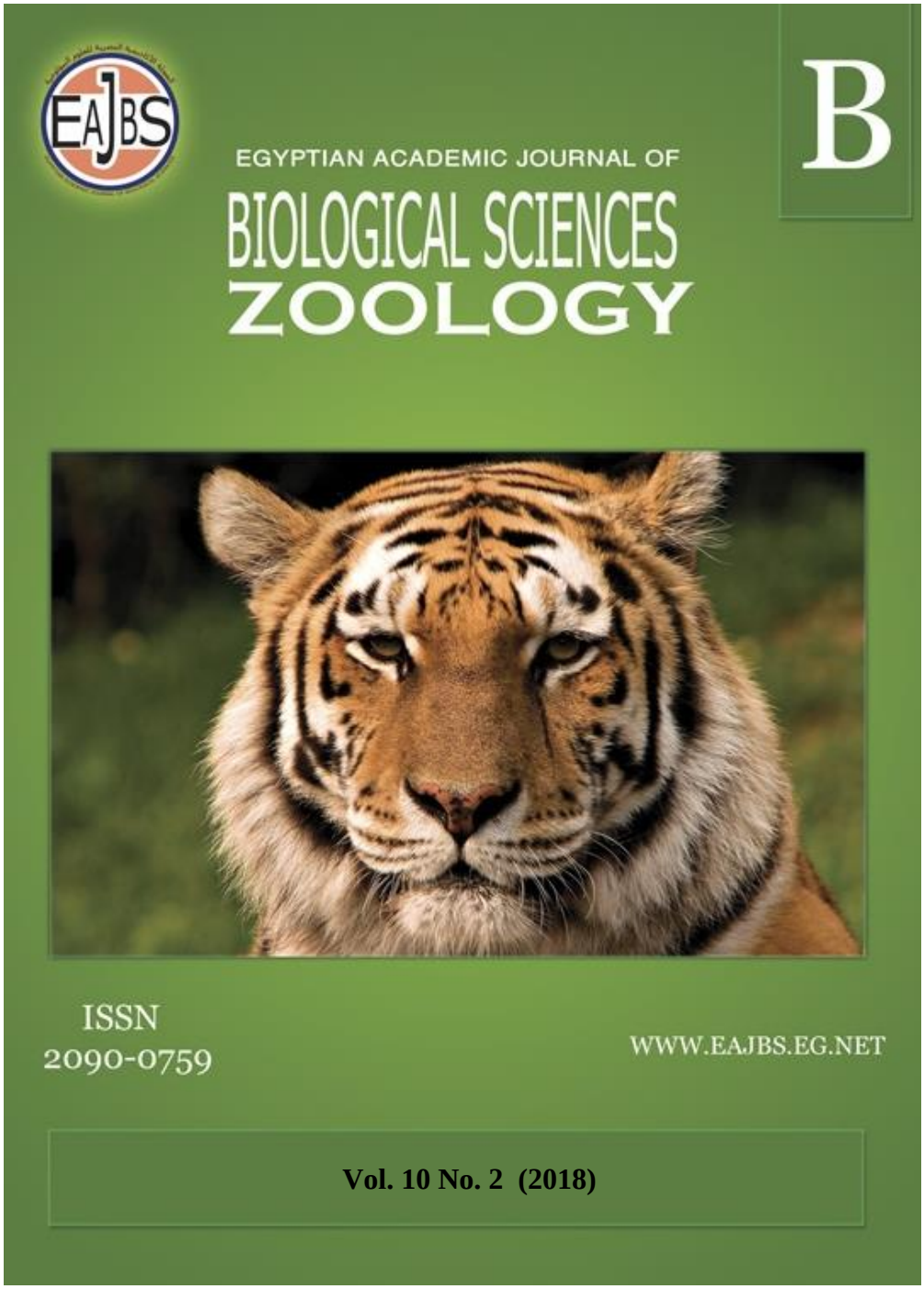

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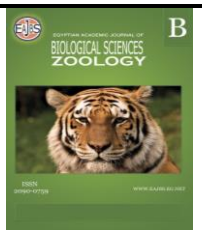

Ecotoxicological Effects of Electromagnetic Radiation on the Wild Rat, *Rattus norvegicus*

Zeinab Z. K. Khater[1]; Hend Sh. Ghareeb[2] and Mona H. Ibraheim[3]

[1] Department of Zoology, Faculty of Science, Zagazig University, Egypt
[2] Plant Protection Research Institute, Dokki, Giza, Egypt
[3] Department of Physics, Faculty of Science, Zagazig University, Egypt

E.Mail:: z_sci.egy@zu.edu.eg

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ABSTRACT

The effect of electromagnetic radiation at frequencies (50, 100, 200 & 250 Hz) and intensity of 2 mT on the mortality of *Rattus norvegicus norvegicus* rat by oral administration of water exposed to this radiation for four hours was investigated. Effect of the sublethal frequency on the physicochemical parameters of water, count of bacteria in water and liver tissues of the rat were also studied. After one month of the experiment, the highest frequency 250 Hz recorded 80% mortality of rats. While, the lowest frequency 50 Hz not exhibited any mortality till the end of experiment. This sublethal frequency caused an increase in temperature, a decrease in total dissolved solids and no change in pH value, salinity, chlorinity and alkalinity of water. It showed also observed bactericidal effect against the water bacteria. It is significantly reduced the number of *Escherichia coli* and *Serratia marcescens* colonies and completely prevent the growth of *Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria in comparing to the unexposed water (control). Destroying in the liver tissue cells was also recorded by this frequency.

