAPD) analysis method developed by Williams *et al.* (1990) and Walsh and McClelland (1990) is one of these techniques. The main advantages in the use of the RAPD method lie in its rapidity, applicability to any organism without prior information on the nucleotide sequence, cell cycle,

karyotype and the potential detection of a variety of DNA damage and mutations at the whole genome level (Rong and Yin, 2004).

Recent studies revealed that magnetic field can influence the biophysical properties of Oxyhemoglobin (HbO<sub>2</sub>), as one of the most physiologically active macromolecule. They studied the effect of SMF on function of hemoglobin and its conformational stability, autoxidation kinetics, bioenergetics and viscosity (Atef *et al.*, 1995). Also osmotic fragility and dielectric properties were measured (Ali *et al.*, 2003).

Viscometric technique has been applied to investigate hydrodynamic properties of dilute solutions i.e., properties related to the shape and motion of macromolecules in solution. The principle of using dilute solution viscometry to measure the miscibility characteristic is based on the fact that molecules of both components may exist in a molecularly dispersed state. They under go a natural attraction or repulsion which has a noticeable influence on the viscosity (Harding, 1997; Monkos, 2005). Intrinsic viscosity depends on the properties of the isolated macromolecule, its shape and its specific volume. Since effects of interaction have been eliminated by extrapolation (Gennaro, 1996; Monkos, 2004). Refractometry is the determination of the optical refractive index "n" of a substance or mixture of substances. The refractive index of solutions is the sum of refractive index of the solvent and the content-dependent refractive index contributions of the dissolved components (Born and Wolf, 1999). Refractometry as a purely physical measurement method allows a very sensitive detection (down to 10<sup>-8</sup>) and quantification of dissolved substances (Weast *et al.*, 1987) and for determination of blood glucose level (Zirk and Poetzschke, 2004). Friebel and Meinke (2006) determined the refractive index of oxygenated native hemoglobin solutions. They found that a model function of Hb solutions could be translated to rheological properties of the erythrocyte cytoplasm.

The present study is designed to investigate the effect of subchronic exposure to 3 mT SMF for various periods on biophysical properties of hemoglobin (by measuring viscosity parameters and refractive index) and on changes at the DNA level in somatic and germ tissues of adult male rats using RAPD method. Also to study the possibility of SMF to induce delayed effects.

## MATERIALS AND METHODS

**Animals:** Thirty two male Swiss albino rats (2-3 months old) weighing 200-250 g at the beginning of the experiment were equally divided into four groups. Group A was used

as control, group B was exposed to 3 mT SMF two hours per day for a period of 15 days. Groups C and D were exposed to the same field two hours per day for a period of 30 days. Group C was used for direct effects studies, while group D was used subsequently housed in a cages away from the magnetic field (in animal house of National Research Center) for a period of 45 days and then was used for delayed effects studies. Animals from all groups were kept under similar environmental conditions of temperature and 12/12-h light dark cycle, ventilation and humidity. Animals were provided with food and water *ad libitum* in their home cages and during exposure. During exposure rats were housed in a plastic cage (length 15 cm, width 12 cm, height 20 cm) fitted with a Styrofoam cover. The cage was placed at the center plane of the gap between the two poles. Two animals were exposed in a cage at a time. Control group was sham exposed to magnetic field (without applying magnetic field).

**In vivo magnetic field exposure system:** 150 MM electromagnet Model 3473-50 was used to generate uniform magnetic field. The magnet has two coils which are connected in series, with resistance at 20°C of 0.87 ohm and power 20 A/17V (0.5 kW). To achieve the uniform field a cylindrical cap of pole diameter 15 mm was used. The gap between the two poles 0-127 mm. The power supply cables were connected directly to the dc current terminal with standard copper of 25 mm<sup>2</sup> cross section. Heat generated was dissipated by using the cooling system which provides clean, cool (16-20°C) water at 3 L min<sup>-1</sup> at 0.8 bars. The magnetic field during exposure was monitored by the input current to the coils and measuring the magnetic flux density over the exposure area with the digital teslameter (Phywe, 13610. 93, Göttingen, Germany).

**Blood collection and hemoglobin extraction:** Animals from different groups were anaesthetized with ether and then blood samples were collected from the eye vein by heparinized capillary tubes on to heparin. Purified aqueous stock Hb solutions were extracted from the collected blood according to the method described by Trivelli *et al.* (1971) for viscometric and refractive index measurements.

