Research Inventy: International Journal Of Engineering And Science
Vol.4, Issue 12 (December 2014), PP 25-37
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

Effects of electric field on histopathological study, electrical properties and enzymes function of liver of albino rats

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ABSTRACT - *The present work was undertaken in order to investigate the effects of electric field (EF) of strength 50Hz-3KV/m on the histopathology, dielectric properties and liver function tests in albino rats. Fifty male albino rats were equally divided into three groups namely A, B, and C. Animals of group A used as control group which didn't receive any treatment . Animals of group B was divided into two subgroups namely B_1 and B_2 which were discretely exposed to 50HZ, 3KV/m electric field for a period of 15 day (8 hours/day, 5day/week). Group B_2 animals were left to survive and housed at normal environmental conditions similar to control group A for a period of 15 day post exposed. Animals of group C are divided into two subgroups namely C_1 and C_2 were discretely exposed to the electric field for a period of 30 day (8 hours/day, 5day/week). Group C_2 animals were left to survive and housed at normal environmental conditions similar to control group A for a period of 15 day post exposed. At the end of this period, blood and tissues samples were collected from all groups for experimental investigations. The dielectric constant (ε), electrical conductivity (σ) was measured in frequency range 42Hz-5MHz to investigate any changes in liver structure through studding histopathological examination. Also, the liver function was studied through analysis of glutamic oxaloacetic transaminase (GOT), glutamic pyruvie transaminase (GPT) and total protein (TP) after exposure to electric field this biochemical parameters have been evaluated in the blood serum of rats. The obtained results show high significant changes in the value of ε and σ of liver tissues for all groups exposed to EF as compared with control group. The levels of GOT and GPT were increased up to four times their values during the period of exposure to EF. These variations were recovered during two week after stopping exposure but they did not return to its original control values before exposure. On microscopic level; liver histological observations in liver cells which revealed some alterations including hepatic tissue with two portal tracts showing mild florous expansion and a dilated central vein, also ghosts of hepatocytes denoting necrotic changes also shows hepatic tissue with dilated central veins engorged with blood and splitting out to adjacent hepatocytes.*

KEYWORDS *- electric field, histopathology, liver enzymes, dielectric constant, conductivity.*

I. INTRODUCTION

Electromagnetic fields (EMFs) exposure exists at home, workplaces as a result of all types of electrical equipment and building wiring as well as a result of nearby power lines. It represents one of the invisible environmental pollutant factors that affect animals and human health **[1]**. During the past decade considerable evidence has been accumulated with regard to the biological effects, both in vivo and in vitro, of extremely low frequency electric and magnetic fields, such as those originating from residentially proximate power lines, household electrical wiring and diagnostic apparatus and therapy devices. Electric and magnetic fields associated with production transmission, and use of electricity is ubiquitous in industrialized societies. Electric fields exist whenever there is electric potential in a line. However, because reductions in field strength occur as electric fields pass through walls and other objects, the potential for human exposure to electric fields in a home environment is modest **[2]**.

Several studies on animal cells have also shown that EMFs influence a large variety of cellular functions **[3]**. The mechanisms (or some) of interaction with living cells involve, as reported, changes in the intracellular levels of Ca^{2+} **[4]**. However, many of the proposed hypotheses assume 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 **[5].**

It is likely that the disturbances lead to adaptive changes, which in turn result in altered lactate dehydrogenase activity and accelerated transamination processes. EMFs penetrate human body and act on all

organs, altering the cell membrane potential and the distribution of ions and dipoles. These alterations may influence biochemical processes in the cell, thus changing both biochemical parameters and enzyme activities of serum **[6]**.

Automation medical and research instruments which generate (EMFs) are widely diffused in recent years, and the people are frequently exposed to it. Despite that the study of the effect of (EMFs) on living organisms is a complex problem, but it is of more interest to give insight into the expected hazards and the proper ways of its use and protection. The EMF penetrates the human body and act ions on all organs, altering the cell membrane potential and the distribution of ions and dipoles **[7]**.

The effects of the EMFs on living organisms are based on the molecular interaction between many tissues and cellular systems, as well as on the level of cell organelles. The explanation of these mechanisms may be the subject for further investigations in this area, and may constitute a basis for the effective and safe application of EMFs in therapy **[8, 9].**

The mechanism of the interaction of EMFs with biological tissue associated with the changes in the permeability of cell membranes as a result of the change in the concentration of ions in the extra- and intracellular environment. The concentration changes are induced by the EMFs, which results from Lorentz force causing the motion of charged particles in the magnetic field **[10]**. Under the effect of electromagnetic field, the researchers observed the changes concerning both enzymatic complexes **[11, 12, 13]** and the coagulation complexes **[13]**.

EMFs 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 **[14, 7]**. Furthermore, alterations may influence biochemical processes in the cell, thus changing both biochemical parameters and enzyme activities of the blood serum.

Recent electron microscopy studies on hepatocytes and liver tissue have shown that constant magnetic fields exhibited structural changes in hepatocytes, primarily in the mitochondria and also split cell membrane **[15]**. Moreover, constant and low frequency magnetic fields exert a preponderant controlling influence on the thermoregulation, metabolism and hematology in rats **[16].**

The exposition of rats 1 hour/day for 10 consecutive days to a static magnetic field of 128 mT induced an increase in hematocrit, hemoglobin, plasma fuel metabolites and tissue enzymes releases within the blood **[17]**.Several authors suggested that chemical and physical processes at the atomic level are the bases of reactions between biomolecules in an EMF, since the field can magnetically affect the chemical bonds between adjacent atoms with consequent production of free radicals **[7,18]**.The magnetic field effects seem to be an ideal means for investigating biological function. Significant area would be better understood if knowledge on magnetic field effects on biological membrane is measured by physical parameters such as dielectric parameter (ε) and conductivity (σ).The physical mechanism for the effects of weak EMFs ranging from microwave to radio waves had been discussed **[7].**by the dielectric nature of all biological molecules especially those constituting the biological membrane.

