



Effect of Microwave Exposed Feed in Different Containers on Histological Structure of Liver and Kidney of Adult Mice

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ABSTRACT

Microwave technology has been widely used in different fields of life including cooking. The present study was designed to investigate the effect of feed directly exposed to microwaves or in different containers on the histology of the liver and kidney of mice. Forty adult male mice were randomly divided into four groups each of 10: Control group was given normal untreated feed, Direct group was fed on food pellets exposed to microwaves in oven tray directly, Glass group was given feed processed in the microwave oven within a glass container and Plastic group was given feed exposed to microwaves in a plastic container. The microwaves exposed feed of all treated groups was heated at 250°C, 2450 MHz frequency for 15 min and was provided for 4 weeks. Intake of microwave exposed feed caused a highly significant increase ($P < 0.001$) in mean body and liver weight of mice of all treated groups as compared to control. Histopathological alterations were observed in sections of liver and kidney of mice that were given microwave processed feed. Statistical analysis revealed that the average cross-sectional area (ACSA) of Bowman's capsule was significantly ($P < 0.05$) increased in mice raised on feed that is directly exposed to microwaves radiations while other groups showed no significant change in Bowman's capsule as compared to control. ACSA of glomeruli and renal tubules significantly increased in direct ($P < 0.001$) and in the plastic ($P < 0.01$) group as compared to the control group. A significant decrease in the mean number of mononuclear hepatocytes was observed in all treated groups as compared to control. While, ACSA of mononuclear hepatocytes increased significantly ($P < 0.001$) in the direct and glass container group as compared to control. Relative nucleio-cytoplasmic index of hepatocytes and mean number of oval and kupffer cells per unit area was significantly decreased in all treated groups as compared to control. Our findings indicated that the severity of degenerative changes was highest in the glass container group.

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Authors' Contribution

SB, SS, and KM performed all the experimental work and prepared the first draft. AI helped in the statistical analysis of the data. SS helped in reviewing data and analysis of results.

Key words

Liver, Kidney, Microwave processed food, Hepatocytes, Glomeruli

INTRODUCTION

Numerous studies revealed the changes in biological systems as a result of exposure to electromagnetic radiation in living organisms. Various sources of electromagnetic radiation, for example, mobile phone, microwave oven, and wireless networks are responsible for influencing the biological parameters (Batool *et al.*, 2019). According to a current study, the most favorite method of cooking and heating foods after grilling and roasting is the use of microwaves (Diplock *et al.*, 2019).

Evidence showed that microwaves (MW) depending on their extent and frequency generate biological alteration

that may be damaging for humans (Zhi *et al.*, 2017). Metals reflect the microwaves; however, these can pass through glass, plastic, ceramics, paper and integrate with water and food contents (Parveen and Archana, 2014). MW radiations are ranked as the fourth-largest source of pollution after water, air, and noise that can generate much biological impairment. Furthermore, microwave-based cooking and heating in plastic containers are most common, whereas plastic wrappers are made up of polyvinyl chloride (PVC) and polyethylene with the addition of plasticizers like di-(2-Ethylhexyl) adipate (DEHA) to increase the flexibility of PVC films. DEHA is thought to be a potential carcinogen as it might leach into food (Meadows, 2002).

Within an organism, these radiations induce multidimensional effects on various systems involving nervous (Li *et al.*, 2012), cardiovascular (Sylvester *et al.*, 2018), immune (Esmekaya *et al.*, 2011), reproductive (Shahin *et al.*, 2013) and hematopoietic (Guo *et al.*, 2011) systems. In addition to structural disintegration in all foods, exposure to microwave radiation also badly affects important nutritious constituents such as nitriliosides,

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vitamins, and galactosides (Hao *et al.*, 2015). The oxidative state of hemoglobin, kidney, and liver is highly susceptible to microwave radiations exposure (Reisz *et al.*, 2014).

Liver and renal disorders are growing issues in developing countries, mostly related to a sedentary lifestyle dependent on the use of appliances including microwave oven in practice for heating and cooking food. It is of great public concern because of the health hazards related to it (Zhi *et al.*, 2017). It has been previously reported that the exposure of MW radiation and microwave heated food on mice for 8 weeks resulted in the loss of regular structure of liver including hepatocytes vacuolization and hyalinization with pyknotic nuclei, change in hepatic sinusoids, cellular intrusion, necrotic cells, hypertrophied kupffer cells and distention of central vein (El-Ghazaly *et al.*, 2014). Various other studies also investigated the effects of X-ray film developer and gamma rays on rat's kidneys and observed changes including edema, Bowman's capsule hypertrophy, tubular destruction, necrosis, infiltration of inflammatory cells, basement membrane thickening (Ugwuanyi *et al.*, 2016), and marked alterations in glomeruli and blood vessels (Mossua, 2009).

