Effect the Microwave X-bandUsing Frequencies with (8.5),(9.5)and (10.5)GHz on Liver Enzymes in AlbinoRats

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Abstract

Background: The growing usage of mobile phones in daily life, combined with the growing negative effects of electromagnetic radiation (EMR) emitted by mobile phones on some physiological systems, has raised worries about their impact and complications on human health, therefore, this work was designed to study the effects ofexposure tothe microwave X-band usingfrequencies with (8.5),(9.5)and (10.5)GHz on liver enzymes(ALT, AST) and histologic changes of liver tissuein experimentalAlbinoRats.**Methods**:Total (20) healthy male rats were randomly divided into two groups (control and radiated) groups as following: control group (5rats)without exposure to MW, and exposure groupwhich subdivided into three subgroups(5 in each group)exposed to (8.5GHz,9.5GHz,10.5GHz)with X-band for(3 h/d for 14 d)consecutively. **Results and discussion:**After one month from the date of exposure to the microwave, the enzymes AST, ALT were tested and statistically analyzed.It was noted from the results that the enzyme AST at the frequency (9.5)GHz is higher than its rates in the control group and other groups of frequencies,As for the enzyme ALT, it decreased at the frequency(10.5) GHz of the control group and the other frequencies,

KeyWords: Liver Enzymes,x-band,Biological Materials,Liver.

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Introduction

Understanding the Microwave Frequencies range from 0.3 to 300 GHz, while wavelengths range from one meter to one millimeter. Earlier this year, Over the past several decades, there has been an increase in the number of devices that emit microwaves. These devices are largely used in telecommunications, but they are also used in other areas of society including in residences. The public is becoming concerned about the potential effects of microwave radiation to people. The most common public fear has been that microwaves may cause cancer. Some encouraging results for leukemia and brain tumors have been published in epidemiological research evaluating possible links between MW exposure and an increased risk of cancer, although the data remain equivocal[1].

EMR's biological impacts can be divided into two categories: thermal and non-thermal. The thermal effects are linked to local heat production, similar to how a microwave oven works. The non-thermal process is initiated by a certain quantity of energy absorption, which is not directly related to temperature change but rather to other tissue changes. [2].

EMR may have an impact on biological systems by generating free radicals, which promote lipid peroxidation (LPO), and modifying antioxidant activity, which can contribute to oxidative stress, because of oxidative stress [3].

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The liver is a huge, complicated organ that is well-suited to its job as the primary regulator of glucose, protein, and lipid metabolism, It is the location where metabolic waste products are detoxified via processes such as amino acid deamination, which ultimately leads to the creation of urea. In conjunction with the spleen, it plays a role in the removal of dead red blood cells and the retrieval of the contents of those cells. A blood test may identify and evaluate enzymes produced by the liver. Increased enzyme levels may point to a problem with the liver. When attempting to diagnose liver illness, clinicians examine enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). When AST and ALT are compared, toxins in the liver, as well as liver disease or injury, can be detected[4].

Anincrease in ALT might occur when you have a short-term infection or illness. Long-term increases are particularly hazardous. This is because it could signal an underlying problem that puts your liver at risk.Although a high ALT level may appear to be all that is required to identify liver disease, this is not the case, the connection between ALT and AST can provide important indications as to what is causing the problem. It will also inform you whether the problem is acute (i.e., appearing suddenly and rapidly) or chronic (i.e., occurring over time) (long-standing or persistent) [5].

When dealing with cancer, radiation therapy is often an essential aspect of the therapeutic process. Over 60% of all cancer patients will receive radiation therapy. Radiation therapy kills tumor cells and uninvolved normal cells alike, with the former having obvious advantages (through the elimination of the tumor cells), and the latter having serious drawbacks (by the emergence of side effects). Alterations in hepatic morphology and function have been documented following radiation therapy, challenging the long-held belief that the liver is radioresistant. Most cases of hepatic radiation are inadvertent and occur when the liver is unavoidably included in the treatment portal for lower breast and lung, distal esophageal, gastric, bile duct, pancreatic cancer or lymphoma, and entire body[6].

