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Changes in the levels of LPO and GSH in Swiss albino mice liver after continuous intake of food exposed to Microwave radiations

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ABSTRACT

With the development of technology the dependence of human on the electronic gadgets has increased sharply. The innovation of 90's which has reduced the cooking time significantly and thus cooking habit of urban household is Microwave oven. The microwave oven cooks food using 2.45 GHz microwaves .In the study conducted, the male Swiss Albino mice were given food pellets in the fixed amount as dose. It was given as their normal dietary intake for 2 weeks, 3 weeks 4 weeks and 4 weeks recovery (for 4 weeks micro waved food and 4 weeks normal food) after which autopsies were performed. Animals were divided into 3 groups namely Experimental, Control, and Sham. The present study suggests that microwave cooked food leads to lowering of GSH and increasing of LPO in experimental group till 2 week and 3 week as compared with control and sham. The 4 week showed increased level of GSH and decreased level of LPO. There are sign of oxidative stress due to deleterious imbalance between production and removal of free radicals.The 4 week recovery group shows remarkable recovery indicating that changes are recoverable with the substitution of microwave food with normal food.The present study is applicable for continous intake of microwave food ,there is no other food given except to week recovery group.

Keywords: Microwavefood. Oxidative stres,LPO,GSH

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INTRODUCTION

One of the most concerned topic of discussions these days are the intensive use of electromagnetic radiations. Microwave (non-ionizing radiation) technology has been widely used in national defense, industrial and agricultural production, transportation, communications, information industry, medical and scientific research fields. Microwaves in microwave oven are generated by Magnetron. A magnetron is a tube in which electrons are subjected to both magnetic and electrical fields, producing an electromagnetic field with a microwave frequency of about 2450 MegaHertz (MHz), which is 2.4 GigaHertz (GHz).

Once microwave energy is absorbed, polar molecules such as water molecules inside the food will rotate according to the alternating electromagnetic field. The water molecule is a "dipole" with one positively charged end and one negatively charged end. Similar to the action of magnet, these "dipoles" will orient themselves when they are subject to electromagnetic field. The rotation of water molecules would generate heat [1, 2] for cooking. In addition to the dipole water molecules, ionic compounds (i.e. dissolved salts) in food can also be accelerated by electromagnetic field [1] and the collided with other molecules to produce heat. Hence the composition of a food will affect how it will be heated up inside the microwave oven. George et al [3] have reported that microwave exposure causes a higher degree of protein unfolding than usual thermal stress at the same temperature.

In 1989, the Lancet medical journal reported that heating baby formula in a microwave changed its chemistry. Dr. Lita Lee found that microwaving converts some trans-amino acids into synthetic substances similar to unhealthy trans-fatty acids; one amino acid, L-proline, reportedly converted to a substance that's reputed to be toxic to the nervous system and kidneys. [4]

Histological studies [5] with micro waved broccoli and carrots have revealed that the molecular structures of nutrients are deformed by high-frequency reversal of polarity, even up to the point of destroying the cell walls, whereas in conventional cooking the cell structures remained intact. The microwaves-induced reversal of the polarity causes the cells in the nutrients to become destructively polarized, possibly allowing for the creation of free radicals [5].

A free radical is defined as any atom or molecule possessing unpaired electrons. Reactive oxygen species are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of LP in the membranes [6].These highly ROS can cause extensive tissue damage through reaction with all 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) [7-9]. Free radicals can also cause oxidation of sulphydryl groups in proteins and strand scission in nucleic acids is also possible. [10] The accumulation of MDA in tissues or biological fluids is indicative of the extent of free radical generation, oxidative stress and tissue damage. [11] Due to their highly reactive

and non-specific nature, ROS can attack almost all biomolecules including lipid membranes [12].Oxidative stress ensues when ROS evade or overwhelm antioxidants. [13] Antioxidant enzyme represents an important defense mechanism against oxidative stress. There are some antioxidant mechanisms against free radical damage. The antioxidant mechanisms are mainly divided into two groups; enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase, and nonenzymatic antioxidants such as vitamin E (Vit E; á-tocopherol), ascorbic acid and â-carotene as well as reduced glutathione (GSH) and uric acid [14-16]. Glutathione is a well known antioxidant. Glutathione-dependent antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species [17-19].The reduced form of the tripeptide glutathione is the most abundant low molecular weight thiol in all mammalian cell system [19]. GSH protects cell against free radical injury and toxic effects of chemicals [20].

MATERIALS AND METHODS

Sexually mature male mice (Mus musculus) weighing between 25 to 30 g were randomly selected .They were housed separately in plastic cages under controlled condition of temperature and light. The animals were divided into 3 groups Control, Sham and Experimental.The experimental mice were given food pellets (Hindustan Lever Pvt. Ltd.) heated in microwave at 320° C for 10 minutes. The sham group was given the normal food in low quantity whereas control was given normal food in sufficient amount.The experimental group was administered with fixed amount of microwave cooked mice pellets daily for 2 week (Experiment 1), 3 week (Experiment 2), 4 weeks (Experiment 3).The recovery group (Experiment 4) was given the microwave pellets for 4weeks and after that they were given normal mice fed for 4 weeks. After the termination of each of experimentation group, the treated and control males were sacrificed by cervical dislocation and the liver was perfused carefully with cold saline.

