



## Effects of radiation leakage of microwave oven on male Swiss albino mice

ElGhazaly, N. A.<sup>1</sup>, Abdel Gawad, H. S.<sup>2</sup>, Radwan, E.H.<sup>3\*</sup>, Barakat, A.<sup>4</sup> and Kamel, K.<sup>5</sup>

E mail: \*Dr\_Eman\_Hashem@yahoo.com

<sup>1,2</sup> Zoology Department, Faculty of Science, Alexandria University, Egypt.

<sup>3\*,4,5</sup> Zoology Department, Faculty of Science, Damanhour University, Egypt.

### Abstract

Continuous microwave radiation leakage from microwave oven may be harmful for users who have high exposure to them. The aim of this work was to assess the effects of microwave leakage on blood, liver, kidney and testis of pre-and post-pubertal male Swiss albino mice. An experimental study was conducted on control and experimental groups from both stages of mice. The experimental groups were exposed to leakage waves from oven three times a day for 30 minutes each. At the end of experiment (8weeks) blood and organs were collected for physiological, biochemical analyses. The results revealed a decrease in the experimental groups Hb and RBCs count ( $p < 0.05$ ) with an increase in WBCs count ( $p > 0.05$ ) compared to their controls. Albumin, bilirubin and protein indicated some changes ( $p > 0.05$ ) meanwhile protein decreased significantly ( $p < 0.05$ ) in post-pubertal experimental group. ALP, ALT and AST values were increased ( $p < 0.001$ ) in both stages. Triglycerides were increased in both stages ( $p < 0.001$ ). Testosterone and LH decreased in the experimental groups than the control ( $p > 0.05$ ). There was a significant decrease in activity of GPx and SOD and an increase in MDA activity among the experimental groups. The present study concluded that the regular use of microwave ovens leads to negative impact upon physiological activities.

**Key word:** Microwave leakage; mice; ALP; testosterone; GPx; SOD.



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## INTRODUCTION

The microwave radiation exposure causes biological effects in living organisms. The increase usage of microwave radiation equipment at home and industry makes adverse concern about the effect of microwave leakage on biological systems. The most frequency commonly used in domestic and industrial food preparation is 2.45 GHz microwave radiation. The radiation Leakage from improperly maintained ovens is a source of environmental pollution and may make a risk on human health (Parker et al., 2010). The enormous amount of energy going into the food molecules from microwave radiation is sufficient to break protein molecules. The molecular structure of the food is changed furthermore, the producing molecules unnatural in the body and consider being carcinogenic substance (Lita lee, 2001). Parker et al. (2010) recorded an elevation in some haematological parameters as white blood cells and hematocrit after exposure to electromagnetic waves.

The exposure to electromagnetic field has a deleterious on the haematological parameters; this effect is more severe in immature animals (Moussa, 2009). The experimental studies on animals which were affected by electromagnetic waves showing a wide range of damage which occurred on the testicular function, the exposure to microwave radiation leads to infertility in male mice (Davoudi et al., 2002). Testes are active body organ which affect by microwave radiation as well as the change which occurred in the concentration of testosterone and luteinizing hormones (Roosli et al., 2007). Reactive oxygen species (ROS) which produced from the process known as oxidative stress resulting from many environmental factors as the use of cell phones have adverse effect on the quality of semen which contributes on male infertility. Furthermore, imbalance in spermatozoa cell cycle, gonadal dysfunctions and poor sperm motility (Agarwal et al., 2008).

There were many changes occurred in the levels of the antioxidant enzymes as superoxide dismutase (SOD) and glutathione peroxidase (GPx) which play a major role in protecting the cells by removal the free radical which were generated by electromagnetic radiation (Kesari et al., 2011). The widespread leakage of microwave radiation gave public and scientific discussion about the possible health effect on organs as liver and testes according to the interaction between the electromagnetic radiation and the vital organs (Jauchem, 2008). The present study aimed to evaluate the effects of the leakage of microwave radiation on some physiological parameters as well as histological examination for liver and testes. These evaluations were done on male Swiss albino mice (pre (one month) and pre (three months)-pubertal ages). The microwave ovens have effects on people who ingest the microwaved foods and also, for their users. The oven door is the most dangerous place for microwave leakage as well as magnetic fields can be present around the oven (IEEE, 2002). The health effects of microwave radiation which can be induced in the tissue dependent on the condition of the exposure (Kundi et al., 2004).

There are two types of exposure according to the time of exposure, the first is short-term (acute-exposure which has exposure time lower than 4 months) and the second ones is prolonged (chronic-exposure which has exposure time rather than 4 months) (Grigoriev, 2004). The dose of radiation is the accumulated absorbed energy measured in radiobiology multiplied by the exposure time (Grigoriev et al., 2005). Junhua et al. (2013) analyzed the blood serum components of experimental rats which had being exposed to microwave radiation leakage of microwave heated food and found that, there were significant changes in the blood content, causes a broken in red blood cell and change in blood circulation. The decrease of ALP activity in vascular smooth muscle cells inhibits artery calcification (Negrao et al., 2006). The increase in ALP concentration is associated with clinical conditions (Sattar et al., 2004). The elevation of serum (ALT) activity in blood is widely used as a marker for tissue damage after irradiated by electromagnetic waves (Su et al., 2006). Moussa, (2009) studied the effect of microwave radiation on serum transaminases (AST& ALT) and alkaline phosphatase (ALP) of Swiss albino mice that exhibited significant increase in the liver enzymes after comparing with control mice.

High blood concentrations of uric acid lead to gout as well as kidney stones (Ford et al., 2007). Elevated urea levels may be used as an indicator of dehydration, starvation or shock. Urea levels below the normal physiological range indicate over hydration, malnutrition and liver injury/disease. Adaptation may also occur in response to increased or decreased urea concentrations within physiological range of homeostasis (Ortolani et al., 2000). The urea formed in the body from protein and amino acid catabolism, is eliminated via the urinary system and accounts for about half of the total urinary salts (Nomura et al., 2006). A rise in blood creatinine level is observed with marked damage in nephrons function. Therefore, the elevation of creatinine concentration is indicator to kidney disease (Chen et al., 2006). There are adverse effects on the biological system as kidney function by increasing the creatinine and urea concentrations of the mice after exposure to microwave radiation (Moussa, 2009). Oktem et al. (2005) examined the damage which occurred in the kidney of mice after inducing the tissue by 900 MHz mobile phone radiation. The level of creatinine is a significant marker of renal function in cirrhosis. An important finding is that the serum of creatinine is an independent predictor for early mortality in patients who had being exposed to long-term electromagnetic waves (Chen et al., 2006).

Testosterone is an androgen sex steroid hormone which made from cholesterol. Testosterone has two different kinds of effects, the first effect is anabolic this effect causes growth of muscle and bone. The second ones is androgenic these effects are responsible for male characteristics (Desai et al., 2011). The reduction in testosterone hormone makes the pituitary gland does not produce normal amounts of some or all of its hormones. Increased testosterone levels may mean cancer in testes and androgen resistance (Cheah and Yang, 2011). De-Luliis et al. (2009) studied human sperm obtained from 2110 patients attending clinics from 1993 to 2007. Semen analysis was performed in all patients. Serum free testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH) and found that patients using cell phone; showed a significant higher T and lower LH levels than those who did not use a cell phone. Trosic et al. (2002) studied the effect of cell phone frequency 900 MHz radiofrequency radiation on rabbits. Some hormonal and biochemical parameters were investigated as LH which was any significant difference when comparing with control groups. Gandhi and Anita, (2007) studied the effect of mobile phone on lipid profile and found that there were some changes in the concentrations of



some parameters as cholesterol and triglycerides. The exposure of electromagnetic field from (0-300 GHz) makes increase in low density lipoprotein, high density lipoprotein, triglyceride, and total cholesterol (NRPB, 2004). The decrease of GPx concentration depends on the exposure to oxidative stress (Perricone et al., 2009).

The production of spermatozoa is called spermatogenesis, a process that includes cell division through mitosis and meiosis and the final differentiation of spermatozooids, which is called spermiogenesis (Terra et al., 2009). Forgacs et al. (2006) studied the effect of 1800 MHz microwave radiation on testicular morphology of mice and revealed that there were many alternations in histological examination in the Sertoli cells as well as abnormality in the morphology of the sperm.

## MATERIALS AND METHODS

The present study deals with the investigation of the impact of exposure to radiation leakage from microwave oven (2.45 GHz). The experimental animal of this study is male Swiss Albino mice were used with body weight ranged from  $17.7 \pm 0.8$ g (prepubertal animals, age 1 month) to  $35.5 \pm 0.7$ g (pubertal animal, age 3 month) were obtained from the animal house of faculty of Medicine-Alexandria University. Animals were housed under optimal environmental conditions, water and natural food with available to the mice ad libitum. The used microwave oven in the present study has model number NGM-123E, power in 1400 W, power out 900W, 23 liters, weight 13.6Kg and radiation leakage of microwave oven was measured by (2.45 GHz meter for microwave). Radiation leakage was measured by Microwave oven meter.

A-The first group is (Control groups):-Animals in this group reared in normal condition and fed on natural food without exposed to any microwave radiation. This group contains two subgroups according to the maturation of animals:-

A-1.Pre-pubertal subgroup:- Numbers of animals in these group ten animals, average weight ( $17.7 \pm 0.8$ g) and fed on natural food without exposed to any microwave radiation.

A-2.Post-pubertal subgroup:-Animals in this group are ten animals, average weight ( $27.3 \pm 0.7$ g) and fed on natural food with any exposed to microwave radiation leakage.

B-The second group is (Experimental groups):-This group was investigating the effect of exposure of the experimental animals to microwave radiation leakage from the door of microwave oven.

The experimental group was divided into two subgroups according to the maturation of animals.

B.1-Pre-pubertal subgroup:-Animals in this group aged one month, average weight ( $17.7 \pm 0.8$ g) and exposed to microwave radiation leakage from the door of microwave oven three times daily, each time thirty minutes for eight weeks.

B.2-Post-pubertal subgroup:-Animals in this group aged three months (number=10), average weight ( $27.3 \pm 0.7$ g) and were exposed to microwave radiation leakage three times daily, each time thirty minutes for eight weeks.

-Determination of mortality rate and survival rate:- $(\text{Total number of animals} - \text{number of survived animal}) \times 100 / \text{total number of animals}$ .

$(\text{Total number of animals} - \text{number of dead animal}) \times 100 / \text{total number of animals}$ .

-physiological studies:-Haematological measurements according to Gartner (1980) method; this study was terminated twenty four hours after last dosing. Mice from the control and experimental groups were sacrificed by slaughtering, and the blood samples were collected from the neck blood vessels into clean, dry, sterile container containing EDTA (1 mg/ml fresh blood). Anticoagulated blood samples were used for the determination of erythrocytic count, leukocytic count, haemoglobin content, and haematocrit value. All the measurements were examined within two hours after blood collection

**-Biochemical assessments:-**Estimation of the liver function and some marker enzymes; in this part of study; albumin by colorimetric method of Tietz (1990): bilirubin, total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated after exposing mice to microwave radiation for 8 weeks.

-The estimation of kidney function:-Uric acid (enzymatic colorimetric method of Tietz, 1990), urea (enzymatic colorimetric method of Marini et al., 2006) and creatinine (kinetic method of Henry, 1974) were estimated.