The aim of the present work is to study the effect of electric field on histopathology, electrical properties and enzymes function of liver of albino rats the study pays attention to patients under investigation to these tests to be protected against exposure to any source of electric field.

II. MATERIALS AND METHOD

2.1. EXPERIMENTAL ANIMALS

The experimental animals kept in the same conditions for 2 weeks for adaptation. In the present work 50 male albino rats were used, each of average weight 170±10gm .The animals were housed in the same environmental conditions in plastic cages, and feed with constant balanced diet and tap water. Which were equally divided into three groups namely A, B and C. Animals of group A are used as a control group and didn't receive any treatment and housed at normal environmental conditions (the temperature inside the lab varied between 22 ̊and 25 ̊C, lighting condition are natural light from large windows during the day and complete darkness during the night). Animals of group B was divided into two subgroups namely B_1 and B_2 which were discretely exposed to 50HZ, 3KV/m electric field for a period of 15 day (8 hours/day, 5day/week). Group B_2 animals were left to survive and housed at normal environmental conditions similar to control group A_1 for a period of 15 day post exposed. Animals of group C are divided into two subgroups namely C_1 and C_2 were discretely exposed to the electric field for a period of 30 day (8 hours/day, 5day/week). Group C_2 animals were

left to survive and housed at normal environmental conditions similar to control group A_1 for a period of 15 day post exposed. At the end of this period, blood and tissues samples were collected from all groups for experimental investigations.

2.2. ELECTRIC FIELD EXPOSURE FACILITY

The exposure cage consisted of Perspex chamber, with an exposure volume of dimension 100x30x35 cm^3 located between two parallel cupper plates, which extended vertically along two parallel sides of the exposure cage as shown in figure (3-1). In order to prevent any animal shock from direct contacts with the electrodes, the cupper plates were covered by a sheet of Polymethyl methacrylate. It is worthy to mention that, the Perspex material has a negligible effect on the field homogeneity **[19].** The two electrodes were connected to a step up transformer with an output voltage of 3Kv when connected to the main supply. For more precautions an electric timer was used to adjust the exposure times specially when mains fall. The electric field inside the chamber was measured through the use of field meter and was found to be homogeneous and reads 3Kv/m.

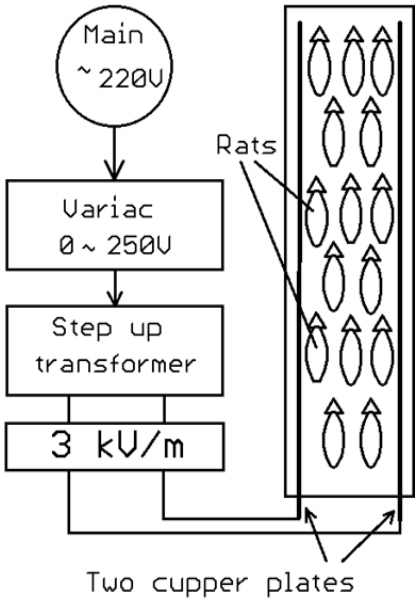

Fig. 1. Schematic diagram for exposure facility system.

2.3. BIOCHEMICAL ANALYSIS

Liver tissues of the experimental animals were immediately removed after exposure to EF. Weighed tissue samples were homogenized by a glass homogenizer after dilution by distilled water then the supernatant fluid was separated by centrifugation at 3000 rpm for 15 minutes, and stored at –20ºC for biochemical analysis. Liver glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminases (GPT) were determined using the method adapted by Fischbach and Zawta **[20]**. Alkaline phosphatase was determined using the method adapted by Bessey *et al*. **[21]**, and total protein content was determined using the method adapted by Henery **[22]**. Also blood serum was collected after blood centrifugation and stored at -20 ºC for biochemical analysis **[7]**.

2.4. THE DIELECTRIC AND CONDUCTIVITY MEASUREMENTS

The dielectric measurements were carried out for the liver samples in the frequency range 42Hz-5MHz using a loss Factor Meter type HIOKI 3532 LCR Hi TESTER; version 1.02, Japan as shown in Fig.2, and cell types (PW 950/60) manufactured by Philips show Fig.3. Animals were sacrificed then the liver was immediately excised and placed between a pair of 1cm diameter black platinum circular electrodes for dielectric measurements, the sample between the electrodes was maintained at constant pressure, and the distance between the electrodes was measured through the use of a micrometer, while the liver sample was filling the whole volume between the electrodes. During measurements, the sample between the electrodes was kept at a constant temperature of 24 ± 0.1^0C the capacitance (C) of the tissue was measured at each frequency and the resistance (R) was

recorded each run was repeated three times .The relative permittivity έ of the sample was calculated for each frequency using the relation:-

$$\acute{\varepsilon} = \frac{Cd}{\varepsilon_0 A} \qquad \textbf{(1)}$$

Where **A** is the area of electrode, **d** the distance between the two electrodes, ε_o is the permittivity of free space and $\acute{\varepsilon}$ is the dielectric constant. The dielectric loss ε'' is calculated from the relation:-

$$\varepsilon'' = \varepsilon' \tan\delta = \frac{\varepsilon'}{2\pi f R C} \qquad \textbf{(2)}$$

Where **f** is the applied frequency in Hertz, **R** and **C** are the resistance and capacitance of the sample at resonance and δ is the loss angle. The electric conductivity σ is given by:

$$\sigma = \frac{d}{RA} \qquad \textbf{(3)}$$

Where ***R*** is the resistance of the sample

Fig.2. Hioki 3532-50 LCR Hitester Bridge.

