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RESEARCH ARTICLE

Harmful Effects of the Interaction Between Microplastic Container, Aluminum Foil and Hot Water on Some Physiological Parameters Using Pigeon Model

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ABSTRACT

Due to common used of disposable food containers currently. This study evaluated the toxicity resulted from the interaction between the boiling tap water, microplastic container, aluminum foil with or without microwave radiation on some physiological and histological parameters in Pigeons. Forty pigeons were classified into four groups. The birds in group 1 drink tap water without boiling placed in the usual drinking bowl. The birds in group 2 received tap water in thermoplastics containers previously boiled in microwave oven for 5 minutes, whereas in group 3, the birds drink boiled tap water that put in thermoplastics containers for 5 min and group 4 the birds drink boiled water placed in a covered bowl completely with aluminum foil for 5 minutes, in the last three groups when the water temperature drops to 27°C placed in the usual drinking bowl. The experimental continued for 30 days. The blood test findings hemoglobin levels dramatically dropped in G2, G3, and G4 compared to G1. Granulocytes Percentage dramatically decreased at all test groups compared to G1, WBCs, Lymphocytes and Monocytes significantly increased in all treated groups. Lipid profile results showed LDL levels increased statistically significant in G2 than in G1, while liver profile only increased in ALT value at G3 compare G1. Histopathological sections of liver, kidney and testicular tissues revealed pathological changes in whole treated groups compared to control. It can be concluded that using of plastic container and Aluminum foils can be toxic when increased temperature with or without using microwave oven.

Keywords: Aluminum foil, Blood parameters, Electromagnetic wave, Lipid profile, Liver function pigeon, Plastic container

Introduction

The spreading and large quantity of microplastics into the world are so wide that researchers practice them as main signs of the current and modern dated important a novel historical period.¹ Traditional glass and steel containers are rarely used compared to disposable plastic food containers after becoming the most common. The reason is that disposable food containers are cheap, simply obtainable, and can be used without fear of breaking. Most importantly, it is used only once, so there is no necessity to have

cleaning at all. Although disposable food containers make our lifespan informal, they also carry excessive health risks. Disposable containers are made up of plastic. This material is usually shown to leak poisonous chemicals after heated, polycarbonate which filters bisphenol A, while Polystyrene leaches styrene and polyvinyl chloride (PVC), which break in vinyl chloride in addition to phthalates, which can filter into several chemicals.² Plastics have two major types: thermosets and thermoplastics. Thermosets are polymers that cannot be remolded, which means they fixed irreversibly when heated and cannot be used

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in food packaging applications. Thermoplastics can easily be shaped and molded into numerous products, such as plastic films that softened by heat and returned to their original form at room temperature; therefore, they are a model for food packaging.³ One of the most commonly used plastics is polyethylene terephthalate (PET), which contains ethylene glycol and terephthalic acid as repeating units.⁴ Polyethylene terephthalate (PTF) is a public plastic in various products. Plastic uptake by individuals induces several disease conditions in humans by decreasing migration and mesenchymal stem cells proliferation of bone marrow and endothelial precursor cells.⁵ Metal is the most adaptable of all packaging forms. Furthermore, it is widely employed as aluminum foil used for food preparation, cooking, and also for foodstuff storage and packaging.⁶ It provides a combination of physical shield and barrier properties; recycling ability and consumer. Metals predominantly used in wrapping are aluminum (AL) and steel. Aluminum is a silvery white metal and lightweight. Magnesium and manganese are commonly added to increase Aluminum strength properties.⁷ Aluminum makes laminated paper or plastic packaging, foil, and cans. Aluminum foil (ALF) is a very thin sheet made by rolling pure aluminum, followed by hardening to achieve properties of dead-folding, which means a fold is made in the film and stays in place, then it can be folded tightly. ALF is available in various ranges of thicknesses: thicker foils are used for platters, and thinner foils are used as food wrappers. ALF has numerous characteristics, such as thermal conductivity, being two times faster than ordinary metal, and being easy to use and dispose of, so it is broadly used in cooking and food wrapping. Furthermore, moisture, odor, air, light and microorganisms are prevented by aluminum foil.⁸ The entire ALF food content includes aluminum which is naturally present; as a contaminant, in food additives, and from food contact materials such as food containers, cookware and food wrappings. Further exposure can arise from drinking water. Aluminum has no identified biological function. Reproductive toxicity studies in male mice and rabbits through the administration of aluminum nitrate or chloride have established the ability of AL to cause declined sperm quality and decreased fertility. The particular mechanism of AL toxicity is not completely understood. It is considered that AL is possibly cell and neurotoxic. Disrupted enzyme activity and impaired mitochondrial function also produce oxidative stress. It has been proposed that AL has a role in the incidence of diseases for example breast cancer and Alzheimer's dementia. The initial direct connections, that revealed between AL and hemodialysis, osteomalacia

