Study the effects of magnetic field on biochemical and histological changes in liver tissue of albino rats

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Abstract: To study the effects of different intensities of magnetic field on biochemical parameters, and histological changes of liver of albino rats. Forty eight of male albino rats were used of average weight of about 150-160gm, the animals were housed in the same environmental conditions in plastic cages and feed with balanced diet and tap water. The animals were divided into two groups as follows: Group C: Consists of 8 animals used as a control group and housed at normal environmental conditions of pressure and temperature. The lab temperature varied between 22^{o}C and 25^{o}C during the experimental period, lighting condition was day light and darkness during night. Group E: Consists of 40 animal was divided into five subgroups (8 animals for each group) namely E_1, E_2, E_3, E_4 and E_5, which were exposed to different magnetic flux density (2, 4, 6, 8 and 10 mT at 50Hz) for a period of 30days (8hours/day,5days/week). Biochemical parameters (Alanine aminotransferase, Aspartate aminotransferase,Serum alkaline phosphatase and total protein were measured in plasma samples obtained after centrifugation of blood samples. The results show that there is a marked change in the liver enzymes due to exposure to different strengths of magnetic field. From liver histology for animals exposed to different intensities of magnetic field showing heavy portal inflammation, congestion, atypia, microvesicular steatosis and lyfocal inflammation. We conclude from this study that it is necessary to conduct some tests for patients to measure the chemical changes of liver enzymes and also study the histological shape of the body's various tissues to protect them from exposure to any source of electromagnetic radiation.

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1. Introduction

In the present era, the era of modern technology through which are dealing with multiple devices emitted by electromagnetic waves such as mobile phone, computer, microwave and television. In modern society, people are exposed to electromagnetic fields EMFs, including the low-frequency magnetic field, which is usually emitted from electricity lines and many kinds of electrical devices (Strasak & Smarad, 2002; Sabo et al., 2002 and Xu & Okano., 2002). They are also emitted from natural and modern sources, which play an important role in our daily lives. Previous studies have indicated that exposure to the low magnetic field has a biological effect on cells and living tissues. In recent years, many studies have shown the potential biological effects of magnetic fields on human health (Jolanta et al., 2001).

The exposure to EMFs can damage biological tissues by inducing changes, which can be explained in terms of thermal or non-thermal mechanisms (Challis.,2005).Thermal effects can occur with the conversion and absorption of heat by the body's electromagnetic energy, increased body temperature is stabilized and alleviated by blood circulation, although non-thermal effects do not raise the body temperature sufficiently to impair the structure of tissues, their effects can still be seen as an increase in free radical production in tissues (Challis., 2005). EMFs are reported to cause a rise in levels of oxygen free radicals in an experimental environment in plants and humans (Georgiou.,2010).

Throughout the evolution processes, the living organisms had developed numerous adaptation mechanisms to natural EMFs that enabled them to survive under the changing environmental conditions. Long-term exposure to this factor may produce a number of biological effects, these being dependent both on the radiation parameters and the properties of biological structures.

Electromagnetic waves (EMWs) damage tissues of the body through heating and changing chemical reactions. High EMWs cause damage by heat; hazardous effects appear on the tissues by long-term exposure to low EMWs because of chemical changes. Some energy spreaded by EMW due to the

heat effect is absorbed by the human body, and heat accumulation occurs inside the body (Ghodbane et al., 2013).Moreover, the biological effects of extremely low frequency magnetic field have been concerned that people living in homes with an excess of electrical wiring configuration suggestive of high current flow had a higher incidence of cancer (Chen et al., 2000), depression (Lyer et al., 2003) birth and reproduction anomalies (Blaasaas et al., 2002), brain tumor, leukemia, miscarriage, chronicfatigue, headache, cataracts, heart problems, stress, nausea, chest pain, forgetfulness and other health problems (Mercola., 2009). Biochemical studies have been carried out to evaluate the effects of magnetic field on the metabolism of cell cultures, animals and humans (Kula et al., 1991).Magnetic fields affect the chemical bonds between adjacent atoms (Rollwitz et al., 2004), resulting in the production of free radicals. Recent studies have shown that exposure to the magnetic field increases the default age of free radicals. These free radicals are responsible for damage the biological systems of the body cells (Lee et al., 2004 & Yokus et al., 2005).