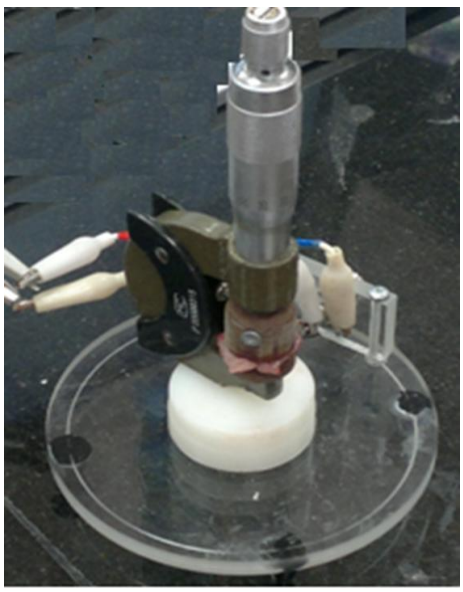

Fig.3. Cell used for measurement dielectric of biological liver tissues

2.5. HISTOLOGY ANALYSIS

Specimens of liver tissues were taken from all groups and prepared for the histological and histopathological sections following Banchroft and Stevens work, 2006 **[23]**. All of them were fixed in 10% buffered formalin (10ml formalin in 30ml normal saline or sterilized distilled water).The tissues were subsequently dehydrated in upgraded concentrations of alcohol (70% alcohol) cleansed in xylene. Several sections of 3-6 micrometer thickness were cut, dried with blotting paper **[24]**, using microtome then embedded in paraffin and sections stained with Hematoxylin and Eosin (H&E) **[25, 26]**. The slides were then evaluated for pathological changes under light microscope (100 x). Photographs were taken using Kodak digital 10.3 mega pixels camera **[27]**.

2.6. STATISTICAL EVALUATION

All results are presented as mean ± standard error of the mean. Statistical significances of the differences for all groups of samples were assessed using Student's t test. Differences were considered to be statistically significant at $p < 0.05$, high significant at $p < 0.01$ and very high significant at $p < 0.001$, not significant at $p > 0.01$.

III. RESULTS AND DISCUSSION

EMF exposure exists at home, workplaces as a result of all types of electrical equipment and building wiring as well as a result of nearby power lines. It represents one of the invisible environmental pollutant factors that affect animals and human health **[1]**. How induced EMF can affect organ function and induced cellular changes? Is a question, which has no definite answer? However, various mechanisms have been suggested. EMF might amplify electric currents in tissues and cells or affect these currents through resonance with local field focus **[28]**.

In the present work, the low frequency EMF was chosen because it has been encountered in many work places, medical practice and new technologies in use nowadays **[29]**. The rat liver function was studied through analysis of glutamic oxaloacetic transaminase (GOT), glutamic pyruvie transaminase (GPT) and total protein (TP) after exposure to electric field. The same biochemical parameters have been evaluated in the blood serum of rats. The levels of GOT and GPT were increased to up to four timed their values during the period of exposure to EF. Also, a recovery was carried out after 15 day from stopping the exposure to electric field. The changes in liver enzymes and total protein from blood serum analysis are shown in Table.1.In a previous study on the effect of static EMF on the liver, kidney and spleen tissues of rat showed that the liver tissue is more affected by EMF than the other tissues.

Table .1

Average values of total protein TP, the glutamic oxaloacetic GOT and glutamic pyruvic GPT transaminases of blood serum for all groups A ,B_1,B_2,C_1 and C_2 respectively. Values are the average of 10 experiments and $P<0.001$ as compared to values for the control group.

Groups	GOT U/L	GPT U/L	TP g/dL
A	**43**	**66**	**6.4**
B1	**259**	**84**	**7.3**
B2	**128**	**207**	**7.8**
C1	**86**	**184**	**8.7**
C2	**79**	**177**	**9.2**

The obtained data showed that EF produced alteration in biochemical parameters of the liver transaminases GOT and GPT which have been widely utilized in mammalian toxicology as biomarkers of specific organ dysfunction. In general the increase in transaminases activity is usually associated with hepatocyte damage. These results are in agreement with the results recorded by Sihem **[17]**. The authors studied the effects of sub-acute exposure to magnetic field on blood hematological and biochemical parameters in female rats and found that the serum GPT activity remained unchanged in treated rats, while GOT activity was increased, our present results agree with observations obtained by many authors **[17, 30, 31].**

The level of serum total proteins (TP) is significantly increased after exposure to EF. These results were agreement with other findings reported by other works that in vivo exposure to a pulsed magnetic field at 1.5mT caused significant changes on plasma proteins in rats, difference in levels of plasma proteins were observed between the control groups of the two studies. This observation supports the hypothesis that the state of physiological equilibrium of a biological system is crucial to its response to a potentially effective EMF **[2].** Valberg *et al.*, **[32]**, showed that the exposure to time varying magnetic field induces EF and this in turn may cause large structural changes of the protein molecules imbedded in the cell membrane forming a new membrane conformation. In this new conformation, the ions are able to pass through the membrane by binding temporarily with the protein molecule, thus "hopping" through the membrane. Watanabe et al., 1997 **[33]** showed that activities of GOT and GPT in the plasma, as indicator of hepatotoxicity, may alter the cell membrane potential and distribution of ions and dipoles. Kula et al, 1999 **[34]** reported that the physicochemical action of an EMF consists of electron, ion, dipolar, macrostructural and electrolytic polarization. Other factors may also play a role, such as molecular excitation, biochemical activation, generation of radicals, weakening of chemical bond and hydration change may alter relaxation protein fractions of serum.