INTRODUCTION

Wild rat, *Rattus norvegicus* is one of the most important pests in rodents. It is the transmitter of a large number of infectious diseases to domestic animals (Dedovic *et al*., 2012). The chain of toxicoinfection to farmer's food infected by rat urine and feces and also contaminated forage which poses a risk to animal health (Daniels *et al*., 2003). Different methods were used for reducing economic damage caused by this rodent species. Magnetic radiation is one of the most potent methods for control rats (Pierre *et al*., 2007). The effects of electromagnetic radiation in biology have been intensively studied on animals (Zwingelberg *et al*., 1993). It is not shown any toxic effect against rats at low intensity (Boorman *et al*., 1997). Acute effect on rats become apparent at the higher radiation strengths (Houpt *et al*., 2003). Moreover, magnetism sciences have developed and become

more complicated when its properties were found to have linkage with all fluid, solid and gaseous matters in addition to living creatures. Electromagnetic radiation is known to make the asymmetry of hydrated shells because of its impact on water molecules arranged around the charged particles (colloid). Exposure to electromagnetic radiation would lead to a higher electro-dynamic movement among the colloid. This will build the probability of attracting particles to cloak with one another (Fadil *et al*., 2001). In addition, magnetic radiation strongly affected on the count of bacteria in water (Khazan and Nasser, 2010). The ability of bacteria to grow and form colonies decreased with increasing the magnetic radiation intensity (Ludek *et al*., 2002). On the other hand, any change in the electrochemical microenvironment of the cell can cause modifications in the structure of its electrically polarized membrane by changing the concentration of a specifically bound ion or dipole that may be accompanied by alterations in the conformation of lipids, proteins and enzymes (Brighton, 1991). It was shown that the exposure to the electromagnetic radiation slows down the erythrocyte sedimentation rate, changes the number of leukocytes and phagocytic activity of leukocytes(PhAL) and their luminescence, Orientation of sickle-shaped erythrocytes of human blood across magnetic lines of force was discovered. It was shown that electromagnetic radiation change osmotic processes in muscles, affect the permeability of the cellular membrane, and disturb the hydration ability of tissues in animals (Kholodov, 1974).

This research was performed to study the effect of different magnetic radiation frequencies on the mortality of *Rattus norvegicus* rat by oral administration of water exposed to these frequencies. The effect of sublethal frequency on physiochemical parameters of water, count of water bacteria and liver tissue of rat were also investigated.

MATERIALS AND METHODS

Rats:

Adult males of the wild rat, *Rattus norvegicus* were trapped and collected from plantation area at Zagazig district, Sharkia Governorate, Egypt. The rats were transported to the laboratory and maintained in metallic cages. Then there were supplied with enough food (crush maize) and water for acclimatization. All rats were observed daily for two weeks before any experiments (Abdel-Azeem, 2013).

Toxic Effect of Magnetic Radiation on Rats:

Healthy animals were divided into two groups. The 1st group was served as a control consists of three replicates; each one contains 10 rats received normal distilled water. The 2nd group received the treated water with an electromagnetic radiation by drinking in four frequencies (50, 100, 200& 250 Hz). Three replicates were used for each frequency; each comprising of 10 rats. Before any treatment, animals fasted for 6 hrs. The rats were treated again with magnetized water another one time after two weeks of the experiment. Mortality percents of animals were recorded daily for one month (Kumar *et al*., 2017). At the end of the experiment, animals which administered with water treated with the sublethal frequency were anaesthetized and sacrificed by decapitation to remove their liver for the histological studies.

Sampling Site and Analytical Methods:

Water samples were taken from a tape water of Faculty of Science, Zagazig University. Water samples were subjected to the electromagnetic radiation at the sublethal frequency (Fig. 1). The water samples (control and treated) were analyzed for water quality (chemical and physical characteristics of water).

Fig. (1): The electromagnetic radiation exposure system was manufactured locally, in the Faculty of Science Cairo University.

Physico-chemical Analysis of Water:

***Temperature**:

The temperature was measured at the sampling site, using a mercury thermometer of 0 to a 50˚C range.

***pH**:

pH has been measured by using a glass electrode pH-meter (Digital Mini-pH-Meter model 55).

***Total dissolved solids:**

Total dissolved solids have been measured according to Ibraheim and Khater (2013).

***Chlorinity**:

Chlorinity was measured by a digital chlorimeter (model HI 93711).

***Salinity:**

Salinity was measured by a digital salinometer (model Atago Hand Refractometer).

***Alkalinity:**

Alkalinity has been determined according to Gupta (2000).