-The estimation of testis function:-The hormonal levels of testosterone (enzyme-linked immunosorbent assay (ELISA) of Zirkin and Chen, 2000) and luteinizing hormone (quantitative ELISA method of Shioya and Wakabayashi, 1998) were determined in the experimental groups after exposed to microwave radiation for 8 weeks.

-Estimation of lipid profile:-Lipid profile as cholesterol (quantitative enzymatic colorimetric method of Cohn et al., 1988) and triglycerides (enzymatic colorimetric of Kaplan, 1984) were determined in the experimental groups.

-Estimation of antioxidant enzymes in the liver tissue:- Estimation of Glutathione peroxidase (GPx) by UV method of Ellman's method (1958), malondialdehyde (MDA) by colorimetric method of Drury et al. (1997) and superoxide dismutase (SOD) by colorimetric method of Verma et al. (2010). Estimation of total protein by Lowry's method (1951).

- Histological studies:-Histological examination on liver and testes were carried out according to (Culling, 1974) in order to investigate the histo-pathological effect of the radiation leakage on these organs.

-Statistical studies:- The results were analyzed using SPSS program -10 package (release 3, SPSS Inc., Chicago III) to evaluate the significance of the difference between mean value of the measured parameters in experimental groups and control groups, all the data were expressed by (mean value  $\pm$  standard deviation).



## RESULTS

In the serum of experimental animals haematological measurements and biochemical studies were established to determine the concentration of liver enzymes, renal function (urea, uric acid and creatinine) and hormonal level of testis (testosterone and LH) as well as antioxidant stress on liver tissue. The mean value of Hb of experimental group with age 1 month was  $10.78 \pm 1.2$  g/dl and in 3 months old was  $11.02 \pm 0.92$  g/dl. In the control groups (pre and post-pubertal stages) were  $13.46 \pm 0.68$  g/dl and  $12.54 \pm 0.52$  g/dl respectively. From the above cited data it was noticed that there was a significant decrease ( $p \leq 0.05$ ) in Hb content (g/dl) after exposure to microwave radiation leakage (table, 1).

The data of haematocrit value (%) which recorded in table (1) showed that the values of control groups with age 1 and 3 months were  $43.2 \pm 1.3\%$  and  $42.8 \pm 3.7\%$ , respectively. After exposure to 2.45 GHz from microwave radiation for 8 weeks the Ht for experimental groups was decrease to  $34.4 \pm 3.36\%$  in pre-pubertal stage and  $34.4 \pm 2.79\%$  for post-pubertal stage. From the previous data it was concluded that there was a highly significant decrease in two subgroups ( $p \leq 0.001$ ). The data of RBCs count were recorded in table (1) showed that the red blood cell count was decreased in exposed groups after 8 weeks (experiment period). In pre-pubertal stage there was a significant decreases in mean values and reached to  $3.94 \pm 0.35 \times 10^{12}/L$  ( $p \leq 0.05$ ). While in post-pubertal stage it showed that there was insignificant changes ( $p \geq 0.05$ ) between their values  $3.9 \pm 0.29 \times 10^{12}/L$  and the mean value of control groups with age 1 and 3 months  $4.68 \pm 0.31 \times 10^{12}/L$  and  $4.02 \pm 0.48 \times 10^{12}/L$ , respectively.

After 8 weeks for exposure the experimental groups to microwave radiation the mean values of white blood cells for both ages had the same value  $1.56 \pm 0.2 \times 10^9/L$  which was higher than (insignificant difference,  $p \geq 0.05$ ) the mean values of the control groups ( $1.41 \pm 0.9 \times 10^9/L$  and  $1.30 \pm 0.6 \times 10^9/L$ ) for pre and post-pubertal stage, respectively (Table, 1).

**Table (1):-Complete blood count (CBC) in male mice after exposed to microwave radiation leakage for 8 weeks.**

Groups	Age	Hb (g/dl)	RBC ( $\times 10^{12}/L$ )	Ht (%)	WBCs ( $\times 10^9/L$ )
Control Groups	Pre-pubertal stage Mean $\pm$ S.D	$13.46 \pm 0.68$	$4.68 \pm 0.31$	$43.2 \pm 1.3$	$1.41 \pm 0.9$
	Post-pubertal stage Mean $\pm$ S.D	$12.54 \pm 0.52$	$4.02 \pm 0.48$	$42.8 \pm 3.7$	$1.30 \pm 0.6$
Exposed groups	Pre-pubertal stage Mean $\pm$ S.D	** $10.78 \pm 1.2$	** $3.94 \pm 0.35$	*** $34.4 \pm 3.36$	NS $1.56 \pm 0.2$
	Post-pubertal stage Mean $\pm$ S.D	** $11.02 \pm 0.92$	NS $3.9 \pm 0.29$	*** $34.4 \pm 2.79$	NS $1.56 \pm 0.2$

Statistical analysis: (\*\*) significant ( $p \leq 0.05$ ). (\*\*\*) highly significant ( $p \leq 0.001$ ) (NS) insignificant changes ( $p \geq 0.05$ ).

### Determination of liver function and marker enzymes:

The mean values of albumin levels in the control groups were  $2.44 \pm 0.29$  g/dl and  $3.2 \pm 0.47$  g/dl for pre and post-pubertal stages, respectively (Table, 9). It was observed these data were more than control groups as follow:  $2.76 \pm 0.46$  g/dl for pre-pubertal stage as well as  $3.66 \pm 0.38$  g/dl for post-pubertal ones. In table (2) the recorded data of bilirubin concentrations for both pre ( $0.334 \pm 0.11$  mg/dl) and post ( $0.278 \pm 0.10$  mg/dl) -pubertal stages of experimental groups showed that insignificant difference ( $p \geq 0.05$ ) between the experimental and control groups ( $0.244 \pm 0.09$  mg/dl and  $0.242 \pm 0.09$  mg/dl) for the pre and the post-pubertal stages of control groups. It was noticed that there was an increase in bilirubin levels of irradiated groups.

The protein concentration in the serum of exposed mice in table (2) it had a decrease value in the pre and post-pubertal stages as comparing with control groups. The protein levels in pre-pubertal stages were  $6.12 \pm 0.46$  g/dl and  $5.50.67$  g/dl for control and exposed groups, respectively. In post-pubertal stage of control group, protein level was  $6.42 \pm 0.56$  g/dl, after 8 weeks for exposure to microwave radiation leakage (period of experiment) protein concentration attained to  $5.22 \pm 0.60$  g/dl. The concentrations of ALP showed a highly significant increase ( $p \leq 0.001$ ) in experimental groups which exposed to microwave radiation for 8 weeks when compared to that of the control groups. In pre-pubertal stage (1 month old) of the control group the mean value of ALP concentration was  $64.8 \pm 3.70$  U/L while for post-pubertal stage (3 months old) was  $74.8 \pm 5.44$  U/L. In exposed groups for microwave radiation leakage the mean values of ALP concentrations which recorded in table (3) were  $137.6 \pm 5.50$  U/L and  $139 \pm 2.34$  U/L for pre and post-pubertal stages, respectively.

The mean values of ALT concentration (Table, 3) showed that there were highly significant increases ( $p \leq 0.001$ ) in ALT concentration of exposed groups which irradiated by 2.45 GHz microwave radiation leakage as compared to the data of the control groups. In pre-pubertal stages the ALT concentrations were  $73.6 \pm 4.03$  U/L and  $123.2 \pm 4.91$  U/L for the control



and exposed groups, respectively. The mean data of the ALT concentrations of the post-pubertal stages were 71.8±3.49 U/L and 145±10.51 U/L for the control and exposed groups, respectively. The data of both pre and post-pubertal stages of experimental groups which exposed to 2.45 GHz were recorded in table (3), shows that there were highly significant differences (p≤0.001) in AST concentration as comparing with control groups as following: 117.8±4.1 U/L and 232.4±8.96 U/L for pre-pubertal stages of control and exposed groups, 123.8±4.76 U/L and 234.4±3.64 U/L for post-pubertal stages of control and exposed groups, respectively.

**Table (2):-Determination of albumin, bilirubin and protein serum of mice after exposure to microwave radiation leakage for 8 weeks.**

Liver function				
Groups	Age	Albumin (g/dl)	Bilirubin(mg/dl)	Protein (g/dl)
Control group	Pre-pubertal stage Mean ± S.D	2.44±0.29	0.244±0.09	6.12±0.46
	Post-pubertal stage Mean ± S.D	3.2±0.47	0.242±0.09	6.42±0.56
Exposed groups	Pre-pubertal stage Mean ± S.D	2.76±0.46	0.334±0.11	5.5 ±0.67
	Post-pubertal stage Mean ± S.D	3.66±0.38	0.278±0.108	** 5.22±0.60

Statistical analysis:-(\*\*) significant (p≤0.05).

**Table (3):-Determination of liver marker enzyme in serum of mice after exposure to microwave radiation leakage after 8 weeks.**

Liver marker enzyme				
Groups	Age	ALP(U/L)	ALT(U/L)	AST(U/L)
Control Groups	Pre-pubertal stage Mean ± S.D	64.8±3.70	73.6±4.03	117.8±4.1
	Post-pubertal stage Mean ± S.D	74.8±5.44	71.8±3.49	123.8±4.76
Exposed Groups	Pre-pubertal stage Mean ± S.D	*** 137.6±5.5	*** 123.2±4.91	*** 232.4±8.96
	Mean ± S.D Post-pubertal stage	*** 139 ±2.3	*** 234.4±3.64	*** 234.4±3.64

Statistical analysis:(\*\*) significant (p≤0.05).(\*\*\*) highly significant (p≤0.001).

### .Renal function

It is clear from table (4), that the concentration of uric acid shows an increase in pre- and post-pubertal stages of experimental mice as compared to the control groups. The mean values of control groups were 4.74±0.98 mg/d and 4.06±0.45 mg/dl for the pre and post-pubertal stages, respectively while the data raised in experimental groups and mg/dl 4.92±0.4 for pre-pubertal stage and 6.68±0.8 mg/dl (highly significant change, p≤0.001) for post-pubertal stage.



The urea concentration of the experimental groups with age 1 and 3 months old were increased when compared with control mice. In control groups the mean values of urea concentrations were 36.2±5.76 mg/dl for pre-pubertal stage and 33.8±3.7 mg/dl for post-pubertal stage. The mean values of exposed group were 47.8±11.21 mg/dl and 40.8±4.32 mg/dl for pre and post-pubertal stages, respectively (Table, 4). The creatinine concentrations in both pre and post-pubertal stages of exposed group were 0.50±0.15 mg/dl and 0.53±0.11 mg/dl, respectively. As well as in control group with age 1 month the mean value was 0.31±0.08 mg/dl and for 3 months old was 0.38±0.05 mg/dl. From the above data, it was noticed that, there was an elevation in creatinine levels of exposed groups more than the values in the control mice (Table,4).

**Table (4):-Determination of renal function in the serum of mice exposed to microwave radiation leakage for 8 weeks:**

Renal function				
Groups	Age	Uric acid(mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control groups	Pre-pubertal stage Mean ± S.D	4.74±0.98	36.2±5.76	0.31±0.08
	Post-pubertal stage Mean ± S.D	4.06±0.45	33.8±3.7	0.38±0.05
Exposed groups	Pre-pubertal stage Mean ± S.D	4.92±0.4	47.8±11.21	0.50±0.15
	Mean ± S.D	*** 6.68±0.85	40.8±4.32	0.53±0.11

Statistical analysis:(\*\*) significant (p≤0.05).(\*\*\*) highly significant (p≤0.001).