The dielectric relaxation spectroscopy study showed a dielectric dispersion in the frequency region from 42 KHz to 5MHz for both control and exposed groups to 50Hz–3KV/m electric field. In this frequency range (β-dispersion) the relaxation mechanism is due to the counter ion molecules and proteins at the cellular membrane. Fig.4. shows the variation of the dielectric constant έ with frequency of liver tissue for rats exposed to EF.

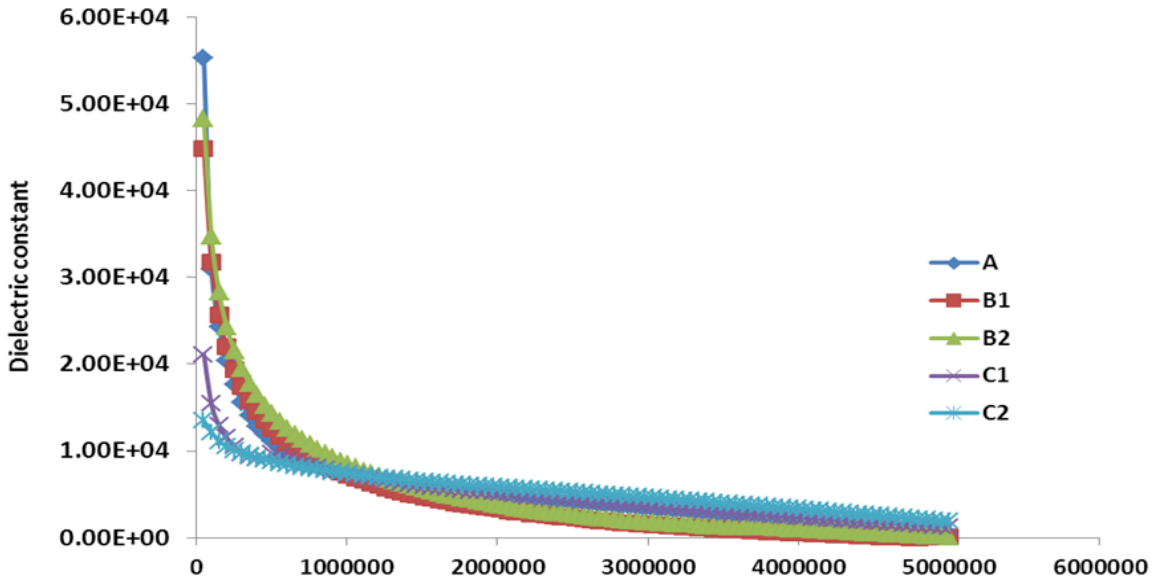

Fig.4. revealed the variation of Permittivity with frequency within the range 42Hz, 5MHz of the liver tissue suspension after exposure to 50Hz-3Kv/m electric field and recovery values after two weeks.

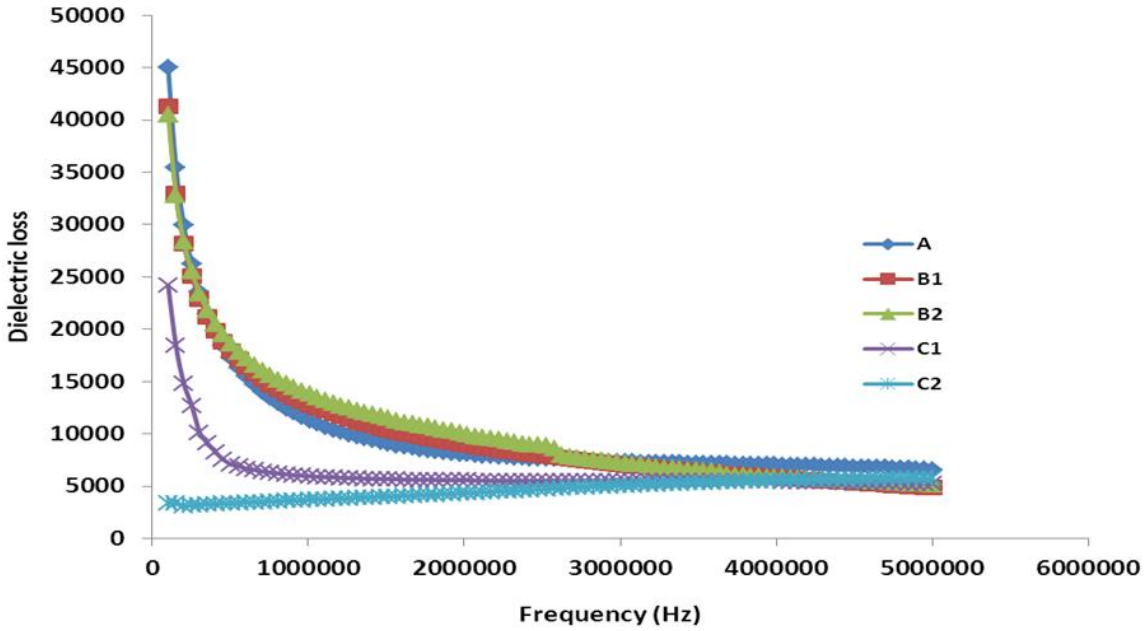

Fig.5. revealed the variation of dielectric loss with frequency within the range 42Hz –5MHz of the liver tissue suspension after exposure to 50Hz-3Kv/m electric field and recovery values after two weeks.

