



Impact of microwave heated food on health

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ABSTRACT

Microwave has attracted a great deal of attention due to its increase usage in occupational environment, which leads to a large number of publications regarding health hazards of microwave. The aim of this research was to investigate the biological effects of microwave heated food on the blood and organs of the experimental mice. The present study had one goal is to evaluate the effects of feeding on microwave heated food. These evaluations were done on male Swiss albino mice (pre (one month) and post (three months)-pubertal ages). All the results of albumin and bilirubin showed that an elevation in the levels of two parameters while, the protein concentration was decreased. The results showed decline in glutathione peroxidase and superoxide dismutase as well as increase in malondahyde concentrations according to the oxidative stress which leads to physiological disturbances. The results of the present study suggests that the microwave radiation has adverse effects on liver functions leading to histological and physiological impairment.

Indexing terms/Keywords

Microwave, oxidative stress, heated food.



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1-INTRODUCTION

Microwaves are very short waves of electromagnetic energy and part of Mother Nature's energy spectrum. This spectrum contains frequencies with wave length from the longest to the shortest: radio waves, microwave, infrared, optical, ultraviolet, X-rays and gamma rays. High frequency electromagnetic field (EMF) is generated from different sources such as radar installations, radio and television transmitters and microwave ovens. Microwave radiation is a type of non-ionizing electromagnetic radiations and considered as environmental pollutant (Paulraj and Behari, 2004). 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 (Parkar et al., 2010).

Microwave ovens heat food volumetrically by electromagnetic radiation. The process of microwave heating food has been extensively studied (Nott and Hall, 2005). The enormous amount of energy going into the food molecules from microwave radiation is sufficient to break protein molecules so; a lot of strange new molecules have been created from the denaturation of protein. The molecular structure of the food is changed furthermore, the producing molecules unnatural in the body and consider being carcinogenic substance. The disorders in the digestive system are resulting from eating the microwave heated food as well as nutritional quality of food decreased by 60% to 90% (Lita lee, 2001). The liver biomarker enzymes as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly changed under the effect of 50 Hz magnetic field (Sert et al., 2002).

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 the tissues and organs as liver according to the interaction between the electromagnetic radiation and the vital organs (Jauchem, 2008).

2-The aim of the present study

The aim of this research was to investigate the biological effects of microwave radiation on the blood and organs of the experimental animals. The present study had one goal is to evaluate the effects of demonstrating the effect of feeding on microwave heated food. These evaluations were done on male Swiss albino mice (pre (one month) and post (three months)-pubertal ages) answered on the following questions; what happens to mice who ingest microwaved food and speculate from the results on human being and takes place by determination of the studies on , physiological assessments on the serum and liver tissue of experimental mice.

The environment of human has been changed fundamentally by increasing the applications of electrical systems in households during leisure time and at work places. The widespread use of cellular phones and microwave oven as high frequency electromagnetic fields have been increased rapidly. There is a need to study the health risks of electromagnetic field because the corresponding number of the people which exposed to these waves (Weiss and Landauer, 2003). The microwave ovens have effects on the food which heated and people who ingest the microwaved foods and also, for their users. Kids who are residing near their mothers and love to watch the foods bubbling inside the microwave exposed to electromagnetic fields. The oven door is the most dangerous place for microwave leakage as well as magnetic fields can be present around the oven (IEEE, 2002).

-Biochemical assessments: -Liver function and marker enzymes

Human serum albumin (HSA) is the most abundant protein in human blood plasma, constitutes about half of the blood serum protein and soluble molecule as well as produced in the liver as prealbumin. Albumin transports hormones, fatty acids and other compounds as well as maintains osmotic pressure. Serum albumin level has been linked in clinical practice to several diseases. Low albumin levels can indicate liver disease, and high albumin levels usually reflect dehydration condition, and liver dysfunction (Novikov et al., 1999). Bilirubin is mainly formed from the haeme portion of aged or damaged red blood cells, combines with albumin to form a complex in the liver. Elevated levels of bilirubin are found in liver diseases, excessive haemolysis (Daniel and Marshall, 1999). Hyperbilirubinemia is produced from overproduction of bilirubin from hepatocytes to bile ducts after irradiated by mobile phones radiation (Friedman et al., 2003). Attia and Yehia (2002) stated that the liver damaged in the case of hypoalbuminemia after irradiated by electromagnetic waves. The major measured serum total proteins are divided into two groups, albumin and globulins and the optimal range is 7.2-8.0 g/100ml. The concentration of total protein may be elevated due to chronic infection, liver dysfunction, dehydration. The decrease of total protein according to malnutrition, malabsorption, liver disease, and low albumin (Zare et al., 2007). The apoptotic pathways which induced by electromagnetic waves change the activity of protein as well as decrease of its concentration (Desai et al., 2011). The damage which occurs in some types of the cells after feeding on microwave heated food can release enzymes into the bloodstream as indicator to cell damage. ALT is one of such enzymes which elevated in hepatitis and from other acute liver damaged (Wu, 2006). The damage which occurs in hepatocytes of the liver after feeding on microwave heated food can release ALP enzyme in blood stream (Gaskill et al., 2005). The increase in ALP concentration is associated with clinical conditions as diabetes (Sattar et al., 2004). The elevation of serum (ALT) activity in blood is widely used as a surrogate marker for tissue damage after irradiated by electromagnetic waves (Su et al., 2006). Aspartate aminotransferase (AST) is a cellular enzyme which found in the highest concentration in the cells of liver, red blood cells. (Wu, 2006).