Bacteriological Studies:

Effect of magnetic radiation at the sublethal frequency on the bacterial counts of water was investigated. Each of the exposed water to this frequency and other unexposed tap water (control) was serially diluted from 10 to 1 as described by Collins and Lyne (1985). One ml was taken from each dilution and spread on to the nutrient agar plates. The plates were incubated at 37ºC for 24 h. Bacterial colonies were enumerated, characterized and recorded. The number of colonies was expressed as colony forming units (CFU/ml). The colonies were purified on sterilized nutrient agar by using the streaking plate method. Then the all isolates were Gram stained and subjected to the biochemical identification tests (Taiwo *et al*., 2002).

Electron Microscopic Studies on the Liver of the Studied Rats:

Specimens of the liver were taken from the control and treated rats with sublethal frequency for one month and prepared for electron microscopic studies (at central laboratory of Faculty of Agriculture, Mansoura University) according to Ali (2013).

Statistical Analysis:

Results are statistically analyzed and the difference between means was tested by using the analysis of one way ANOVA (Costat, 2005) statically program analysis, computer program software. The least significant differences (L.S.D.) were also calculated. Tables and graphics were done using Microsoft Word (2007) and Origin 8. The methods used for analysis of the results were done according to Bishop (1980) and McCreadie *et al*. (2006).

RESULTS AND DISCUSSION

Toxic Effect of Magnetic Radiation on the Wild Rat:

As shown in Table (1) mortality of *Rattus norvegicus* rats increased with increasing the frequencies of magnetic radiation. The frequencies 100 and 200 Hz recorded their first effect against rats after three days of administration with mortality 23.33 and 46.66%, respectively. While, the highest frequency 250 Hz showed 70% mortality after only one day of administration. After 30 days of administration; 100, 200 and 250 Hz frequencies recorded 36.66, 60 and 80% mortality, respectively. The lowest frequency 50 Hz not recorded any mortality till the end of the experiment. There is a high significant difference in the effect of magnetic radiation frequencies against rats compared to the untreated individuals.

Table (1): Effect of magnetic radiation at different frequencies on the wild rat .

Frequencies (Hz)	Mortality percent at days					
	1	**3**	**7**	**14**	**21**	**30**
50	0.00	0.00	0.00	0.00	0.00	0.00
100	0.00	23.33	30.00	30.00	36.66	36.66
200	0.00	46.66	46.66	60.00	60.00	60.00
250	70.00	70.00	73.33	73.33	80.00	80.00
Control	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}
P	$.0000^{***}$	$.0000^{***}$	$.0000^{***}$	$.0000^{***}$	$.0000^{***}$	$.0000^{***}$
L.S.D. $_{0.05}$	0.94	1.21	0.76	0.54	1.08	1.08

These results were strongly agreed with Trezeciak *et al*. (1993) revealed that the low magnetic radiation frequency 50 Hz was not showed any significant or observed effects on the male and female rats. However, it is possible that exposure to 60 Hz magnetic radiation cause cancer to rats which may be lead to a future death

(Boorman *et al*., 1997). Moreover, this low intensity of magnetic radiation has an inhibitory effect on the lethality and seizures in rats (Klaus and Donald, 1991). On the other hand, Pierre *et al*. (2007) indicated that is unexpected increase in mortality (76%) was observed for young rats that had been exposed to 7 Hz magnetic radiation with maximum intensities around 5 nT. Other report cited by Jelenkovic *et al*. (2006) showed that exposure of rats to the low-frequency magnetic radiation does not lead to rapid death but having a harmful and dangerous effect on the brain which adversely affects the movement and all vital functions of rats.

Physico-chemical Parameters of Water:

Comparing the average means of the same physico-chemical parameters of water samples in the pre and post- exposure to sublethal frequency (50 Hz) of magnetic radiation, the data recorded in the Table (2) determined significant differences in temperature and total dissolved solids. It is thought that the modifications to the properties of solutions through the electromagnetic radiation changes the molecular structure of liquids, polarization, resulted from the arrangement of particles and finally from changes of the electric potential (Lebkowska, 1991; Szczypiorkowski and Nowak (1995) and Krzemieniewski *et al*., 2004).

Table (2): The physico-chemical parameters (mean ± SD) of water samples before and after exposure to magnetic radiation frequency (50 Hz).

Electromagnetic / Parameters	Pre-exposure	post-exposure
Temperature (℃)	**17±0.35[a]**	**23±0.35[a]**
pH	**7.150 ± 0**	**7.150 ± 0**
Chlorinity (%)	**0.177 ± 0**	**0.177 ± 0**
Salinity (%)	**0.32 ± 0**	**0.32 ± 0**
Total dissolved solids (ppt)	**0.4 ± 0.007[a]**	**0.300 ± 0.007[a]**
Akalinity (ppm)	**124 ± 0**	**124 ± 0**

- Data are represented as mean ± SD, (n = 12).
- Means with the same letters in the same row are significantly different ($p < 0.05$), using Independent t-test.

Temperature:

In this study, the fluctuation in water temperature caused by exposure to the electromagnetic radiation leading to an increase in it (Shatalov, 2009, and Ibraheim and Khater, 2013).

pH- value:

In the present study, the pH values at exposure to electromagnetic radiation were always on the neutral side and there was no effect of the electromagnetic radiation on it. It disagrees with that of Alkhazan and Sadddiq (2010) who stated that electromagnetic radiation caused increasing of the pH- value and Shatalov (2009) who observed a decrease in the pH value with the electromagnetic exposure. It may be attributed to the short period (4 hours) of the exposure in this study.

