THE INFLUENCE OF 50 Hz MAGNETIC FIELD ON LIVER FUNCTION

M. IBRAHIM[#], M. EL-ASHRY*, E. ALI***

*Physics Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
**Faculty of Medical Science, University of Science and Technology, Sana'a, Yemen
[#]Present address: King Abdulaziz University, Faculty of Science, Physics Department, Arar, KSA

Abstract. The present study has been conducted to evaluate the influence of different intensities of 50 Hz magnetic field on some liver function tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin and protein. The study also involved the effect of magnetic field on the oxidative state of the liver tissue. The study was conducted on about 80 Sparague-Dawely male rats of an average weight of 140–160 g. The exposure period was 2 hours/day for two weeks. The sera taken from control and exposed rats were also examined spectroscopically. The results showed an increase ($p < 0.05$) in the concentration of all investigated liver enzymes. Both bilirubin and aspartate aminotransferase were mostly affected with exposure (with a percentage > 90%), while albumin and alanine aminotransferase changed with about 11% and 22% respectively. The applied field also caused an increase in the liver lipid peroxidation and a decrease ($p < 0.05$) in glutathione concentration. Results consequently suggest that the redox potential of both glutathione (GSH/GSSG) and nicotinamide dinucleotide (NADH/NAD) are disturbed as a result of exposure. Precursors of NADH, as a result of its exhaustion, such as kynurenine, tyrosine and tryptophan, might be also affected.

Key words: oxidative, enzymes, absorbance, NADH.

INTRODUCTION

A number of epidemiological studies have suggested an increased risk for cancers, particularly leukemia and brain and breast cancer, with residential or occupational exposure to 50/60 Hz magnetic fields (MFs) [31, 37]. MFs were observed to influence enzyme action, signal transduction, protein synthesis and gene expression. These activities play an important role in regulating cell growth and processes important to promotion. Furthermore, some studies have suggested that the genotoxic potential of certain chemical mutagens or ionizing radiation may be affected by co-exposure to MFs [26].

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The beneficial responses described in humans are counterbalanced by a number of epidemiological studies suggesting adverse health effects, e.g. promotion of certain cancers [7]. Several studies on bacterial and animal cells have also shown that electromagnetic fields influence a large variety of cellular functions [18]. The mechanisms (or some) of interaction with living cells involve, as reported, changes in the intracellular levels of Ca^{2+} [23]. However, many hypotheses assumed that the cell membrane is most likely the target for the primary impact of the field and that this interaction might affect the signal transduction mechanisms at different levels [4].

Some biochemical studies have been carried out to evaluate the effects of magnetic fields on the metabolism of cell cultures, animals and humans. They showed significant disturbances in the metabolism of carbohydrates, lipids and proteins reflected by altered blood glucose levels and by accelerated glycolysis and glycogenolysis with a metabolic block of conversion of pyruvic acid to acetylocoenzyme A [17]. The levels of total protein and its fractions were also changed [36]. These disturbances lead to adaptative changes, which in turn result in altered lactate dehydrogenase activity and accelerated transamination processes. Electromagnetic fields penetrate human body and act on all organs, altering the cell membrane potential and the distribution of ions and dipoles. These alterations may influence the biochemical processes in the cell, thus changing both biochemical parameters and enzyme activities of serum [2].

Other studies concluded that exposure to magnetic field induces changes in the activity of some enzymes involved in the antioxidant system and thiol-disulfide exchange in the liver of animals [28]. It was suggested that MF could deteriorate the antioxidant defensive system in the mouse brain [19]. Moreover, MF induces changes in gene expression, membrane structure and functions and causes DNA damage [16, 20]. Cadmium influences many metabolic processes causing great damage in many organs. Ingested cadmium is mainly translocated to the kidney and the liver has been reported to play an active role in rapidly removing cadmium ions from the blood. In the liver, cadmium is retained with a long biological half-life and causes a variety of toxic responses by the hepatic cells [39].