The damage which occurred in the organs as liver in experimental animals after irradiated by radiofrequency field causes an additional AST is released into the blood stream. The amount of AST in the blood directly related to the extent of the tissue damage. AST concentration is elevated in hepatitis, and trauma (Sattar et al., 2004). ALT is a more specific indicator of liver inflammation or damage than AST. The elevation of AST concentration in the serum is not specific to hepatic disease so, it must take other enzymes to diagnostic any diseases as ALT with AST (ALT/AST) ration. The ration of ALT/AST sometimes can help whether the liver has been damaged or not (Hanley et al., 2007). Moussa (2009) studied the effect of microwave radiation on serum transaminases (AST& ALT) and alkaline phosphatase (ALP) of Swiss albino mice and exhibited significant increase in the liver enzymes after comparing with control mice. Morelli et al. (2005) revealed that the electromagnetic fields have extreme effects on membrane-associated enzymes of the liver on experimental animals. Uric acid consists from carbon, nitrogen, oxygen and hydrogen (C₅H₄N₄O₃), forms ions and salts known as urates and acid urates such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides. High blood concentrations of uric acid can lead to gout (excess serum accumulation of uric acid in the blood can lead to a type of arthritis known as gout) as well as kidney stones (kidney stones can also form through the process of formation and deposition of sodium urate microcrystals) (Ford et al., 2007). Urea (CH₄N₂O) is an endogenous product of protein and amino acid catabolism, formed in the liver from ammonia which is a deamination product of amino acids. Approximately 20–35 g of urea is excreted in human urine per day. Urea was the first organic compound synthesized from inorganic reagents (O'Neil et al., 2006).

Elevated urea levels may be associated with gastrointestinal disorders as well as renal disease. Elevated urea levels may be an indicator of dehydration. Urea levels below the normal physiological range may indicate over hydration. 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). Creatinine is breakdown product of creatinine phosphate in muscle, is usually produced at a fairly constant rate by the body. Serum creatinine is an important indicator of renal health. Creatinine is removed from the blood chiefly by the kidneys primarily by glomerular filtration (Mehta et al., 2007). 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). Belyaev et al. (2000) studied the physiological effects of extremely high-frequency microwaves radiation on renal function of the mice. There are adverse effects on the biological system as kidney function by increasing the creatinine and urea concentrations of the mice after exposed to microwave radiation (Mossua, 2009). Oktem et al. (2005) examined the damage which occurred in the kidney of mice after induced the tissue by 900 MHz mobile phone radiation. The level of creatinine is a significant marker of renal function in cirrhosis.

Chaturvedi et al. (2011) observed the effect of 2.45 GHz microwave radiation on some parameters as DNA structure and cholesterol concentration and revealed that there are increased in the cholesterol levels. Gandhi et al. (2005) suggested that the exposure to radiofrequency radiation makes some effects as physiological, neurological, cognitive and behavioral changes as well as to induce, initiate and promote carcinogenesis furthermore alter the lipid profile concentrations. The exposure of electromagnetic field from (0-300 GHz) makes increase in low density lipoprotein, high density lipoprotein, triglyceride, and total cholesterol (NRPB, 2004). Microwave radiation (MW) is considered as a type of non-ionizing electromagnetic (EM) field present in the environment and may pose a potential threat to human health. Because of that, there has been a growing public concern regarding the potential health hazard of exposure to microwave frequencies. It is already known that aging, several diseases and the exposure to various toxic substances as well as radiation increase production of reactive oxygen species (ROS). The increase of ROS production with the consequent disturbance of the oxidative balance in the cell, called oxidative stress, disturbs the metabolism of macromolecules. The oxidative stress causes the damage to membrane lipids which manifests as the increase of the malondialdehyde (MDA) concentration (Ahlbom et al., 2008). MDA is one of the better-known secondary products of lipid peroxidation. Products of lipid peroxidation are released into plasma as result of membrane damage, and MDA can be used as an indicator of cell membrane injury. Although its low chemical reactive at physiological pH, the MDA molecule is able to interact with nucleic acid bases to form several different adducts (Marnett, 2002). The main antioxidant in the organism is GPx which plays central role in defense against different diseases and cell insults and its concentration may serve as an indicator of disease risk in humans thus, the decrease of GPx concentration depends on the exposure to oxidative stress (Perricone et al., 2009). Irmak et al. (2002) found that radiation of 900 MHz did not increase the MDA concentration in serum and brain of rabbits. Meral et al. (2007) analyzed the effect of microwave radiation in the brain of guinea pigs and found that the increased MDA concentration. Ayata et al. (2004) recorded the reduction in antioxidant enzymes GPx and SOD concentrations in the kidney organ of treated mice. The lipid peroxidation as an index to the production of reactive oxygen species (ROS) as well as oxidative stress was monitored by Ayata et al. (2004) observed an elevation in MDA level in experimental rats which exposed to 150 KHz magnetic fields long-term exposure.

The liver is the second-largest organ of the body and the largest gland is situated in the abdominal cavity beneath the diaphragm. The liver is the organ in which nutrients absorbed in the digestive tract are processed and stored for use by other parts of the body. All the materials absorbed via the intestines reach the liver through the portal vein. The position of the liver in the circulatory system is optimal for gathering, transforming, and accumulating metabolites and for neutralizing and eliminating toxic substances. Elimination occurs in the bile, an exocrine secretion of the liver that is important for lipid digestion. The liver also has the very important function of producing plasma proteins, such as albumin, other carrier proteins. The liver is covered by a thin connective tissue capsule. The basic structural component of the liver is hepatocyte. The epithelial cells are grouped in interconnected plates and constitute two-thirds of the mass of the liver. In light-microscope sections, structural units called liver lobules. Hepatocytes are polyhedral in the structure and the cytoplasm of the hepatocyte is eosinophilic, mainly because of the large number of mitochondria (Terra et al., 2009). In liver, portal spaces are regions located in the corners of the lobules, containing connective tissue, bile ducts, lymphatics,



nerves, and blood vessels. The space between hepatocytes contains capillaries; the liver sinusoids. The sinusoid is surrounded and supported by a delicate sheath of reticular fibers. In addition to the endothelial cells, the sinusoids contain macrophages known as Kupffer cells. These cells are found on the luminal surface of the endothelial cells, within the sinusoids. Their main functions are to metabolize aged erythrocytes, digest hemoglobin, secrete proteins related to immunological processes, and destroy bacteria that eventually enter the portal blood through the large intestine. Kupffer cells account for 15% of the liver cell population. Most of them are located in the periportal region of the liver lobule, where they are very active in phagocytosis (Gartner and Hiatt, 2001).