**.Determination of testes function**

In control groups, the mean values of testosterone were 5.6±1.03 ng/ml and 6.94±1.48 ng/ml for pre-and post-pubertal stages, respectively. After 8 weeks (the experimental period) of exposure to microwave radiation the hormone level was decreased to 5.1±0.86 ng/ml for the pre-pubertal stage and 6.74±1.15 ng/ml for post-pubertal stage (Table, 5). Table (5), shows that the mean values of the control groups of pre- and post-pubertal stages were 3.76±1.78 ng/ml and 5.517±2.07 ng/ml, respectively while in the exposed groups which irradiated by 2.45 GHz from microwave oven the LH levels were decreased insignificantly and attained to 2.98±0.59 ng/ml and 4.21±1.22 ng/ml for pre- and post-pubertal stages, respectively.

**Table (8):-Determination of testis function of mice after exposure to microwave radiation leakage for 8 weeks.**

Testis function			
Groups	Age	Testosterone (ng/ml)	LH (ng/ml)
Control Groups	Pre-pubertal stage Mean ± S.D	5.6±1.03	3.767±1.78
	Post-pubertal stage Mean ± S.D	6.94±1.48	5.517±2.07
Exposed groups	Pre-pubertal stage Mean ± S.D	5.1±0.86	2.98±0.59
	Post-pubertal stage Mean ± S.D	6.74±1.15	4.21±1.22



### .Estimation of lipid profile

The mean value of cholesterol concentration in post-pubertal stage of exposed groups had a significant increase ( $141.2 \pm 9.57$  mg/dl,  $p \leq 0.001$ ) after 8 weeks for exposure to radiation leakage, while in pre-pubertal stage showed insignificant change ( $105.6 \pm 7.7$  mg/dl) when compared to control groups of the same ages ( $96 \pm 6.16$  mg/dl and  $106 \pm 5.97$  mg/dl for pre and post-pubertal stages, respectively) (table,6). There was a highly significant increase difference ( $p \leq 0.001$ ) in irradiated group. The mean values of triglycerides in the pre-pubertal stages were  $136 \pm 6.38$  mg/dl and  $219.6 \pm 5.41$  mg/dl for the control and experimental groups, respectively. Furthermore, in post-pubertal stages the mean values were  $119.2 \pm 3.11$  mg/dl for the control group and  $215.4 \pm 7.76$  mg/dl for exposed group (Table, 6).

Table (6):-**Determination of lipid profile of mice after exposure to radiation leakage from microwave oven for 8 weeks**

Lipid profile			
Groups	Age	CH (mg/dl)	TC (mg/dl)
Control Groups	Pre-pubertal stage Mean $\pm$ S.D	$96 \pm 6.16$	$136 \pm 6.38$
	Post-pubertal stage Mean $\pm$ S.D	$106 \pm 5.97$	$119.2 \pm 3.11$
Exposed Groups	Pre-pubertal stage Mean $\pm$ S.D	$105.6 \pm 7.7$	*** $219.6 \pm 5.41$
	Post-pubertal Stage Mean $\pm$ S.D	** $141.2 \pm 9.57$	*** $215.4 \pm 7.76$

Statistical analysis:(\*\*) significant ( $p \leq 0.05$ ). (\*\*\*) highly significant ( $p \leq 0.001$ )

### .Biochemical markers of oxidative stress in the liver tissue of mice :

As seen in table (7) the experimental groups have significant decreases ( $p \leq 0.05$ ) for pre and post-pubertal stages ( $26.4 \pm 6.61$  nmol/mg tissue,  $28.8 \pm 2.58$  nmol/mg tissue). In control groups, the mean values for GPx were  $44.2 \pm 5.35$  nmol/mg tissue for pre-pubertal stage,  $44.2 \pm 4.96$  nmol/mg tissue for post-pubertal stage. The mean values of MDA concentrations in control groups are  $4.86 \pm 0.66$  nmol/mg tissue and  $4.46 \pm 1.10$  nmol/mg tissue for pre and post-pubertal stages, respectively. In exposed groups of 1 month old the MDA level was  $7.34 \pm 0.95$  nmol/mg tissue and for 3 months old the mean value was  $7.98 \pm 0.88$  nmol/mg tissue. From the above cited data it was noticed that there was significant increase in MDA concentration in experimental group as compared to the control groups which irradiated by 2.45 GHz from microwave oven (Table, 7) .

This parameter showed a highly significant decrease ( $p \leq 0.001$ ) in SOD concentration of irradiated mice when compared to control groups. The mean values of SOD for pre-pubertal stages were  $52.4 \pm 3.34$  nmol/mg tissue and  $36.4 \pm 5.128$  nmol/tissue for control and experimental groups, respectively. In post-pubertal stage of control group the SOD level is  $56.8 \pm 6.22$  nmol/mg tissue while the SOD concentration for exposed group is  $35.6 \pm 7.36$  nmol/mg tissue (Table,7).In the control group, the protein concentration was  $260.6 \pm 3.78$  nmol/mg tissue for pre-pubertal stage and  $244.2 \pm 5.31$  nmol/mg tissue for post-pubertal stage. In irradiated groups which exposed to 2.45 GHz from microwave radiation leakage from oven, the protein levels were  $224 \pm 4.84$  nmol/mg tissue and  $266.6 \pm 4.66$  nmol/mg tissue for pre and post-pubertal stages, respectively (table, 7) it was noticed that there was insignificant difference ( $p \geq 0.05$ ) between two groups.



Table (7):-Determination of oxidative stress of liver tissue of mice after exposed to microwave radiation leakage for 8 weeks.

Oxidative stress					
Groups	Age	GPx(nmol/ mg tissue)	MDA(nmol/ mg tissue)	SOD(nmol/ mg tissue)	Protein(nmol/ mg tissue)
Control Groups	Pre-pubertal stage Mean ± S.D	44.2±5.35	4.86±0.66	52.4±3.34	260.6±3.78
	Post-pubertal stage Mean ± S.D	44.2±4.96	4.46±1.10	56.8±6.22	244.2±5.31
Exposed groups	Pre-pubertal stage Mean ± S.D	** 26.4±6.61	** 7.34±0.95	*** 36.4±5.12	224±4.84
	Post-pubertal stage Mean ± S.D	** 28.8±2.58	** 7.98±0.88	*** 35.6±7.36	266.6±4.66

Statistical analysis:(\*\*) significant ( $p \leq 0.05$ ).(\*\*\*) highly significant ( $p \leq 0.001$ )

### -Histological results

Some organs are selected in this part to estimate the effect of microwave radiation leakage. In control group with both ages (pre and post-pubertal stages) the examination of light micrographs reveals that the structural components of the liver are the liver cells called hepatocytes which are radically arranged in anatomizing and branching plates separated by wide vascular channels known as blood sinusoids. Hepatocytes may contain one nucleus (mononucleated) or two nuclei (binucleated). The blood sinusoid is lined with endothelial cell and contains many triangle kupffer cells. The central vein is lined by endothelium with flattened nuclei as well as hepatocytes are polyhedral in outline and exhibited distinct bounders and all hepatocytes have centrally placed spherical basophilic nuclei with prominent nucleolus. In 3 months age of experimental groups which exposed for 2.45 GHz microwave radiation for 8 weeks, the inspection of liver tissue demonstrated the presence of many pathological effects in liver tissue as cellular infiltration, disappear of normal blood sinusoids and foamy area are present. There are disappear in normal architecture of liver as well as the dilation and congestion of hepatic vessels with increase the number of kupffer cells. From the above cited observation it was reflected the harmful effect of microwave radiation and these results are agreement with the physiological data which recorded in table (5) of liver marker enzymes which recorded an increase in the concentrations. In 5 months age experimental group, there is an increase in binucleated hepatocytes as well as degeneration of some hepatocytes nucleus with cytoplasmic vacuolization.

The testis of control group is surrounded by a thick capsule of connective tissue called the tunica albuginea. The testis consists of a number of seminiferous tubules with complete spermatogenesis process separated by loose connective tissue (interstitial tissue) contained polyhedral cells (Leydig cells). The seminiferous tubule had central lumen filled with spermatozoa and lined with a complex thick stratified epithelium. The spermatogenic lineage occupies the space between the basal lamina and the lumen of the tubule. With higher magnification, each seminiferous tubule contained various developmental stages of germ cells which consists the spermatogenic lineage and supporting Sertoli cells with long extension with prominent nucleolus to which elongated spermatids are directed. These spermatids transformed to spermatozoa through the process of spermiogenesis. In 3 months age of experimental group which irradiated by 2.45 GHz microwave radiation, the light microscope showing deformed and necrotic germinal cells with damage in the basal lamina. Pathological effects were detected with the presence of deformed Sertoli cell with disappearance of its cytoplasmic extensions with accumulation of residual bodies inside the tubular lumen as result of decrease in the number of Sertoli cells which lead to absence of phagosomatic activity. There are some morphological differences between the sizes of seminiferouse tubules in 5 months age of experimental group according to the deformation which occurred in intra tubular vacuolation as result from the exposure to microwave radiation. There was a decrease in number of spermatozoa with sloughing of germinal cells, seminiferous tubules with abnormal contour with epithelial out growth. The germinal lyses and the congestion in intra tubular connective tissue from previous results it was clear that the adverse effect of radiation leakage.





## 5-DISCUSSION

In the present study, some questions will be answered on particularly; physiological parameters which changed according to the exposure to microwave radiation leakage and feeding on microwave heated food. In this part of the research some parameters were determined.

### 5-1-Determination of total body weight of mice:-

The results of the present study show there were an increase in the body weight of all experimental mice but the percentage of body weight gains in subgroups of age 1month (pre-pubertal stage) were higher than those of subgroups with 3 month age (post-pubertal stage). A number of works provide some examples of physiological trails, measurable in the first half of the life span, young stages that can provide significant prognostic information about life expectancy in animals (Miller et al., 2002). Determination of body weight gain in mice of this work disagrees with Lita, (2001) who revealed that there was a decrease in the body weight with life span. In contrast to the observation of this work because there was a positive correlation between body weight and life span across mammalian species. In the present result there was an increase in the body weight in pre-pubertal stage of exposed mice to microwave radiation leakage.

The result of this work assured that there was an increase in percentage of body weight in pre and post-pubertal stages. This study agrees with Kesari et al.(2011) they showed that there was a significant decrease in body weight of exposed animals as comparing with unexposed animals. The results of the present study disagrees with the works of other authors as Cosquer et al. (2005) they found that radiofrequency radiation exposure had no influence on several parameters as the body weight gains. Chaturvedi et al.(2011) stated that there was no significant difference in the body weight of mice which exposed to 2.45 GHz microwave continuous radiation(2hours/day for 30 days) when comparing with control groups as well as chronic exposure to microwave radiation had no effect on the body weight.