encephalopathy, anemia, and aluminosis (pathological changes to the lungs).⁹ With current society development, microwaves are commonly used in various traits, like medical, manufacturing and many other scopes. Microwaves MWR get assistance but generate deliberations about the risks of electromagnetic situations. World Health Organization, besides the International Agency for Research on Cancer, regards electromagnetic influences as the fourth pollutant, also classified as "possibly carcinogenic to humans."¹⁰ Birds have been utilized in Ecotoxicological research since the 1960s because they have a number of qualities that make them ideal for this type of study. Contaminant exposure in birds has been thoroughly documented compared to other animal models due to many factors, including migratory skills, worldwide distribution, prominent nature, various foraging patterns, and environmental pollutant sensitivity.¹¹ Since the blood has varied functions, which can be generally classified into transport, protection, and homeostasis. It confirms the resource of oxygen, nutrients, and whole hormones to the sites where they are required and carry carbon dioxide and metabolites to their port of removal. This study aimed to evaluate toxic effects which resulted from the interaction between the boiling tap water, microplastic container, aluminum foil with or without microwave radiation on some physiological and histological parameters in Pigeons.

Materials and methods

Preparing birds for the study

Forty Pigeons (*Columba livia domestica*) with average body weights of (220 ± 15) g were bought from the Basrah local market. All were sexually inactive and had mature plumage at around the same age. All birds perform one week of adaptation following a clinical examination upon arrival. The pigeons were housed separately in $140 \times 110 \times 100$ cm³ cages.¹²

Study design

Pigeons were haphazardly assigned into 4 groups with six of them in each group. Birds are treated as the following:

- G1: The birds drink tap water without boiling, placed in the usual drinking bowl.
- G2: The birds were treated with tap water in thermoplastics containers previously boiled in microwave oven for 5 minutes, then put in the normal drinking bowl when the water temperature falls to 27°C.

- G3: The birds drink boiled tap water in thermo-plastic containers for 5 min, and when the water temperature drops to 27°C placed in the usual drinking bowl.
- G4: The birds drink boiled water placed in a covered bowl completely with aluminum foil for 5 minutes, then the aluminum cover is removed, and when the water temperature becomes 27°C, it is transferred to the usual bowl.

During the 30 days of the trial, the environmental conditions were maintained at around 23°C with a 12/12-hour light/dark cycle. Pelleted pigeon food was freely available for the birds. All experiment stages began with the birds fasting for 12 hours before treatment. Throughout the period of adaption, no deaths were noted; all methods employed in this investigation were approved. The number granted by the institution's ethics committee was 045 A Pharmacology & Toxic Department Pharmacy College. Thirty days later, 3 ml of blood samples were collected from the wing vein and kept in a gel tube for biochemical test and 2ml kept at EDTA containing tube for measuring blood cells and parameters. The birds were synthesized via ether to obtain livers and kidneys, and the test is for histopathological evaluation.¹³

Biochemical analysis

Liver Function Tests (LFT) are useful screening tool, they are very effective modality to find hepatic dysfunction,¹⁴ which include: aspartate aminotransferase (AST) and alanine transaminases (ALT). The measurements were made using a JOURILABS, Ethiopia kits for colorimetric analysis.

Profile of lipids

Using a specialized colorimetric JOURILABS, Ethiopia kits, triglyceride, total cholesterol, and high-density lipoprotein levels were assessed. Friedwald et al's formula for calculating LDL cholesterol is as follows:¹⁵

Low Density Lipoprotein (LDL)

$$= \text{total cholesterol} - \text{HDL} - \text{Triglycerides}/5$$

Very Low-Density Lipoprotein (VLDL)

$$= \text{Triglycerides}/5$$

Histopathological investigation

Organs were removed from the body and rinsed with regular saline after the pigeon was euthanized

with chloroform. The samples were transported to the pharmacy college central lab after being fixed in 10% formalin. The samples for histopathological evaluation were prepared in embedded paraffin. Hematoxylin and eosin stained the slides.