Salinity& Alkalinity:

From the present data, it is clear that the salinity and alkalinity contents were not changed in the exposure to electromagnetic radiation. These findings disagreed with Ni´ am *et al*. (2006) and Alkhazan and Sadddiq (2010), and may be related to the short period of the electromagnetic exposure and weak intensity of the electromagnetic radiation.

Total Dissolved Solids (TDS):

From the data reported in this study, it is clear that the values of TDS were changed after the exposure to the electromagnetic radiation and this disagreed with Ni´am *et al*. (2006) and Ibraheim and Khater (2013), and may be related to the short period of the electromagnetic exposure.

Effect of Magnetic Radiation on the Count of Water Bacteria:

Effect of magnetic radiation at the sublethal frequency 50 Hz on the bacterial count of water was studied. As illustrated in Table (3) *Escherichia coli* was the most proliferate isolate in the unexposed water to magnetic radiation (control) recorded 18 colonies (58.06%); *Serratia marcescens*, 5 colonies (16.13%); *Klebsiella pneumoniae*, 3 colonies (9.68%); *Pseudomonas fluorescens*, 2 colonies (6.45%); *Staphylococcus aureus*, 2 colonies (6.45%) and *Staphylococcus epidermidis*, 1 colony (3.23%). But, in the water which exposed to magnetic radiation; *E. coli* and *S. marcescens* number was significantly decreased to 3 and 2 colonies in comparison to 18 and 5 colonies in the (control) water, respectively. Magnetic radiation also completely prevent the growth of the other bacterial isolates; *K. pneumoniae*, *P. fluorescens*, *S. aureus* and *S. epidermidis* in water.

Table (3): Effect of the sublethal magnetic radiation frequency 50 Hz on the bacterial number (CFU / ml) of water

Bacterial species	Unexposed water to magnetic field (control)		Water exposed to magnetic field	
	Total number of strains	% of identification	Total number of strains	% of identification
Escherichia coli	18	58.06	03	60
Klebsiella pneumoniae	03	9.68	0	0
Pseudomonas fluorescens	02	6.45	0	0
Serratia marcescens	05	16.13	02	40
Staphylococcus aureus	02	6.45	0	0
Staphylococcus epidermidis	01	3.23	0	0
Enumeration	**31**	**100**	**5**	**100**

These results were perfectly concurred with Ludek *et al*. (2002) revealed that growth of *E. coli* bacteria was negatively affected by magnetic radiation at the low frequency of 50 Hz. The ability of this bacteria to form colonies decreased with increasing intensity of magnetic radiation and the time of exposure. This cleared that effect of magnetic radiation is probably bactericidal. Additionally, El-Sayed *et al*. (2006) confirmed that when this bacteria subjected to this frequency and electromagnetic waves (2µT) for 6 h., its growth greatly inhibited and also the length

of bacterial cell significantly decreased. In the same direction, Pengfei *et al*. (2007) stated that growth and concentration of *Pseudomonas aeruginosa* bacteria were inhibited by using a wireless magneto elastic. Moreover, magnetic radiation was significantly reduced the number of *S. epidermidis*, *Streptococcus parasanguinis* and *Rhodococcus equi* bacteria (Brkovic *et al*., 2015) and caused inhibition of *S. marcescens* at 80 ± 20 gausses (Piatti *et al*., 2002). The numbers of heterotrophic bacteria were decreased by the increase of magnetic radiation intensity. It was recorded 240×10^{-3} and 133×10^{-3} CFU / ml for both static and shaking cases of magnetic intensity (130 μT). This is due to the effect of the magnet on the metals, organic substances, nitrogen and phosphorus which are essential in the bacterial metabolism reactions. In addition, water form 80% of bacterial cells, so when its chemical and physical properties changed by the magnetic force, the growth of bacterial cells inhibited as their composition changed (Molouk and Amna, 2010).

Electron Microscopic Studies on the Liver of Studied Rats:

The electron microscopic studies indicated the dangerous effects of water exposed to the electromagnetic radiation at sublethal frequency (50 Hz) on the liver of the wild rats, as shown in Figs. (2-7). The frequency and severity of histological alterations in the liver were increased in treated rats compared to the control.

The present results indicate the very dangerous effect of magnetized water on the experimental animals as it damaged the liver tissue cells of the experimental rats (Fig. 2 - 7). The magnetized water causes risk impacts on the examined rat's liver as it harms the tissues prompting putrefaction and liver disappointment. This may be attributed to the role of electromagnetic radiation on the hydration of ions of the exposed water which affect on molecules arrangement around charged ions in the experimental animal cells causing disruption and deformation of animal tissue cells. This concurred with Kholodov (1974), Mc Dwell (1974), Ali (2013), Türker and Yel (2014) and Khater and Ibraheim (2016).

CONCLUSION

The results of this study concluded that magnetic radiation at the frequencies of 50, 100, 200 and 250 Hz and intensity of 2 mT can be used as the successful method for control the wild rats. In addition, the magnetized water by the lowest frequency (50 Hz) has observed the harmful effects on the liver of treated rats.