Liver function tests are an effective ways to detect liver function abnormalities enzymes that detect hepatocellular necrosis – aminotransferases. The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes- aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase-SGOT) and alanine amino transferase (ALT, formerly serum glutamic pyruvate transaminase-SGPT). ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver (Friedman et al.,2003 ,Rosen &Keefe.,2000).

Both ALT and AST are useful in establishing the presence of liver cell injury of any cause. ALT is the more sensitive test in acute and obstructive liver disease, whereas AST is more sensitive in chronic and infiltrative lesions so that the aim of the present study is to investigate the effects of different intensities of magnetic fields on biochemical parameters and histological changes of liver tissue of albino rats.

2. Materials and Methods

2.1. Chemicals

All reagents were of the highest purity available. All chemicals for biochemical analysis were purchased from Sigma Chemical Co.

2.2. Experimental animals and study design

The animals under the study were exposed to different magnetic flux intensity (2, 4, 6, 8 and 10mT at 50Hz) for a period of 30days (8hours/day, 5days/week). Homogenous magnetic field generated by four solenoids of 1500 turns each of electrically insulated 2.2mm copper wire, wound around a copper cylindrical chamber of 17 cm external diameter. Water was pumped in a copper jacket separating the wire winding and the chamber in order to keep the temperature of the chamber fixed during the exposure period. The temperature of the flowing cooling water at the outlet of the jacket and the temperature inside the irradiation chamber were periodically measured through the use of thermocouple thermometer, which can give readings for the temperature variations within $\pm 0.1^{o}$C. There was no measurable difference in temperature between the room and the chamber. The actual current passing in the solenoids was about 1A.The animals were kept in special plastic cages that permit normal ventilation and daylight. The coils were connected to a variac fed from the mains (220 V and 50 Hz). The magnetic field exposure system was locally manufactured.

2.3. Protocol of Study

The used rats are sourced from Medical Research Institute, Alexandria University. Forty eight of male albino rats of average weight of about 150-160gm were used .They were housed in the same environmental conditions in plastic cages, and feed with balanced diet and tap water. The animals were divided into two groups as follows: Group C: Consists of 8 animals used as a control group and housed at normal environmental conditions of pressure and temperature. The temperature inside the lab varied between 22^{o}C and 25^{o}C during the experimental period and lighting condition was day light and darkness during night. Group E: Consists of 40 animal was divided into five subgroups (8 animals for each group) namely E_1, E_2, E_3, E_4 and E_5, which were exposed to different intensities of magnetic field (2,4, 6, 8 and 10mT at 50Hz) for a period of 30 days (8hours/day,5days/week). After four weeks blood samples were obtained using cardiac puncture method. Following serum reparation, level of enzymes was measured using automated biochemical analyzer, also the histological study were performed for liver tissue.

2.4. Biochemical analysis

Biochemical analysis was performed through 24h after the irradiation with different intensities of magnetic fields. The rats were anesthetized by inhalation of diethyl ether until muscular tonus relaxed. After sacrificing the animals, 2 ml of blood samples were collected and placed in chilled non-heparinized tubes, centrifuged at 3000rpm for 10min at 4°C (Muhammad et al., 2013). Blood serum was collected after blood centrifugation and stored at -20 ºC for biochemical analysis. The serum levels of the activity of ALT,

AST, and TP were measured using automated biochemical analyzer (Type 7170, Hitachi).

2.5. Histological Study

Histological sections were prepared according to the method of (Luna., 1968) which included the following processes

A. Fixation: After animals have been sacrificed, liver were quickly excised and saved in 10% formalin for 24 hours.

B. Dehydration: Tissues dehydrated in an ascending grade of propanol (30, 50, 70, 80, 90, 95 and 100%) for two hours to each.

C. Clearing: After dehydration, tissues cleared with chloroform for 12-24 hours.

D. Infiltration: This process was done by using paraffin wax for 6 hours. In the first two hours the wax was renewed to riddance chloroform twice.