**Viscosity measurements:** Viscosity measurement was carried out at (20±0.1°C) with a capillary type Ostwald viscometer which allows reproduction of the flow times with an accuracy of 0.03S. The temperature was controlled with circulating water through a jacket around the viscometer. The instrument was equipped with a model

U/UH thermostat, manufactured by VEB MLW company, in Berlin, Germany. The flow time (t) of the hemoglobin solution of density  $\rho$  was measured and the specific viscosity  $\eta_{sp}$  was calculated by the equation

$$\eta_{sp} = (\rho - \rho_0) (t - t_0) - 1 \quad (1)$$

Where,  $\rho_0$  is the density of distilled water at temperature 20°C and  $t_0$  is the measured flow time of distilled water.

The solution density  $\rho$  was calculated using equation:

$$\rho = \rho_0 + C (1 - \bar{v} \rho_0) \quad (2)$$

Where, C is the concentration in g dL<sup>-1</sup>,  $\bar{v}$  is the partial specific volume for hemoglobin and was taken as 0.749 (Desnica, 1979).

The intrinsic viscosity  $[\eta]$  of hemoglobin solution was calculated by means of the Huggins equation,

$$\eta_{sp}/C = [\eta] + k' [\eta]^2 C \quad (3)$$

Where the Huggins coefficient  $k'$ , proportional to the "crowding factor" represents a measure of intermolecular interaction -for different liquids and solutions-these interactions can be different, in each case,  $k'$  has to be calculated separately (Monkos, 2004). A graph of  $\eta_{sp}/C$  against Hb concentration was plotted, from the gradient the Huggins coefficient  $k'$  was calculated.

**Refractive Index measurements:** Optical refractive index was determined by using manually held refractometer (Eclipse from Bellingham and Stanly, Turn bridge wells, UK, reading accuracy:  $5 \times 10^{-4}$ ) of which the display was calibrated to the value of distilled water ( $n = 1.3329$ ) at constant temperature 30°C and set with its attached temperature control unit.

**DNA isolation:** After blood collection animals of each group were sacrificed and then testis and liver were rapidly dissected out. High molecular weight genomic DNA was isolated from liver and testis of each rat using phenol/chloroform extraction and ethanol precipitation method with minor modification (Sambrook *et al.*, 1989). The concentration of DNA and its relative purity were determined using a spectro-photometer based on absorbance at 260 and 280 nm, respectively (Aquardo *et al.*, 1992). The quality of DNA was checked by ethidium-bromide staining after resolution on a 1% agarose gel. Only high quality genomic DNA was used for the RAPD analysis.

**RAPD-PCR:** A set of ten primers, procured from the Operon Technologies was selected to generate different profile patterns including OPA10 (5'-GTGATCGCAG-3'), OPA20 (5'-GTTGCGATCC-3'), OPB09 (5'-TGG GGG ACTC-3'), OPB10 (5'-CTG CTG GGAC-3'), OPB12 (5'-CCT TGA CGCA-3'), OPC03 (5'-GGGGGTCTTT-3'), OPC05 (5'-GATGACCGCC-3'), OPC07 (5'-GTCCCGACGA-3'), OPC09 (5'-CTCACCGTCC-3'), OPC15 (5'-GACGGATCAG-3').

DNA amplification reactions were performed under conditions reported by Williams *et al.* (1990) and Plotsky *et al.* (1995). PCR amplification was conducted in 50  $\mu$ L reaction volume containing 100 ng genomic DNA, 100  $\mu$ M dNTPs, 40 nM primer (Operon, Alameda, CA, USA), 2.5 units of Taq DNA polymerase and 5  $\mu$ L promega 10X Taq DNA polymerase buffer. The reactions were carried out in Thermocycler (Perkin-Elmer 9700) programmed with a first denaturation of 5 min at 94°C, followed by 45 cycles of 1 min denaturation at 95°C, 1 min annealing at 36°C and 2 min extension at 72°C. Final extension at 72°C for 5 min was allowed before holding the reaction at 4°C for 10 min. Reaction products were stored at 4°C prior to electrophoresis. The PCR product was analyzed by electrophoresis 25  $\mu$ L of the amplified mixture on agarose gel. The Gel-Pro Analyzer (Media Cybernetics) was used to document ethidium bromide DNA gels. For accuracy in the comparison of the results, the controls and the exposed DNA extract were run in the same gel. A negative control reaction without template DNA and a molecular weight marker (marker VI, Roche, Germany or X174-Hae III EURO BIO, France) were also included in the same gel.