Magnetic fields can also enhance the concentration of free radicals in living cells. Transition metals, e.g. iron or copper, are among the most important agents that can cause damage of DNA, RNA and other macromolecules through the production of oxygen free radicals by Fenton reactions or by interaction with cellular thiols. When these radicals react with non radicals, new free radicals can be formed, which leads to chain reactions, i.e. lipid peroxidation [40]. One of the most important biological consequences of the macromolecules damage by free radicals could be cell death. They may, directly or indirectly, participate in the initiation of apoptotic or necrotic cell death. Cell death induced by either physiological or non-physiological agents, including chemical (drugs, environmental toxicants) and physical (ionizing radiation) factors, could be related to free radicals production [10].

Liver function tests represent a broad range of normal functions performed by the liver. The diagnosis of liver diseases depends on a complete history, complete physical examination and evaluation of liver function tests. Inflammation of hepatic cells results in elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and possibly of the bilirubin. Inflammation of the biliary tract cells predominantly results in an elevation of alkaline phosphatase (ALP). In liver diseases, there are crossovers between purely biliary and hepatocellular diseases [1, 30].

This paper is interested in examining the changes of liver function tests due to 50 Hz magnetic field. The paper also tries to interpret these changes by linking them to some redox systems such as GSH/GSSG and NADH/NAD. The study, therefore, shows how important for any person under investigating these liver tests is to be protected against any magnetic, even induced, fields.

MATERIALS AND METHODS

The exposure system was Helmholtz two coils, the coils were distant by 15 cm, and each one was 30 cm in diameter and of 250 turns. The wire of the coil was 0.7 mm in diameter and its resistance was 13 ohm. The field was probed by a magnetic flux meter (ELWE 8533996, Cerligene, Germany) [15]. The flux meter was used to map the magnetic field in the area between the Helmholtz coils. The area of constant magnetic field was chosen to be the exposure area in which the cage was located. The investigated rats, except the controls, were all exposed to a 50 Hz magnetic field of different intensities (0.2, 0.6, 1 and 1.4 mT). The control group was exposed to a sham (not energized) field.

The experiments were carried out on about 80 male Sprague-Dawely rats, of about 140–160 g. They were obtained from a known breeding unit. The rats were housed four per cage in a well ventilated room (25 ± 2 ºC), and 12 hours light and dark cycle at the animal house where they were regularly fed on a standard diet *ad libitum* providing the principles of laboratory animal care (NIH publication No. 86–23, revised 1985) and the specific national laws (e.g. the current version of the German Law on the Protection of Animals). The rats were divided into five main groups (16 rats/each) as follows:

Group 1: The control group was exposed to a sham (not energized) field.

Group 2: Rats were exposed to a magnetic field of intensity 0.2 mT (2 hours/day) for seven days.

Group 3: Rats were exposed to a magnetic field of intensity 0.6 mT (2 hours/day) for seven days.

Group 4: Rats were exposed to a magnetic field of intensity 1.0 mT (2 hours/day) for seven days.

Group 5: Rats were exposed to a magnetic field of intensity 1.4 mT (2 hours/day) for seven days.

After exposure, rats were decapitated and blood samples were collected and the livers were removed as well. To obtain serum, the collected blood was incubated at 37 °C for one hour in clean dry test tubes, then centrifuged at 3000 rpm for 20 min. Sera were aspirated and stored in deep freezer at –20 °C until used. The serum was used to measure the liver function tests; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Serum bilirubin, Serum albumin and serum total protein. The sera were also investigated by a double-beam UV-visible spectrophotometer (V-570, Jasco, Japan).

The livers were removed and perfused with cold KCl of concentration 0.15 M, then blotted between filter papers. Liver homogenate was used for the determination of liver lipid peroxidation and liver glutathione (GSH).

The liver function tests were measured by using kits obtained from Roche, Germany.

Determination of lipid peroxidaton of liver tissue was based on the measurement of thiobarbituric acid (TBA) reactants [34] as follows:

(1) 0.5 ml of serum was mixed with 3 ml of 1.0% phosphoric acid (pH 2.0) and 1 ml of 0.6% TBA in air-light tubes and then put in boiling water for 45 min.