E. Embedding: Tissues samples have been embedded in paraffin wax at 6°C in suitable blocks. The blocks were left at room temperature until the solidity of the wax. Later they were cooled in a refrigerator and became ready to sectioning.

F. Trimming and Sectioning: By using rotary microtome, blocks were first trimmed for removing excessive wax and then sectioned (3-5 µm thickness). Ribbons resulting from sectioning process were floated in a warm water bath and mounted on clean slides covered with a thin layer of Mayer's albumin after their extension in water bath and left to dry at room temperature.

G. Staining and Mounting: To obtain stained sections, first paraffin must be removed by xylene for 10minutes. The perfused sections were passed by descending concentrations of ethyl alcohol (absolute, 90,70,50,30%) for two minutes for each concentration, stained with haematoxylin stain for 5 min. and extensively washed with tap water. After that they passed by ascending grade of ethanol (30, 50, 70, 90%) for 2min. to each concentration, stained with alcoholic eosin stain for 30sec and then passed by absolute ethanol for 5 min. finally sections have been cleared by xylene (3min), mounted by canda balsam, covered with cover slides and dried at room temperature to be ready for microscopic examination by using light microscope.

2.6. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Differences between groups were determined by one-way analysis of variance (ANOVA, Student t-test). Differences with $P < 0.05$ were considered significant.

3. Results & Discussions

Liver is located under the chest cage in the upper right part of the abdominal cavity. It has many vital functions necessary for life. These important functions include detoxification of blood, albumin production and many important proteins, storage of vitamins, cholesterol, fat, bile and glucose production. Liver is hepatic blood tests are some of the most common blood tests. These tests can be used to evaluate liver function or injury; this is an initial step to detect liver damage. The liver enzymes levels were determined. These enzymes are located inside the liver cells. However, when the liver is injured, no cause is spilled; these enzymes are in the bloodstream and are among the enzymes. From the results obtained from exposure to 50Hz different intensity of the magnetic field ;we note a rise in the liver enzymes levels, as the liver cells leak these enzymes into the blood, the enzyme levels increase in the blood which refers to liver diseases. These liver diseases were confirmed by the study of the histological shape of the liver cells. The results revealed the presence of infections in the histological shape of the liver tissue.

There are some tests of blood chemistry that measure the levels of chemicals, enzymes, organic waste normally found in the blood and damage to biological systems within the body as a result of exposure to the electromagnetic fields present in each a place surrounding us and thus check the toxicity of the liver by determining the levels of liver enzymes in order to take into account the performance of the treatment at the level of liver enzymes.

Results of liver enzymes as shown in table (1) Indicate that enzyme activity of alanine aminotransferase (ALT) ranges from between 62.5±2.6 (u/l) after exposure to 2mT and 96.5±2.6 (u/l) after exposure to10mT of magnetic field. There is a marked change in activity of ALT, this apparent change is associated with increasing in the ALT activity values resulting from exposure to different magnetic field strengths compared to the experimental groups. The percentage of difference between the control group and exposed groups for ALT was ranged between24.3% after exposure to 2mT and 92.2% after exposure to 10mT of magnetic field. The activities of aspartate aminotransferase (AST) of rats exposure to magnetic field was ranged between 53.9±2.3 to 81.5±4.3 (u/l). The percentage of difference for AST between the control group and exposed groups was ranged between16.2% after exposure to 2mT and 75.9% after exposure to 10mT of magnetic field.

Table (1) Effect of different intensities of 50 Hz magnetic field on liver enzymes