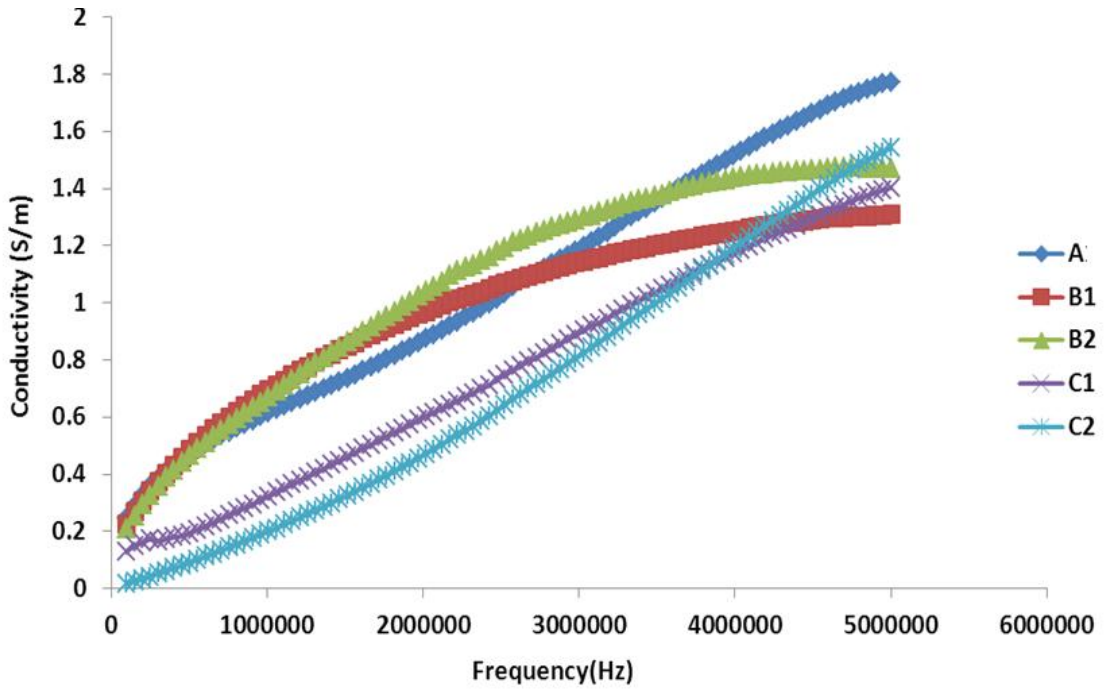

Fig.6. revealed the variation of dielectric loss with frequency within the range 42Hz –5MHz of the liver tissue suspension after exposure to 50Hz-3Kv/m electric field and recovery values after two weeks.

There was a pronounced decrease in conductivity of liver tissue suspension with frequency for all groups as compared with control group as shown in Fig. 6. The decrease in conductivity due to field exposure and the recovery groups not returned to the control value during the recovery period this is an indicator that there is no improvement in the liver state. The relative high control value of hepatocytes membrane permittivity $\acute{\varepsilon}$ and conductivity σ may be attributed to the high value of the membrane capacitance and conductance due to normal activity of GOT and GPT and normal values of cell membrane potential and distribution of ions and dipoles. So, the low values of the membrane permittivity, dielectric loss and conductivity after exposure to electric field are due to the lipid peroxidation, which causes destruction of cell membrane **[15, 36]**. During

recovery, for group B_2 the changes in σ , έ and ε" approximately return to its control value and the conductivity was approximately returned to the control value except for group C_2 it attained a higher value than the control value.

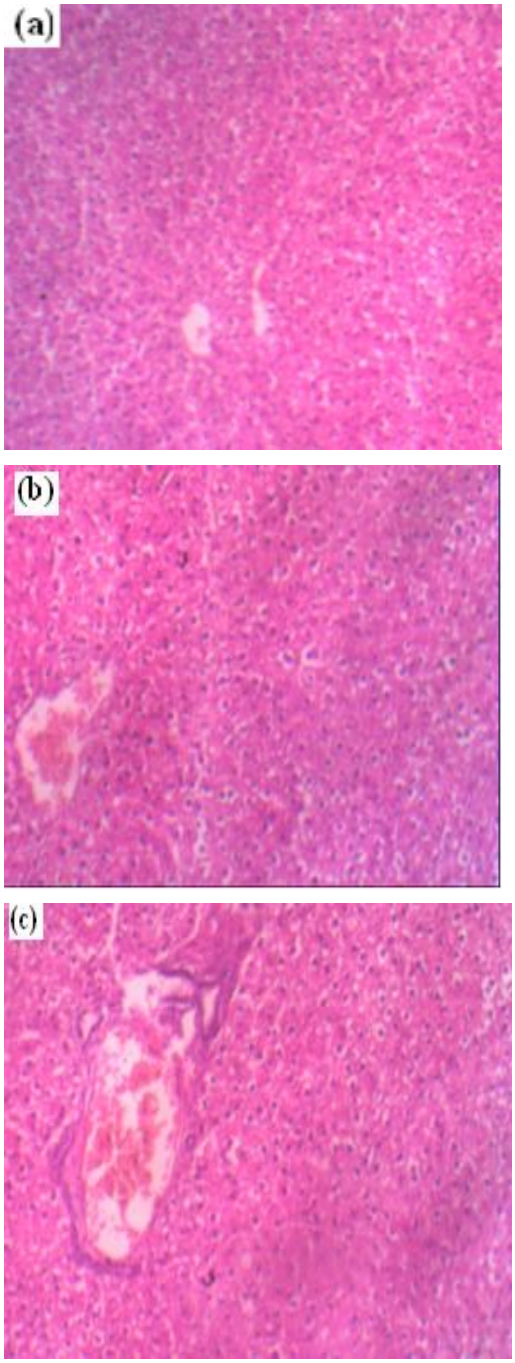

Fig.7. indicates photomicrographs of liver sections for control rat A_1. **(a)** Microscope Examination ME reveals hexagonal hepatocytes separated by sinusoidal spaces.**(b)** ME reveals a central vein as well as a portal tract are seen, cords of hepatocytes separated by sinusoidal spaces.**(c)** ME reveals a dilated central vein engorged with blood is seen also liver tissue with dilated a portal tract to the left (H&E×100).