The exposed to high levels of non-ionizing radiation, sensitive organs might suffer immediate damage. The severity of this effect is largely a function of the radiation's intensity, frequency, and duration [7].

The aim of the recent study is to evaluate the effect different doses of microwaves on some biochemical parameters (liver enzymes)and the histologic changes of liver tissuein laboratory rats exposed to x-band microwaves at various frequencies.

Materials and Methods

The present study was done on (20) healthy adult male albino rats, age (12-14) weeks with average weight (240 _ 250) g.The animals were housed in plastic cages with mesh lids (20 * 30 * 40) cm, The animals were brought from the Colleges of VeterinaryMedicine, and animalhusbandry centers,then transferred to the animal house / College of Science /University of Basra**.**The cages are furnished with sawdust, taking in consideration that they are replaced every week and taking care of the cleanliness and sterilization of the cages, with an adequatetemperature (25-30)°**[12].**

Themicrowave radiation:Rats were exposed to microwave radiation at frequencies (8.5, 9.5, 10.5) GHz by the X-band,the rats divided into four subgroups within each frequency, in addition to the control group.

1. Sacrificed the Experimental Rats

All experimental rats were anesthetized, blood was drawn from the heart and liver from each rat was excised for histological slicing and histological comparison, the rats were sacrificed after one month and samples collected as follows.

Collection of Blood

Rats in all experimental groups were sacrificed at the end of each periods after anesthesia with chloroform, suitable cut in the rat abdomen was done, the samples of blood were collectedfrom the heart by heart puncture with use of disposable syringes of (3-5)cc capacity, blood allowed to clot,through leaving it at room temperature for(15-30) min, (3 to 4)milliliters of blood were put into jelly test tubes without anticoagulant and centrifuged at 3500 rpm for 10 minutes to extract blood serum, which was then transferred into tubes and kept at -20°C until utilized for the testing of liver enzymes (ALT, AST).

1. Tissue samples: Specimens from livers of rats in each experimental group was dissected post (1month), washed with normal saline to removed blood and tissue debris then, cut into suitable size of specimens and transferred to fixed with

(10%) formalin fixative to processing for histological examination, suitable stained section examine to clarified the histologic changes[13].

