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Oxidative Stress and Lipid Profile Alterations in Albino Rat Liver Fed on Microwave Exposed Food.

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ABSTRACT

Background: the health consideration of microwave (MW) radiation has been the subject of scientific investigations particularly in the last decades. There is a little information about the adverse effect of consumption of MW treated food. Objective: this study was designed to find out the effect of continuous intake of MW exposed food on diet composition, lipid peroxidation (LPO), antioxidant enzymes, DNA fragmentation and the lipid profile in liver of albino rats. For this purpose, the rats were fed for 15 weeks on a fixed amount of food exposed to the MW radiation for 10 minutes at 320°C in MW oven. Blood and tissue samples were collected after 3, 6, 9, 12 and 15 weeks. Results: According to our results, MW treatment changed the diet composition. Data showed a significant depletion in reduced glutathione all over the experimental period and elevation in of LPO after 6, 12 and 15 weeks, which sign of oxidative stress. Moreover a significant increase in fragmentation after 9 weeks was detected. A significant increase total cholesterol and LDL-C level and reduction in HDL-C were observed. Conclusion: Feeding of rats on MW exposed food induced hepatotoxicity by increasing LPO and alteration of lipid metabolism particularly in lipoproteins metabolism.

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INTRODUCTION

One of the most concerned topics of discussions these days is the intensive use of electromagnetic radiations. Microwave (MW) technology has been widely used in different fields in our lives (MW oven, WIFI and cell phone) (Raghuvanshi *et al*., 2013). Microwave is an electromagnetic radiation with frequencies between 0.3GHz and 300GHz. The most investigated is the radiation 2.45 GHz because it is absorbed by water molecules present in all live cells (Cretescu *et al*., 2013). It has a thermal effect through rising in temperature produced by the energy absorbed from oscillating electric fields (Khalafallah *et al*., 2009). Microwave oven is a kitchen appliance that heats food by dielectric heating, through using of MW radiation thermal effect (George, 2008). The main advantages of MW oven are the fasting and selective heating ability compared to conventional heating methods (Cretescu *et al*., 2013). On the other hand, the composition of a food is affected by MW oven processing; Kopp (1998) observed that MW exposure adversely affect the essential nutritional components of a food like vitamins, minerals, nucleoproteins. A high degree of protein unfolding was reported by George *et al* (2008). Conversion of some trans-amino acids into synthetic substances similar to unhealthy trans-fatty acids that toxic to various tissues (Lee, 1989). Microwave heating induced reversal of the polarity which causes the cells in the nutrients to become destructively polarized, possibly allowing for the creation of reactive oxygen species (ROS). In addition, the MW radiation itself becomes a carrier and secondary source of generated radiation inside the body (Raghuvanshi *et al*., 2012). Reactive oxygen species can cause extensive tissue damage through its action on biological macromolecules e.g. lipids, proteins and nucleic acids, leading to the formation of oxidized substances such as the membrane lipid peroxidation (LPO) product (malondialdehyde , MDA) (Srivastava *et al*., 2010). Malondialdehyde serves as a reliable marker of oxidative stress (Irmak *et al*., 2003). The accumulation of MDA in tissues or biological fluids is indicative for free radical generation, oxidative stress and tissue damage (Raghuvanshi *et al*., 2013). Elimination and neutralization of free radicals is provided by the antioxidants which represents an important defense mechanism against oxidative tissue damage through prevention of the LPO chain reaction (Aksoy, 2013). The antioxidants are divided into two groups; enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) and non enzymatic antioxidants as reduced glutathione (GSH) (Raghuvanshi *et al*., 2013). When the balance between ROS

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production and antioxidant defense is disrupted the oxidative stress results (Stump *et al*., 2010). Oxidative stress is considered a major factor in the pathogenesis of a lot of chronic diseases (Roy *et al.*, 2009). There is a close relationship between LPO and hypercholesterolemia and/or hyperlipidemia (Bulur *et al*., 1995). There are not enough data on the time-course effects of consumption of food exposed to MW on tissues and biological fluids. On the basis of literature reference this work was planned to explore the effect of continues intake of food treated with MW oven on oxidative stress, antioxidant parameters, DNA fragmentation and lipid profile in the liver of albino rats.