Statistical valuations

The results' mean values and standard deviations (mean \pm SD) were recorded. One-way analysis of variance was used to assess differences between groups (ANOVA) ($p < 0.05$).

Results and discussion

The erythrocyte parameters of domestic pigeons revealed no significant change in RBC count or RBCs indices between test groups. However, there was a significant drop ($p < 0.05$) in hemoglobin values between G2 and G1 (control). Differential white blood cells, including lymphocytes, monocytes, and granulocytes, showed statistically significant alteration among test groups. WBCs count increases in G2 and G4 than in control. G3 exhibits significant alterations with G1. Lymphocyte percentages in G2, G3, and G4 showed highly significant increases compared to G1. A highly significant decline in granulocyte percentage was revealed in G2, G3, and G4 compared to the control group as shown below in [Table 1](#).

Biochemical tests

Liver function test

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were used to assess liver function, according to the data in [Table 2](#). AST levels of all test groups were statistically insignificant compared to those of the control and other treated groups. Additionally, it is evident from the same data that the serum ALT level at G3 increased significantly ($p < 0.05$) in contrast to the control.

Lipid profile

According to the results in [Table 2](#), whole treated groups showed insignificant alteration in the serum cholesterol; Triglyceride, HDL, and LDL, except in LDL, revealed a significant increase in G2 compared to control and other groups.

Histopathological evaluation

Hepatic histopathological assessments

Photomicrograph of G1 exposed integral histological structure through a typical central vein. In addition, normal sinusoids in addition to nearby

Table 1. The effect of microplastic container & aluminum foil on blood parameters (mean \pm SD).

Groups	Blood parameters					LSD
	G1	G2	G3	G4		
WBCs($\times 10^3$)	9.68 \pm 1.24	30.4 \pm 15.49 a	13.8 \pm 2.51	41.34 \pm 3.72 abc	10.94	
Lym.%	30.42 \pm 1.83	93.06 \pm 7.15 a	81.96 \pm 6.56 ab	92.78 \pm 1.92abc	10.82	
Mon.%	1.92 \pm 0.944	2.46 \pm 1.105	2.62 \pm 0.83 a	3.42 \pm 0.86	1.5	
Gran.%	67.4 \pm 1.816	1.78 \pm 0.7 a	4.0 \pm 1.22 ab	2.74 \pm 0.32 a	2.22	
RBC($\times 10^6$)	3.69 \pm 0.22	3.01 \pm 1.6	3.47 \pm 0.995	3.06 \pm 1	NS	
Hb(g/dl)	15.76 \pm 0.74	10.74 \pm 4.56 a	13.44 \pm 13.44	12.04 \pm 4.03	5.02	
PCV(%)	48.52 \pm 3.47	37.96 \pm 19.5	38.04 \pm 9.59	35.92 \pm 15.92	NS	
MCV(fl)	134.34 \pm 3.53	131.58 \pm 3.99	133.02 \pm 6.15	131.66 \pm 2.34	NS	
MCH(pg)	53.6 \pm 1.9	52.78 \pm 3.57	51.76 \pm 5.55	53.74 \pm 1.65	NS	
MCHC(g/dl)	41.06 \pm 0.63	39.96 \pm 1.77	38.0 \pm 4.52	41.34 \pm 1.61	NS	

NS means non-significant; a = significant differences with G1, b = significant differences with G2, C = significant differences with G3.

Table 2. The effect of microplastic container & aluminum foil on lipid profile and liver function tests.