Trosic et al.(2002) observed in their experimental animals which exposed to microwave radiation leakage there were a significant body mass reduction when compared to control animals after 20, 40 and 60 days ( $p < 0.001$ ). Abdel Aziz et al.(2010) reported that no significant difference was found in the groups of mice which exposed to microwave radiation with power density (40 mW/cm<sup>2</sup>) and control mice. Also, Kristic et al.(2005) stated that there was neither reduction in the body weight of control groups nor experimental groups which exposed to microwave radiation leakage (2.45 GHz). The number of mice which exposed to microwave radiation leakage from oven door for 8 weeks decreased when comparing with the control groups so; the mortality rate of the experimental groups were higher than the mortality rate of control mice. Grigor'ev, (2003) reported an increase in mortality rate of chick embryos which has exposed to radiation of mobile phone. Nakamura et al.(2003) discussed the impact of this phenomena according to thermal effect of microwave radiation leakage and recorded that there were fetal malformations leads to death in experimental animals. These studies agree with the present research who found that the number of experimental animals in pre-pubertal stage decrease after exposure to microwave radiation leakage. The current study stated that there was an increase in white blood count and this work agrees with Zeni et al.(2003) who found that there were correlation between the increasing white blood cells (WBCs) count and inflammation which predict cardiovascular disease and high mortality rate as well as the association between WBCs count and mortality rate in the oldest age of the animals.

The results of present research who recorded that the mortality rate in post-pubertal stage was higher than pre-pubertal stage and that in agree with the statement of Tiikkainen et al.(2003) who stated that there was an increase in ages there were an increase in the mortality rate. Ono et al.(2004) observed that the exposure to microwave radiation affects on the mortality rate of irradiated animals. Gorlitz et al.(2005) who showed that there is a significant decrease in the number and weight of exposed animal. Turek et al.(2005) who discussed that the reason of increase the mortality rate that there were damage on the blood which may induce increasing morbidity and even mortality. On the other side, the studies of Wannamethee et al.(2005) were in disagreement with the present study because they found that there was no significant effects of the low-level microwave exposure, 2.45 GHz, at a power density of 1 mW/cm<sup>2</sup> and specific absorption rate of 1.2 mW/g, continuous waves (CW) or pulsed waves (PW), 2.5 h/day through 6 weeks and has been studying on the black mice. There was no observation of tumor development in irradiated animals when comparing with control mice. The same result recorded also on the studies of Paulraj and Behari, (2004) who revealed that there were insignificant differences in the mortality rate among control and exposed groups.

Some physiological parameters were estimated in male mice after exposed to microwave radiation for 8 weeks as hematological estimation, liver function and some marker enzymes, renal and testes function, lipid profile and oxidative stress on liver tissue of the experimental mice. Zare et al.(2007) explained that haemoglobin mass is a key factor for maximal exercise capacity. Blood parameters are believed to be the primary particles that come in contact with microwave radiation. Blood ions are reacted with electromagnetic waves as microwave radiation (Usman et al.,2012). Complete blood count (C.B.C.) gives information about the blood cells which conducted to microwave radiation. The mean values of complete blood count in the present study of the experimental mice which were exposed to microwave radiation as well as feeding on microwave heated food for 8 weeks showed that there were decrease in the concentration of haemoglobin, red blood cells, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and platelets while, an increase in white blood cells and mean corpuscular haemoglobin concentration .

The present results are in agreement with some studies which done by Prisco et al.(2008) who investigated that the microwave radiation leakage makes transformation of lymphocytes and abnormalities in the erythrocyte and lymphocyte precursors in bone marrow. Moustafa et al.(2001) reported that long-term exposure to microwave radiation makes



significant decrease in RBCs, HB, PCV, MCV and MCH in experimental group comparing with the control mice. Irradiation by microwave radiation has low intensity ( $p=0.5\mu\text{W}/\text{cm}^2$ ) induce chromosome aberrations and micronuclei increase in human lymphocytes (Trosic et al., 2002).

Black and Heynick, (2003) stated that the exposure to radiofrequency electromagnetic fields can stimulate lymphocytes to become lymphoblasts (active cells undergoing mitosis). Rusnani et al. (2008) exposed the mice to 0.65 GHz microwave radiation and observed that there were decrease in RBCs count the present results are in line with findings of this work where the irradiation by 2.45 GHz microwave radiation causes decrease in RBCs of mice when comparing with control groups. The data of this research are in line with the results of Abdel Aziz et al. (2010) who stated that a significant increase in some blood parameters like WBC and MCHC comparing with control group and a significant decrease in RBC, HB, MCV and MCH. The long exposure to microwave radiation leads to an increase in WBC number (leukemia) furthermore, chronic exposure to microwave radiation alters the blood picture (Chaturvedi et al., 2011). The current study reported that there was decrease in PCV and RBCs while, the results of Forgacs et al. (2006) disagree with the present work because who recorded that when exposed mice to 1.8 GHz there were decrease in RBCs and PCV values. Usman et al. (2012) studied the haematological changes of Swiss albino mice after long-term exposure to microwave radiation and recorded an increase in PCV, RBC and Hb values, while there were a decrease in MCHC and WBC in all the exposed groups and these findings are disagreement with the data of this research. The current study was disagree with Chaturvedi et al. (2011) they said that microwave radiation increased the number of erythrocytes in irradiated mice this observation called polycythemia (increased number of RBC) as well as no significant change in the haemoglobin content. The data of this work detected that there was difference in complete blood count in control groups with experimental groups while the studies of Usman et al. (2012) who was comparing between the CBC of exposed and unexposed mice and found that all the haematological parameters of both groups in normal range.

Liver function and some marker enzymes were performed as albumin, bilirubin, total protein, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Renal function as urea, uric acid and creatinine were taking placed in experimental groups as well as testis hormonal levels of testosterone and luteinizing hormone were estimated and lipid profile was determined. Some antioxidant enzymes as superoxide dismutase, glutathione peroxidase and malondialdehyde were estimated in liver tissue of experimental mice. The liver is responsible for maintenance many homeostatic and physiological functions in animal body. The biochemical activity of some enzymes were maintained the optimal function of liver. Enzymes play a vital role in biological processes and also make cell-cell communication as well as any alternation in the activity or concentration of the enzymes effect on their functions (Moussa, 2009).

In the present study the albumin, bilirubin and protein as liver function were taking placed in two groups the first, the experimental group which exposed to microwave radiation and the second ones is the group which fed on microwave heated food for 8 weeks. All the results of albumin and bilirubin in the two groups showed that an elevation in the levels of two parameters while, the protein concentration was decreased in exposed and feeding groups. Veneman et al. (2004) discussed that the elevation in the levels of albumin and bilirubin according to the damage which be occurred in the liver cells after feeding the mice on irradiated food or exposed to magnetic field. The data of this part of research agrees with the work of other authors as Lohmann et al. (2000) who found that liver enzymes, albumin and bilirubin were significantly increased under the effect of 50 Hz magnetic field. The current results are agree with Moussa, (2009) who stated that there were an increase in albumin and bilirubin concentrations when exposed the mice for 3.5 GHz microwave radiation. There is association between the elevation of liver marker enzymes concentrations as (alanine aminotransferase and aspartate aminotransferase), albumin, bilirubin and protein levels in treated animals (Clark et al., 2003).

The data of present study agrees with Burgert et al. (2006) reported that the elevation in liver enzyme activity as alkaline phosphatase as well as the increase of albumin and bilirubin concentrations induced liver damage for hepatocytes. The protein concentration is decreased in the present study and this result is in agreement with the works of Bohr and Bohr, (2000) studied the effect of electromagnetic waves exposure on protein alternation. There is an increase in biological activity of protein concentrations according to the effect of microwave radiation leakage. The changes in ligand binding properties of cellular proteins can affect their function, calcium is one of such ligands which may alter the formation of protein. The estimation of these enzymes concentrations in the current research recorded elevation in ALP, ALT and AST levels in the exposed group which irradiated by 2.45 GHz microwave radiation which emitted from the oven as well as the experimental group which fed on microwave heated food for 8 weeks. The present study agrees with Pashovkina and Akoev, (2001) who discussed that there was significant increase in ALP concentration according to the alternation in permeability of the cell membrane of hepatocytes of the liver. Thus, the increase in serum ALP activity observed in the experimental groups indicates hepatocellular injury. Dufour et al. (2000) stated that measurements of some liver marker enzymes concentrations in the blood were simple manner to detect the organ dysfunction as well as the damage which occurred in the liver hepatocytes. So, the alternations in ALP, ALT and AST activities are markers, indicators for liver diseases and hepatocytes necrosis. The mice in the present research which were exposed to microwave radiation for 8 weeks has higher concentration of AST than control group and these results are in agreement with Abdel-Aziz et al. (2010) who recorded that the activities of AST were increased significantly after exposure the rats for electromagnetic fields for two weeks. Oh et al. (2006) recorded that there were an association between serum ALT concentration and non-alcoholic fatty liver disease (NAFLD).

The results of this study show that there was significant increase in alanine aminotransferase activity level in experimental mice. These findings are in agree with Shen et al. (2005) who observed an elevation of ALT level and fatty liver diagnosis were independently associated with increased risk of metabolic syndromes (MetS). The current results were disagreed with Dufour et al. (2000) who observed no relationship between the activity of the liver enzymes and the environmental factors as radiation pollution, photoperiod and temperature. In the present study the final body weight of



experimental mice increase from the initial body weight body. The liver is the primary target organ which can be damaged by electromagnetic waves furthermore, increase the levels of albumin, bilirubin, cholesterol and triglycerides as well as an elevation of AST concentration in blood serum of irradiated mice (Finfer et al., 2006). The liver is exposed to toxicity material as arsenic the leakage of hepatic enzymes as AST is commonly used as a direct biochemical index of hepatocellular damage which occurred by toxic substance (Zotti-Martelli et al., 2005). Moussa, (2009) exposed rats to microwave radiation and found elevation in some liver enzymes as well as there was an association between specific liver enzymes, hepatic diseases and the damage of liver cells.

Kidney is a vital organ with several functions. Some biochemical parameters as urea, uric acid and creatinine were chosen for studying of the renal function of mice after exposed to microwave radiation leakage as well as feeding on microwave heated food. In the current study, the concentrations of urea, uric acid and creatinine for pre and post-pubertal stages of experimental mice (exposed groups) were elevated more than the control mice. The data which recorded in this part of the present research are in agreement with the works of Dasdag et al. (2008) who studied the effect of radiofrequency and microwave radiation (420 MHz, 2 GHz) on human and found that there were significant increase in the urea, uric acid and creatinine levels. The electromagnetic fields originate from man-made sources such as microwave oven increase the public concern about their possible adverse health effects. Radiofrequency radiation (RFR) which generated from these devices on oversensitive to the animals which exposed to this radiation (Collins et al., 2008).

Mehta et al. (2007) revealed that the concentration of creatinine is used clinically to detect and evaluate the acute kidney injury and chronic kidney disease. Furthermore, the increase in creatinine level was associated with dramatic increase in mortality rate of human patients. The creatinine concentration is not only an index of liver function but also it is an indicator of early liver diseases. For example, in a patient with a high bilirubin level as 0.3mg/dl, leading to increase in creatinine level and also kidney damage is present (Guney et al., 2007). In the general population, the level of creatinine was used to estimate the glomerular filtration rate (Schwartz et al., 2009). These findings were in line with preliminary results because the concentration of creatinine is measured more than 280 million times annually in the United States, and more than 80% of clinical laboratories now reported an elevation of glomerular filtration rate when the creatinine levels were increased (Stevens et al., 2008). Creatinine produced from muscle metabolism and secreted into the blood serum at a continuous rate then excreted in the urine. This process occurred naturally in normal individuals. The muscle mass does not change in persons so, the elevation of creatinine indicates the increase in the glomerular filtration rate which already present. When the serum creatinine concentration in steady state the generation of creatinine equal creatinine excretion rate (Matsushita et al., 2010).