parameters	Magnetic field intensities (mT)						
	groups	C (0)	E_1(2)	E_2(4)	E_3(6)	E_4(8)	E_5(10)
ALT (u/l)	Mean value ± SE	50.2 ± 3.3	62.5 ± 2.6[a]	78.7 ± 5.4[c]	88.6 ± 3.1[c]	91.1 ± 4.4[c]	96.5 ± 2.6[c]
	% change	0.0	24.3	56.5	76.6	81.44	92.2
AST(u/l)	Mean value ± SE	46.4 ± 4.2	53.9 ± 2.3[a]	67.3 ± 5.2[c]	71.2 ± 4.2[c]	79.3 ± 5.1[c]	81.5 ± 4.3[c]
	% change	0.0	16.2	45.09	53.66	71.11	75.9
ALP (u/l)	Mean value ± SE	140 ± 5.32	148 ± 4.4[a]	156.7 ± 6.4[a]	168 ± 7.4[a]	172 ± 7.1[b]	188 ± 8.3[c]
	% change	0.0	5.8	11.5	19.9	22.5	33.9
Total protein (g/dl)	Mean value ± SE	5.32 ± 0.5	6.1 ± 0.5[a]	6.8 ± 0.6[b]	7.4 ± 0.6[b]	7.9 ± 0.7[c]	8.4 ± 0.6[c]
	% change	0.0	15.4	27.44	38.4	47.6	57.1

(u/l) refers to (Units/liter) and (g/dl) refers to (gram/deciliter)-Values represented Mean ± standard error mean, small letters a ,b and c refer to difference is statistically significant between groups at level (p<0.01) , difference is highly statistically significant between groups at level (p<0.001) and difference is very highly statistically significant between groups at level (p<0.0001) respectively.

There are clear and very high significant changes in serum aspartate aminotransferase compared to the control group at the level of p< 0.0001 as a result of exposure to 2,4,6,8 and 10mT respectively. Serum alkaline phosphatase activity was significantly increased after exposure to 2mT and 10mT of magnetic field at the level of p< 0.05. The activity of ALP was ranged between 148±4.4 (u/l) after exposure to 2mT and 188±8.3 (u/l) after exposure to 10mT of magnetic field .The percentage change between the control group and exposed group was ranged between 5.8% after exposure to 2mT and 33.9 % after exposure to 10mT of magnetic field. There was significant increase in value of total protein from 6.1±0.5 (g/dl) after exposure to 2mT and 8.4±0.6 (g/dl) after exposure to 10mT of magnetic field. The percentage change between the control group and exposed group was ranged between 15.4% after exposure to 2mT and 57.1% after exposure to 10mT of magnetic field.

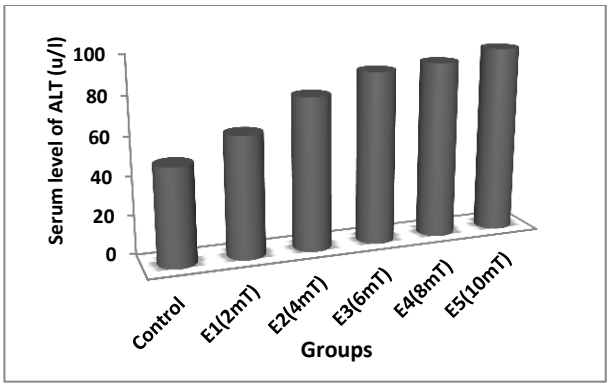

Fig.1. Serum levels of ALT (SGPT) in rats exposed to 50Hz with different intensities of magnetic field for 30days.

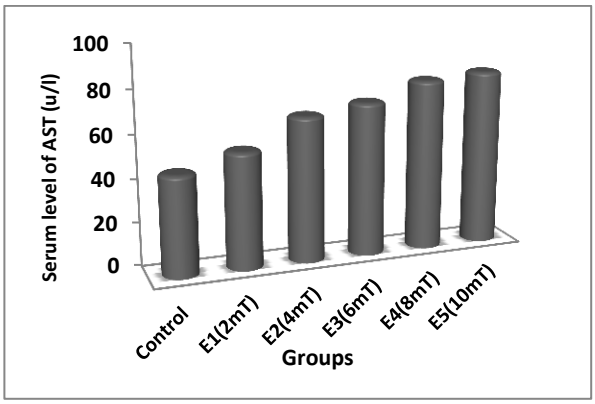

Fig.2. Serum levels of AST (SGOT) in rats exposed to 50Hz with different intensities of magnetic field for 30days.