Greater use of electricity based system in houses and at work place has changed the human environment. High frequency electromagnetic field devices as cellular phones and microwave oven are in extensive use. Because of the corresponding number of the people exposed to these waves, there is a need to study the health risks of electromagnetic field (Weiss and Landauer, 2003).

So the major aim of this research was to evaluate the various biological effect of microwave processed feed in different types of containers on the liver and kidney of experimental animals to utilize the findings in humans.

MATERIALS AND METHODS

Adult male albino mice (*Mus musculus*) were retained in standard conditions at the animal house of the Department of Zoology, University of Sargodha, Sargodha Pakistan. The animals were given drinking water and feed in the form of pellets containing wheat, corn, sorghum, barley, rye, triticale, oat and powder milk. Food and water were provided ad libitum. Forty male mice of 25-30g body weight and 5-6 weeks age were randomly divided (n=10) into four groups.

Control group mice were provided untreated feed during experimental period. Direct group was given microwave exposed feed without any container. Plastic group was provided feed exposed to microwaves in plastic container. Glass group animals were fed on microwaves treated feed in a glass container.

Feed was exposed to 900watt, 2450MHz microwaves

at 250°C for 15 min. Experimental period was of four weeks. The body weight of control and all treated mice was recorded daily with the help of digital balance.

At the end of the experimental period, animals were dissected to collect their liver and kidneys. Organs were then weighed and fixed in Carnoy's fixative. Random sections of 5µm thickness from liver and kidney were then processed through the routine procedure of hematoxylin and eosin staining for histological studies. Digital microphotographs of stained sections were taken by Huawei company's digital camera (Model no DSC-W35) affixed on a trinocular microscope (Labomid CXR2) at 400×. Micrometric data was obtained using Corel DRAW 11 software. While the number of mononucleated, binucleated, and oval cells per unit area was counted randomly within a quadrant of 10 × 10cm in addition to the diameter of the nucleus of hepatocytes, central vein, and nucleocytoplasmic index from photomicrographs of liver sections.

Statistical analysis

Statistical analysis of data was performed by One Way Analysis of Variance (ANOVA) followed by Tukey's post hoc analysis by the help of software Graph Pad Prism version 5.00. Data of all parameters related to control and treated groups are expressed as Mean ± SEM.

RESULTS

Body and organs weights

One way analysis of variance (ANOVA) showed a significant ($P < 0.001$) increase in mean body weight of mice of all treated groups as compared to control after 28 days intake of microwaved exposed feed with highest weight gain in glass container group. Statistical analysis (ANOVA) showed a non-significant difference in mean kidney weight of all treated groups as compared to the control group. Whereas highly significant ($P < 0.001$) increase in mean liver weight of mice belonging to all treated groups was noticed as compared to control with highest mass gain in glass container group (Table I).

Table I. Effects of microwave processed feed (4 weeks) in different containers on body and organs weight of adult male mice.

Groups	Body weight (g)	Kidney weight (g)	Liver weight (g)
Control	29.36±0.16	0.36±0.01	1.77±0.03
Direct	30.89±0.18 ^{a***}	0.37±0.01	2.20±0.05 ^{a***}
Glass	32.33±0.21 ^{ab***}	0.38±0.03	2.34±0.04 ^{a***}
Plastic	31.74±1.22 ^{a***b*}	0.34±0.01	2.08±0.04 ^{a***c**}

Values are presented as Mean ± SEM, a, Control group vs treated groups; b, Direct group vs glass and plastic groups and c, Glass group vs plastic group. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Histopathological observations

Kidney

Histological analysis of kidney sections in the control mice showed all signs of normal structures having well-organized Bowman's capsule and round glomeruli confined in proper peri-glomerular space. The healthy proximal and distal tubules with appropriate luminal space and well-aligned nuclei in the epithelium of tubules were observed (Fig. 1A). While kidney sections of direct and glass group (Fig. 1B, C) showed edematous shattering of Bowman's capsule with thickened parietal layer, swollen and deformed glomeruli, wide peri-glomerular spaces and dilation of the proximal tubule. Tubular degeneration (Td) was also observed. Whereas the plastic group showed almost normal Bowman's Capsules, glomeruli, and peri-glomerular spaces, dilation of proximal and distal tubules with narrow luminal space was observed (Fig. 1D).