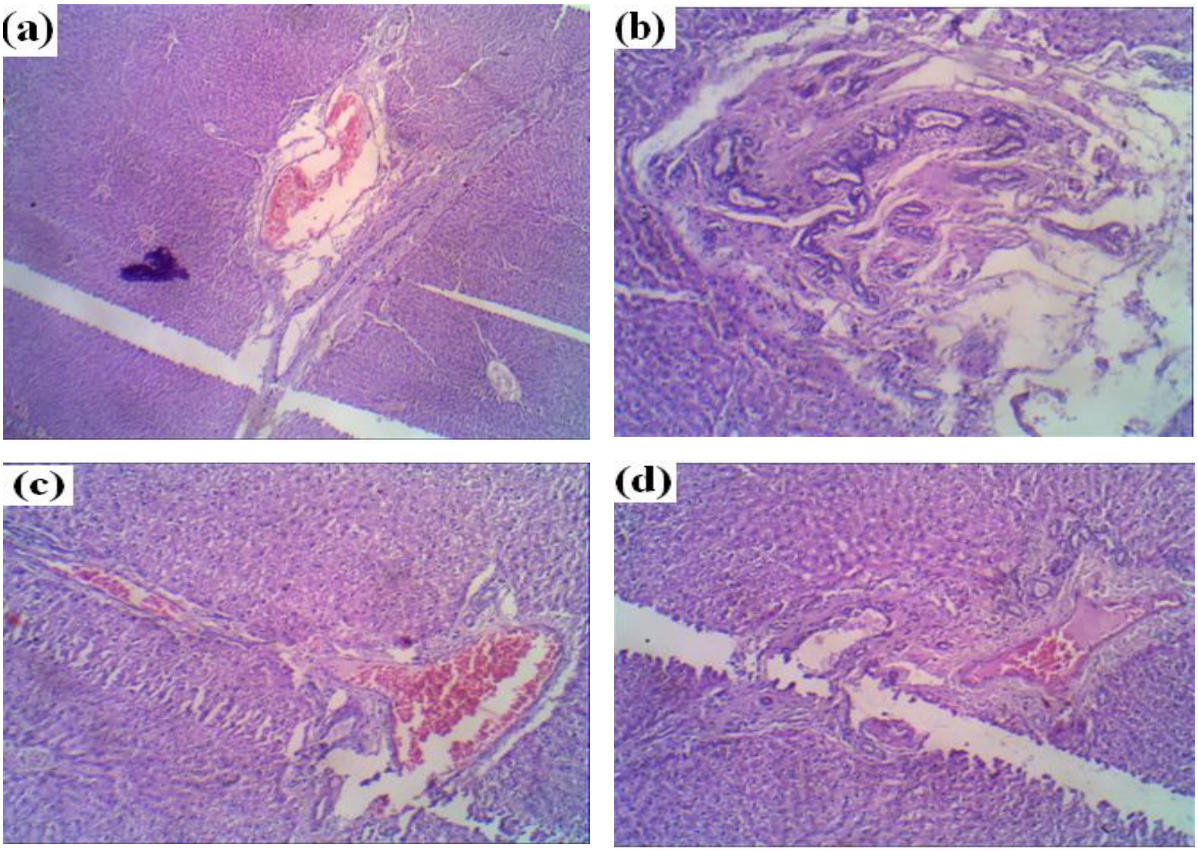

Fig.8. indicates photomicrographs of liver sections for group B_1 **(a)** ME reveals a widely expanded portal tract by fibrous tissue, with dilated congested portal vein and a dilated proliferating bile duct (H&E×40) **(b)** ME reveals an area of bile duct proliferations as well as fibrous tissue bands (H&E×100) **(c)** ME reveals a portal tract expanded by fibrous tissue and shows dilated congested portal vein and bile duct proliferations (H&E×100) **(d)** ME reveals a rudely expanded portal tract with abundant proliferating bile ducts and a dilated congested portal vein (H&E×100).

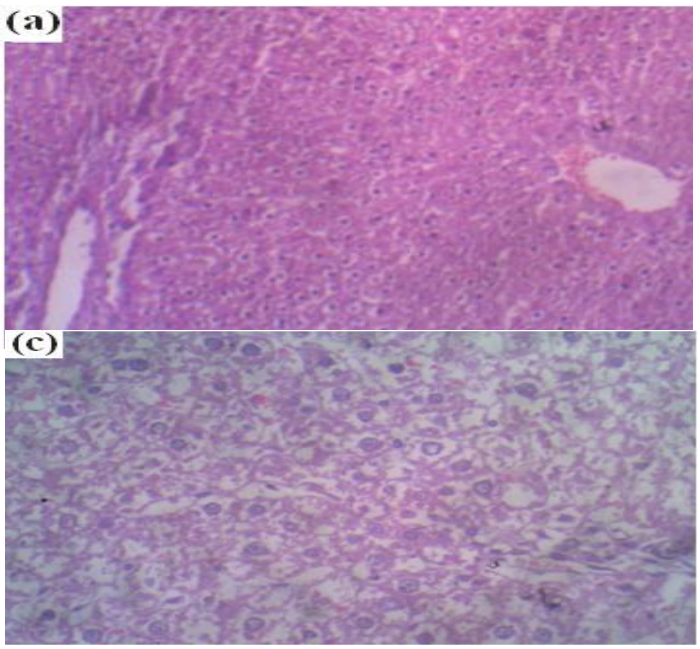

Fig.9. indicates photomicrographs of liver sections for group B_2. **(a)** Shows hepatic tissue with two portal tracts showing mild florous expansion and a dilated central vein (H&E×100). **(b)** Shows hepatic tissue with focus of inflammations (H&E×400) **(c)** Shows ghosts of hepatocytes denoting necrotic changes (H&E×400).