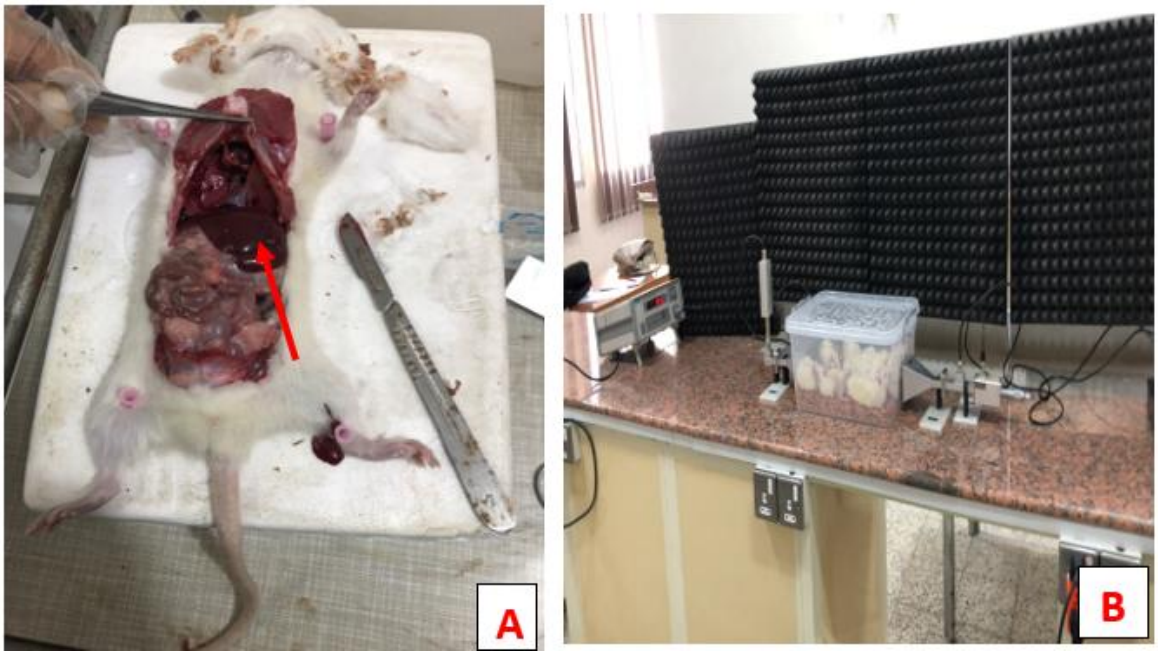

Fig. 1. A-Image of rat to excised liver(red arrow), B- Microwave irradiation system

2. Biochemical Study

Serum Aspartate Aminotransferase (AST) Estimation (IU)

This estimations were done by using the colorimetric determinations of aspartate amino transferase activity (Randox,UK).

Principle: Aspartate aminotransfe is measureds by monetoring the concentration of oxaloacetates hydrazine formed with 2,4-dinitrophenyl-hydrazine [10].

α- oxoglutarite + L-aspartate AST ⟶ L -glutamates + Oxaloacetates

Table 1. Measurement Liver Enzyme (AST)

Reagents	**Reagent blank**	**Sample**
Sample	……	0.1ml
Buffer	0.5ml	0.5ml
Distilled water	0.5ml	……
Test tubes Mixed and incubatedfor exactly 30min at 37ºC		
2,4-DNP(R2)	0.5ml	0.5ml
Test tubes Mixed and allowed to standfor exactly 20min at 20 to25ºC		
Sodium hydroxide(R3)	0.5ml	0.5ml

Test tubes After 5 minutes, the absorbance of the sample (Sample) is compared to the absorbance of the reagent blank (blank) at wave length (546)nm.

Calculations

Activity of AST in the serums can be obtained by plotting the absorbance againstthe transaminases activities.

Serum Alanine Aminotransferase (ALT) Estimation (IU)

This estimations was done by using the colorimetric determinations of alanine aminotransferase activity (Randox,UK).

Principle

Alanine aminotrensferase is measured by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-dinitrophenyl-hydrazine [10].

α-oxoglutirate+ L-alenine ALT ⟶ L-glutemate+pyruvate

Procedure

Measurement against reagent blank.

Table 2. Measurement Liver Enzyme (ALT)

Reagents	Reagent blank	Sample
Sample	.....	0.1ml
Buffer	0.5ml	0.5ml
Distilled water	0.1ml	.....
Test tubes Mixed and incubated for exactly 30min at 37ºC		
2,4-DNP(R2)	0.5ml	0.5ml
Test tubes Mixed and allowed to stand for exactly 20min at 20 to25ºC		
Sodium hydroxide(R3)	0.5ml	0.5ml

Results and Discussion

Electromagnetic radiation has several applications, especially medical applications with different frequencies according to the need of each device. Micro frequencies have been used in medical devices such as devices for eradicating tumors and fibrosis from the internal organs of the body, but we do not forget that there are also side effects on some members of the body as a result of exposure to this radiation, which is what is done Highlighting it in this research paper. We noticed from the results that exposure of rats to microwaves caused a significant increase in the values of ALT and AST enzymes, As shown in the tables(3,4) below.