(2) The samples were cooled and added to 5 ml butanol.

(3) The butanol phase was separated by centrifugation at 10000 rpm for 10 minutes and transferred to glass cuvettes.

(4) The color of TBA chromogen was measured at 520 nm and 532 nm by the spectrophotometer.

(5) The difference between absorbance at 520 nm and 532 nm gives rise to the TBA value, which primarily represents the malondialdehyde concentration which is taken as a measure of lipid peroxidation.

(6) The level of lipid peroxidation was expressed as micromoles MDA per milligram of tissue weight**.**

Determination of liver reduced glutathione (GSH) was done [5] as follows:

(1) 0.2 ml of blood was added to 1.8 ml distilled water.

(2) 3.0 ml of precipitating solution was added and the samples were immediately vortexed. The precipitating solution was 1.67g glacial metaphosphoric acid, 0.2 g ethylene di-amine tetra acetic acid (EDTA) and 30 g NaCl in 100 ml of distilled water.

(3) The mixture was centrifuged at 2200 rpm for 15 minutes at room temperature.

(4) 1.0 ml of supernatant was added to 4 ml of $Na_2 HPO_4$ of concentration 0.3 M.

(5) One half ml of DTNB reagent (40 mg 5, 5’ dithiobis-2-nitrobenzoic acid in 100 ml 1% sodium citrate) was added and absorbance was immediately measured at 412 nm.

(6) Glutathione concentration was calculated by using a standard one (Sigma, USA).

The spectroscopic investigation was done by mixing 0.5 ml of the serum with phosphate buffer (0.1 M of disodium monohydrogen phosphate Na_2HPO_4 and potassium dihydrogen phosphate KH_2PO_4, pH ≈ 7.5). One cuvette was then filled

with 1 ml of 0.1 M phosphate buffer and the second one was filled with the sample under the spectroscopic investigation.

The data were statistically analyzed using SPSS, statistical Package for Social Science Version12, software [6, 32].

RESULTS

The results showed that the activities of all examined function tests increased, if compared to control, as a result of increase in the intensity of magnetic field as Table 1 shows. The activity of ALT increased from 40.88 U/L (the control value) to 76.25 U/L with a percentage of about 86% when the intensity enhanced to 1.4 mT. Similarly, the activity of AST increased with a percentage of about 93%, due to exposure to the applied field of intensity 1.4 mT. The activity of ALP increased also with a percentage of about 22%. The values of bilirubin, albumin and total protein enhanced also with about 95%, 11% and 39%, respectively, as a result of the magnetic field intensity 1.4 mT.

Table 1

Effect of 50 Hz magnetic field on liver function tests

Exposure (mT)	0.0	0.2	0.6	1.0	1.4
ALT (U/L)	40.88±8.7	47.88±9.9	59.62±10.8*	69.75±10.3*	76.25±11.9*
% change	0	17.1	45.8	70.6	86.5
AST (U/L)	44.25±6.2	51.50±11.9	61.00±10.1*	73.50±8.7*	85.75±11.4*
% change	0	16.4	37.9	66.1	93.8
ALP (U/L)	159.88±12	165.13±11	167.00±16.4	188.50±13*	194.75±12*
% change	0	3.3	4.5	17.9	21.8
Bilirubin (mg/dL)	0.525±0.1	0.538±0.1	0.600±0.2	0.813±0.2*	1.025±0.1*
% change	0	2.5	14.3	54.9	95.2
Albumin (g/dL)	3.762±0.3	3.850±0.3	3.938±0.2	4.000±0.2*	4.175±0.2*
% change	0	2.3	4.7	6.3	10.9
Protein (g/dL)	6.525±0.8	6.675±0.6	7.038±0.3	7.988±0.5*	9.050±0.5*
% change	0	2.3	7.9	22.4	38.7

The data are represented as mean values ± standard deviation.
*Significant difference between control and exposed groups at the level of $p < 0.05$.