**Statistical analysis:** Student's t-test was used for the evaluation the data of refractive index experiment. The data was expressed as mean  $\pm$  standard error. Differences were considered significant at  $p < 0.05$  level and high significant at  $p < 0.001$ .

## RESULTS

**Biophysical changes of hemoglobin:** Figure 1 displays the variation of the reduced viscosity,  $\eta_{sp}/C$  in dL g<sup>-1</sup> of hemoglobin extracted from control and exposed rats to 3 mT SMF plotted as a function of protein concentration, C in g dL<sup>-1</sup>.

Intrinsic viscosity  $[\eta]$  was obtained from the Huggins plots of the reduced viscosity  $\eta_{sp}/C$  versus protein concentration, by the extrapolation to infinite dilution (zero solute concentration) with an uncertainty of less than  $\pm 0.01\%$ . The values of intrinsic viscosity  $[\eta]$ , the slopes and the Huggins coefficient  $k'$  for hemoglobin of 3 mT magnetic field exposed rats as compared to control were showed in Table 1.

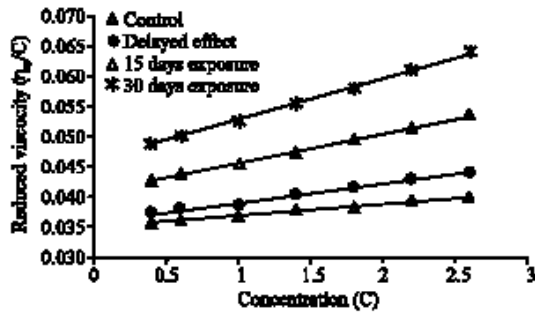


Fig 1: Variation of reduced viscosity of hemoglobin extracted from rats exposed to 3 mT magnetic field against protein concentration, as compared to control

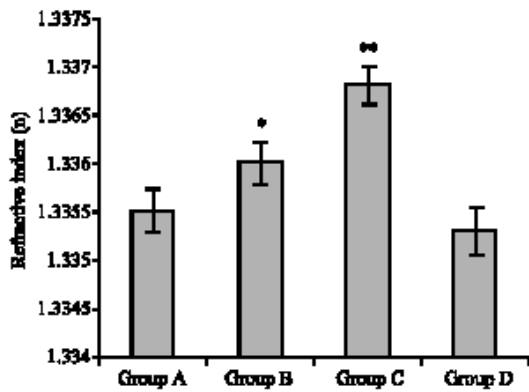


Fig 2: Changes in refractive index for hemoglobin extracted from rats exposed to 3 mT magnetic field as compared to control. Group A: control, Group B: after 15 days of exposure to SMF, Group C: after 30 days of exposure to SMF, Group D: delayed effect study, \*p < 0.05, \*\*p < 0.001

Table 1: Viscometric measurements for Hb of control and 3 mT SMF exposed rats

Groups	Intrinsic viscosity $[\eta]$ dL g <sup>-1</sup>	Slope	Huggins constant (K')
Control	0.0357	0.0021	1.633
15 days exposure	0.0411	0.0047	1.218
30 days exposure	0.0465	0.0063	0.981
Delayed effect	0.0364	0.0026	1.604

pH = 5.87, Concentration range 0-2.6 g dL<sup>-1</sup>, at temperature t = 20 ± 0.05°C

The data shows a remarkable increase in the values of intrinsic viscosity, slope and decrease in Huggins coefficient k' as a direct effect of exposure to magnetic field. The magnitude of change depends on the periods of exposure (15 and 30 days exposure) that the increase in  $[\eta]$  and slope with decline in k' values are more observable at 30 days than at 15 days of exposure to SMF