Table 3. Serum ALT (U/ml) in all studied groups

group	Control	8.5 GHz	9.5 GHz	10.5 GHz
Mean	59.25	185.3	213.1	93.81
SD	/	2.124	6.108	2.804
P value	/	<0.0001	<0.0001	<0.0001

Table4. Serum AST (U/ml) in all studied groups

group	control	8.5 GHz	9.5 GHz	10.5 GHz
Mean	41.17	184.6	388.9	189
SD	/	6.25	9.716	2.131
P value	/	<0.0001	<0.0001	<0.0001

The significant increase in serum ALT and AST indicates that there are chemical changes that have occurred as a result of exposure to microwaves, and it is likely that they have caused oxidative stress and the generation of reactive oxygen species, which in turn leads to the production of free radicals and lipid peroxide that damages liver tissues, and this is consistent with Musaa(2009).
The results of the statistical analysis of the studied enzymatic changes of (ALT) shown in the figure (2A) showed that there was a clear significant difference in the first month of the study and for all groups Compared to the control group. Moreoverthe results of the statistical analysis of the

studied enzymatic changes of (AST) shown in figure (2B) showed that there was a clear significant difference in the first month of the study and for all groups.

Recent study clarified the histologic observations in liver sectionsof the negative control ratswhich showed normalhepatic lobules each lobule formed of hepatocytes arranged regularlyaroundthe central vein incenter lobular area,normal hepatic plates separated by sinusoids (fig. 3A,B).

Liver of the(8.5 GHZ) group reveals to normal hepatocytes with nuclei, the hepatocytes arranged radially around central vein and the structure resemble the architecture of normal liver(fig 4), while in comparison to liver sections regarded to(9.5 GHZ microwave treated group which revealsto normal hepatocytes in the center lobular area, normal central vein lining with endothelial layer, mild inflammation in the portal area and mild dilation of sinusoids(fig 5).

Liver sections of the (10.5 GHZ group (after one month) of exposure illustrated normal hepatocytes in the centrilobular area, congestion of the portal vein, mild inflammation in the peri-portal area and moderate infiltration of inflammatory cells(fig 6A,B). Radiation damage is largely produced by the overproduction of ROS, such as superoxide anion (O2), hydroxyl radical (OH), and hydrogen peroxide (H2O2), which reduces antioxidant levels and causes oxidative stress and cellular damage. ROS damage cells by interacting with biological macromolecules such nucleotides in nucleic acids, polyunsaturated fatty acids in cellular membranes, and sulfhydryl bonds in proteins. If the damage is irreversible, it can result in injury, mutagenesis, cancer, accelerated senescence, and cell death[8,11].

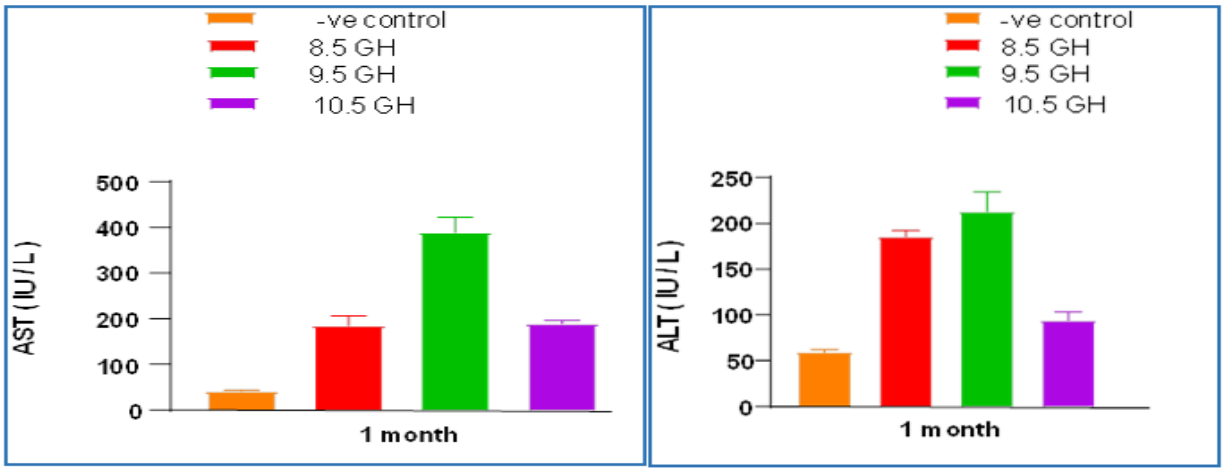

Fig. (2A,B). Illustrated the level of (AST,ALT)in all treated group compared to control