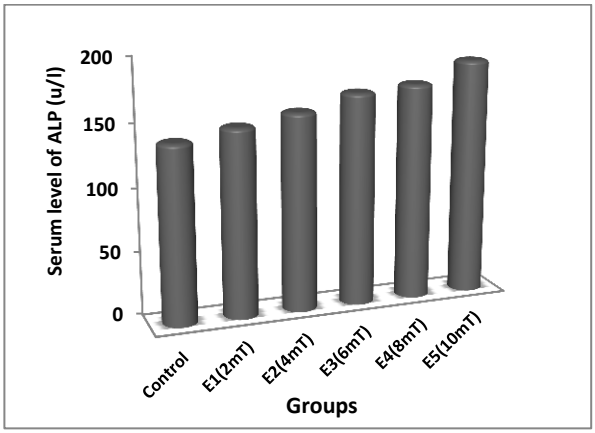

Fig.3. Serum levels of ALP in rats exposed to 50Hz with different intensities of magnetic field for 30days.

The ALT values significantly increased with increasing the intensities of magnetic fields for rat blood serum as compared with the control group (Fig.1). This study suggests that the liver might be damaged with irradiation to magnetic field. The liver enzyme ALT rearranges the building blocks of proteins. The AST values significantly increased after irradiation with different intensities of magnetic field

when compared to the control group (Fig.2)., also ALP significantly increased as the intensity of magnetic field increased (Fig.3).The serum ALT , AST and ALP levels are common markers for hepatic toxicity; levels of these proteins were rapidly increased by increasing the magnetic field intensities.

From the studding of liver histology, the samples obtained from the animals included in the control group (group C) and the microscopic images revealed a normal liver and hepatocytes have polygonal shapes most mononucleate hepatocytes separated by sinusoidal spaces. A central vein as well as a portal tract are seen, cords of hepatocytes separated by sinusoidal spaces. We found no evidence of pathological lesions: steatosis, necrosis or degenerative changes (Fig.4).

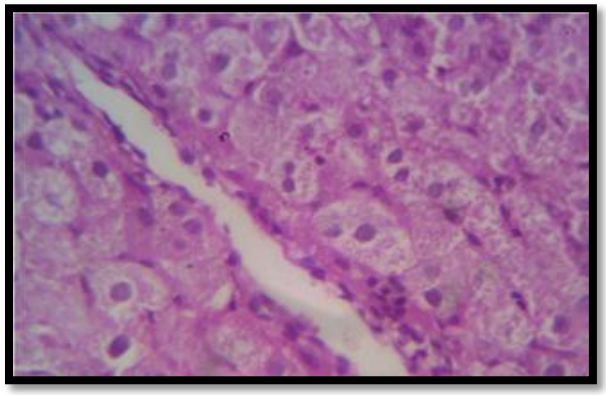

Fig.4. Group C, HE staining, 400X – liver normal structure.

In the experimental groups (E_1&E_2) which were exposed to 2mT &4mT respectively of intensities of magnetic field for a period of 30days we noted that cell and nuclear changes, as well as structural changes in the liver heavy portal inflammation, were recorded (Fig.5,6) respectively.

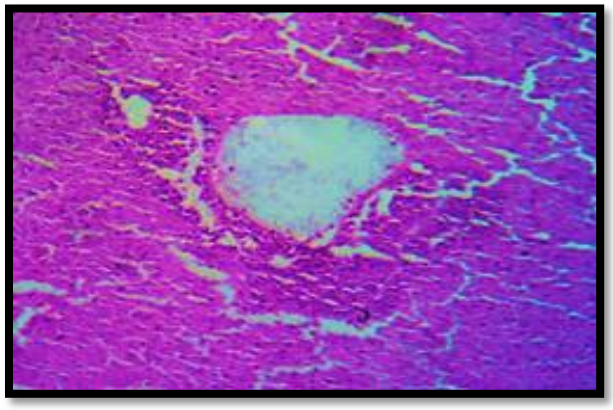

Fig.5.Group E_1,HE, 100X, heavy portal inflammation.