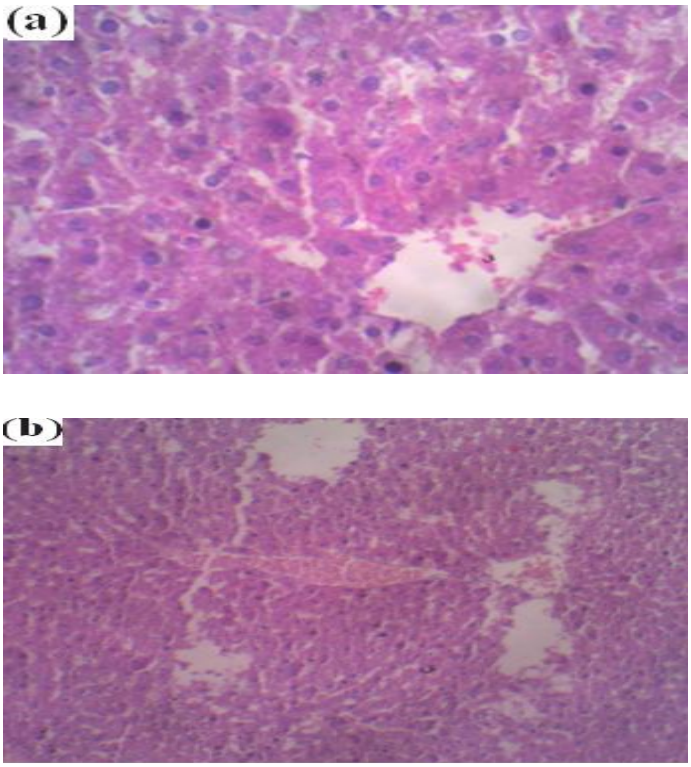

Fig.10. indicates photomicrographs of liver sections for group C_1. **(a)** Shows hepatic tissue with dilated central vein and engorged sinusoids (H&E×400). **(b)** Shows hepatic tissue with dilated central veins engorged with blood and splitting out to adjacent hepatocytes (H&E×400).

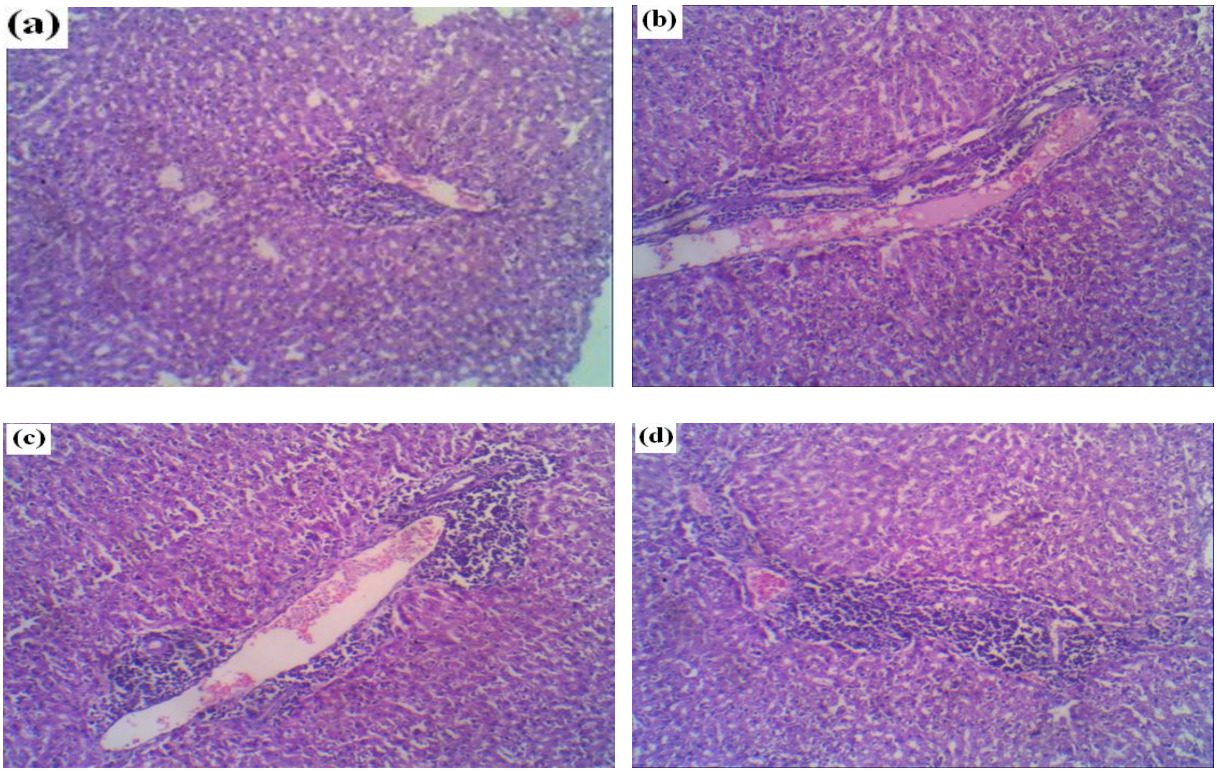

Fig.11. indicates photomicrographs of liver sections for group C_2 **(a)** ME reveals a portal trace infiltrated by moderated amount of lymphocytes with moderate spilling out to adjacent hepatocytes, moderate spotty necrosis is noted (H&E×100) **(b)** ME reveals a portal tract with fibrous tissue expansion and is moderately infiltrated by lymphocytes with mild to moderate spilling into adjacent hepatocytes, a dilated congested portal vein is seen (H&E×100) **(c)** ME reveals a portal tract which is expanded by fibrous tissue and shows bile duct proliferations

and is moderately to heavily infiltrated by lymphocytes with moderato spilling into adjacent hepatocytes, a dilated congested portal vein is seen (H&E×100) **(d)** ME reveals a portal tract heavily infiltrated with lymphocytes with moderate spilling into adjacent hepatocytes , moderate to heavy spotty necrosis is seen (H&E×100).