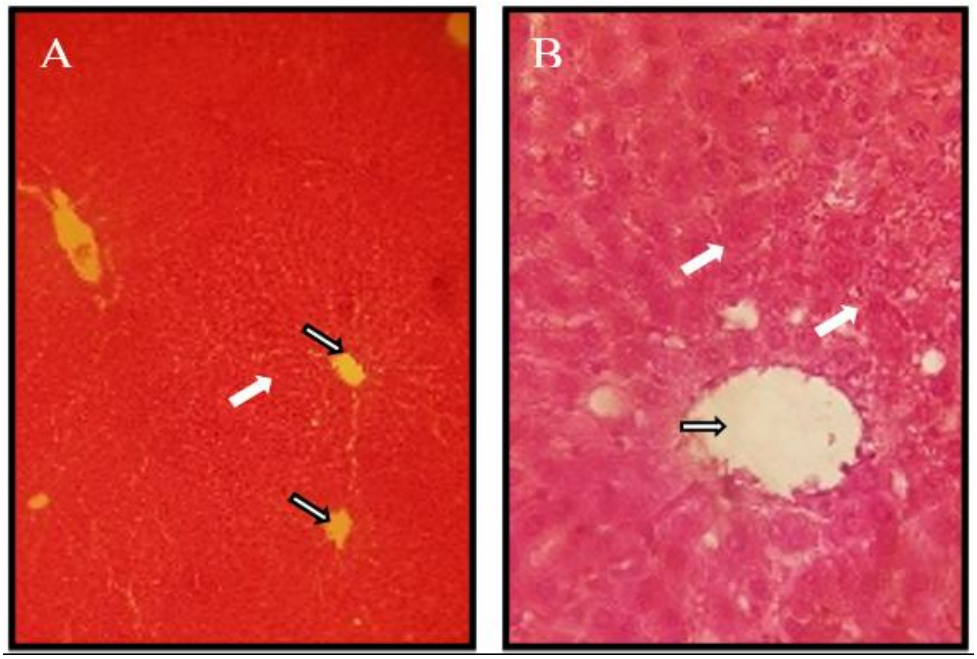

Fig. 3. Liver of the negative control group reveals normal hepatocytes in the centri-lobular area (white arrow), normal central vein (black arrow). H&E, A) 125X, B) 500X

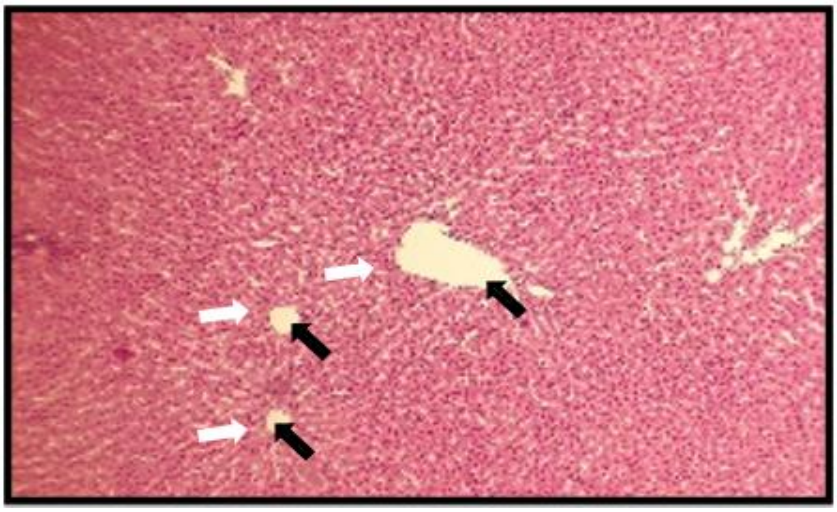

Fig. 4. Liver of the 8.5 GH microwave group reveals normal hepatocytes in the centri-lobular area (white arrow), normal central vein (black arrow). H&E 125X