Groups	Lipid profile					Liver profile	
	TC Mg/dl	TG Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl	AST IU/L	ALT IU/L
G1	226.66 \pm 46.82	187.24 \pm 59.81	126.04 \pm 66.71	63.16 \pm 49.3	43.04 \pm 13.85	33 \pm 2.73	66 \pm 22.03
G2	326.26 \pm 162.1	212.04 \pm 86.33	122.24 \pm 100.79	161.6 \pm 82.03 a	42.42 \pm 17.28	45.6 \pm 24.35	88.4 \pm 7.95
G3	162.1 \pm 71.65	182.34 \pm 43.47	178.32 \pm 44.31	88.54 \pm 50.36	36.48 \pm 8.66	48.4 \pm 17.5	94 \pm 0.01 a
G4	218.18 \pm 37.74	142.92 \pm 15.96	80.736 \pm 47.77	108.87 \pm 20.31	28.57 \pm 3.21	32.4 \pm 4.92	86.2 \pm 9.17
LSD	NS	NS	NS	98.44	NS	NS	28

NS = means non-significant; a = significant differences with G1.

hepatocytes in the parenchyma, were observed. Each G2 & G3 displays congestion inflammatory cell intrusion in the portal area. Photomicrograph of G4 revealed degeneration of the hepatocytes in the hepatic parenchyma related to dilation in the central vein, all lesions are illustrated in Fig. 1.

Renal histopathological assessments

Normal glomeruli, proximal tubules, and distal tubules were visible in the first group's kidney histology. Bowman's space enlargement and glomerular shrinkage were visible in all treated groups compared to the control group Fig. 2.

Histopathological evaluation of testis

Histopathological changes in the Testis of the pigeon after treatment compared to the control. At G1(control) a typical seminiferous tubule, showing sperm, germinal epithelium, and interstitial connective tissue, was observed. While G2, G3, and G4 groups showed spermatogenic cells separate, interstitial connective tissue is lost, foci of congestion, numerous seminiferous tubules degenerate, and there is evident vacuolization Fig. 3.

Pigeons were used as test subjects for basic science research as early as the 19th century. Pigeons were used by Josef Breuer, Sigmund Freud's mentor and one of the pioneers of psychoanalytic thought, to study semicircular canal function. B.F. Skinner,

a US psychologist, introduced pigeons to experimental psychology in the 1930s. Since pigeons live longer than rats, Skinner moved from using them to studying the processes of operant training. It is impossible to make definite inferences about quantitative variations in absorption across various chemicals because of sensitivity limitations in analytical methods, methodological variations between experiments, including the supplied doses, and inter-animal variation. Pigeons have the latent to assist as an opening species for researchers to discover the connection people have with their native environment. As we increase our collective thought of our environment, we can investigate the upcoming use of the pigeon as an influential lens to study the built-up dynamics of ecological requirements.¹⁶

Plastic contamination in many forms has developed as the most severe environmental threat. Small plastic pieces, such as microplastics and nanoplastics derivative from primary and secondary sources, are a major concern locally due to their antagonistic effects on the environment and public health.¹⁷

Through the results of our current study, a significant effect was observed for each treatment group compared to the control group on some blood parameters for pigeons, particularly the differential WBCs, white blood cells, and hemoglobin concentration. However, the interaction of hot water with plastic food containers and the effect of the