MATERIAL AND METHODS

One hundred of male albino rats weighing 150 ± 10g were used in this study. They were housed in plastic cages under controlled condition of temperature and light in accordance with the Guide for the Care and Use of Laboratory Animals (Guide for the care and use of laboratory animals, 1995). The animals were housed in Department of Biochemistry, Faculty of Veterinary Medicine, Cairo University, Egypt. Rats were divided into two equal groups (control and MW treated group). The rats were fed on experimental diet consist of (potato 57.4 %, beef meat 17.3 %, vegetable 20.8% and vitamin 1% and mineral mixture 3.5% according to Jonker and Till (1995). The diet was prepared by conventional method then divided into 2 equal portions one used as it for the control group and the second was exposed to MW radiation in MW oven (Daewoo KOR-1AOA 31 liter) at 320°C for 10 minutes which used for MW treated group.

***Diet analysis*:**

The freshly prepared and MW-treated diets were analyzed for macronutrient composition (crude protein, fat, ash, crude fiber and carbohydrate) in laboratory of nutrition, Department of Nutrition, Faculty of Veterinary medicine, Cairo University, while calcium, sodium, selenium, vitamin E and vitamin C were analyzed in laboratories of chemistry, Complex Research Laboratories of the Faculty of Agriculture, Cairo University.

Sampling*:*

Ten rats from each group were anesthetized and decapitated for collection of blood and liver samples after 3, 6, 9, 12 and 15 weeks. The blood samples were collected in plain tubes that were centrifuged at 10000 rpm for 10 minutes and the serum were stored at - 20°C for subsequent lipid profile analysis. Livers were removed and washed using cold saline. The liver was divided into two portions one for biochemical analysis and the other for DNA fragmentation. The liver specimens were homogenized in ice cold 0.1M phosphate buffer saline (pH 7.4) using a Teflon tissue homogenizer. The crude tissue homogenate was centrifuged at 14,000 rpm, for 15 minutes at 4°C. Supernatant was collected and subjected for determination concentrations of MDA according to Kerman and Senol (2012),reduced glutathione (GSH) according to Mailankot *et al* (2009), total protein by Bradford's method (1976) and estimation the activities of glutathione peroxidase(GSH-Px) (Deng *et al*., 2000), superoxide dismutase (SOD) (Marklund and Marklund, 1974) and Catalase (CAT) (Shahin *et al* ,2013). Quantitation of DNA fragmentation was determined by colorimetric diphenylamine (DPA) assay as described by Diab *et al* (2012). Serum samples were analyzed for determination of triacylglycerol (TAG) (Trinder, 1969), total cholesterol (TC) (Richmond, 1973), Low density lipoprotein cholesterol (LDL-C) (Wieland and Seidel, 1983) as well as high density lipoprotein cholesterol (HDL-C) (Wybenga *et al*., 1970) by using assay kits (purchased from Bio diagnostic company, Egypt). The very low density lipoprotein cholesterol (VLDL-C) was calculated using following formula (TAG/5) (Friedwalds *et al*., 1972).

***Statistical Analysis*:**

All values are given as the mean ± S.E. Significant difference between the means of the control and MW exposed samples for each group was statistically analyzed using independent t-test and one way ANOVA test by SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA).

Results:

Microwaves are known to induce compositional, nutritional and functional changes in majority of the food constituents. The effects of cooking method (microwaving) on the proximate composition of micronutrient of examined diet were investigated in the present study and shown in table (1). Significant reduction in the percentage of fat and ash concentration was observed. Also MW treatment caused dramatic alterations in the antioxidant properties of the food through decreasing the percentage of vitamin E, C and selenium. On the contrary, MW increased significantly the carbohydrates and crude fiber percentage.

Table 1: Effect of MW treatment on proximate composition of the diet.