The microwave radiation affects the oxidative state of the liver, kidney and hemoglobin macromolecule (Moussa, 2009). The acute effect of microwave radiation consists of accumulation of lipids and the appearance of degenerative processes leading to the death of the cell. The necrotic process can affect small groups of isolated parenchymal cells ("focal necrosis"), groups of cells located in zones ("centrilobular, mid zonal or periportal necrosis") or virtually all the cells within an hepatic lobule (massive necrosis). Altered hepatic cell membrane permeability can lead to increased enzyme activity in plasma (Gokcimen et al., 2002). Microwave has attracted a great deal of attention due to its increase usage in occupational environment, which leads to a large number of publications regarding health hazards of MW (Chou, 2007). Despite large number of researches regarding biological effects of MW on the cell and DNA of organs as liver cells and concluded that the feeding on microwave heated food makes damage in the nitrogen base of DNA as well as in the cell membrane of the hepatocytes (Verschaeve, 2005).

3- MATERIALS AND METHODS

3-1 experimental animal

The experimental animal of this study was male Swiss Albino mice (body weight ranged from 17.7 ± 0.8 : 27.3 ± 0.7 g for pre- (1 month) and post-pubertal (3 months) stage, respectively) which obtained from the animal house of faculty of Medicine-Alexandria University.

3-2 Methods

3-2-a-Experimental tools: The tools which used in this experiment were microwave oven, microwave meter, cages and digital balance.

3-2-a-1- Microwave oven : Modern life style of human dependent on electric appliances such as televisions, computers, microwave ovens, mobiles and many other devices. Some of these devices emit electromagnetic fields which are probably risky to health and may cause an effect to some biochemical processes. 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. Radiation leakage of microwave oven was measured by (meter for microwave radiation). Microwave oven consists of 6 microwave power levels, pull handle door, steel cavity, class turntable, timer 30 min, cooking end signal, defrost function and express cooking .

3-2-a-2-Microwave oven meter: This meter is specially designed for measuring or monitoring RF emissions from microwave ovens reliably. Microwave oven meter measures 2.45 GHz (± 50 MHz) frequencies from other analog signal sources which have residual sensitivity from 30 MHz to 3 GHz so other frequencies can be detected, but for reference only.

A-The first group is control group :Animals in this group were reared in normal conditions and fed on natural food without exposure to any microwave radiation. This group contains two subgroups according to the maturation phase of animals:-

A-1.Pre-pubertal subgroup: Numbers of animals were ten animals, average weight (17.7 ± 0.8 g) and fed on natural food without exposure to microwave radiation.

A-2.Post-pubertal subgroup: Animals in this group are ten animals, average weight (27.3 ± 0.7 g) and fed on natural food without exposure to microwave radiation

B-The second group is experimental group: This group was done to investigate the effect of microwave heated food. The experimental group was divided into two subgroups according to the maturation phase of animals.

B.1-Pre-pubertal subgroup:Animals in this group one month old (number=10), average weight (17.7 ± 0.8 g) and fed on microwave heated food three times daily, each time ten minutes for eight weeks.

B-2.Post-pubertal subgroup:Animals in this group were three months old (number=10), with average weight (27.3 ± 0.7 g) and fed on microwave heated food three times daily, each time ten minutes for eight weeks.

-Estimation of the liver function and marker enzymes

In this part of study; albumin, bilirubin, total protein, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated after feeding on microwave heated food for 8 weeks.

-Estimation of albumin by colorimetric method of Tietz (1990): Albumin is serum hepatic protein, the most abundant protein in serum and contributes to the maintenance of osmotic pressure as well as to the transport of hydrophobic molecules. Serum albumin level has been linked in clinical practice to several diseases.

Principle of the assay: The Assay Max Human Albumin ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative competitive enzyme immunoassay technique that measures albumin in human plasma and serum in less than 2 hours. A polyclonal antibody specific for human albumin has been pre-coated onto a 96-well microplate with removable



strips. Albumin in standards and samples is competed with a biotinylated albumin sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

-Estimation of bilirubin by quantitative method of Jendrassik and Grof's (1938): Bilirubin is mainly formed from the heme portion of aged or damaged red blood cells. It then combines with albumin to form a complex, which is not water-soluble. This is referred to as indirect or unconjugated Bilirubin. In the liver this Bilirubin complex is combined with glucuronic acid into a water-soluble conjugate. This is referred to as conjugated or direct Bilirubin. The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyperbilirubinemia.

Principle of the assay: Bilirubin reacts with diazotised sulphanilic acid to form a coloured azobilirubin compound. The unconjugated bilirubin couples with the sulphanilic acid in the presence of a caffeine-benzoate accelerator. The intensity of the color formed is directly proportional to the amount of bilirubin present in the sample.

-Estimation of total protein by Biuret method of Henry et al. (1974): Proteins are essential parts of organisms and participate in virtually every process within the cells.

Principle of the assay: Proteins form a colored complex with cupric ions in alkaline solution.

-Estimation of alkaline phosphatase by colorimetric kinetic determination of Wan et al. (2007): Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones.