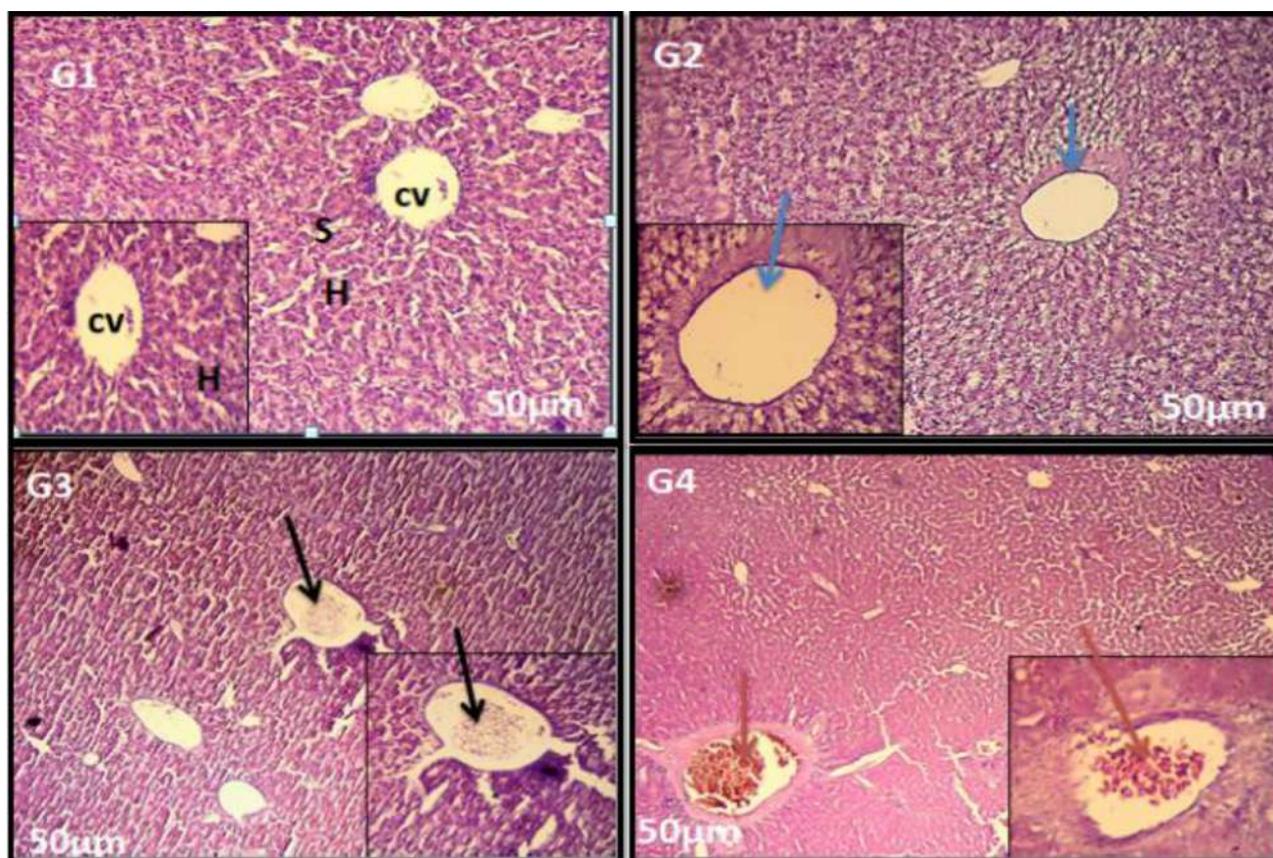


Fig. 1. Photomicrographs of the liver tissue in pigeons. (G1) control group showing whole histological structure with normal central vein normal sinusoids, besides surrounding hepatocytes in the parenchyma. G3 and G4 Photomicrograph of pigeons, each group displaying congestion inflammatory cell infiltration in the portal area (black arrow). G2 Photomicrograph of pigeon group M showing degeneration of the hepatocytes with dilation in the central vein (blue arrow) and Stain: hematoxylin and eosin; magnifications: X100 and X400 for chief and inset images correspondingly. (CV: central vein, H: hepatocytes, S: blood sinusoids).

electromagnetic wave of microwaves is the most harmful to the blood of the rest of the groups. The immune system is impacted by microwave radiation, which can influence cellular, humoral, and non-specific immunity. Depending on the power density and duration of the exposure, microwave radiation has varying effects on immune function. According to another study, immune cells exposed to microplastics will perish three times as rapidly as those that are not. However, 60% of the cells died within the same time frame when they came into contact with microplastics. Numerous animal studies have demonstrated that contact with nano- and microplastics induces disruptions in the gut's epithelial permeability as well as disturbances in the balance between oxidative and inflammatory processes. Dysbiosis (changes in the gut micro biota) and immune cell toxicity are two other prominent impacts of nano- and microplastic exposure.¹⁸

An enormous number of chemical compounds with endocrine-disrupting activity have been recognized. These chemicals are universal and extensively used

in various products of our daily lives. Bisphenol is amongst the most public endocrine disrupting chemical that has been used for a lot of years in the manufacture of polycarbonate plastics and epoxy resins.¹⁹ Packaging food in plastic or warming food in the microwave, bisphenol-A, besides phthalates escape into the diet.²⁰ Among the results of the current study, aluminum foil had a clear effect on white blood cell count, which caused a significant increase compared with the control. The explanation may be due to the increased leakage of aluminum ions from aluminum foil, which increases with the rise in water temperature. Certain investigational results acquired by prior studies reveal that the complex effect plays a significant role in the route of aluminum leaching from food preparation instruments. Augmented concentrations of complex ions such as organic acid, fluoride ions, or OH significantly improve aluminum discharge, as increased cooking temperature causes extra aluminum ions to escape. The leakage relies greatly on the pH value of the diet solution, spices and salt addition to the food solutions. Aluminum