Macronutrient composition	Groups	
	Ordinary Cooked Diet	MW-treated Diet
Moisture%	10	10
Crude protein%	13.97±0.77	11.49±0.63
Fat %	2.99±0.088	2.29±0.057*
Crude Fiber%	0.64±0.08	0.89±0.011*
Ash%	1.01±0.012	0.89±0.011*
Carbohydrate %	71.39±0.73	74.44±0.79*
Vitamin C (mg\100g)	3.69±0.60	1.58±0.50*
Vitamin E (IU\100mg)	90.11± 0.54	29.42± 0.46*
Ca (g\100g)	0.137±0.60	0.158±0.55
Na (g\100g)	0.907±0.26	0.945±0.25
Se (ppb)	9.09±0.57	4.69±0.64*

Every value represented as mean values ± S.E: Micronutrient analysis was calculated on dry matter while for vitamins and minerals were calculated on fresh sample. The star superscripts (*) indicate significant differences between MW treatment and the ordinary cooking method by t-test at $P < 0.05$.

We examined ROS–scavenging enzymes, reduced GSH and oxidant marker (MDA) responsible for oxidative stress. The MDA accumulation in liver was increased significantly ($p < 0.05$) in rats fed on MW treated food after 9, 12 and 15 weeks in comparing with control one. There is a significant accumulation in MDA concentration in time course manner in 6, 9 and 12 weeks. The level of reduced GSH showed significant reduction throughout the experimental period. No significant differences were observed in hepatic SOD, CAT and GSH-Px activities between MW treated and conventional fed one (table 2).

Table 2: Effect of MW treated food on the oxidant and antioxidant parameters in liver of albino rats.

Weeks	groups	MDA (µM/mg)	GSH (µM/mg)	SOD (U/g)	CAT (U/g)	GSH-Px (U/mg)
3 weeks	Control	2.01±0.11	6.7±0.20	33.5±1.15	64.9±2.8	1.4±0.11
	Treated	2.12± 0.08^{bc}	4.3±0.15*^{a}	32.1±0.98^{c}	69.5±4.8^{ab}	1.4±0.09^{d}
6 weeks	Control	2.15±0.08	4.5 ±0.24	35.3±0.51	65.5±2.9	2.05±0.16
	Treated	1.92±0.16^{c}	3.7±0.37*^{a}	32.5±0.14^{c}	67.3±2.3^{ab}	2.2 ±0.11^{ab}
9 weeks	Control	1.43±0.08	5.42±0.19	41.7±0.54	54.1±2.9	1.8± 0.19
	Treated	2.41±0.09*^{b}	4.1±0.16*^{a}	38.7±0.83^{b}	55.4±1.6^{c}	1.9 ± 0.17^{bc}
12 weeks	Control	1.7±0.27	7.2 ± 0.56	33.1±0.82	67.7±3.1	1.7± 0.16
	Treated	3.03±0.09*^{a}	4.3±0.16*^{a}	37.2±1.26^{a}	60.9±3.04^{bc}	1.8 ± 0.09^{c}
15 weeks	Control	2.24±0.21	6.2±0.32	32.5±1.69	69.7±4.3	2.3 ± 0.09
	Treated	2.94±0.17*^{a}	4.6±0.16*^{a}	36.2±2.02^{b}	71.6±3.2^{a}	2.3 ±0.08^{a}
LSD		0.42	0.80	3.61	8.75	0.34

MDA = Malonaldehyde, SOD = superoxide dismutase, GSH= reduced glutathione, CAT = Catalase and GSH-Px = glutathione peroxidase.
Every value represented as mean values ± S.E. (n = 10).
The star superscripts (*) indicate significant differences between MW treated group and the control at the same week by t-test at $P < 0.05$.
Different small letters indicate significant variation between different weeks in MW-treated group by ANOVA test at $P < 0.05$.

Rats fed on MW-exposed food showed significant increase in DNA fragmentation percentage in comparison with the control one after 9 weeks, but there was non-significant increase during other weeks. There are significant increases in DNA fragmentation percentage in time course manner throughout the experimental period (except at last week) as observed in table (3).

Table 3: Effect of MW treated food on the DNA fragmentation percentage in liver of albino rats.