Principle of the assay: The improved method utilizes P-nitrophenyl phosphate that is hydrolyzed by ALP into P-nitrophenol a yellow colored product (maximal absorbance at 405 nm) and inorganic phosphate. The rate of the reaction is directly proportional to the enzyme activity.

P-Nitrophenyl phosphate (PNPP) → P-nitrophenol + phosphate

-Estimation of alanine aminotransferase (ALT) by Tietz (1995) method: The alanine aminotransferase is a cellular enzyme found in highest concentration in the liver and kidney.

Principle of the assay: Alanine aminotransferase catalyzes the reversible transfer of an amino group from alanine to α -Ketoglutarate forming glutamate and pyruvate.

-The estimation of aspartate aminotransferase (AST) by colorimetric method of Bowers and McComb. (1984): Aspartate aminotransferase (AST), also known as serum glutamic oxaloacetic transaminase (GOT) or aspartate aminotransferase (ASAT/AAT), facilitates the conversion of aspartate and α -ketoglutarate to oxaloacetate and glutamate.

-The estimation of kidney function: Uric acid, urea and creatinine were estimated in this part of the research.

-The estimation of uric acid by enzymatic colorimetric method of Tietz (1990): Uric acid is an important metabolite in living organism. It is an intermediate product in the biological nitrogen process.

The principle of assay: Uric acid is transformed by uricase into allantoin and hydrogen peroxide under catalytic influence of peroxidase.

-The estimation of urea by enzymatic colorimetric method of Marini et al. (2006): Urea is an endogenous product of protein and amino acid catabolism, and consequently 20-35 g of urea is excreted daily in human urine.

-The creatinine in alkaline solution reacts with picrate to form a colored complex. The rate of complex formation is measured photometrically at 492 nm.

-Estimation of antioxidant enzymes in the liver tissue:

-Estimation of Glutathione peroxidase (GPx) by UV method of Ellman's method (1958): Cellular glutathione peroxidase is a member of a family of GPx enzyme whose function is to detoxify peroxides in the cell. Because peroxides can decompose to form highly reactive radicals, the GPx enzymes play a critical role in protecting the cell from free radical damage, particularly lipid peroxidation. The GPx enzymes catalyze the reaction of H₂O₂ to water and organic peroxides (R-O-O-H) using glutathione (GSH) as a source of reducing equivalents.

The principle of assay: This assay is indirect measure of the activity of GPx. Oxidized glutathione (GSSG) produced upon reduction of an organic peroxide by C-GPx is recycled to its reduced state by the enzyme glutathione reductase (GR).

-Estimation of malondialdehyde (MDA) by colorimetric method of Drury et al. (1997): Thiobarbituric acid (TBA) reacts with malondialdehyde in acid medium at temperature of 95°C for 30 minutes to form thiobarbituric acid, reactive product can be measured at 534 nm.

-Estimation of superoxide dismutase (SOD) by colorimetric method of Verma et al. (2010): Superoxide dismutase is metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defence mechanism.

$2O_2 + 2H + SOD \rightarrow H_2O_2 + O_2$

The principle of assay: This enzyme inhibits the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

-Estimation of total protein by Lowry's method (1951):



Principle of assay: The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin-Ciocalteu reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of color depends on the amount of these aromatic amino acids present. Most proteins estimation techniques use Bovin serum Albumin (BSA) universally as a standard protein.

- Histological studies: Liver was selected in this part of the study. Histological examination on liver was carried out according to (Culling,1974) in order to investigate the histopathological effect of the radiation leakage from oven as well as the feeding on microwave heated food. The organs removed from the experimental mice and fixed in formaldehyde 10% for 24 hours then placed in 70% ethanol till processing. After fixation, the specimens were dehydrated in ethanol series. Then cleared the tissues by transferring them into methyl benzoate. After clearing the process of embedding took place by passing the material through successive series of benzene paraffin 2:1, benzene paraffin 1:1 then pure paraffin with three changes in an oven adjust at 58c°. After embedding the specimens were supported by paraffin as a block and transversely cut at thickness 5 μ . Sections were stained with eosin and haematoxylin then mounted with Canada balsam.

3-2-f-Statistical studies: The obtained results measurements from the two experiments were analyzed using SPSS program -10 package (release 3, SPSS Inc.,Chicago Ill) to evaluate the significance of the difference between mean values of the measured parameters in experimental and control groups. All the data were expressed (mean value \pm standard deviation).

4-RESULTS

The present work was carried out to study the biological effect of feeding of mice on microwave heated food. This study is accomplished by performing determination of biochemical studies on serum and liver tissue as well as histological studies of liver. This study contains two major groups according to the maturation of the animals (pre and post-pubertal groups).

4-1-Results of experiments of feeding on microwave heated food

.Physiological assessments: 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) as well as antioxidant stress on liver tissue.

.Biochemical assessments: The biochemical measurements consist of studies as determination of liver function and marker enzymes, renal function, and antioxidant enzymes were performed in two groups (control and feeding groups).

.Determination of liver function and marker enzymes:Liver function which estimated in this part of the study included albumin, bilirubin and protein as well as marker enzymes were taken place as alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase for male mice of experimental groups with pre and post-pubertal stages after exposure to microwave radiation leakage for 8 weeks.

.Estimation of albumin: There was a significant increase ($p \leq 0.05$) in albumin concentration of the post-pubertal stage of experimental group which fed on microwave heated food for 8 weeks. In the control groups with age 1 and 3 months the mean values of the albumin were 2.44 \pm 0.29 g/dl and 3.2 \pm 0.47 g/dl, respectively. While, in the experimental groups albumin level was 2.58 \pm 0.40 g/dl in the pre-pubertal stage and increased to 4.12 \pm 0.51 g/dl in the post-pubertal stage (table, 1).