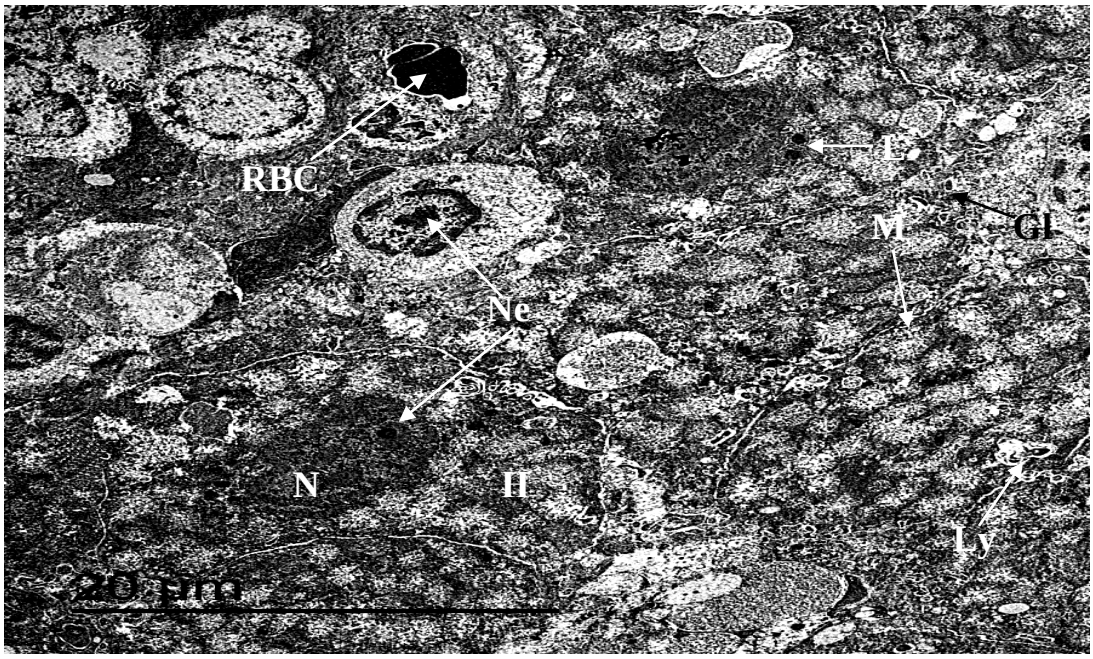

Fig. 2: TEM photomicrograph of the liver from a normal wild rat showing the general morphology of hepatocytes (H). The liver cell has a round nucleus (N) with nucleolus (Ne), centrally placed in the cytoplasm packed with various organelles and inclusions. These organelles comprise lipids (L), mitochondria (M) which are numerous exhibiting round or elongated shapes and Lysosomes (Ly) which appear as rounded vesicles bounded by a single membrane. Abundant glycogen particles (Gl) are scattered in the ground cytoplasm in the form of rosette-shaped particles. (X 10132).