In the current study there was an increase in the mortality rate of the experimental mice which irradiated by 2.45 GHz microwave radiation. These data are in line with the observations of Stevens et al. (2006) who recorded an elevation in the mortality rate of experimental animals due to the increase in creatinine concentration without excretion of any amount of the creatinine. Guyton et al. (2006) studied the effect of 1800 MHz radiofrequency on male rats after 2 hours for exposure and found elevation on some parameters of the kidney as the creatinine, urea and uric acid according to the presence of carcinogenic cells by radiation. Testicular function is controlled by central nervous system through gonadotropin releasing hormone which promoter testosterone and luteinizing hormone as the key of hormonal signals. Testosterone promotes sexual differentiation in the fetus in addition to the presence of secondary sexual maturation and spermatogenesis processes in the adult (Cheung et al., 2005). Reed et al. (2006) defined testosterone as a steroid hormone related to androgen group. In mammals testosterone is primarily secreted in the testis of males by Leydig cells and in small amount in ovaries of females. In men, testosterone plays a principle role in the development of male reproductive tissues such as the testis as well as promoting the secondary sexual characteristics. Desai et al. (2011) demonstrated that testosterone deficiency causes impairment of sperm production. The present study demonstrated that there were significant decrease in testosterone hormone level in serum of the first group of experimental male mice which irradiated by 2.45 GHz microwave radiation from oven as well as the second ones which feeding on microwave heated food for 8 weeks.

Testosterone is a primary male gender hormone and any change in normal levels may be leading to disorder of reproduction. The effect of electromagnetic field radiation (EMFR) on testosterone levels were examined by Stephen et al. (2008) who exposed Wistar albino rats by radiation leakage for 60 minutes/day for 3 months and observed that there was significant decrease in the testosterone concentrations when comparing with control rats. In the present research, degeneration were observed in the cells of the testes due to the remarked decrease in the serum testosterone level which produce from Leydig cells and this damage were observed also in mice which feeding on microwave heated food. The findings of some authors as George et al. (2008) are in line with the above data who studied the effects of 900 MHz microwave radiation on testosterone concentration and recorded decline in the hormone levels.

Saraiva et al. (2008) revealed that the changes which occurred in the morphology of leydig cells under experimental conditions as irradiation by microwave radiation make decrease in testosterone concentrations. Sadeghi et al. (2006) investigate the effect of long-term exposure to microwave radiation on male reproduction in rats and found that there was a significant decrease in serum testosterone concentration after 28 days as well as presence of injury to male reproduction organs which is positively correlated with the radiation dose. The findings of Vangelova et al. (2007) are in disagreement with the data of this work who exposed the rats to microwave signal generator (900 MHz), 12 hours/day for 2 weeks and found that there are insignificant changes when comparing with control groups in the levels of testosterone.

As concerning the estimation of luteinizing hormone concentration it was found that, in the current study the concentration of luteinizing hormone was decreased in male mice after exposed male mice to 2.45 GHz microwave radiation for 8 weeks (experiment period). The findings of many authors as Yasmina et al. (2008) were matched with the present results who investigated the effect of 950 MHz magnetic field on testosterone and luteinizing hormone levels on



male rabbits and found that there were decline in the concentrations of both hormones. Ozguner et al.(2005) stated that electromagnetic fields have adverse effects on the concentration of luteinizing hormone which has lower level in experimental animals which affected by electromagnetic radiation which leakage from an oven when comparing with control groups. The present results is observed in the histological structure of testis in animals which irradiated by microwave radiation. This damage interpreted by many authors as Khavanin et al.(2007) who associated the effect of irradiation and vitamin B12 and in turn on the activity of LH. They illustrated that, the heated food on microwave oven loss 30-40% from the amount of vitamin B12 in the food as well as makes degradation in the structure of vitamin molecules. Furthermore, the conversion of vitamin to inactive molecules so; the activity of the luteinizing hormone and male infertility reduced in mice which feeding on microwave heated food.

The pituitary gland secretes the gonadotropins hormones as testosterone and LH which regulate the testicular spermatogenesis and steroidogenesis processes. The effect of electromagnetic waves on gonadotropins hormones levels have been studied in humans and other animals by many authors as Alhekkail, (2001) who examined the concentrations of LH in 21 healthy males after applying 900 MHz radiofrequency radiation which emitted from a cell phone (2 h/day for 1 month) and found no effect has occurred in the hormone level of experimental animals. Aghdam et al.(2008) evaluated the effects of 2.45 GHz electromagnetic field (EMF) radiation on germ cell spermatogenesis processes and observed that the levels of serum LH does not show any significant difference between the exposed and control groups.

In the present study the concentration of total cholesterol and triglycerides were increased after exposed to microwave radiation for 8 weeks. Dasdag et al.(2008) investigated the effects of radiofrequency and microwave on the people occupationally exposed to non-ionizing radiation. An increase in cholesterol level was observed in experimental groups when comparing with control mice. High concentration of cholesterol causes hemodialysis. Precipitation of excess cholesterol in bile as solid plate-like monohydrate crystals is an indicator for the formation of cholesterol gallstones (Portincasa et al.,2006). The liver plays as a central role in cholesterol homeostasis and lipoprotein metabolism (Tsai et al.,2004). Weihs et al.(2005) performed some toxicology studies in rats and recorded high levels of cholesterol in treated rats.

ALP level is recognized as a clinical marker of liver injury and Non-alcoholic fatty disease (NAFLD). NAFLD is the most common liver disease, the pathogenesis of this disease result from accumulation of triglycerides in hepatocytes and subsequent lipid peroxidation and after that oxidative stress (Farrell et al., 2007). Non-alcoholic fatty disease is characterized by accumulation of fat in liver with or without inflammation, fibrosis and cirrhosis according to the exposure to microwave radiation (Su et al.,2006). Shahryar et al.(2009) studied the effect of radiation which leakage from the cell phone and found that there are changes in the lipid profile of male medical students after using their phones. The findings of Igel-Korcagova et al.(2003) are in disagreement with the results of this research who observed the decline of the cholesterol concentration in experimental animals after exposure to direct solar radiation when comparing with the morning levels.

Oxidative stress is toxicological activities which induced through cellular damage by producing the free radicals (Wu et al., 2008). Several epidemiological studies suggest a link between electromagnetic field exposure which is resulting from the use of electric devices as microwave oven (Roosli et al.,2007). In the current study the results of GPx and SOD concentrations showed significant decrease in groups which exposed to microwave radiation as well as feeding on microwave heated food as comparing with the control mice. Belyaev, (2000) said that the exposure to microwave radiation induced decline in the concentration of GPx and SOD in liver tissue. Fang et al.(2002) homogenate the liver tissue of mice after treatment and estimated the enzymatic antioxidant as GPx and SOD and recorded the reduction in concentrations of these parameters. Banerjee et al.(2003) studied the effect of mobile phone and microwave radiation (2.45 GHz) on liver tissue of male rats and found that there was decline in GPx and SOD concentrations. The findings of some authors are disagree with the results of this research as Stopczyk et al.(2005) they studied that the impact of 150 KHz electromagnetic fields on male rats which showed significant increase ( $p \leq 0.01$ ) in SOD concentration in brain tissue of rats.

The results of the present work indicate there was a significant increase in MDA concentrations in the liver tissue of male mice which irradiated by microwave radiation for 8 weeks as comparing with the control mice as well as there was decline in the protein level in experimental groups. Belyaev et al.(2000) exposed the animals to microwave radiation and found that there was an increase in MDA concentration as an index to lipid profile. Moussa, (2009) discuss that the electromagnetic fields as microwave radiation affect biological systems by increasing the free radicals which enhanced the lipid peroxidation and change the concentration of antioxidant enzymes. The lipid peroxidation level was significantly increased which indicated that there is an association between the exposure to microwave radiation and oxidative stress leading to physiological disturbances. The increased level of lipid peroxidation was an induction of free radicals during the microwave exposure. These free radicals affect the lipid membrane and protein content and elevated the chance of diseases.

The microwave radiation can work as environmental pollutant which cause genetic and cell alternation furthermore, oxidative stress (Lykkesfeldt,2007). Yurekli et al.(2006) investigated the effects of microwave radiation and effects on oxidative stress in rats. When microwave radiation well below current exposure limits the MDA level was increased from the normal concentration. The exposure to 900 MHz and 1800 MHz microwave radiation (30 days) lead to significant increase ( $p \leq 0.05$ ) in MDA concentration in brain tissue of rats as a marker of lipid peroxidation as compared to control mice (Ilhan et al., 2004). The investigation of biological effects of microwave radiation on the brain and liver tissue of experimental animals showed that the MDA concentration was significantly higher in the brain and liver tissues of MWR-exposed rats so there are significant increase in lipid peroxidation as a direct result (Zotti-Martelli et al.,2005). The findings



of some authors are disagree with the results of this research as Irmak et al. (2002) found that the exposure to radiation of 900MHz not effect on the MDA concentration in serum and brain tissue of rabbits.

Histological examination of the liver cells revealed that there were loss of radial arrangement in addition there are hypertrophy, vacuolization and hyalinization of hepatocytes with dilated central vein and sinusoids after the exposure the experimental mice to the microwave radiation and also feeding on microwave heated food for 8 weeks. The findings of some authors are agreement with the observation of this research as Verschaeve, (2009) who investigated the effects of 2.45 GHz microwave radiation on the vital organ of mice as liver, kidney and testis and found that in liver cells there are some histopathological evidence of cell injury when compared to control ones. The liver cell look cloudy in their appearance and there are aedema in some cells. The liver cells show isolated pyknotic hepatocytes, increased mitotic figures and there were increased hyperchromasia in occasional cell. Also, narrow blood sinusoids and cell necrosis were observed. The present study also reported that there was alternation in hepatic sinusoid of the liver which is the smallest vessel which plays an important role in hepatic microcirculation. The structure of these sinusoids has an effect on the functions of the liver. The results of the current study are in concomitant with the observation of Usman et al.(2012) who found significant disorders in function and structure of liver cell in the experimental mice which exposed to 0.9 GHz and 1.8 GHz microwave radiation for 8 weeks (experiment period). There is inflammatory response.

Liver histopathology has been used as an indicator of environmental stress, since it provides a definite biological end point of historical exposure (Fernandes et al.,2008). As well as the kind injury of damage is often dependent upon the time of exposure to any pollutants (Gaskill et al.,2005). In the present research, there are liver alternations as a ballon shape of hepatocytes in pre and post-pubertal stages of experimental mice which fed on microwave heated food. Lysosomal membrane is more sensitive to the microwave radiation and lead to the release of the enzymes from lysosome and caused degeneration as well as appears of vacuolation of the hepatocytes of liver (Mohamed and Gad,2005). The liver consider as the target organ for irradiated heated food on microwave oven. These food substances are absorbed in blood circulation especially via portal vein of liver this leads to adverse effect on the health of liver in turn, there are presence of steatosis of red blood cells which consider the characteristic features of chronic hepatitis C (Au,2004). It can be concluded that the results of the present study suggests that the microwave radiation has adverse effects on liver functions leading to histological and physiological impairment.

The results which obtained in the present study revealed that the exposure mice to microwave radiation and feeding on microwave heated food for 8 weeks induced various histological changes in the testis which indicated inhibition of spermatogenesis processes (in pre-pubertal stage rather than post-pubertal stage of experimental group). These changes include alternations in both germ and Sertoli cells. The seminiferous tubule showed irregularly in shape, degeneration and reduction in number of elongated spermatids as well as in interstitial tissue appeared lytic. In agreement with these changes in the testis many authors reported similar findings under experimental conditions as Lucke and Coffy, (1999) who considered that the presence of intracellular vacuolization and reduction in number of spermatids which are similar to the changes in the testis of the present results may be due to impairment of androgen hormone in testis.