.Estimation of bilirubin: In the control groups, the mean value was 0.244 \pm 0.09 mg/dl for both ages (pre-and post-pubertal stages). While, in the experimental group with 1 month age the bilirubin level raised to 0.908 \pm 0.13 mg/dl as well as 0.628 \pm 0.07 mg/dl for 3 months old. The above data which were recorded in table (1) observed that there was a highly significant increase ($p \leq 0.001$) in bilirubin concentration.

.Estimation of protein :Total protein concentrations in experimental group which recorded in table (1) showed that there was an insignificant decrease in protein level after fed on microwave heated food for 8 weeks. The mean values of protein concentrations in the control and experimental groups were 6.12 \pm 0.46 g/dl, 6.42 \pm 0.56 g/dl and 5.66 \pm 0.52, 5.22 \pm 0.33 g/dl for pre and post-pubertal stages, respectively.

.Estimation of alkaline phosphatase (ALP): When comparing the data of pre-pubertal stage of experimental group with the same age of control mice it found that the ALP concentration increased in experimental group from 64.8 \pm 3.7 U/L to 177.4 \pm 4.87 U/L. While in post-pubertal stages the ALP concentrations were 74.8 \pm 5.44 U/L and 129 \pm 2.34 U/L for control and experimental mice. From the above data it was noticed that there were a highly significant increases ($p \leq 0.001$) in both ages of experimental groups (table, 2).

.Estimation of alanine aminotransferase (ALT): The mean values of ALT levels in control groups with age 1 and 3 months were 73.60 \pm 4.03 U/L and 71.8 \pm 3.49 U/L, respectively. The data were recorded in (table, 2). In the experimental groups which were fed on microwave heated food for 8 weeks the ALT concentration were 169.8 \pm 3.7 U/L in 1 month old and 137.4 \pm 5.59 U/L in 3 months.

.Estimation of aspartate aminotransferase (AST):The AST concentration which recorded in table (2) it was clear that there was a highly significant increase ($p \leq 0.001$) in experimental mice. The mean values of pre-pubertal stages were 117.8 \pm 4.1 U/L and 298.2 \pm 3.63 U/L for control and experimental groups, respectively. While in the post-pubertal stage AST were 123.8 \pm 4.76 U/L for the control group 222 \pm 3.8 U/L for the experimental group.



Table (1):- Albumin, bilirubin and protein serum of mice after feeding on microwave heated food 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
Feeding groups	Pre-pubertal stage Mean ± S.D	2.58±0.40	0.908±0.13***	5.66±0.52
	Post-pubertal stage Mean ± S.D	4.12±0.51**	***0.628±0.07	5.22±0.33

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

Table (2):-Liver marker enzyme in the serum of mice after feeding on microwave heated food for 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±3.7	73.60±4.03	117.8±4.1
	Post-pubertal stage Mean ± S.D	74.8±5.44	71.8±3.49	123.8±4.76
	Pre-pubertal stage Mean ± S.D	177.4±4.87***	169.8±3.7***	298.2±3.63***
	Post-pubertal stage Mean ± S.D	129±2.34***	137.4±5.59***	222±3.8***

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

.Renal function: Estimation of uric acid: Feeding group: The recorded data in table (3) show that the mean values of uric acid of experimental groups revealed that there was a highly a significant increase in pre-pubertal stage (7.52±1.21 mg/dl, p≤0.001) as compared to the control group (4.74±0.98 mg/dl) while, there was a significant increase for post-pubertal stage of the experimental groups (5.18±0.68 mg/dl, p≥0.05) and 4.06±0.45 mg/dl for control ones.

.Estimation of urea: Feeding group: The data in table (3) illustrate the effect of feeding the mice on microwave heated food showed that the mean values of urea concentrations of control groups were 36.2±5.76 mg/dl and 33.8±3.7 mg/dl for pre and post-pubertal stages, respectively. The calculated data of urea level of pre-pubertal stage of experimental mice attained to 60.2±11.51 mg/dl as well as in post-pubertal stage the mean value was 48.6±6.65 mg/dl. From the above mean values it was noticed that there was a significant increase in urea levels in both ages of experimental groups (p≤0.05).

.Estimation of creatinine: The mean values of creatinine concentrations in blood serum of the experimental mice reveals that pre and post-pubertal stages have a highly significant increase (p≤0.001) after 8 weeks for feeding on microwave heated food. The mean values of pre-pubertal stages for control and experimental groups were 0.31±0.08 mg/dl and 0.76±0.18 mg/dl, respectively. While, in the post-pubertal stage the creatinine level was 0.38±0.05 mg/dl in the control group and attained to 0.64±0.1mg/dl in the experimental mice (table, 3).

Table (3):-Renal function in the serum of mice which feeding on microwave heated food for 8 weeks

Renal function				
Groups	Age	Uricacid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control Groups	Pre-pubertal stage Mean ± S.D	4.74±0.98	36.2±5.762	0.31±0.08
	Post-pubertal stage Mean ± S.D	4.06±0.45	33.8±3.7	0.38±0.05
Feeding on groups	Pre-pubertal stage Mean ± S.D	7.52±1.21***	60.2±11.51**	0.76±0.18***
	Post-pubertal stage Mean ± S.D	5.18±0.68	48.6±6.65**	0.64±0.1**

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

Biochemical markers of oxidative stress in the liver tissue of mice

Estimation of glutathione peroxidase (GPx): In the experimental group with age 1 month old the GPx concentration were 25±6.74 nmol/mg tissue and for 3 months old 26.8±5.54 nmol/mg tissue. In control groups, the mean values were 44.2±5.35 nmol and 44.2±4.96 nmol/mg tissue for pre and post-pubertal stages, respectively. From the above results it was noticed that there was a significant decrease (p≤0.05) in GPx levels. The data were recorded in (table, 4).