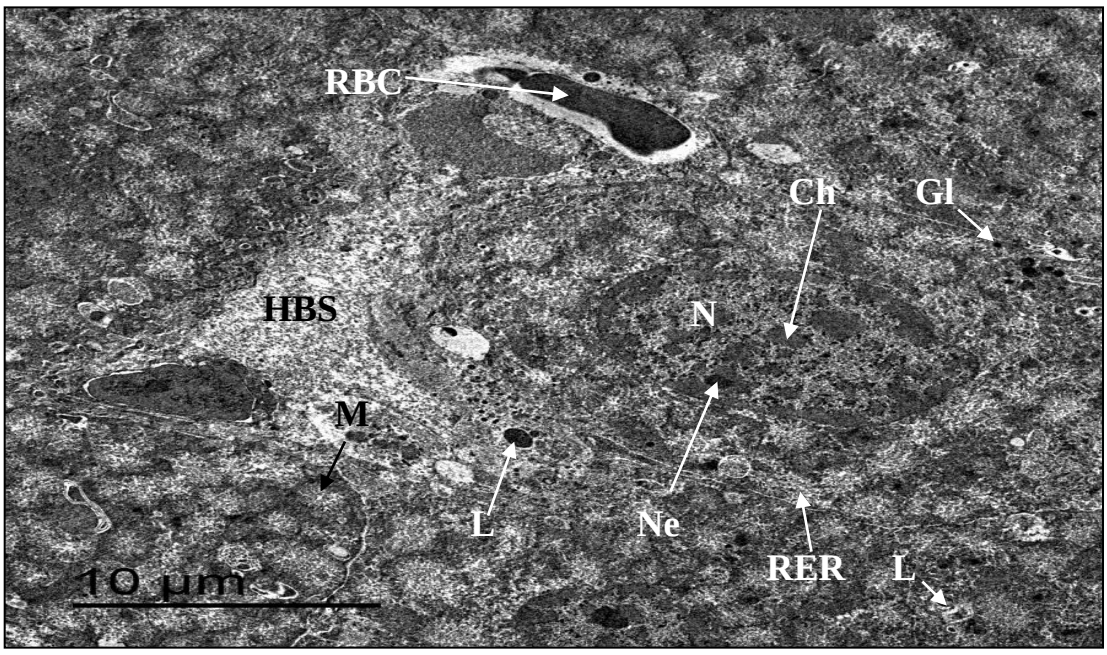

Fig. 3: TEM photomicrograph of the liver cells of a normal wild rat showing the general morphology of hepatocytes (H) and hepatic blood sinusoid (HBS) containing RBCs (RBC). The liver cell has a round nucleus (N) with peripherally located nucleolus (Ne) and homogenously distributed chromatin (Ch), centrally placed in a cytoplasm packed with various organelles and inclusions. These organelles comprise the rough endoplasmic reticulum (RER), mitochondria (M) are numerous exhibiting round or elongated shapes, Lysosomes (Ly) appear as small rounded vesicles bounded by a single membrane, lipids (L) and abundant glycogen particles (Gl) in the form of rosette-shaped particles (X 10138).

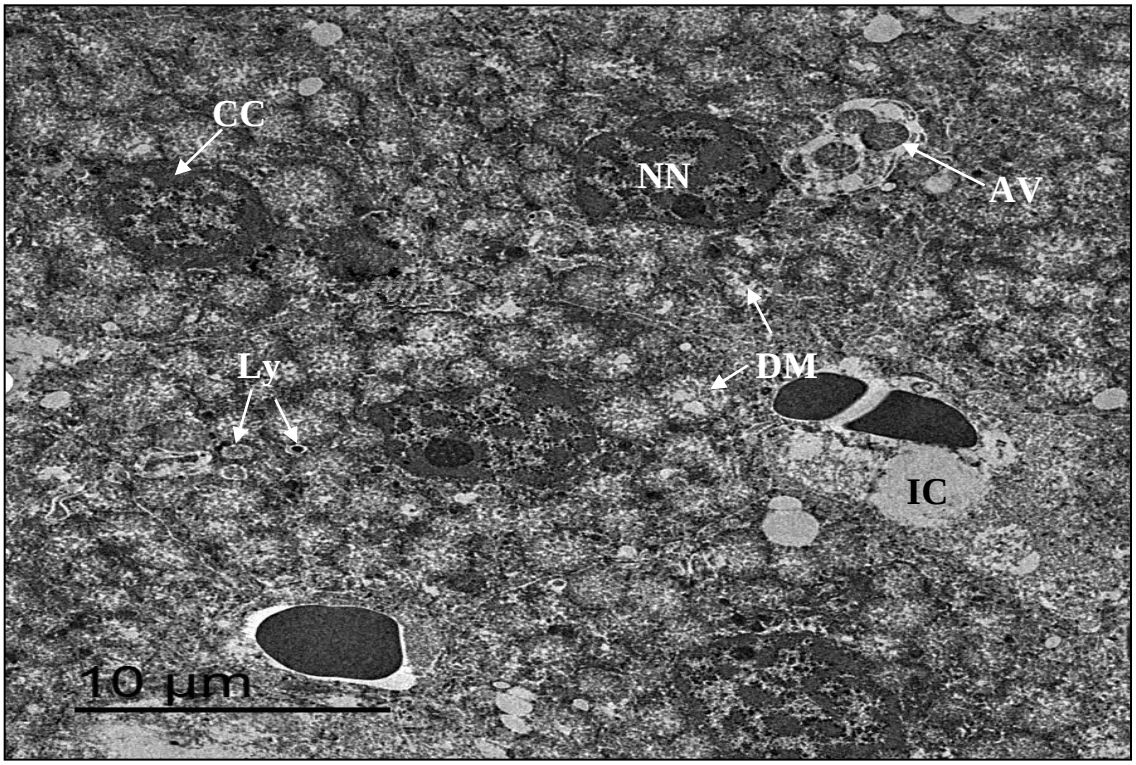

Fig. 4: TEM photomicrograph of a liver cell from a wild rat treated with magnetized water for a month (30 days) showing necrotic nuclei (NN) with condensed chromatin (CC), degenerated mitochondria, great number of lysosomes (ly), inflammatory cell (IC) and autophagic vacuole (AV) (X 10143).