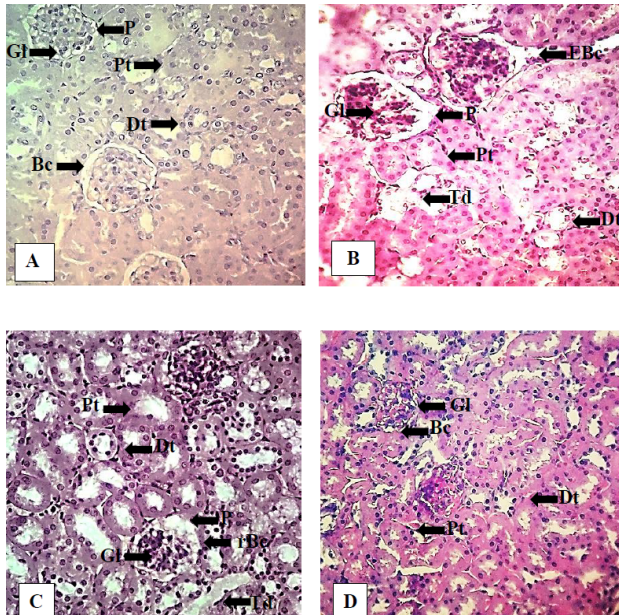


Fig. 1. Effect of exposure of feed to microwaves in different containers on histological structure of kidney of male mice. A: Control showed well organized Bowman's capsule (Bc) with narrow peri-glomerular spaces (P) compact glomerulus (Gl), normal proximal (Pt) and distal tubules (Dt) with slight lumen. B: Direct group showed edematous Bowman's capsule (EBc), increased peri-glomerular space, with swollen glomeruli (Gl), tubular degeneration (Td), and dilation of proximal and distal tubules with increased lumen. C: Glass group showed ruptured Bowman's capsule (rBc), swollen glomeruli, wide peri-glomerular space. Proximal and distal tubules showed dilation and tubular degeneration. D: Plastic group renal sections showed almost normal peri-glomerular spaces, Bowman's capsules and proximal tubules. Stain: hematoxylin and Eosin Magnification: 400X.

Liver

Histological analysis of liver sections of the control group presented normal anatomical structure as most of the hepatocytes showed a continuous array of one cell thick hepatic chord around the compact central vein (Cv). Main parenchyma comprised of mononucleated and binucleated hepatocytes. Sinusoidal spaces of usual size were observed between the hepatic cords with the lining of elongated kupffer cells and free oval cells (Fig. 2A). Various histological alterations were observed in liver sections of all groups given microwaved processed feed as reduced hepatocytes number with deformed and large nuclei, loosening of hepatic tissues with irregular arrangement of hepatocytes. Dilation of Cv with cellular infiltration was observed. Sinusoidal spaces were decreased. A decrease in the mean number of kupffer and oval cells per unit area was also noticed (Fig. 2B). The striking feature in these sections was the area of regeneration of hepatocytes (Fig. 3B, C) that pointed toward the natural recovery process in the damaged liver.

Micrometric analysis of kidney

According to statistical analysis the average cross-sectional area of Bowman's capsule ($P<0.05$) and glomeruli ($P<0.001$) (10cm^2) was significantly increased in mice raised on feed that is directly exposed to microwaves as compared to control and other treatment groups (Table II).

Analysis through ANOVA showed highest increase in average cross-sectional area of proximal and distal tubule in glass ($P<0.001$) container used group as compared to control while dilation of tubules in other treatments was also obvious (Table II).

Table II. Average cross-sectional area of glomeruli, Bowman's capsule, proximal, and distal tubule in control and treated groups after 28 days microwaves-exposed feed intake in adult mice.