Estimation of malondialdehyde (MDA): In control groups, the MDA concentration in pre-pubertal stage were 4.86±0.66 nmol/mg tissue and 4.46±1.10 nmol/mg tissue for post-pubertal stage. In experimental groups which fed on microwave heated food for 8 weeks, the mean values were 8.32±1.24 nmol/mg tissue and 8.38±1.43 nmol/mg tissue, for pre and post-pubertal stages, respectively. The above data show a significant increase (p≤0.05) in MDA level in experimental groups when compared to control mice and recorded in (table, 4).

Estimation of superoxide dismutase (SOD): After feeding the mice on microwave heated food, the SOD concentration were recorded in (table, 4). It was noticed that there was a highly significant decrease (p≤0.001) in SOD concentrations. The mean values were 30.4±5.68 nmol/mg tissue and 34.8±4.817 nmol/mg tissue for pre and post-pubertal stages of experimental groups, respectively. In control mice, the mean values of SOD levels were 52.4±3.34 nmol/mg tissue for pre-pubertal stage as well as 56.8±6.22 nmol/mg tissue for post-pubertal stage.

.Estimation of protein concentration : There was a decrease (p≥0.05) in protein concentration in experimental groups which fed on microwave heated food. The data were recorded in table (4). The mean values of protein levels in pre-pubertal stages were 260.6±3.78 nmol/mg tissue and 246.2±6.87 nmol/mg tissue for control and experimental groups, respectively. While in post-pubertal stage of control group the protein concentration was 244.2±5.31 nmol/mg tissue and decreased to 222.2±4.14 nmol/mg tissue for experimental group.

Table (4):-Oxidative stress of liver tissue of mice after feeding on microwave heated food for 8 weeks

Oxidative stress					
Groups	Age	GPx (nmol/mg tissue)	MDA (nmol/mg tissue)	SOD (nmol/mg tissue)	Protein (nmol/mgtissue)
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
Feeding on group	Pre-pubertal stage Mean ± S.D	25±6.74**	25±6.74**	30.4±5.68***	246.2±6.87
	Post-pubertal stage Mean ± S.D	26.8±5.54**	8.38±1.43**	34.8±4.81**	222.2±4.14

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

-Microscopical examination of the liver

In control groups, the normal structure of liver section is seen in (figure 1). In the experimental group which fed on microwave heated food for 8 weeks there were pathological effects in the micrograph section of 3 months age. In some areas there were disappearances of normal architecture of the liver which involved cellular infiltration, shrank blood sinusoids, vacuolization of hepatocytes with pyknotic nuclei and increased number of binucleated hepatocytes. Foamy area and congested of central vein was recorded (figures 6, 7). In 5 months age of experimental mice which fed on microwave heated food it was found that dilated portal vein, cellular infiltration around bile ductile as well as increased number of binucleated and noticeable degeneration of hepatocyte cytoplasm. Many areas appeared with complete disappearance of blood sinusoids, hypertrophied kupffer cells and foamy area (figures 8, 9).

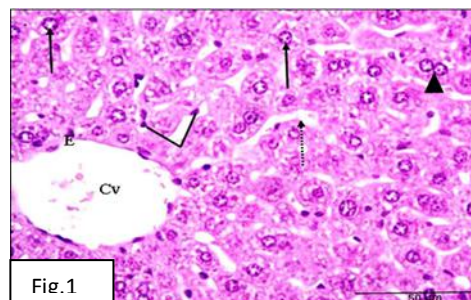


Figure (1):-Light micrograph of liver section of control male mouse, showing blood sinusoid (dashed arrow), binucleated hepatocytes (head arrow), mononucleated hepatocytes (arrow) and kupffer cell (Kc) (H&E stain, X400).

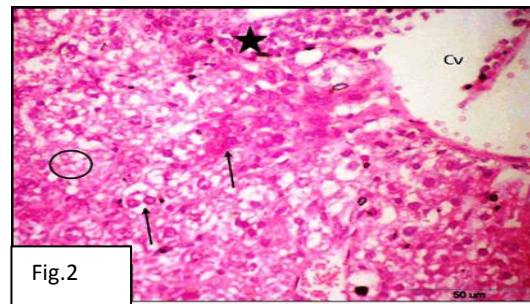


Figure (2):-Light micrograph of liver section of male mouse (3 months age) topically exposed to microwave radiation for 8 weeks, showing cellular infiltration (star), cytoplasmic vacuolization (circle), increase number of hepatocytes (arrow) and congested central vein (Cv) (H&E stain, X400).

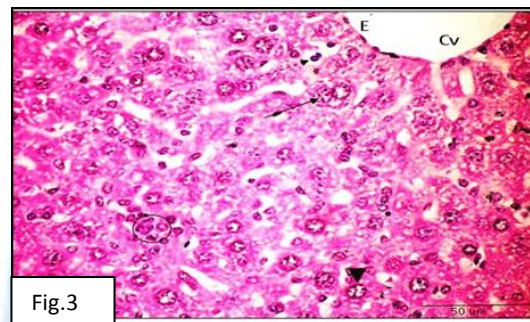


Figure (3):-Light micrograph of liver section of male mouse (3 months age) topically exposed to microwave radiation for 8 weeks, showing increase number of binucleated hepatocytes (arrow), cellular infiltration (circle), Kupffer cell with shrunken nuclei (dashed arrow) and apoptotic hepatocytes (head arrow) (H&E stain, X400).

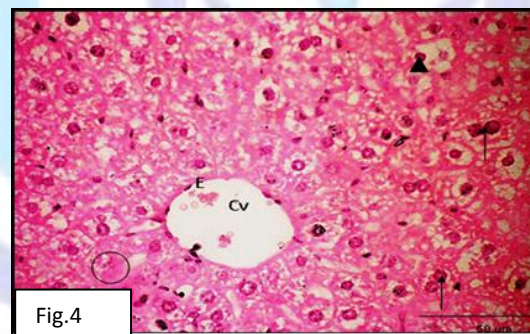


Figure (4):-Light micrograph of liver section of male mouse (5 months age) topically exposed to microwave radiation for 8 weeks, showing increase number binucleated hepatocytes (arrow), congested central vein (Cv) and degenerated hepatocytes with cytoplasmic vacuolization (head arrow) (H&E stain, X400).