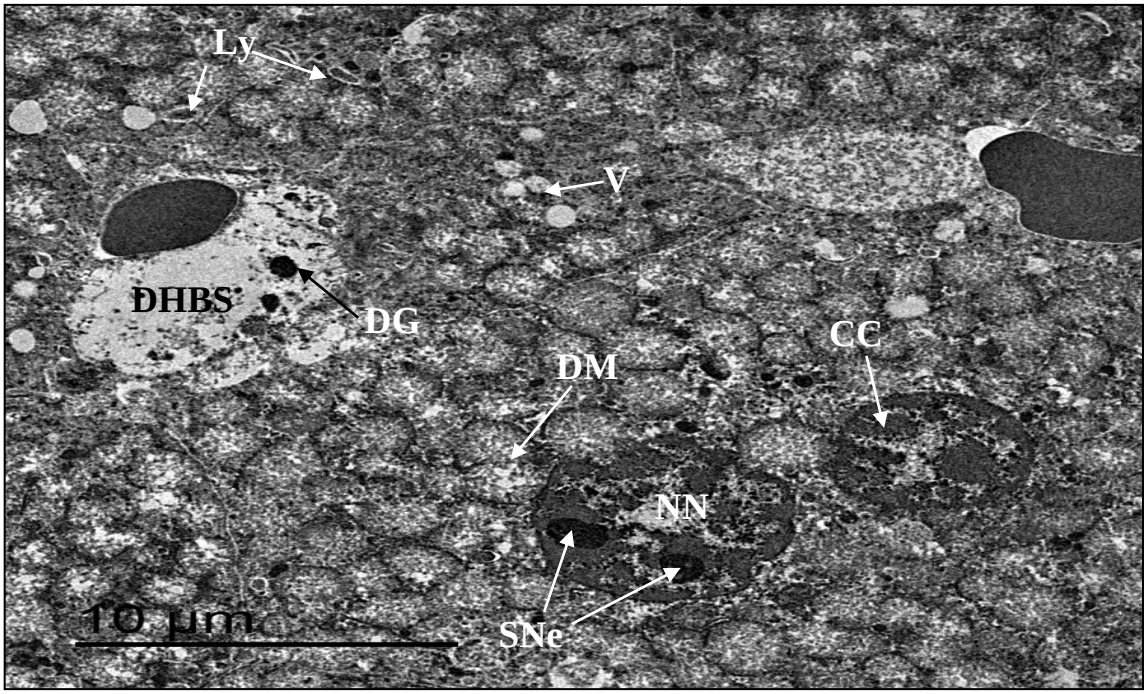

Fig. 5: TEM photomicrograph of a liver cell from a wild rat treated with magnetized water for a month (30 days) showing necrotic nuclei (NN) with segregated nucleolus (SNe) and condensed chromatin (CC), dilated hepatic blood sinusoid (DHBS), great number of lysosomes (Ly), degenerated mitochondria (DM), vacuolation of the cytoplasm (V) and dark granules (DG) (X 10141).

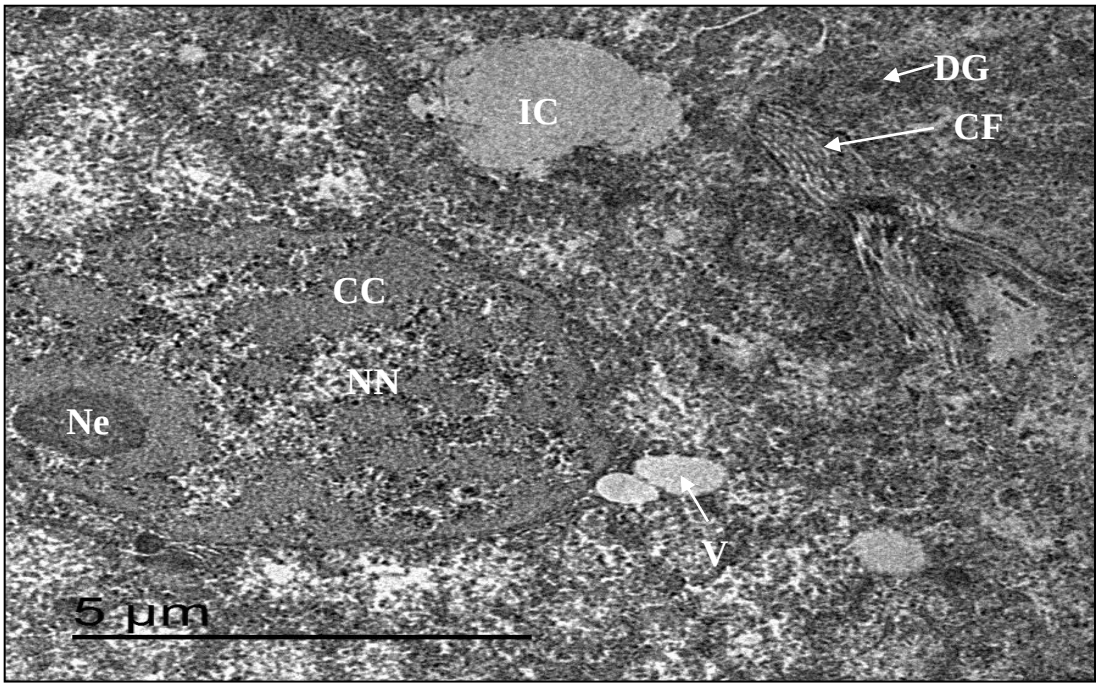

Fig. 6: TEM photomicrograph of a liver cell of a treated wild rat with magnetized water for a month (30 days) showing necrotic elongated nuclei (NN) with condensed chromatin (CC) and large nucleoli (Ne), degeneration of the cytoplasmic organelles (DO), dilated inflammatory cell (IC) and vacuolation of the cytoplasm (V), dark granules (DG) and marked deposition of collagen fibres (CF) in portal area (X10142).