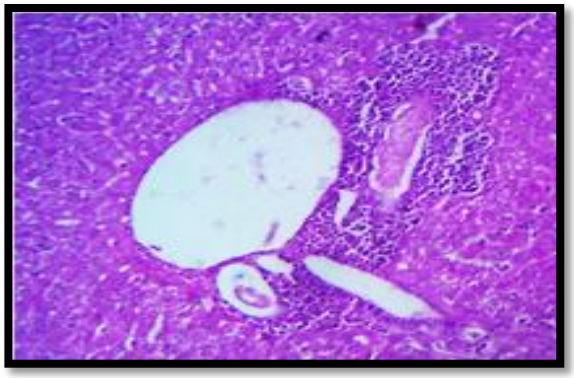

Fig.6. Group E_2, HE, 100X, heavy portal inflammation

In the experimental group (E_3), which was exposed to 6mT of intensities of magnetic field for a period of 30days, we noted congestion, atypia (Fig.7). In the experimental group (E_4), which was exposed to 8mT of intensities of magnetic field for a period of 30days, we noted micro-vesicular steatosis and lyfocal inflammation (Fig.8). In the experimental group (E_5), which was exposed to 10mT of intensities of magnetic field for a period of 30days, we noted portal inflammation (Fig.9).

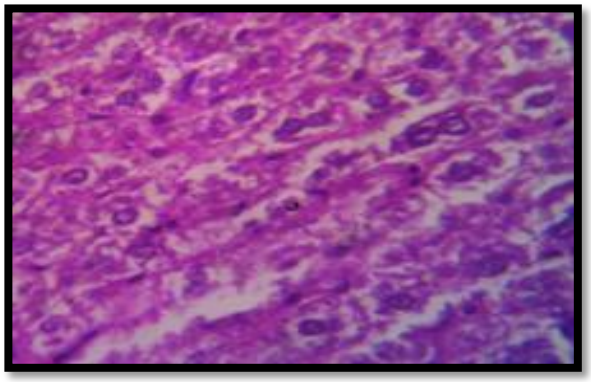

Fig.7. Group E_3, HE, 1000X, congestion, atypia.

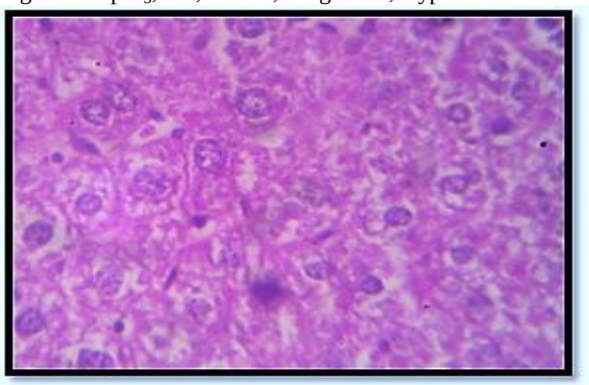

Fig.8. Group E_4, HE, 1000X, microvesicular steatosis and lyfocal inflammation

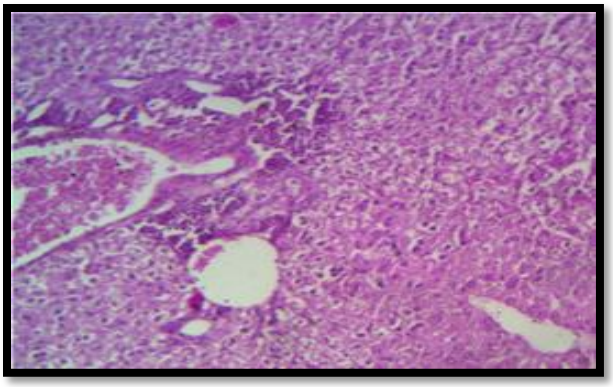

Fig.9. Group E_5, HE, 100X, portal inflammation

4. Conclusion

We concluded that the exposure to magnetic fields results in increased serum levels of liver enzymes, also the exposure to magnetic fields for a long period of time may adversely influence heart and/or liver function or structure and/or other tissues. However, further research is needed to clarify the effects of magnetic fields on body organs or tissues at cellular and molecular level. Liver was damaged with irradiation with different magnetic radiation intensities.

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