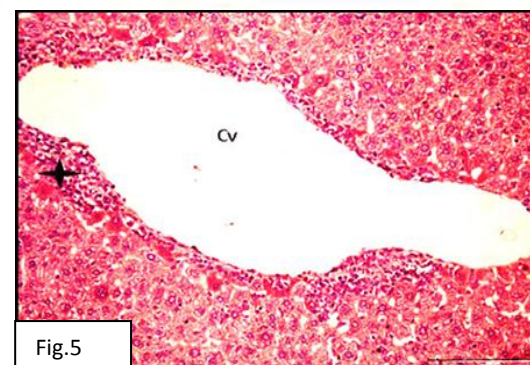


Figure (5):-Light micrograph of liver section of male mouse (5 months age) topically exposed to microwave radiation for 8 weeks showing, dilatation of central vein (Cv) and cellular infiltration (star) (H&E stain, X200).

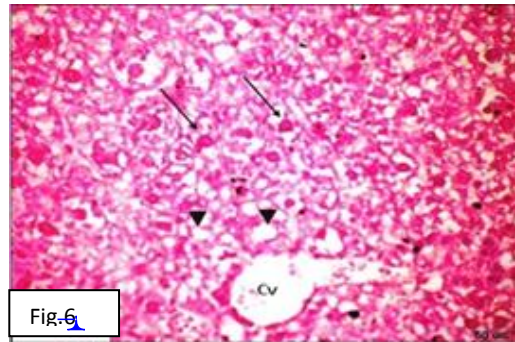


Figure (6):-Light micrograph of liver section of male mouse (3 months age) after feeding on microwave heated for 8 weeks, showing congested central vein (Cv), sever hepatocytes degeneration (arrow) and complete disappear of normal blood sinusoid (head arrow) (H&E stain,X400).

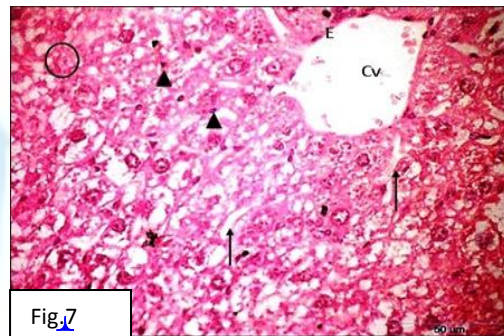


Figure (7):-Light micrograph of liver section of male mouse (3 months age) after feeding on microwave heated food for 8 weeks, showing dilation of central vein (Cv), increase number of binucleated hepatocytes (arrow), foamy area (circle) and cellular infiltration (star) (H&E stain, X400).

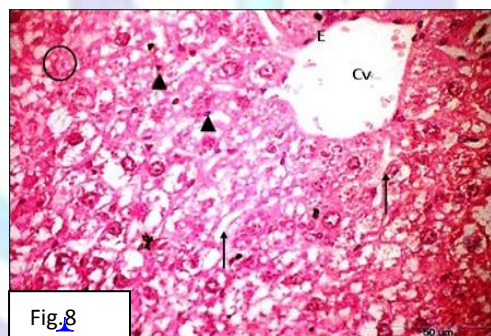


Figure (8):-Light micrograph of liver section of male mouse (5 months age) after feeding on microwave heated food for 8 weeks, showing deformed liver with congested central vein (Cv), increase number of kupffer cell with shrunk nucleus (head arrow), foamy area (circle) and disappear of normal blood sinusoid (arrow) (H&E stain, X400).

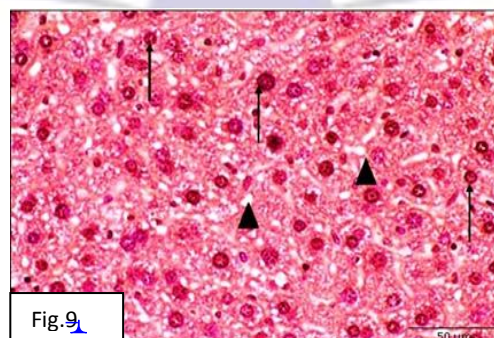


Figure (9):-Light micrograph of liver section of male mouse (5 months age) after feeding on microwave heated food for 8 weeks, showing completely disappear of normal architecture of hepatic strands with degeneration of hepatocytes (arrow) and abnormal blood sinusoid (head arrow) (H&E stain, X400).



5-DISCUSSION

In the present study, two questions were answered on particularly; physiological parameters which changed according to feeding on microwave heated food.

-Biochemical assessments: Liver function and 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 taken place in experimental groups. Some antioxidant enzymes as superoxide dismutase, glutathione peroxidase and malondialdehyde were estimated in liver tissue of experimental mice.

- Liver function and marker enzymes: The liver is responsible for maintenance, homeostatic and physiological functions in the animal body. The biochemical activity of the 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 and bilirubin as liver function were taken place in two groups the first one 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 the feeding group. 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 in agreement 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), who 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 findings of Moussa (2009) disagree with the result of the present study who found that there was an increase in protein levels of experimental mice when exposed to microwave radiation. The estimation of the liver 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.