Groups	Average cross sectional area (10cm^2)			
	Bowman's capsule	Glomeruli	Proximal tubule	Distal tubule
Control	22.91 \pm 0.37	18.91 \pm 0.32	13.61 \pm 0.17	9.27 \pm 0.10
Direct	24.34 \pm 0.40 ^{a*}	21.15 \pm 0.41 ^{a***}	14.35 \pm 0.13 ^{a*}	9.21 \pm 0.10
Glass	23.78 \pm 0.40	20.52 \pm 0.37 ^{a**}	15.08 \pm 0.26 ^{a***b*}	11.07 \pm 0.11 ^{ab***}
Plastic	22.12 \pm 0.32 ^{b***c*}	19.47 \pm 0.27 ^{b**}	14.15 \pm 0.17 ^{c**}	10.21 \pm 0.15 ^{abc***}

Values are presented as Mean \pm SEM; a, Control group vs treated groups; b, Direct group vs glass and plastic groups and c, Glass group vs plastic group. *** $P<0.001$, ** $P<0.01$, * $P<0.05$

Micrometric analysis of liver

One way analysis of variance showed that microwaved exposed feed in all groups caused a highly significant decrease in the mean number of mononuclear hepatocytes per unit area (10cm^2) as compared to control. Highest ($P<0.001$) decrease was noticed in the mononuclear hepatocytes number of mice in the glass container group (Table III).

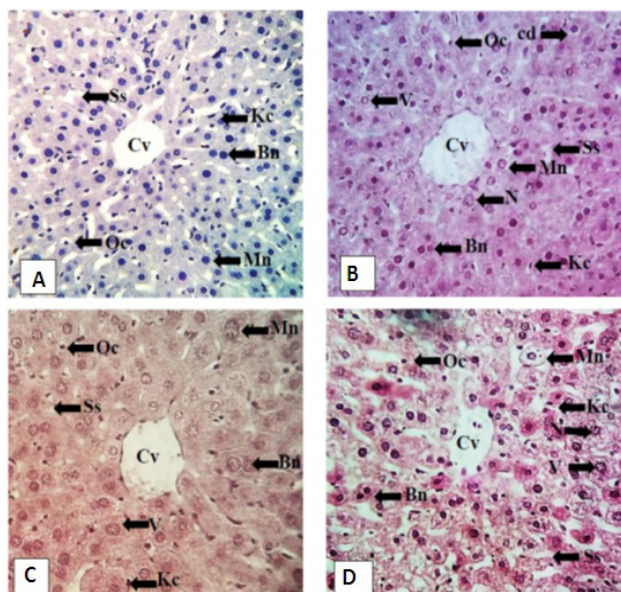


Fig. 2. Effect of exposure of feed to microwaves in different contains on histological structure of liver of male mice. A: Control showing well organized mononucleated (mn) and bi nucleated (bn) hepatocytes of similar size in cord like structure having round nuclei. Sinusoidal spaces (Ss) of equal width lies between hepatic cords, round oval cells (Oc) observed in sinusoidal spaces. Central vein (Cv) is compact. Kupffer cells (Kc) elongated in shape lining the hepatocytes. B: Direct group represented decreased number of hepatocytes as well as nuclear vacuolation (V), necrosis (N) and cellular degeneration (cd). C: Glass group showed decreased number of hepatocytes (Bn and Mn) of variable size and shape, nuclear vacuolation and necrosis. Dilated central vein with infiltrated cells in its lumen. Sinusoidal spaces became wider. D: Plastic group also showed dilated sinusoidal spaces and central vein having infiltrated cells. Number of oval and Kupffer cells was decreased and nuclear vacuolation was also observed. Stain: hematoxylin and Eosin Magnification: 400X.

According to statistical analysis, the average cross-sectional area of mononuclear hepatocytes and their nuclei (10cm^2) increased significantly ($P<0.001$) in direct and glass container groups as compared to that of control and the effect was highest in the glass group.

Whereas statistically microwave exposed feed adversely affected the cross-sectional area of nuclei of hepatocytes in all treated groups as compared to control. The highly significant increase in average cross-sectional area of nuclei of mononuclear hepatocytes was noticed in direct ($P<0.001$), glass ($P<0.001$) and plastic ($P<0.01$) groups as compared to control (Table III).

Table III. Mean number of mononuclear hepatocytes, ACSA of mononuclear hepatocytes, and their nuclei in control and treated groups after 28 days exposure of microwaves treated feed in different containers.