Liver histopathology for control animals A**,** the hepatic lobule appeared consisted of numerous lobules bounded together with connective tissue. The portal areas appeared at the peripheries of hepatic lobules, each containing a branch of the portal vein, a branch of the hepatic artery and branch of bile ductile embedded in connective tissue. The hepatocytes had polygonal outlines with relatively large, rounded vesicular and central nuclei. The blood sinusoids were seen alternate with the liver cell strands and were lined with Kupffer and endothelial cells (Fig.7).

Liver histopathology for animals of group (B_1) showing a portal-portal bridging septa containing hyperplastic bile ductules embedded in fibrous tissues connecting between two portal areas which shows numerous dilated, thin and elongated biliary proliferated ductile were observed at the peripheral areas of the hepatic lobules which contained different sized surviving hepatocytes and fibroblasts also reveals a widely expanded portal tract by fibrous tissue, with dilated congested portal vein and a dilated proliferating bile duct , shows an area of bile duct proliferations as well as fibrous tissue bands (Fig.8).

Liver histopathology for animals of group (B_2) showing well developed fibrous septa containing proliferated ductular structure surrounded by infiltrated inflammatory cells, also shows hepatic tissue with two portal tracts showing mild florous expansion and a dilated central vein, shows hepatic tissue with focus of inflammations shows also ghosts of hepatocytes denoting necrotic changes (Fig.9).

Liver histopathology for animals of group (C_1) showing partial disappearance of proliferated biliary epithelial cells and ductules and a conspicuous increase of the regenerating and surviving hepatocytes, shows hepatic tissue with dilated central vein and engorged sinusoids and hepatic tissue with dilated central veins engorged with blood and splitting out to adjacent hepatocytes (Fig.10).

Liver histopathology for animals of group (C_2) reveals a portal trace infiltrated by moderated amount of lymphocytes with moderate spilling out to adjacent hepatocytes, moderate spotty necrosis is noted also appear portal tract with fibrous tissue expansion and is moderately infiltrated by lymphocytes with mild to moderate spilling into adjacent hepatocytes, a dilated congested portal vein is seen, a portal tract which is expanded by fibrous tissue and shows bile duct proliferations and is moderately to heavily infiltrated by lymphocytes with moderato spilling into adjacent hepatocytes, a dilated congested portal vein is seen and also a portal tract heavily infiltrated with lymphocytes with moderate spilling into adjacent hepatocytes , moderate to heavy spotty necrosis is seen.

In the present study the most conspicuous histological change of rat's livers after exposure to electric field was the proliferation of bile duct epithelium - like cells as well as the distinct capacity of these cells to differentiate into hepatocytes and/or biliary epithelial cells. These proliferated bile ductular cells were not usually seen in normal liver but were observed in all the examined groups after exposure to EF to repopulate the destroyed hepatic cells. Thus, the proliferating ductular cells might engage in hepatocyte regeneration **[37, 38**]. In this respect, numerous histological studies on the liver showed hyperplastic reactions with proliferation of bile duct epithelium - like cells in the periportal areas of diseased livers **[39, 40]**. Moreover, other investigators recorded that the prolonged exposure to EMF increased ductular proliferation in the liver **[38, 39]**.

The present study also revealed that the ductular proliferated cells were often seen in the periportal areas after hepatic injury in close association with proliferated fibroblasts. Also, the degree of activation of ductular proliferated reaction was positively correlated with the degree of inflammatory activity and liver damage. It was previously reported that areas of more severe injury were more closely associated with ductular reaction **[41, 42]**. So, other workers recorded that the proliferating ductular cells were observed at the portal areas after hepatic injuries in association with areas of necrosis, inflammation, malignant transformation **[43].** However, other investigators reported that there was a negative correlation between the cell proliferation activity of the bile ducts and the number of fibroblast like cells which were seen to be increased in number with time after bile duct legation and liver injury **[44]**.

In this respect, It was mentioned that ductular cells underwent hepatocytic differentiation and might be considered as an early form of regenerating hepatocytes **[45, 46]**. Thus, the ductular reactions were observed around the wound areas and the ductules extended to the injured areas to repopulate the injured hepatocytes **[47]**. In the present study extended biliary ductules were observed surrounded by survived hepatocytes after thirty days following the end of exposure to electric field.

IV. CONCLUSION

In conclusion, the results demonstrated that

1- This study suggests that, in humans under investigation, the activities of liver enzymes GOT and GPT may increase and the conductivity may decrease by exposure to electric field generated during magnetic resonance imaging or nuclear magnetic resonance procedures.
2- The decrease in conductivity due to field exposure and the recovery groups not returned to the control value during the recovery period this is an indicator that there is no improvement in the liver state.
3- The prolonged exposure to electric field enhanced and increased ductular proliferation in the liver. The hepatic regeneration was related to ductular proliferated cells. The histological investigation of the nature of these cells would help to understand the mechanism of hepatocytes regeneration. Moreover, biliary epithelial cells and ductular proliferated structures could be utilized as an early histological mark to describe the diseased liver and might be considered as a prognostic indication for assessing the grading of the severity of the hepatic injury and might open a way to cell based therapy for liver diseases.

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