The role of androgenic steroids as testosterone hormone in spermatogenesis stages is proved by Isoken and Chijioke, (2010). The alternations which occurred in seminiferous tubules as consequential effect of hormone secretion. Decreased number of spermatids in mice testis due to the exposure to microwave radiation in present research may indicate that there were general toxic effects occurred in the testis with the resultant interference with spermatogenesis. The necrosis of interstitial cells probably would have resulted in decreased synthesis of testosterone hormone concentration which is well known to support spermatogenesis and might be a possible explanation of pathological changes seen in seminiferous tubules (Singh and Bansode,2011). From the previous results of physiological studies it can be found that there is decrease in the testosterone hormone level in mice after exposure to microwave radiation in turn affected the testicular blood barrier. This interpretation in confirmed by (Monsees et al.,2001). In the current studies, there is inhibition of spermatogenesis and the function of seminiferous tubule in pre-pubertal stage of experimental group was affected by interfering of the environmental pollutants as microwave radiation with the testicular blood barrier of young stages. These observations are concomitant with the studies of (Raychoudhury and Kubinsk,2003).

Electromagnetic waves radiation alter Leydig and Sertoli cell function, leading to decreased hormone secretion as testosterone which may lead to altered cell proliferation (Roosli et al.,2007). Leydig cells are known as interstitial cells of the testis, are responsible for secretion of androgen under the effect of luteinizing hormone. Testosterone secretion is essential for initiation and maintenance of spermatogenesis. Generally, Leydig cells are known for their resistance to the effects of ionizing radiation across a wide range of exposed doses. However, the impact of EMW on the function of Leydig cells has been examined in a variety of animal studies and observed that there are injury to these cells which may affect spermatogenesis (Shahryar et al.,2009). Sertoli cells are found in between the spermatogenic cell based on basement membrane and have process that extends between the germ cells of seminiferous tubules. These cells have many functions during spermatogenesis as supporting and nutrition of the formed germ cells (Raychoudhury and Kubinsk,2003) and it have important role in the formation of testicular blood barrier which protects the new formed cells from the toxic material in blood that surround the testis (Gartner and Hiatt,2001).

Testosterone has been essential role for normal spermatogenesis because it stimulates the conversion of round spermatids into elongated spermatids. Androgen deficiency disturb the spermiation process by altering spermatid Sertoli cells and seminal epithelium (Beardsely and O'Donnell,2003). The histological examination of testis revealed degeneration of the extensions of Sertoli cells. Therefore, there are number of residual bodies accumulated in the lumen of seminiferous tubules due to inability of Sertoli cells to make their normal function in engulfing these residual bodies



which separated from spermatids during transformation to spermatozoa. The above cited views confirm the statement of Orisakwe et al.(2004) who said that disruption of Sertoli cells affects the functional status of the testis.

### Conclusions:-

From the above mentioned results it was concluded that:

- 1-Microwave radiations must be considered potentially environmental pollutant.
- 3-The leakage of microwave radiation has marked physiological disturbances as hematological measurements, liver function and enzymes, renal function, hormonal levels of male reproductive organs as well as lipid profile.
- 5-Microscopical examination of some organs as liver and testis showing many changes in structures and functions of these organs.

### -REFERENCES.

- 1-Abdel Aziz, I.; El-Khozondar, H. J.; Shabat, M.; Elwasife, K. and Mohamed-Osman, A. (2010). Effect of electromagnetic field on body weight and blood indices in albino rats and the therapeutic action of vitamin C or E," Romanian J. Biophys., Vol. 20, 235-244.
- 2-Abraham, W.T. and Rina, C. (1991). Population distribution profile of the activities of blood alanine and aspartate aminotransferase in normal F344 inbred rat by age and sex. Lab. Anim.25, 263–271.
- 3-Adey, W.R. (1993). Electromagnetics in Biology and Medicine, in Modern Radio Science (ed. H.Matsumoto), University Press, Oxford, pp. 231-249.
- 4-Agarwal, A.; Deepinder, F.; Sharma, R.K.; Ranga, G. and L. J. (2008). Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertility Sterility, 89:124–128.
- 5-Aghdam, H.; Lotfi, A.R.; BahoJb, M. and Karami, A.R. (2008). Effects of electromagnetic fields of cellular phone on cortisol and testosterone hormones rate in Syrian hamesi. Int J. Zool. Res. 4(4): 230-233.
- 6-Alhekail, Z. O.(2001). "Electromagnetic radiation from microwave ovens", J. Radiol. Prot., Vol. 21, pp. 251-258.
- 7-Attili, A.F.; Carulli, N.; Roda, E. (1995). Epidemiology of gallstone disease in Italy: prevalence data of the multicenter italian study on cholelithiasis (M.I.C.O.L.). Am J Epidemiology,141:158–65.
- 8-Au, D.W.(2004). The application of hist-cytopathological biomarkers in marine pollution monitoring: a review. Marine pollution Bulletin. 48:817-834.
- 9-Banerjee, S.K.; Mukherjee, P.K. and Maulik, S.K.( 2003). Garlic as an antioxidant: the good, the bad and the ugly. Phytother. Res. 17, 97–106.
- 10-Belyaev, I.Y.; Shcheglov, V.S.; Alipov, E.D.; Ushakov, V.D.(2000). "Nonthermal effects of extremely high-frequency microwaves on chromatin conformation in cells in vivo-dependence on physical, physiological, and genetic factors," IEEE Transactions on Microwave Theory and Techniques, vol.48, no.11, pp. 2172- 2179.
- 11-Bohr, H. and Bohr, J. (2000).Microwave enhanced kinetics observed in ORD studies of protein, Bioelectromagnetics 21: 68–72.
- 12-Boldt, J; Knothe, C and Zickmann, B. (1993). Influence of different intravascular volume therapies on platelet function in patients undergoing cardiopulmonary bypass. Anesth Analg; 76:1185–1190.
- 13-Burgert, T.S.; Taksali, S.E.; Dziura J.; Goodman, T.R.; Yeckel,C.W. and Papademetris, X. (2006). Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab;91:4287–4294.
- 14-Burtis, C.A.; Ashwood, E.R. (Ed).(1999). Tietz textbook of Clinical Chemistry, Third Edition W.B.Saunders Co.,Philadelphia, PA.
- 15-Chaturvedi, C. M.; Singh, V.P.; Singh, P. Basu, P. and Singaravel,M. (2011). 2.45 (CW) microwave irradiation alters circadian organization, spatial memory, DNA structure in the Brain cells and blood cell counts of male mice, *mus musculus*, Progress in Electromagnetics Research B, Vol. 29, 23-42.
- 16-Cheah, Y. and Yang, W.(2011).Functions of essential nutrition for high quality spermatogenesis. Advances in Bioscience and Biotechnology, 2011; 2:182-197.
- 17-Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y., Jia, G., Wang, T., Yuan, H., Ye, C., Zhao, F., Chai, Z., Zhu, C., Fang, X., Ma, B., Wan, L., (2006).Acute toxicological effects of copper nanoparticles in vivo.Toxicol.Lett. 163 (2), 109–120.
- 18-Chon, J.S.; McNamara, J.R. and Schaefer, E.J.( 1988).Lipoprotein Cholesterol Concentrations in the plasma of Human Subjects as Measured in the fed and Fasted States. Clin. Chem;34:2456-2459.
- 19-Clark,J.M; Brancati,F.L. and Diehl, A.M.( 2003). The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol, 98:960–7.



- 20-Collins, A.R.; Oscoz, A.A.; Brunborg, G.; Gaivo, I.; Giovannelli, L.; Kruszewski, M.; Smith, C.C. and Stetina, R. (2008). The comet assay: topical issues. *Mutagenesis* 23, 143–151.
- 21-Cosquer, B.; Kuster, N. and Cassel, (2005). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter 12-arm radial maze with reduced access to spatial cues in rats," *Behavioural Brain Research*, Vol. 161, 331-334.
- 22-Culling, C.F. (1974). *Hand book of histopathological and histochemical techniques*. P.150.
- 23-d'Ambrosio, G.; Lioi, M.B.; Scarfi, M.R. and Zeni, O.(1995). Genotoxic effects of amplitude-modulated microwaves on human lymphocytes exposed in vitro under controlled conditions, *Electro-Magnetobiol.* 14, 1995.157–164.
- 24-Dasdag, S., Bilgin, H.M., Akdag, M.Z., Celik, H., and Aksen, F. (2008). Effect of long term mobile phone exposure on oxidative–antioxidative processes and nitric oxide in rats. *Biotechnol. Equip.* 22, 992–997.
- 25-Davoudi, M., Brossner, C. and Kuber, W.(2002). The influence of electromagnetic waves on sperm motility. *Urol Urogynacol*, 19:18-22.
- 26-De-Iuliis, G.N.; Newey, R.J.; King, B.V., Aitken, R.J. (2009). Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. *PLoS One*; 4(7):6-446.
- 27-Desai, A., Shirode, A.R., Mittal, B. and Kadam, V.J. (2011). Assisted Reproductive Technology (ART): Combating Infertility. *Asian J Pharm Clin Res.*, 2011;4(1):18-21
- 28-Drury, J.A., Nycyk, J.A., Cooke, R.W.I. (1997). Comparison of urinary and plasma malondialdehyde in preterm infants. *Clin.Chim. Acta* 263, 177–185.
- 29-Dufour, D.R., Lott, J.A., Nolte, F.S., Gretch, D.R., Koff, R.S., Seeff, L.B. (2000). Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin.Chem.* 46, 2027–2049.
- 30-Ellman, G.L. (1958). A colorimetric method for determining low concentrations of mercaptans. *Arch. Biochem. Biophys.* 74, 443–450.
- 31-Fang, Y.Z., Yang, S., Wu, G., (2002). Free radicals, antioxidants, and nutrition. *Nutrition* 18, 872–879.
- 32-Fernandes, C., Fontainhas-Fernandes A., Rocha, E., Salgado, M.A.(2008). Monitoring pollution in Esmoriz-Paramos Lagoon, Portugal: Liver histological and biochemical effects in Liza. *Environmental Monitoring and Assessment* 145(1-3):315-322.
- 33-Finfer, S., Bellomo, R., McEvoy, S. (2006). Effect of baseline serum albumin concentration on outcome of resuscitation with albumin or saline in patients in intensive care units: Analysis of data from the saline versus albumin fluid evaluation (SAFE) study. *BMJ* 2006; 333:1044.
- 34-Fridovich, I. (1975). Superoxide dismutases. *Annu. Rev. Biochem.*, 44: 147-159.
- 35-Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* 64, 97–112.
- 36-Ford, E.S., Li, C., Cook, S., Choi, H.K.( 2007). Serum concentrations of uric acid and the metabolic syndrome among US children and adolescents. *Circulation*; 115: 2526-32.
- 37-Forgacs, Z., Somosy, Z., Kubinyi, G., Bakos, J. Akos, Hudak, A.(2006). Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice, *Reproductive Toxicology*, 22, 111–117.
- 38-Gandhi G, and Anita, (2007). Genetic damage in mobile phone users: some preliminary findings. *Int J Hum Genet* 11(2): 99-104.
- 39-Gartner, K.(1980). Stress response of rats to handling and experimental procedures," *Lab. Animals*, Vol. 14, 267-274.
- 40-George, D.F.; Bilek, M.M. and McKenzie, D.R.(2008). Non-thermal effects in the microwave induced unfolding of proteins observed by chaperone binding. *Bioelectromagnetics*; 29(4):324-30.
- 41-Grigor'ev Iu.G.(2003). Biological effects of mobile phone electromagnetic field on chick embryo (risk assessment using the mortality rate). *Radiat Biol Radioecol.*; 43:541–543.
- 42-Grigoriev, Y. G.(2004). "Role of modulation in bioeffects of electromagnetic fields (summary of Russian studies) (review in Russian)," *Annals of the Russian National Committee for Non-Ionizing Radiation Protection*.
- 43-Grigoriev, Y., Nikitina, V., Rubtcova, N. Pokhodzey, L., Grigoriev, O. Belyaev, I and Vasin, A.(2005). "The Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP) and the radiation guidelines," presented at Transparency Forum for Mobile Telephone Systems, Stockholm.
- 44-Guney, M., Ozguner, F., Oral, B., Karahan, N., and Mungan, T., (2007). 900MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. *Toxicol. Ind. Health* 23, 411–420.
- 45-Guyton, Arthur; Hall, and John (2006). "Chapter 26: Urine Formation by the Kidneys: I. Glomerular Filtration, Renal Blood Flow, and Their Control". In Grulow, Rebecca (Book). *Textbook of Medical Physiology* (11th ed.). Philadelphia, Pennsylvania: Elsevier Inc.. pp. 308–325. ISBN 0-7216-0240-1.