Table 1 also shows that serum bilirubin, albumin and total protein levels were also affected by exposure to 50 Hz magnetic field. The levels of serum bilirubin, albumin and total protein after exposure were increased compared to control at the level at $p < 0.05$.

Concerning the oxidative state of liver tissue, the results showed that the lipid peroxidation (Table 2) raised from 1.175 μmol/mg MDA (the control value) to 2.188±0.2 μmol/mg MDA, i.e. with a percentage of about 86%, following the increase in intensity 1.4 mT. On the other hand, the concentration of GSH (Table 2) decreased from 1271 μg/g fresh tissue (the control value) to1138 μg/g fresh tissue, i.e. with a percentage of about 10%, following the magnetic field intensity 1.4 mT.

Table 2

Effect of 50 Hz magnetic field on lipid peroxidation (μmol/mg MDA) and GSH (μg/g fresh tissue)

Exposure (mT)	0.0	0.2	0.6	1.0	1.4
Lipid perox	1.175±0.1	1.225±0.1	1.638±0.3*	1.912±0.2*	2.188±0.2*
% change	0	+4.3	+39.4	+62.7	+86.2
GSH conc.	1271±20	1230±45	1204±32*	1165±29*	1138±27*
% change	0	–3.2	–5.3	–8.3	–10.4

The data are represented as mean values ± standard deviation ($p < 0.05$).

The spectroscopic investigation of the sera taken from control and exposed rats, as figure 1 illustrates, showed three peaks at about 409, 540 and 577 nm in addition to a shoulder at 340 nm. This shoulder gave rise to a clear peak at high intensities of the used field. The absorbance of spectra also decreased, indicating that the concentrations of the molecules which are responsible for these peaks decrease, due to the increase in the intensity of the applied field.

DISCUSSION

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as reported, are specific liver enzymes that increase in hepatic diseases and toxic damage of liver cells [29]. It was found that these enzymes increase under the effect of 50 Hz magnetic field which also enhanced the concentration of alkaline phosphatase [21, 33]. On the other hand, the elevation in the levels of serum bilirubin, albumin and total protein, observed in the present study, may result from the damaged cells which leak into circulation after exposure to magnetic field [27]. It was also found that *in vivo* exposure to a pulsed magnetic field at 1.5 mT caused significant changes on plasma proteins in rats [14]. This observation supports the hypothesis that the state of physiological equilibrium of a biological system is crucial to its response to a potentially effective electromagnetic field [35].

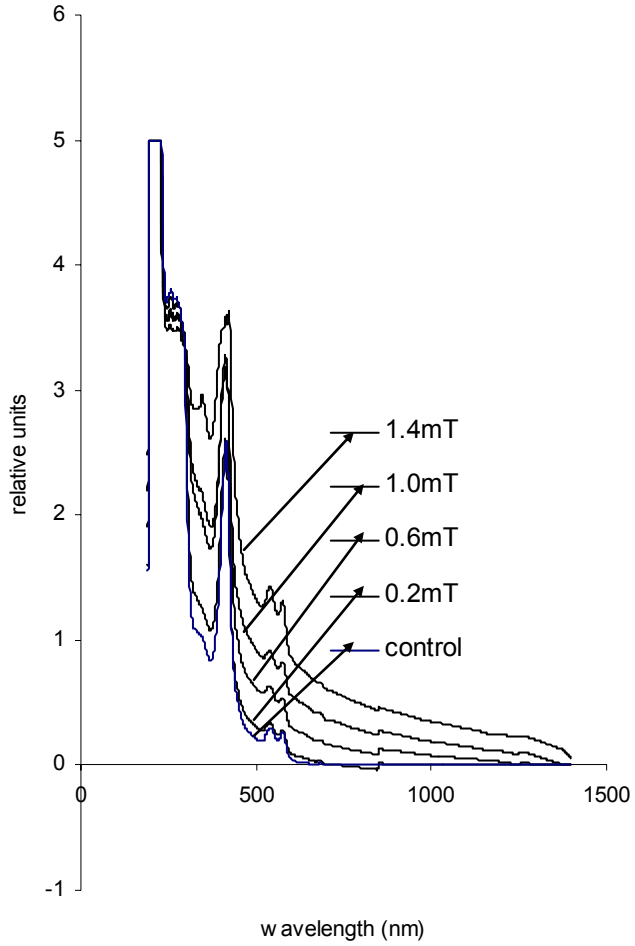

Fig. 1. Absorption spectra (absorbance (relative units) on y-axis versus wavelength (nm) on x-axis) of sera taken from control and rats exposed to a magnetic field of different intensities.