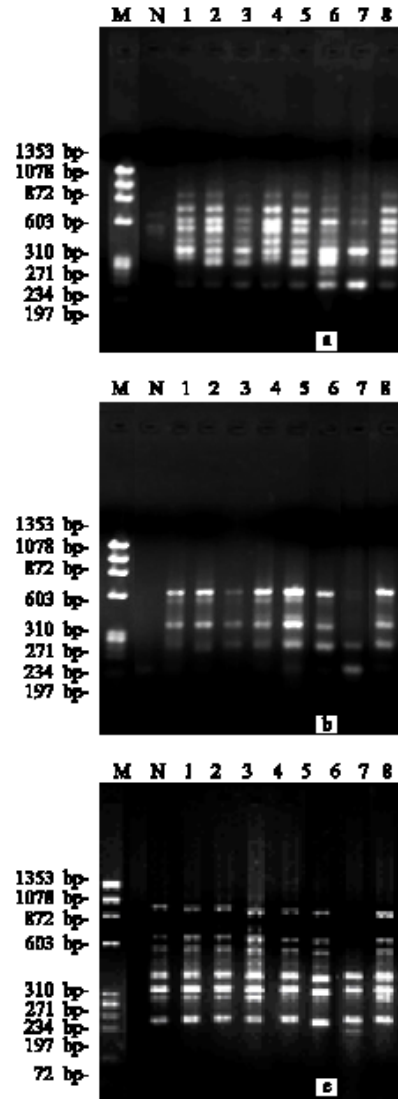


Fig 3: Comparison of RAPD fingerprinting profiles of different rat genomic DNA (a) represents PCR products with primer OPA10, (b) represents PCR products with primer OPB10, (c) represents PCR products with primer OPC5, Lane M:  $\phi$ X174 DNA marker (bp). Lane N: Negative control reaction without DNA template. Lane 1 represents liver DNA extract from control, lane 2 represents liver DNA extract from 15 days of exposure, Lane 3 represents liver DNA extract from 30 days of exposure, lane 4 represents liver DNA extract from delayed effect, lane 5 represents testis DNA extract from control, lane 6 represents testis DNA extract from 15 days of exposure, Lane 7 represents testis DNA extract from 30 days of exposure, lane 8 represents testis DNA extract from delayed effect

on comparing with control. While in the delayed effect experiment these values tend to be normal.

The Fig. 2 illustrates significant increase in the RI values for group B ( $p < 0.05$ ) and high significant increase for group C ( $p < 0.001$ ) which exposed to magnetic field for 15 and 30 days, respectively. While values of the delayed effect study, show non significant difference in RI as compared to control.

Table 2: The total No. of bands, polymorphic bands and polymorphism between 3 mT SMF exposed and control male rats using different random primers

Primer	DNA bands		Size of bands (bp)	Testis			
	Total	Polymorphic		GA	GB	GC	GD
OPA10	9	4	889	+	+	+	+
			725	+	+	+	+
			603	+	+	+	+
			511	+	+	-	+
			403	+	+	-	+
			341	+	+	+	+
			290	+	+	-	+
			216	-	+	-	-
			181	+	+	+	+
			181	+	+	+	+
OPA20	4	3	501	+	+	+	+
			449	+	+	-	+
			310	+	+	-	+
			278	+	+	-	+
OPB9	6	4	1033	+	+	+	+
			781	+	+	-	+
			560	+	+	-	+
			432	+	+	-	+
			310	+	+	-	+
			294	+	+	+	+
OPB10	7	4	478	+	+	+	+
			432	+	+	-	+
			343	+	+	+	+
			280	-	-	+	-
			223	-	+	+	-
			207	+	-	-	+
			129	+	+	+	+
OPB 12	5	4	637	+	+	-	+
			562	+	-	-	+
			341	+	+	-	+
			216	+	+	+	+
			131	-	-	+	-
OPC 0 5	9	5	962	+	+	-	+
			656	+	+	-	+
			564	+	+	-	+
			447	+	+	-	+
			398	+	+	+	+
			331	+	+	+	+
			293	+	+	+	+
			221	+	+	+	+
			187	-	-	+	-
OPC 0 7	8	5	1315	+	-	-	+
			1046	+	-	-	+
			545	+	+	+	+
			419	+	+	+	+
			249	+	-	-	+
			220	+	+	+	+
			145	+	+	-	+
48	-	+	+	-			

GA: group A (control), GB: group B (15 days of exposure), GC: group C (30 days of exposure), GD: group D (delayed effect), t: Present, -: Absent