- 46-Guy, A.W.; Chou, C.K.; Kunz, L.L.; Crowley, J.; Krupp, J. (1985). Effects of long-term low-level radiofrequency radiation exposure on rats. US Air Force School of Aerospace Medicine Brooks Air Force Base, Texas TR-85-64,
- 47-Henry, R.J., Cannon, D.C., and Winkelman, J.W. (1974). "Clinical Chemistry-Principles and Techniques", Harper&Row., 2 nd Ed.
- 48-Institute of Electrical and Electronics Engineering (IEEE), (2002). IEEE Recommended Practice for Measurements and Computations of Radio Frequency Electromagnetic Fields with Respect to Human Exposure to Such Fields, 100 kHz–300 GHz. IEEE Std C95.3-, The Institute of Electrical and Electronics Engineers, New York.
- 49-Irmak, M.K., Fadillioglu, E., Gulec, M., Erdogan, H., Yagmurca, M. and Akyol, O. (2002). Effect of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. *Cell Biochem.Funct.* 20, 279–283.
- 50-Isoken, E. M. and Chijioke, A.K. (2010). Serum testosterone and s in wistar rats following chronic garlic feeding. *Journal of Physiological and Pathophysiology* Vol. 1(3):39-43.
- 51-Jauchem, J.(2008). Effects of low-level radio-frequency (3 kHz to 300 GHz) energy on human cardiovascular, reproductive, immune, and other systems: a review of the recent literature. *Int. J. Hyg. Environ. Health.* 211, 1–29.
- 52-Junhua, A; Yun, J; Zhenzhou, W; Ling, Y and Ding, L.(2013). Treatment of Malignant Liver Tumors by Radiofrequency Ablation Combined with Low-Frequency Ultrasound Radiation with Microbubbles. *PLoS ONE* 8(1): e53. doi:10.1371/journal.pone.0053351.
- 53-Kalphan, A. (1984). Triglycerides. *Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton;*437 and *Lipids* 1194-1206.
- 54-Kesari, K.K., Kumar, S., and Behari, J.(2011).Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male wistar rats. *Appl Biochem Biotechnol* 164(4):546-59.
- 55-Khavanin, A., Zaroushani, Y., Mortazavi, S.B., Mirzaie, R., and Hassani, J. (2007).Investigation of biological effect of microwave mobile phone on antioxidant in rabbit blood. *Inranian J. Basic Med. Sci.*, 4(32): 244-249.
- 56-Kowalczuk, C.I.; Saunders, R.D. and Stapleton, H.R. (1983).Sperm count and sperm abnormality in mice after exposure to 2.45 GHz microwave radiation, *Mutation Res.* 122\_.155–161.
- 57-Kristic, D. D.; Dindic, B. J.; Sokolovic, D. T. ,Markovic, V. V. Petkovic, D. M. and Radic, S. B. (2005).The result of experimental exposition of mice by mobile telephones," *TELSIKS 2005 Conference, Serbia, Montenegro.*
- 58-Kundi, M; Mild,K ; Hardell,L. and Mattsson ,M. O. (2004). "Mobile telephones and cancer - a review of epidemiological evidence," *J. Toxicol Environ Health B Crit Rev.*, vol. 7, pp.351-384.
- 59-Laskey, J; Dawes, D. and Howes, M. (1970).Progress report on 2450 MHz irradiation of pregnant rats and the effects on the fetus.In *Radiation bio-effects summary report.* Rockville, MD: Public Health Service. U.S. Department of Health, Education and Welfare;167-13.
- 60-Lita Lee.(2001).Microwaves and Microwave oven (ph.D in Colorado university-Boulder).
- 61-Lohmann,C.H.;Z.Schwartz,Y.LIU,Guerkov,H.D.Dean,B.Simon,B.Boyan,(2000).Pulsed electromagnetic field stimulation of MG63 osteoblast-like cells affects differentiation and local factor production,*J.orthop.Res.*,18(4),637-646.
- 62-Lowry, O.H.,Resebrough, N.J., Farr, A.L., and Randall, R.J.(1951) *J. Biol. Chem* 193:265.
- 63-Lucke, M.C. and Cffy, D.S. (1999). The male sex accessory tissue structure, androgen action and physiology of reproduction. *Knobil E, Neil J.D.(Ed). New York: Raven-Press, 1435-1487.*
- 64-Lykkefeldt, J. (2007). Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking.*Clin.Chim. Acta* 380, 50–58.
- 65-Maes, A.; Collier, M.; Slaets, D. and Verschaeve, L. (1995). Cytogenetic effects of microwaves from mobile communication frequencies (954 MHz). *Electro Magnetobiology* 14: 91-8.
- 66-Magras, I.N. and Xenos, T.D. (1997).RF radiation-induced changes in the prenatal development of mice. *Bioelectromagnetics.*;18:455–461
- 67-Malyapa, R. S., E. W.; Ahern, W. L.; Straube, E. G. ;Moros,W. F. ;Pickard, and J. L. Roti Roti (1997). Measurement of DNA damage after exposure to 2450MHz electromagnetic radiation," *Radiation Research*, Vol. 148, 608-617.
- 68-Marini, J.C.; Lee, B.; and Garlick, P.J. (2006) .In vivo urea kinetic studies in conscious mice. *J Nutr* 136:202–206.
- 69-Matsushita, K.; Selvin, E.; Bash, L.D.; Astor, B.C. and Coresh ,J. ( 2010). "Risk implications of the new CKD Epidemiology Collaboration (CKD- EPI) equation compared with the MDRD Study equation for estimated GFR: the Atherosclerosis Risk in Communities (ARIC) Study". *American Journal of Kidney Diseases* 55 (4): 648–59.
- 70-Mehta, R.L.; Kellum, J.A.; Shah, S.V.; Molitoris, B.A. Ronco, C. Warnock, D.G. and Levin, A.(2007). Acute kidney injury network: Report of an initiative to improve outcomes in acute kidney injury.*Crit Care* 11: R31.





- 71-Miller, R.A.; Harper, J.M.; Galecki, A.; and Burke, D.T. (2002). Big mice die young: early life body weight predicts longevity in genetically heterogeneous mice. *Aging Cell* 1, 22–29.
- 72-Mohamed, F.A. and Gad, N.S. (2005). Distribution of some Heavy metals in tissues of *Oreochromis niloticus*, *Tilapia zillii* and *Gilchristia lazera* from Abuza'baal lakes and their impacts on some biochemical parameters and on the histological structures of some organs. *Egyptian Journal of Aquatic Biology and Fisheries*. 9(1):41-80.
- 73-Monsees, T., Hinkel, S., Miham, K., Endo, F., Schill, W and Hayatpour, J. (2001). Effects of environmental hormones on the function of testicular cells. *Proceeding of the second status seminar "Endocrine disruptors" Berlin*. Umwelt bumsamt Berlin, 133-136.
- 74-Moussa, S.A. (2009). Oxidative stress in rats exposed to microwave radiation. *Romanian J. Biophys.*, 19(2):149-158.
- 75-Moustafa, Y.M., Moustafa, R.M., Belacy, A., Abou-El-Ela, S.H., and Ali, F.M., (2001). Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. *J. Pharm. Biomed. Anal.* 26, 605–608.
- 76-Nageswari, K.S. (1990). Biological effects of Microwaves-A Review. *Ann. Natl. Acad. Med. Sci. (India)* 26(1&2):1-16.
- 77-Nakamura, H.; Matsuzaki, I.; Hatta, K.; Nobukuni, Y.; Kambayashi, Y, and Ogino K. (2003). Nonthermal effects of mobile-phone frequency microwaves on uteroplacental functions in pregnant rats. *Reprod Toxicol.*; 17:321–326
- 78-Negrão, M.R.; Keating, E. and Faria, A. (2006). Acute effect of tea, wine, beer, and polyphenols on ecto-alkaline phosphatase activity in human vascular smooth muscle cells. *J Agric Food Chem* ; 54:4982–4988.
- 79-Nomura, N; Matsumoto, S; and Nishimura, Y. (2006) .Disposition of exogenous urea and effects of diet in rats. *Arzneimittel* .56(3):258–266.
- 80-Oh, S.Y.; Cho, Y.K.; Kang, M.S.; Yoo, T.W.; Park, J.H. and Kim, H.J. (2006) .The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease, *Metabolism* 55:1604-1609.
- 81-Oktem F., Ozguner, F., Mollaoglu, H., Koyu, A., and Uz, E. (2005). Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone radiation and its protection by melatonin. *Arch Med Res*. 36:350-355.
- 82-Ono, H., Ito, T., Yoshida, S., Takase, Y., Hashimoto, O., and Shimada, Y. (2004). Noble magnetic films for effective electromagnetic noise absorption in the gigahertz frequency range. *IEEE Trans Magn.*; 40:2853–2857.
- 83-Ortolani, E.L.; Mori, C.S.; and Rodrigues Filho, J.A. (2000) .Ammonia toxicity from urea in a Brazilian dairy goat flock. *Vet Hum Toxicol* 42(2):87–89.
- 84-Ozguner. M., Koyu, A., Cesur, G., Ural, M., and Ozguner, F. (2005). Biological and morphological effects on the reproductive organs of rats after exposure to electromagnetic field. *Soudi Med. Z.*, 26(3): 405-10.
- 85-Parkar, M.A, Ahmed, R. Abdullah, B. B, Patil, B. S, and Das, K. K. (2010). Effect of cell phone exposure on physiologic and hematologic parameters of male medical students in Bijapur (Karnataka) with Reference to Serum Lipid Profile. *Journal of Basic and Clinical Physiology and Pharmacology*. Vol. 21 (2): 201-210.
- 86-Pashovkina, M.S., and I.G. Akoev (2001). The liver enzymes which increase in hepatic diseases and toxic damage of liver cells, *Biol. Radioecol.*, 41(1), 62-68.
- 87-Paulraj, R. and J. Behari (2004). Radiofrequency radiation effect on protein kinase C activity in rats' brain," *Mutation Research*, Vol. 545, 127-130.
- 88-Perricone, C., De Carolis, C., and Perricone, R. (2009). Glutathione: a key player in autoimmunity. *Autoimmun. Rev.* 8, 697–701.
- 89-Phillips, J. I., Vaschuk, Ishida-Jones, T. Jones, R. Campbell-Beachler, M. and Haggren, W. (1998). DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. *Bioelectrochemistry and Bioenergetics* 45: 103-110.
- 90-Portincasa, P., Moschetta, A., and Palasciano, G. (2006). Cholesterol gallstone disease. *Lancet*; 368(9531):230–9. [PubMed: 16844493]
- 91-Preece, A.W.; Wesnes, K.A. and Iwis, G.R. (1998). The effect of 50 Hz magnetic field on cognitive function in humans. *International Journal of Radiation Biology* 74: 463-470.
- 92-Radovanovic, J., Winterhalter, M. Jadric, R. and Gornjakovic, S. (1994). Effect of microwave irradiation on biological systems, *Med. Arh.* 48 (3) 101–104.
- 93-Raychoudhury, S. and Kubinski, D. (2003). Polycyclic aromatic hydrocarbon induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. *Environ Health Perspect* Jan, 111(1):33-38.
- 94-Reed, W.L.; Clark, M.E.; Parker, P.G., Raouf, S.A., Arguedos, N., Monk, S.D., Snajdr, E., Nolan, V.; and Ketterson, E.D. (2006). Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. *Am. Nat.*; 167(5):667-83.