Alanine aminotransferase, as reported [3], catalyzes the reaction between L-alanine and 2-oxoglutarate forming pyruvate. This pyruvate is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) producing L-lactate and NAD. The rate of NADH oxidation which is directly proportional to catalytic ALT activity is determined by the decrease in absorbance at 340 nm which appeared as a result of exposure especially at 1.4 mT. Oxidation of NADH can also take place as a result of catalytic activity of AST. The alkaline phosphatase, as also reported [3], hydrolyses the colorless 4-nitrophenyl phosphate to 4-nitrophenoxide and phosphate. The rate of nitrophenoxide formation, which is directly proportional to the catalytic activity of ALP, is determined by the absorbance at 409 nm which appeared in the spectra of the present study.

The peaks observed at wavelengths 540 and 577 nm might be due to NADH precursors such as kynurenine, tryptophan and tyrosine which are supplied by the body as a result of NADH exhaustion [22].

The results of this study also showed a significant decrease in liver GSH, at the level of $p < 0.05$ accompanied by an increase in liver lipid peroxidation after exposure to 50 Hz magnetic field. GSH depletion could be related to its involvement in the detoxification of the deleterious effects of increased free radical reduced within the cell, the reason which Lipid peroxidation highly produced after exposure to the magnetic field [13]. It was also observed that when free hydroxyl radicals increased, the activities of antioxidant enzymes decreased resulting in a decrease in GSH/GSSG ratio. This was accompanied by an increase in the content of lipid peroxidation resulting in an oxidative stress [8]. This might be due to the increase in the level of oxidized form of GSSG which inhibits the glucose monophosphate pathway which is responsible for the continuous supply of GSH within the cell [9]. The failure of continual supply of GSH and the increase in the levels of free radical due to magnetic field might increase erythrocyte fragility *via* inhibition of glucose-6-phosphate dehydrogenase activity and the concomitant decrease in NADPH concentrations, which is essential to maintain normal levels of GSH [24]. Reduced glutathione depletion renders the animal more susceptible to free radicals-mediated damages, especially the damage induced by cellular lipid peroxidation. It has been observed that reduced glutathione depletion is accompanied by an increase in the amount of total lipid peroxide in experimental animals due to the deflator of GSH [25].

The observed increased level of total lipid peroxidation, in this study, is an indication to a high production of free radicals during the exposure magnetic field. These free radicals are highly oxidative moieties which affect directly the lipid membrane producing oxidative products such as GSSG and lipid peroxidation which increase also in many diseases and in tissues poisoned by a variety of toxins [11]. This, consequently, is followed by an inactivation of some antioxidants, leakage of antioxidants from the cell and the release of metal ions (especially iron and copper) from storage sites and from metalloproteins hydrolyzed by enzymes released from damaged lysosomes [12].

Lactate dehydrogenase which catalyzes the reduction of pyruvate by NADH forming L-lactate and NAD was reported to be a novel marker to study many physiological and pathological conditions, such as liver diseases and cancer [38].

CONCLUSION

All liver function tests are influenced as a result of exposure to extremely low frequency magnetic fields. Bilirubin and aspartate aminotransferase were mostly affected (with a percentage > 90%), while albumin and alanine aminotransferase changed with about 11% and 22%, respectively.

The field also affects the oxidative state of liver tissue. The redox potential of both glutathione (GSH/GSSG) and nicotinamide dinucleotide (NADH/NAD) are disturbed as a result of exposure. Precursors of NADH, as a result of its exhaustion, such as kynurenine, tyrosine and tryptophan, are also affected.

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