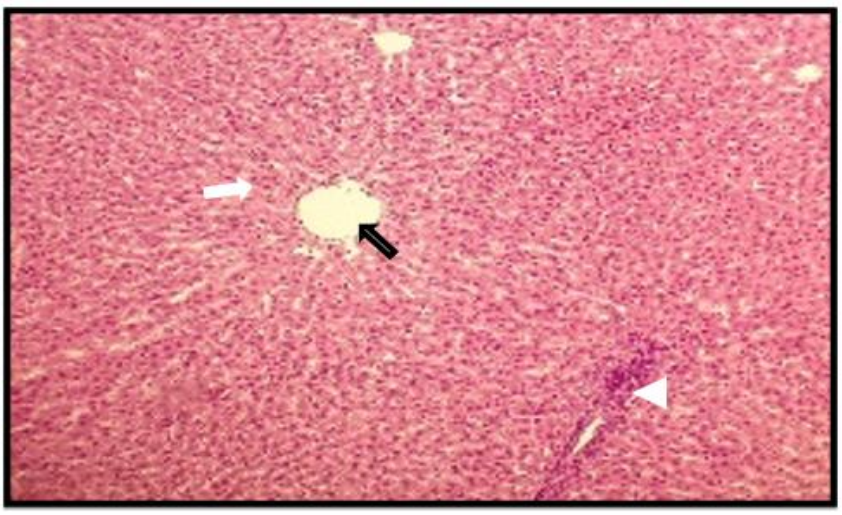

Fig. 5. Liver of the 9.5 GH microwave group reveals normal hepatocytes in the centri-lobular area (white arrow), normal central vein (black arrow), mild inflammation in the peri-portal area (white arrow head). H&E 125X

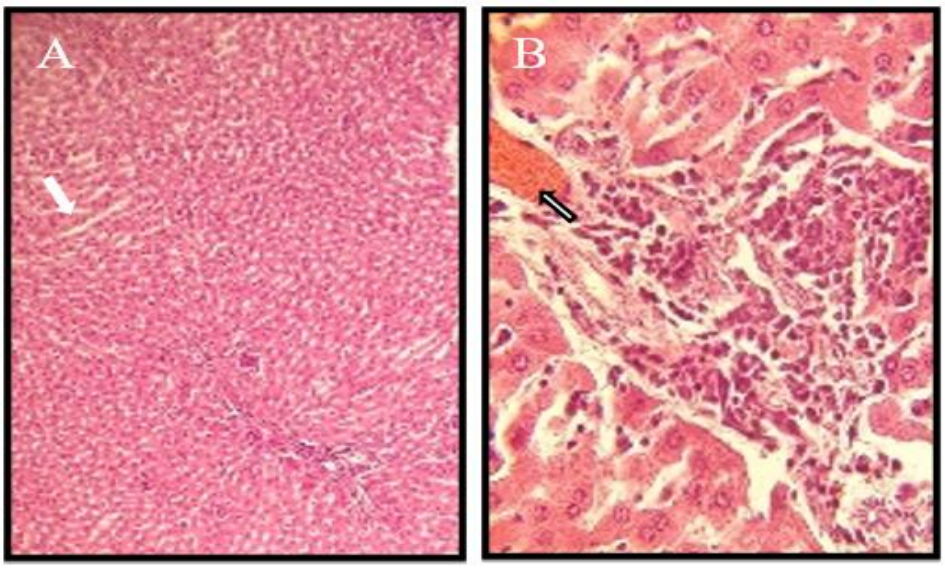

Fig. 6. Liver of the 10.5 GH microwave group (after one month) reveals normal hepatocytes in the centri-lobular area (white arrow), congestion of the portal vein (black arrow), mild inflammation in the peri-portal area (white arrow head). H&E A) 125X B) 500X

Conclusion

Significant increase in liver enzymes (AST, ALT)in all experimental rats exposed to radiation at different doses compared to control, in addition to mild histologic changes in liver tissueafter one month for end of the experiment.

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