Lipid per oxidation (LPO)

Lipid peroxidation was estimated by the method as described by Ohkawa et al. [14]

Reduced glutathione (GSH)

The hepatic level of reduce glutathione (GSH) was determined by the method as described by Moron et al [21].

RESULTS

Significant increases in LPO level occurs in the sham group after 2 weeks as compared to control; there is insignificant increase in level of LPO in experimental group at 2 weeks. The level of LPO increased significantly in Sham ($p<.05$) and experimental group($p<.01$) at 3 week autopsy interval indicating toxicity .LPO decreases significantly at 4 week corresponding to high

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GSH in both the groups. The recovery group in sham shows significant increase whereas experimental group indicate the sign of recovery with low levels of LPO. (Table 1)

Table 1: Effect on LPO Concentration in Liver of Male Swiss Albino Mice Fed on the Food Exposed to Microwave Radiations

Autopsy Interval	Control (µ mol/gm of tissue)	Sham (µ mol/gm of tissue)	Experiment (µ mol/gm of tissue)
2 Week	38.07±.098	42.18±0.12*	39.42±0.17
3Week	79.86±2.73	84.85±1.15*	130.9±0.85**
4Week	21.69±1.98	52.28±5.07	60.0±2.10***
4Week Recovery	37.77±1.83	195.6±2.08**	29.57±11.4*

Significance in relation to control,* p<0.05, ** p<0.01, *** p<0.001

The level of GSH shows exactly the reverse trends to the LPO. There is significant decline by p<0.05 in GSH at 2 week. The level of GSH decreased significantly in Sham (p<.05) and experimental group(p<.01) at 3 week autopsy interval indicating toxicity .GSH decreases significantly at 4 week corresponding to high LPO in both the groups. The recovery group in sham shows significant decrease whereas experimental group indicate the sign of recovery with low levels of LPO and high level of GSH. (Table 2)

Table 2: Effect on GSH Concentration in Liver of Male Swiss Albino Mice Fed on Food Exposed to Microwave Radiations

Autopsy Interval	Control (µ mol/gm of tissue)	Sham (µ mol/gm of tissue)	Experiment (µ mol/gm of tissue)
2 Week	3.878±.02	1.992±.012***	5.025 ±0.48***
3Week	4.459±0.04	5.843±0.03**	3.848±0.07**
4Week	2.577±0.14	2.304±0.15*	4.154±.0.1**
4Week Recovery	2.925±0.67	0.095±0.02***	0.538±0.13***

Significance in relation to control,* p<0.05, ** p<0.01, *** p<0.001

The difference within control group is attributed to increasing age of mice.

DISCUSSION AND CONCLUSION

Oxidative stress is caused by imbalance between the production of reactive oxygen and biological systems ability to readily detoxify the reactive intermediate or easily repair the resulting damage [22-26]. ROS produced in excess may cause toxic effects by oxidative damage of molecules, membranes, and tissues. Free radicals can attack almost any component of the cell, but lipids, proteins, and nucleic acids are particularly important targets. Lipids of cell membranes and organelles are frequently damaged, resulting in LP. [27] Injury to mitochondria induced by lipid peroxidation can direct to further ROS generation [28]. Lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue. Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. GSH provides

powerful antioxidant protection to body systems heavily exposed to reactive oxygen species [29, 30]. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides [31]. Its deficiency causes oxidant damage and greater lipid peroxidation which in turn leading to cell damage [32-35].

It is important to note that shifting the GSH/GSSG redox toward the oxidizing state activates several signaling pathways (including protein kinase B, protein phosphatases 1 and 2A, calcineurin, nuclear factor κB, c-Jun N-terminal kinase, apoptosis signal- regulated kinase 1, and mitogen-activated protein kinase), thereby reducing cell proliferation and increasing apoptosis [36]. Thus, oxidative stress (a deleterious imbalance between the production and removal of reactive oxygen/nitrogen species) plays a key role in the pathogenesis of many diseases. Because glutathione is part of a cellular mechanism concerned with defense against peroxidative attacks [37].The microwave exposed food induced lipid peroxidation can be explained by the depletion of GSH.

Yadav et al [38] reported that decrease in LPO by GSH shows that GSH status in the cell is an important factor to reduce peroxidation. Microwave exposed food decreased glutathione reductase (GR) activity within a 2 week of exposure, indicating the generation of oxidative stress in animal at an early stage. Glutathione protects hepatocytes by uniting with reactive metabolites, and thus prevents them from binding covalently to liver proteins. Intracellular decrease of the reducted GSH exposes the cell to the destructive effects of the oxidative stress [39]. A drastic elevation in the LPO levels and a further decrease in glutathione (GSH) levels were observed. Reduced levels of GSH confirm an increased susceptibility to oxidative damage and this observation is an agreement with the reports that inverse relationship exists between LPO and glutathione status [40]. After 4 weeks there is increase in glutathione (GSH) which probably resulted from an attempt by the organism to control or reverse the biochemical lesion. [41]. The 4 week recovery shows significant increase in GSH level reveals be recovery of hepatic cells from oxidative stress induced by microwave exposed food .

The results suggest microwave exposed food exhibit hepatotoxicity by increasing free radical generation. The observed abnormalities in the liver is probably due to the alteration in membrane property and function, changes in the activity of anti peroxidative enzymes and GSH and increased LPO. Lipid peroxides could be a part of the cytotoxic mechanisms leading to the hepatic injury. It further indicates the oxidative stress recoverable by the substitution of microwave food by normal food.

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