Mononuclear hepatocytes			
Groups	Mean number	ACSA	ACSA of nuclei
Control	8.51 ± 0.25	7.51 ± 0.09	2.92 ± 0.03
Direct	$6.01 \pm 0.31^{a***}$	$10.21 \pm 0.20^{a***}$	$4.37 \pm 0.08^{a***}$
Glass	$4.00 \pm 0.24^{ab***}$	$11.16 \pm 0.20^{ab***}$	$5.13 \pm 0.11^{ab***}$
Plastic	$7.02 \pm 0.30^{a**b*c***}$	$07.94 \pm 0.16^{bc***}$	$3.35 \pm 0.07^{a**bc***}$

Values are presented as Mean \pm SEM; a, Control group vs treated groups; b, Direct group vs glass and plastic groups and c, Glass group vs plastic group. *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

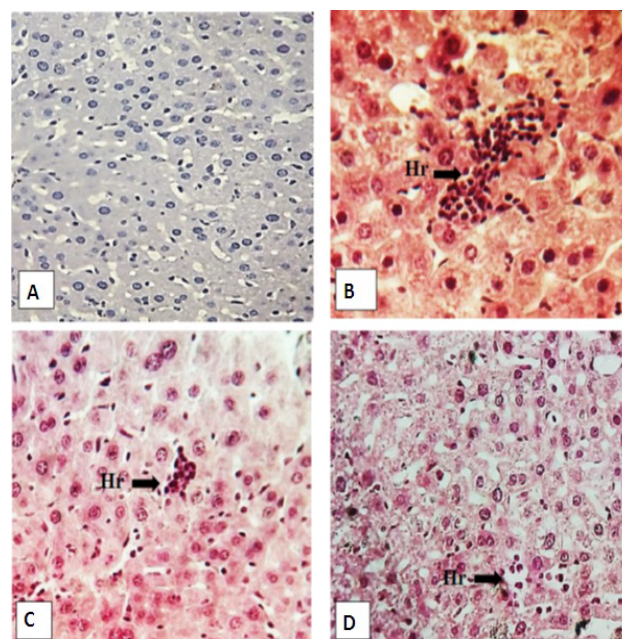


Fig. 3. Effect of exposure of feed to microwaves in different contains on histological structure of male mice liver. A: Direct group showed hepatocytes regeneration (Hr) and disorganized structure of sinusoids (Ss) similarly glass. (B) and plastic group (C) also represented the hepatocytes regeneration zone and disorganized sinusoidal spaces. Stain: hematoxylin and Eosin Magnification: 400X.

Statistically significant decrease in the relative nucleo-cytoplasmic index of hepatocytes in direct exposure, glass ($P<0.001$), and plastic ($P<0.01$) container groups was found as compared to control. Among treatments, significant ($P<0.01$) increase was noticed in the relative nucleo-cytoplasmic index of mice raised on microwave processed feed in a glass container as compared to the plastic group (Fig. 4A).

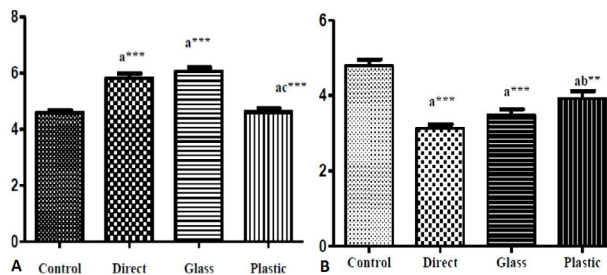


Fig. 4. (A) Relative Nucleo-cytoplasmic index of mononuclear hepatocytes (μm). (B) Effect of microwave exposed feed on mean number of oval cells per unit area (10 cm^2) in control and treated liver of adult mice after 28 days exposure of microwaves treated feed in different containers. Values are presented as Mean \pm SEM, a = Control group vs treated groups, c = Glass group vs plastic group. *** $P<0.001$

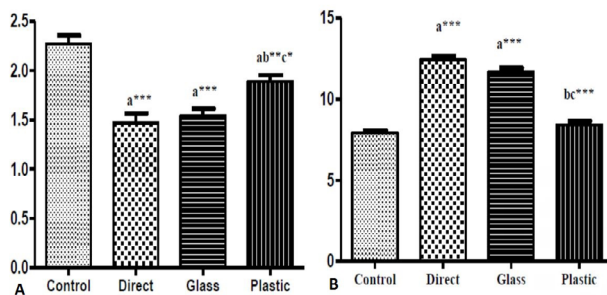


Fig. 5. (A) Effect of microwaves exposed feed on mean number of Kupffer cells per unit area (10 cm^2) in liver of control and treated mice after experimental exposure of 28 days. (B) Effect of microwaved exposed feed in different containers for four weeks on ACSA of central vein (μm) in liver of adult male mice. Values are presented as Mean \pm SEM, a = Control group vs treated groups, b = Direct group vs glass and plastic groups and c = Glass group vs plastic group. *** $P<0.001$, ** $P<0.01$, * $P<0.05$