Groups	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks
Control group	16.1± 3.4	22.1±5.4	17.1±1.8	28.3±1.4	17.2±1.8
Treated group	18.4±1.3^{b}	27.4±3.2^{ab}	28.3±3.7 *^{ab}	31.9±0.22^{a}	22.4±3.4^{b}

Every value represented as mean values ± S.E. (n = 10):
The star superscripts (*) indicate significant differences between MW treated group and the control at the same week by t-test at $P < 0.05$.
Different small letters indicate significant variation between different weeks in MW-treated group by ANOVA test at $P < 0.05$.

Feeding of rats on MW treated diet caused significant increase in TC after 6 weeks as well as LDL-C after 3, 6, 9, 12 and 15 weeks with respect to control one. Significant reduction in HDL-C was observed after 6, 9, 12 and 15 weeks. On the other hand there were no significant differences between MW treated samples and the control for TAG and VLDL-C (table 4). There are significant increasing in time course manner in first 6 weeks for TC, TAG and VLDL.

Table 4: Effect of MW treated food on the lipid profile in serum of albino rats.

Week	Groups	TC (mg /dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
3 weeks	Control	113.36 ± 2.08	63.76±91	48.62±1.4	51.98±1.5	12.75± 1.8
	Treated	122.33 ± 2.1^{b}	67.8±4.6^{b}	45.9.2±0.2^{a}	62.7±0.8*^{ab}	13.5± 0.9^{b}
6 weeks	Control	111.38 ± 4.6	85.8±7.2	46.6.6±0.8	52.6±2.0	17.1± 1.4
	Treated	133.89±3.2^{a} *	98.2±4^{a}	41.5±0.2*^{a}	67.5±2.1*^{a}	19.6± 0.8^{a}
9 weeks	Control	126.16 ± 0.47	69.3±5.2	51.35±1.3	60.9±0.34	13.8± 1.07
	Treated	118.45 ± 3.71^{bc}	57.5±2.0^{b}	40.7±2.8*^{b}	66.2±2.9 *^{a}	11.5± 0.40^{b}
12 weeks	Control	122.38± 1.95	62.9±3.8	52.6±1.06	57.8±1.9	12.5± 0.76
	Treated	123.31± 4.37^{b}	60.9±4.5^{b}	46.9±2.8*^{a}	64.1±2.9*^{a}	12.1± 0.91^{b}
15 weeks	Control	111.31± 5.28	47.4±4.6	48.7±1.0	53.04±1.1	9.4± 0.93
	Treated	112.06± 4.80^{c}	53.7±3.6^{b}	43.1±1.0^{ab}*	58.1±1.2*^{b}	10.7± 0.66^{b}
LSD		9.78	15.09	4.37	5.04	3.00

Triglycerides (TG), total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) as well as very Low density lipoprotein cholesterol (VLDL-C).
Every value represented as mean values ± S.E. (n = 10).
The star superscripts (*) indicate significant differences between MW treated group and the control at the same week by t-test at $P < 0.05$.
Different small letters indicate significant variation between different weeks in MW-treated group by ANOVA test at $P < 0.05$.

Discussion:

A microwave oven, or a microwave, is a kitchen appliance that cooks or heats food by dielectric heating. Microwave cooking requires minimum time and energy. It is also good for domestic purpose, but it had several drawbacks on micronutrient composition of the food (Kushwaha, 2012). In our study we detected significant reduction in the fat and ash percentage in diet which come in accordance with findings of Odura *et al* (2011) and Kushwaha (2012). The increasing in the carbohydrate percentage was in the line with that reported by Kushwaha (2012). Vallejo *et al* (2009) reported that the antioxidant properties of broccoli were decreased about 97% after MW treatment which was agreed with that detected with the present study where we observed significant reduction in vitamin C, E and selenium. The reducing percentage of vitamin E comes in the line with results recorded by Malheiro *et al* (2009). Cooking using MW oven led to deleterious effects on the vitamin C content which were reported previously by Zhang and Hamauzu (2004), Gliszczynska- Swiglo *et al* (2006) and Csapo *et al* (2009). MW ovens appeared to induce free radicals and or ROS through the process of cooking (Raghuvanshi *et al*., 2013). There are some suggestions that ROS are involved in the action of MW radiation on biological system (Raghuvanshi *et al*., 2012; Raghuvanshi *et al*., 2013). The radiation could enhance ROS production in many tissues; however, among the most susceptible tissues are brain, blood and liver (Harakawa *et al*., 2005). In the present study, we used the rodent model to evaluate the effect of continuous intake of MW-exposed food on liver oxidative status, DNA fragmentation as well as serum lipid profile. We detected that, continuous intake of MW- exposed food induced LPO which can be explained by the elevation of MDA level and depletion of GSH content. The hepatic MDA content elevated significantly after 9, 12 and 15 weeks, (table 2). This result was in good agreement with Raghuvanshi *et al* (2013). In contrast Ahmed *et al* (2011) reported that no change was detected MDA level in rats fed on MW treated oil. The observed depletion in GSH content along the experimental period come in the line with that reported by Singh and Ahluwalia (2012) and Raghuvanshi *et al* (2013). Deficiency of vitamins C and E as well as selenium element in MW-treated diet may cause rapid exhaustion of GSH with subsequent increase in MDA content during the last half of the experimental period. The activity of antioxidant enzymes (SOD, CAT and GSH-PX) fluctuated around normal rang. This may be due to short time of feeding on MW-treated diet (15 weeks). Also, the observed lower selenium content in MW-treated diet (4.69 ppb) may fulfill its essential requirement and consequently did not decrease GSH-PX activity during the experiment. Hakkarainen *et al* (1986) recorded that Se deficiency was followed by a subsequent decrease in Se-dependent GSH-Px which was compensated by a simultaneous increase in Se-independent GSH-Px activity. Sunde *et al* (2005) also reported that growth, and mRNA levels for selenoprotein P, 5'-deiodinase, and GPX4 were not decreased by Se deficiency. GPX4 is a phospholipids hydroperoxidase that protects cells against membrane lipid peroxidation, (Esworthy *et al*., 1994). The attained increase in MDA content without activation antioxidant enzymes increased ROS production and caused defect in oxidative / antioxidants balance towards oxidative stress especially in the last 6 weeks of experiment. That may interpret the observed significant increase of DNA fragmentation in liver of rats fed on MW-treated diet in our experiment (table 3). This result is in quit agreement with the results reported by Carta and Desogus (2012). Feeding on MW-treated diet caused non-significant change in serum total cholesterol (except after the $6^{\underline{th}}$ week), TAG and VLDL concentrations (table 4). The symmetric distribution of cholesterol in HDL-C and LDL-C fractions is essential for health equilibrium. Unfortunately, MW-treated diet deteriorated the cholesterol fractionation where it increased LDL-C and decreased HDL-C levels all over the experimental period. That attained disturbance in distribution of cholesterol fractions may attribute to the harmful effect of oxidative stress upon synthesis of apo-lipoproteins. Liu and Lee (1998) and Raghuvanshi and Mathur (2013) recorded increase serum cholesterol in serum of mice fed on MW exposed food. On the other hand, Ahmed *et al* (2011) showed

no differences in plasma TC of rats fed on MW heated corn oil for 5 weeks. Aksoy, (2013) attributed the changes in plasma lipid profile to membrane damage by oxidative stress in rats supplemented with diesel fuel or with opium poppy seed oil biodiesel. Few studies have been focused on the involvement of oxidative stress in adverse effects of MW exposed food consumption (Raghuvanshi *et al*., 2012). Hyperlipidaemia and oxidative stress have been shown to be prognostic in the development of several degenerative diseases such as coronary heart disease (Essien *et al*., 1992).

In conclusion, increased production of free radicals or decreased function of the defense system play an important role in MW toxicity. MW-treated food intake exhibits hepatotoxicity by increasing free radical generation, increased LPO , depletion of GSH and alterations in serum lipid levels. Lipid peroxides could be a part of the cytotoxic mechanisms leading to the hepatic injury. Up to knowledge it may be the first report in Egypt discusses this topic. The present study is applicable for continuous intake of MW treated food only.

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