**RAPD fingerprinting pattern:** RAPD profiles were comparable with DNA samples extracted from different tissues (liver and testis) in exposed and control animals. Which indicated the absence of genetic damage in DNA samples from liver as revealed by appearance of similar "monomorphic" bands in exposed and control animals in all primers used (Fig. 3). However, alterations in RAPD profile were detected in DNA samples extracted from testis that was increased in exposed rats over a time dependent manner. From the ten primers used, seven gave positive and detectable bands. These Seven primers amplified a total of 48 different bands, ranging from 48 to 1315 bp. Of the 48 scorable bands, 28 were polymorphic. At 15 days of exposure to SMF 4 primers out of 7 showed 8 (16.6%) altered bands, while after 30 days 7 primers showed an altered 28 (58.3%) pattern. The major observations involved loss or addition of novel bands in exposed DNA samples compared to control. The DNA of the samples (testis) analyzed with seven primers revealed the appearance of 3 (OPA10-216, OPB10-223, OPC07-48) and 5 (OPB10-223, OPB10-280, OPB12-131, OPC05-187, OPC07-48), new bands after 15 and 30 days of exposure to SMF, respectively, which did not appear in the samples of other groups (Table 2). These new bands could be considered as "genus diagnostic" markers which can attribute to exposure to SMF. Furthermore, some stable bands were lost which occurred in the DNA of the control animals. In the delayed effect experiment, 45 days after 30 days of continuous exposure to 3 mT SMF, no polymorphic bands were detected in this group compared with control one with all primers used.

**DISCUSSION**

In the present study viscometric, refractive index measurements and RAPD assay were used to evaluate the possible harmful effects on hemoglobin and potential genotoxic properties of magnetic field, when male rats were exposed to 3 mT SMF.

Previous studies demonstrated many mechanisms behind the interaction between the magnetic field and biological system. It has been hypothesized that exposure to magnetic fields increases free radical activity (Reiter, 1997; Guler *et al.*, 2006) or free radical life span in organisms and could deteriorate antioxidant defensive system by Reactive Oxygen Species (ROS) which could affect biomolecular processes and cell function (Lee *et al.*, 2004; Lai and Singh, 2004). Fiorami *et al.* (1997) found that magnetic field was able to increase cellular damage of rabbit red blood cells induced by oxidizing agents. This over production of oxygen free radicals can give rise to functional and morphological disturbances in

the hemoglobin molecule. Baureus Koch *et al.* (2003) found that static and time varying magnetic fields in a suitable combination had an effect on a biological system consisting of highly purified plasma membrane vesicles. Their data revealed that the cell membrane is a site of interaction for weak low frequency magnetic field.

The intrinsic viscosities of globular proteins, in aqueous salt solutions and hemoglobin at isosmotic conditions were calculated by Tanford (1961) and Morse and Warth (1990). Gennaro *et al.* (1996) found that macroscopic viscosity depends on the characteristics of the individual macromolecules, as well as on the interactions among them. In present study the values of intrinsic viscosity (Fig. 1 and Table 1) for native protein for control group are consistent with the values obtained by Tanford (1961) and Gennaro *et al.* (1996). Increasing in the macromolecule intrinsic viscosity and slope values in groups exposed to 3 mT SMF for 15 and 30 days indicate molecular changes in dimensions and shape of hemoglobin molecule under the influence of magnetic field. In turn decreasing in the intermolecular interaction, as represented by the Huggins coefficient  $k'$  (Table 1), suggest changes in the tertiary conformation of Hb in accordance with results obtained by previous studies (Atef *et al.*, 1995). These conformational changes may be attributed to the direct effect of SMF on Hb molecule through oxidative stress species or to an indirect effect through interaction of SMF with many enzyme systems related to Hb function in erythrocyte. Concerning refractive index of hemoglobin for SMF exposed animals, there was a significant increase in the values for 15 days exposed group ( $p < 0.05$ ) and high significant increase for 30 days exposed group ( $p < 0.001$ ) indicates molecular changes in the protein conformation (Zirk and Potzschke, 2004) which may be attributed to the formation of new protein molecules different from the native proteins of control under the effect of exposure to magnetic field.