Abdel-Aziz et al. (2010) recorded that the activities of AST were increased significantly after exposure the rats for electromagnetic fields exposure for two weeks. Oh et al. (2006) recorded that there were an association between serum ALT concentration and non-alcoholic fatty liver disease (NAFLD) as well as cardiovascular disease (CVD). Clark et al. (2003) stated that the most common elevation of ALT in NAFLD. Furthermore, Eckel et al. (2005) observed that the leading cause of the mortality in the worldwide is cardiovascular disease (CVD) which occurred in the human. he increase in ALP and AST levels in the blood serum after feeding on microwave feeding not only an indicator for liver damage but also for other tissues (Hajimehdipoor et al., 2006). The results of this study show that there was significant increase in alanine aminotransferase activity level in experimental mice. These findings are in agreed with Maria and Stuchly (1995) stated that there was an elevation in serum ALT activity in experimental mice. Shen et al. (2005) observed that the elevation of ALT level and fatty liver.

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. The ALT activities in blood serum depend upon many factors such as: time of the day, day to day variation, gender, exercise, and sucrose in the diet, insulin levels, hemolytic anemia and muscle injury (Purkins et al., 2004). Quinlan et al. (1998) discuss the major risk factor for the elevation of ALT concentration is the overweight and obesity. 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). Amacher (1998) stated that the concentration of AST is the marker of hepatocyte injury and liver fat accumulation. These findings were in agreement with the result of the examination of liver tissue which lacks normal architecture. 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). The elevation of AST concentration was correlated with fibrosis on liver biopsy. Schindhelm et al. (2007) found that there is a relation between liver dysfunction and Alzheimer's disease.

- Renal function: In the current study, the concentrations of urea, uric acid and creatinine for pre and post-pubertal stages of experimental mice (feeding group) were elevated more than the control mice. The data which are 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 increases in the urea, uric acid and creatinine levels. The electromagnetic fields originate from man-made sources such as mobile phones, base stations and 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 (AKI) and chronic kidney disease (CKD). Furthermore, the increase in creatinine level was associated with dramatic increase in morbidity and 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 (GFR) (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 (GFR) 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 (GFR) which already present and also resulted in the experimental mice which fed on microwave heated food. When the serum creatinine concentration in steady state the generation of creatinine equal creatinine excretion rate (CER) (Matsushita et al., 2010). Stevens et al. (2006) 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. 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.

-Biochemical markers of oxidative stress in the liver tissue of mice

Oxidative stress is toxicological activities which induced through cellular damage by producing the free radicals (Wu et al., 2008). Intracellular antioxidant enzymes as GPx and SOD protect biological macro-molecules from oxidative stress which induced organs pathophysiology as liver tissue. The concentrations of these enzymes were decreased than normal levels in case of the process which called oxidative stress as well as a large amount from these enzymes consumed to accomplish this task. The exposure to microwave radiation increased the reactive oxygen species (ROS) which make liver damage, renal toxicity and apoptosis in kidney tissue. Oxidative stress altered the enzymatic antioxidant defense as GPx and SOD (Garaj-Vrhovac et al., 1996). Several epidemiological studies suggest a link between electromagnetic field (EMF) exposure which resulting from the use of electric devices as microwave oven and neurodegenerative disorders (Roosli et al., 2007). There are various neurodegenerative disorders as Alzheimer's disease and Parkinson's disease resulted from the formation of ROS and oxidative stress as well as the alternation in the concentration of antioxidant enzymes (Brugnara and Mohandas, 2013).

In the current study the results of GPx and SOD concentrations showed significant decrease in the feeding group on microwave heated food as comparing with the control mice. The findings of research of Moustafa et al. (2001) who investigated the effect of mobile phone radiation on human serum and found that there was decrease in SOD concentration which are in line with the data of this study. The oxidative stress which occurred after the exposure to microwave radiation change the activity of SOD and MDA levels in human blood platelets (Valenzuela, 1991). 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 ($p < 0.01$) in SOD concentration in brain tissue of rats. Yurekli et al. (2006) recorded that there was a less significant increase in SOD activity in the treated mice. 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 and feeding on microwave heated food 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.

Oxidative stress altered the phospholipid cell membrane of the hepatocytes and increases the MDA concentration which released into plasma as a result of membrane damage. MDA can be used as an indicator to cell membrane injury (Marnett, 2002). Cell membrane damage and modification of proteins due to oxidative stress which occurred after feeding



on microwave heated food were measured by lipid peroxidation and protein content. The MDA concentration was the end product of lipid peroxidation so; it has been estimated to indicate the degree of the damage. The exposure to microwave radiation increased the levels of MDA and protein content (Boder and Wittrup, 1997). The microwave radiation can work as environmental pollutant which cause oxidative stress (Lykkesfeldt, 2007). The results of Harper and Yoshimura (1993) recorded that the exposure to high frequency fields can promote tumor cell and changed the content of protein are in line with the data of this research. 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. In a similar study, the microwave radiation not increases the MDA concentration in rats brain (Ferreira et al., 2006).

Microscopical examination of the liver: 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 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 were 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. Any damage in the structure of this sinusoid after feeding on microwave heated food makes morphological and pathological alternations in the liver organ (Gorczyńska and Wegrzynowicz, 1991). The liver organ is more sensitive to microwave radiation, this sensitivity in histopathological views as well as causes an increase in the liver weight (Kristic et al., 2005). 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 (i.e) presence of great number of lymphocytes around the central vein furthermore; the exposure of 1.8 GHz is more severe destruction on liver cells than 0.9 GHz.

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 balloon shape of hepatocytes in pre and post-pubertal stages of experimental mice which fed on microwave heated food. Hinton and Lauren (1999) discuss the reason of vacuolation of hepatocytes and revealed that these changes not result of uptake small amount of lipid but the radiation effect on the lipid in the cells which result from dissolving the fat from the hepatocytes. Morsey and Protasowiciki (1990) assured that some histological changes which occurred in hepatocytes structure associated with the response of the cells to the presence of any toxicants or environmental pollutant. 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 is considered 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).



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