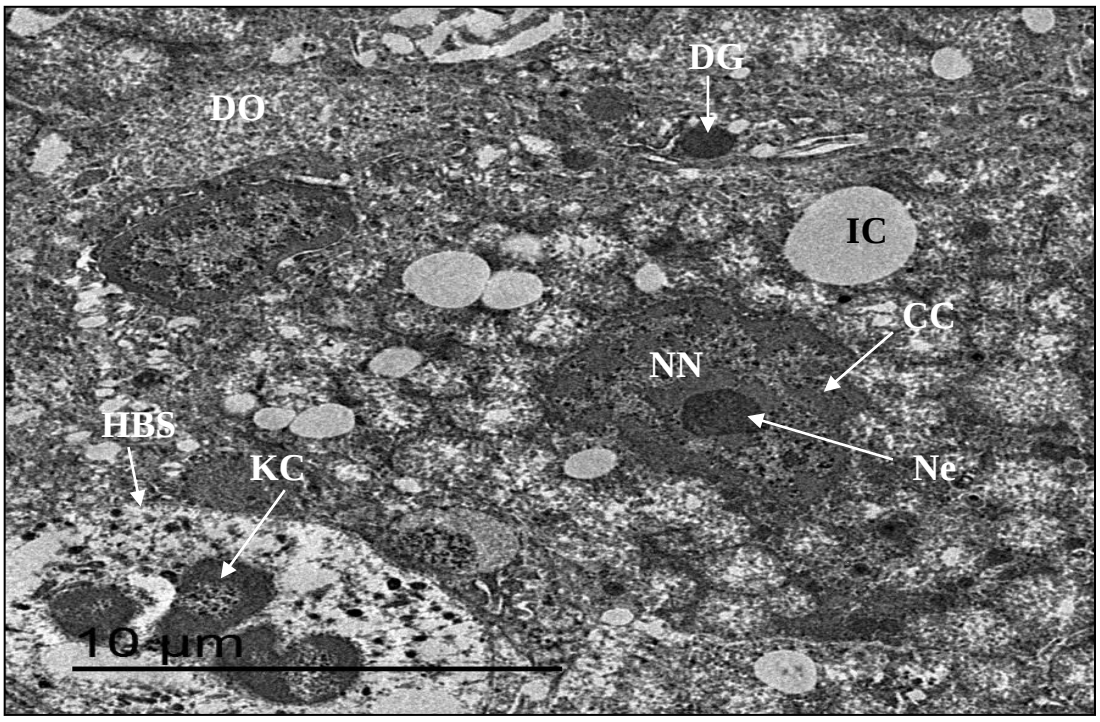

Fig. 7: TEM photomicrograph of a liver cell of a treated wild rat with magnetized water for a month (30 days) showing necrotic nuclei (NN) with condensed chromatin (CC) and large nucleoli (Ne), degeneration of the cytoplasmic organelles (DO), dilated inflammatory cell (IC), dilated blood sinusoid (DBS) with phagocytic kupffer cells (KC) and dark granules (DG) (X10139).

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ARABIC SUMMERY

التأثيرات البيئيه السميه للإشعاع الكهرومغناطيسي على الجرذ البري
Rattus norvegicus norvegicus

زينب زهرى كامل خاطر[1] - هند شكري غريب [2] – منى حسين ابراهيم [3]
1- قسم علم الحيوان - كلية العلوم - جامعة الزقازيق - مصر
2- معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – جيزة – مصر
3- قسم الفيزياء - كلية العلوم - جامعة الزقازيق - مصر

تم إجراء هذه الدراسه بهدف إختبار التأثير المميت للإشعاع الكهرومغناطيسي عند الترددات 50 و 100 و 200 و 250 هرتز ضد الجرذ البري *Rattus norvegicus norvegicus* من خلال تناوله للمياه التي تعرضت لهذه الترددات لفتره تعرض أربعه ساعات. تناولت الدراسه أيضا إختبار تأثير التردد تحت المميت على الخصائص الفيزيائيه و الكيميائيه للمياه التي تناولتها الجرذان و أيضا على عدد المستعمرات البكتيريه في هذه المياه كما تم أيضا دراسه تأثير هذا التردد على أنسجه الكبد للجرذان. أوضحت النتائج أن بزياده تردد الإشعاع الكهرومغناطيسي إزدادت نسبه الموت لتصل إلى 80٪ عند أعلى تردد 250 هرتز بعد شهر من التجربه بينما لم يحقق أقل تردد 50 هرتز أي موت للجرذان خلال هذه الفتره. تسبب التردد تحت المميت للإشعاع الكهرومغناطيسي في زيادة درجة الحرارة وانخفاض المواد الكليه الذائبة بالمياه ولم يحدث أي تغيير في قيمة الرقم الهيدروجيني والملوحة والكلور والقلوية. تسبب هذا التردد أيضا في اختزال عدد مستعمرات بكتريا *Escherichia coli* و *Serratia marcescens* في الماء بشكل كبير كما أنه منع تماما نمو *Klebsiella pneumoniae* و *Pseudomonas fluorescens* و *Staphylococcus aureus* و أيضا *Staphylococcus epidermidis* مقارنه بعددها في المياه العاديه غير المعرضه لأي تردد. كما أدى هذا التردد إلى تدمير خلايا أنسجة الكبد للفئران المعامله.

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