- 95-Richie, J.P., Skowronski, L., Abraham, P., and Leutzinger, Y. (1996). Blood glutathione concentrations in a large-scale human study. *Clin. Chem.* 42, 64–70.
- 96-Robert W, Moore, and Colin R. (1991). 2,3,7,8-Tetrachlorodibenzo-p-dioxin inhibits steroidogenesis in the rat testis by inhibiting the mobilization of cholesterol to cytochrome P450. *Toxicology and Applied Pharmacology*; 109(1):85-97.
- 97-Roosli, M.; Lortscher, M.; Egger, M.; Pfluger, D.; Schreier, N.; Lortscher, E.; Locher, P.; Spoerri, A. and Minder, C. (2007a). Mortality from neurodegenerative disease and exposure to extremely low-frequency magnetic fields: 31 years of observations on Swiss railway employees. *Neuroepidemiology* 28:197–206.
- 98-Roosli, M, Michel, G, Kuehni, C.E, and Spoerri A.(2007b). Cellular telephone use and time trends in brain tumour mortality in Switzerland from 1969 to 2002. *Eur J Cancer Prev*; 16: 77-82.
- 99-Sadeghi, H., Zare, S., Hayatgeibi, H. and Alivandi, S. (2006). Biological effect of power frequency magnetic fields on serum biochemical parameters in guinea pig. *Pak. J. Biol. Sci.*, 9:1083-1087.
- 100-Salford ,L.G.; Brun, A.; Stureson, K.; Eberhardt, J.L. and Persson, .B.R.R. (1994). Permeability of the blood brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50 and 200 Hz. *Microscopy Research and Technique* 27: 535-542.
- 101-Saraiva, K.; Da Sliva, Jr.; Torres.; Donato, Peres, N.; De Souza, J. and Peixoto, C.(2008). Changes in mouse leydig cells ultrastucture and testosterone secretion after diethylcarbazine administration. *Micron*; 39:580-586.
- 102-Sattar, N., Scherbakova, O., Ford, I., O'Reilly, D.S., Stanley, A., and Forrest, E. (2004). Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes*;53:2855–60.
- 103-Saunders, R. D. and C. I. Kowalczyk (1981). Effects of 2.45 GHz microwave radiation and heat on mouse spermatogenic epithelium," *International Journal of Radiation Biology*, Vol. 40, No. 6, 623-632.
- 104-Schwartz ,G.J., Muñoz, A., and Schneider, M.F. (2009). "New equations to estimate GFR in children with CKD". *Journal of the American Society of Nephrology* 20 (3): 629–37.
- 105-Shahryar, H. A., A. Lot, M. B. Ghodsi, and A. R. K. Bonary (2009). Effects of 900 MHz electromagnetic fields emitted from cellular phone on the T3, T4, and cortisol levels in Syrian Hamsters," *Bull. Vet. Inst. in Pulawy*, Vol. 53, No. 2, 233-236.
- 106-Shen, Y.H., Yang, W.S., Lee, T.H., Lee, L.T, Chen, C.Y., and Huang, K.C.(2005). Bright liver and alanine aminotransferase are associated with metabolic syndrome in adults. *Obes Res*;13:1238–1245.
- 107-Singh, R.K. and Bansode, F.W. (2011). Benzene-induced histopathological changes and germ cell population dynamics in testes of Sprague Dawley rats. *J. Environ. Biol.* 32:687-694.
- 108-Stephen, M.E., Agarwal, N. and Qian, K.( 2008) . Hormonal Regulation of Testicular Steroid and Cholesterol Homeostasis. *Molecular Endocrinology*; 22(3): 623-635.
- 109-Stevens, L.A., Coresh, J., Greene, T, and Levey, A.S. (2006). "Assessing kidney function--measured and estimated glomerular filtration rate". *The New England Journal of Medicine* 354 (23): 2473–2483.
- 110-Stevens, L.A., Coresh, J., and Schmid, C.H.( 2008). "Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD". *American Journal of Kidney Diseases* 51 (3): 395–406.
- 111-Stopczyk, D., Gniatecki, W., Buczynski, A., Kowalski, W., Buczynska, M., and Kroc, A.(2005). Effect of electromagnetic field produced by mobile phones on the activity of superoxide dismutase (SOD-1) – in vitro researches. *Ann. Acad. Med. Stetin.* 51,125–128.
- 112-Su, C.C., Wang, K., Hsia, T.L., Chen, C.S., and Tung, T.H.(2006). Association of nonalcoholic fatty liver disease with abnormal aminotransferase and postprandial hyperglycemia. *J. Clin. Gastroenterol*;40:551–4.
- 113-Terra, R.M., Junqueira, J.J., Teixeira, L.R., Vargas, F.S., Pêgo-Fernandes, P.M., and Jatene, F.B. (2009). Is full postpleurodesis lung expansion a determinant of a successful outcome after talc pleurodesis? *Aug*;136(2):361-8.
- 114-Tietz, N.W., ed (1990). *Clinical Guide to laboratory tests*. 2nd ed. Philadelphia: WB Saunders; 26-29.
- 115-Tiikkainen, M., Bergholm, R., Vehkavaara, S., Rissanen, A., Hakkinen, A.M., and Tamminen, M. (2003). Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes* ;52:70-75.
- 116-Trosic, I., Busljeta, I., Kasuba, V., and Rozgaj, R. (2002). Micronucleus induction after whole-body microwave irradiation of rats. *Mutat. Res.*, 521(1-2):73-79.
- 117-Tsai, C.J., Leitzmann, M.F., and Willett, W.C. (2004). The effect of long-term intake of cis unsaturated fats on the risk for gallstone disease in men: a prospective cohort study. *Ann Intern Med*;141(7):514–22.
- 118-Turek, F.W., Joshu, C., and Kohsaka, A.(2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*;308: 1043–1045.



- 119-Usman, W. F. W. Ahmad, M. Z. A. Ab Kadir, M. Mokhtar, and R. Ariffin (2012). "Microwave effect of 0.9 GHz and 1.8 GHz cw frequencies exposed to unrestrained swiss albino mice," *Progress In Electromagnetics Research B*, Vol. 36, 69-87.
- 120-Vangelova, K., Israel, M., Velkova, D., and Ivanova, M. (2007). Changes in excretion rates of stress hormones in medical staff exposed to electromagnetic radiation. *Environmentalist*, 27: 551-555.
- 121-Veneman, T.F., Oude Nijhuis, J., and Woittiez, A.J. (2004). Human albumin and starch administration in critically ill patients: A prospective randomized clinical trial. *Wien Klin Wochenschr*; 116:305-309
- 122-Verma, A.R.; Vijayakumar, M.; Rao, C.V.; and Mathela, C.S. (2010). In vitro and in vivo antioxidant properties and DNA damage protective activity of green fruit of *Ficus glomerata*. *Food Chem. Toxicol.* 48, 704-709.
- 123-Verschaeve, L. (2009). Genetic damage in subjects exposed to radiofrequency radiation. *Mutat. Res.* 681, 259-270.
- 124-Verschaeve, L.; Slaets, D.; Van Gorp, U.; Maes, A. and Vankerkom, J. (1996). In vitro and in vivo genetic effects of microwaves from mobile telephone frequencies in human and rat peripheral blood lymphocytes in: D. Simunic (Ed.) COST 244 Position Papers. COST 244: Biomedical Effects of Electromagnetic Fields, CEC-XIII-PP01/96, European Union.
- 125-Wannamethee, S.G., Shaper, A.G., Lennon, L., and Whincup, P.H. (2005). Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. *Diabetes Care* 28:2913-2918.
- Weihs, D., J. Schmidt, I. Goldiner, D. Danino, M. Rubin, Y. Talmon, and F. M. Konikoff. (2005). Biliary cholesterol crystallization characterized by single-crystal cryogenic electron diffraction. *J. Lipid Res.* 46:942-948.
- 126-WHO Information (1998). Electromagnetic field and public health, "Fact sheet 182.
- 127-Wu, D.M., Lu, J., Zheng, Y.L., Zhou, Z., Shan, Q., and Ma, D.F. (2008). Purple sweet potato color repairs D-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. *Neurobiol. Learn. Mem.* 90, 19-27.
- 128-Yasmina, D., Yvan, T., and De Seze, R. (2008). Influence of Electromagnetic Fields Emitted by GSM-900 Cellular Telephones on the Circadian Patterns of Gonadal, Adrenal and Pituitary Hormones in Men. *Radiat. Res.*, 169(3): 337-343.
- 129-Yurekli, A.I., Ozkan, M., Kalkan, T., Saybasili, H., Tuncel, H., Atukeren, P., Gumustas, K., and Seker, S. (2006). GSM base station electromagnetic radiation and oxidative stress in rats. *Electromagn. Biol. Med.* 25, 177-188.
- 130-Zare, S., S. Alivandi, and A. G. Ebadi (2007). Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in Guinea pig, " *World Applied Sciences Journal*, Vol. 2, 509-511.
- 131-Zecca, L., Mantegazza, C., Margonato, V., Cerretelli, P., Caniatti, M. and Piva, F. (1998). Biological effects of prolonged exposure to ELF electromagnetic fields in rats: III. 50 Hz electromagnetic fields. *Bioelectromagnetics.*; 19:57-66.
- 132-Zeni, O., Schiavoni, A.S., Sannino, A., Antolini, Forigo, D., Bersani, F., and Scarfi, M.R. (2003). Lack of genotoxic effects (micronucleus induction) in human lymphocytes exposed in vitro to 900MHz electromagnetic fields. *Radiat. Res.*, 160(2):152-158.
- 133-Zirkin, B.R. and Chen, H. (2000). Regulation of leydig cell steroidogenic function aging. *Biol. Rerod.* 63(4):977-81.
- 134-Zotti-Martelli, L., Peccatori, M., Maggini, V., Ballardini, M., and Barale, R. (2005). Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 1800-MHz microwave radiation. *Mutat. Res.* 582, 42-52.