Statistical analysis revealed significant ($P<0.001$) decrease in the mean number of oval cells in direct, glass, and plastic ($P<0.01$) container groups as compared to that of control (Fig. 4B).

One way analysis of variance showed that there

was a highly significant decrease ($P<0.001$) in the mean number of Kupffer cells per unit area (10 cm^2) as a result of microwaved processed feed intake in direct, glass, and plastic ($P<0.01$) containers as compared to control group (Fig. 5A).

According to statistical analysis, average cross-sectional area of the central vein (10 cm^2) was significantly ($P<0.001$) increased in direct and glass groups as compared to control, however, this increase was non-significant in the plastic group. Analysis within groups revealed a highly significant ($P<0.001$) increase in ACSA of the central vein of the direct and glass container groups as compared to that of plastic group (Fig. 5B).

DISCUSSION

Scientists alarmed the public about the negative impacts of microwaves on the liver, kidneys, heart, skin, brain, thyroid, reproductive tissues, and eyes (Ayata *et al.*, 2004; Lahkola *et al.*, 2005; Oktem *et al.*, 2005; Wdowiak *et al.*, 2007; Makker *et al.*, 2009). The crucial organ that is targeted and harmed by electromagnetic radiation is the liver (Finfer *et al.*, 2006). Histopathology of the liver has been realized as a marker of environmental strain (Alturkistani *et al.*, 2015). In the present study, the mean body weight and liver weight of all treated mice showed highly significant increase ($P<0.001$) as compared to that of control animals (Table I). This might be attributed to the altered lipid metabolism associated with microwaves exposure (Tian *et al.*, 2019).

Liver is more susceptible to microwaves that can induce histopathological changes and elevation in the weight of the liver (Kristic *et al.*, 2005). Histological analysis of liver sections of the control group in the current study presented normal anatomical structure while continuous intake of microwave oven processed feed for four weeks in glass/ plastic container or on oven tray resulted in many structural anomalies. These radiations are responsible for the vacuolation of hepatocytes by dissolving the lipids from the hepatocytes (Tian *et al.*, 2019). The vacuolation and necrosis of liver hepatocytes are mediated through lysosomal enzymes released out by the effect of radiations on the lysosomal membrane (Wang *et al.*, 2018). So, the liver is the major target organ of the microwave processed food, as the food particles reach the liver through the hepatic portal vein and affect it which is characterized by steatosis of RBC's an indicator of chronic hepatitis C (Au, 2004).

Similarly, the histological analysis of treated groups revealed the loss of normal anatomical structure of the kidney as compared to that of control (Fig. 1). Microwaves exposure affects kidney function by increasing the

creatinine and urea concentrations in the blood of mice (Mossua, 2009), whereas, creatinine level is an important indicator of the functioning of the kidney (Pandya *et al.*, 2016). Molecular alterations due to radiations exposure might be the underlying cause of structural deformities in the liver and kidneys in the present study.

As previously reported the influence of radiofrequency and microwaves of 420 MHz, 2 GHz on humans resulted in a significant elevation in levels of uric acid, urea, and creatinine (Dasdag *et al.*, 2008). It has been investigated that non-thermal forces of MW cause a substantial increase in reactive oxygen species and nitrogen oxide production in cells both in vitro and in vivo conditions (Sokolovic *et al.*, 2008; Agarwal *et al.*, 2009; Grigoriev *et al.*, 2010). Oxidative stress-induced by MW might be the stimulatory factor for cancer (Schuermann and Mevissen, 2021).

These findings indicated that besides its benefits and ease of cooking, microwave ovens might have dangerous effects on human health. As obesity is a growing issue nowadays parallel with the use of electromagnetic devices like microwaves that might contribute to increased body weight. Similarly, liver and kidney anomalies are increasing with the sedentary lifestyle. Radiations exposure seems to be a contributing factor in piling these up.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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