In the current study, It has attempted to study the genotoxic effect of 3 mT SMF exposure on rat tissues. Raylman *et al.* (1996) suggested that the effect of exposure to SMF varies depending on the cell type. The interaction of magnetic field through oxidative stress or deficiency of antioxidant defense mechanism may result in reversible or irreversible cell and tissue injury (e.g., DNA damage) (Sun, 1990). The present results showed that, no alteration on DNA fingerprinting in rat liver at different exposure time was detected (Fig. 3). Therefore, present results suggested that 3 mT SMF was unable to cause DNA damage on liver of rat. This is in consistent with the recent findings of Chater *et al.* (2006), who found that the exposure to SMF (128 mT; 1 h/day) from day 6 to day 19 of pregnancy did not induce oxidative DNA

lesions in liver and kidney of pregnant rats. They also found an increase in liver glutathione which plays an important role in protection against SMF during pregnancy. In addition, Zmyslony *et al.* (2000) demonstrated that Lymphocyte exposure to a SMF of 7 mT did not affect the number of cells with DNA damage in the comet assay. Moreover, our study of SMF exposure showed definite and perceptible clear changes in the profiles between exposed and control DNA samples of testis over a time dependent manner (Table 2). These indicate an interaction between intensity and duration of exposure on biological effects of SMF. Because of the high mitotic rate of germ cells, testis are a vulnerable when exposed to static or time varying magnetic fields (Galaktionova *et al.*, 1985). Amara *et al.* (2006) revealed that SMF exposure induces a depression of testis antioxidant enzymes, glutathione peroxidase and catalase (-34 and -33%), respectively. These antioxidants protect germ cells against oxidative DNA damage and play important roles in spermatogenesis (Fraga *et al.*, 1991).

Present results also show that when the exposure time was increased to 30 days the number of altered bands were increased to (28) 58.3% as compared with group SMF exposed for 15 days (8) 16.6% altered pattern. These convey us to predict that, the SMF seems to interact with the cells in some way, over time, results in an increased the alterations in DNA profiles. The alteration detected in the RAPD profiles generated from rats exposed to SMF revealed the appearance and disappearance of bands in comparison to control patterns. These changes observed in the fingerprints of exposed animals may be due to the presence of DNA adducts, mutations or DNA strand breaks (Singh and Roy, 2001). The DNA damage induced in testis in this study was in consistent with the recent study of Amara *et al.* (2006). They revealed that the increase of 8-oxo-dG level (44%), a biomarker of oxidative stress, in testis indicating DNA damage in adult male rats exposed to SMF (128 mT; 1 h/day for 30 days). Ramadan *et al.* (2002) found that exposure of mice to SMF decrease the sperm count, motility and daily sperm production with marked testicular histopathological changes. In contrast, Heredia *et al.* (2004) have found that no statistically significant differences indicative of a 60 Hz (MF) at 2.0 mT effects on meiotic chromosome aberrations in spermatocytes and sperm morphology on exposed compared with control male mice. SMF fails also to alter the count and motility of epididymal spermatozoa but decreases the testosterone in the plasma (-42%) and testis (-68%) (Elbetieha *et al.*, 2002).

Data obtained from the delayed effect experiment, exposure of male rats to 3 mT SMF for consecutive 30 days and then allowed for further 45 days without

exposure, revealed non significant changes in viscometric and refractive index measurements of hemoglobin as compared to control and results tend to normal values. This indicates non cumulative effect of 3 mT static magnetic field exposure on the new born red blood cells and their precursors, or either that most of the cells died and only those cells whose alterations do not exceed a reversible level were able to repair over time. In addition, present data indicated that no alteration was found on RAPD profiles in this group as compared to control one. The fact that the changes disappeared in this experiment indicates that the DNA damage were fully repaired or reversed over time. *In vitro* Chow and Tung (2000) and *in vivo* Potenza *et al.* (2004) discovered that heat shock proteins are overproduced after exposure to a low frequency magnetic field and the heat shock proteins DnaK/J (Hsp70/40) are involved in improving the efficiency of the process of DNA repair. This observation seems to confirm present findings.

### CONCLUSIONS

The results of this study suggest that subchronic exposure to 3 mT SMF may be biologically toxic in rats. It induces DNA damage in testis, changes in molecular shape and dimensions of Hb and its protein conformation while no effect was detected in liver. This is an interesting finding, which can provide clues to discovery the susceptibility of the target organ involved.

In the delayed effect study, no damage was detected in rat testis and Hb molecule Which indicated that the damage was fully recovered or repaired in rat tissues and blood cells by time.

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