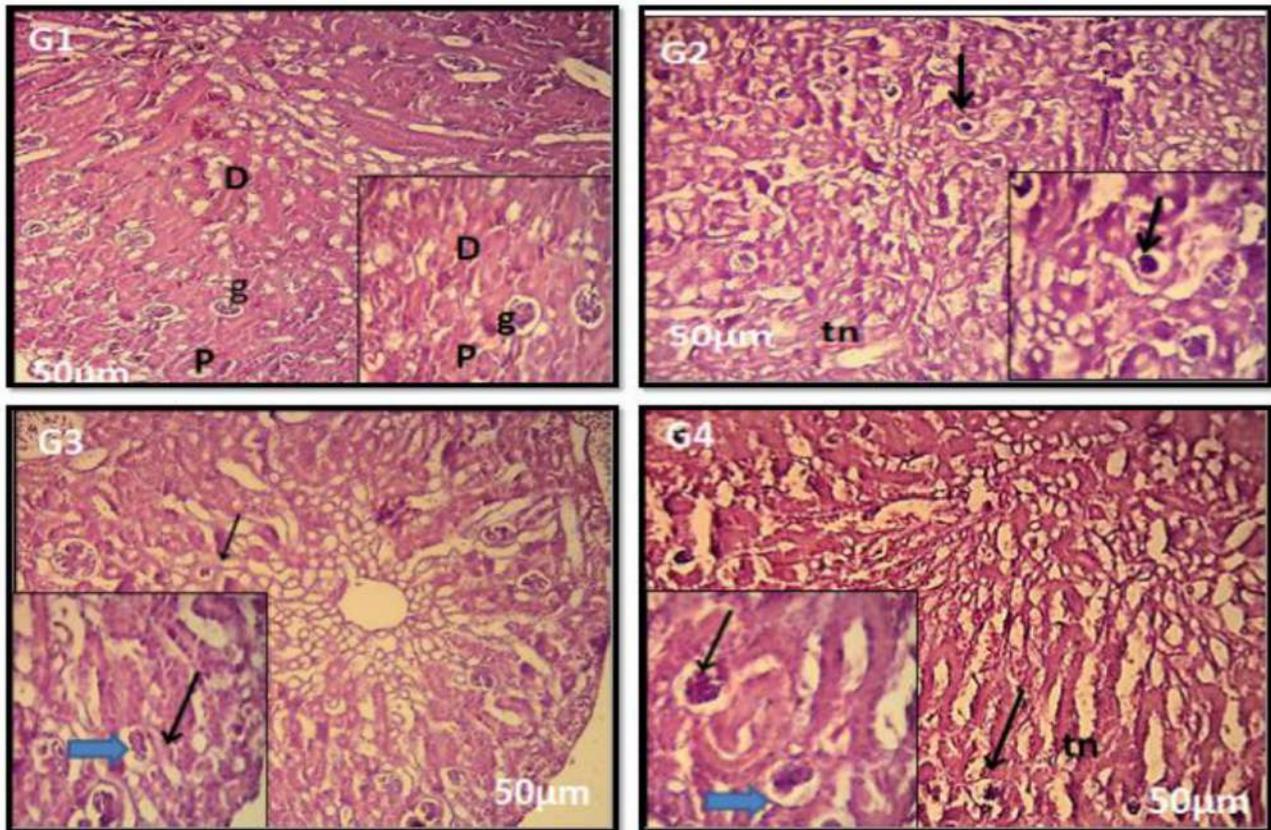


Fig. 2. Photomicrographs of the kidney tissue in pigeons (G1) Control group, glomerulus (g), proximal tubules (p) and distal tubules (d). Each G3, G2, and G4 revealed glomerular shrinkage (black arrow), Bowman's space enlargement (blue arrow), and GM showed tubular necrosis (tn). Using H&E stain and magnifications 100x and 400x.

foil is not fit for cooking generally through bitter food.

Another study has shown that, when the foods are cooked by wrapping aluminum foil leaching occurs with the consumption of these foods.²¹ Alanine aminotransferase (ALT) in addition to aspartate aminotransferase (AST), are normally used as indicators for liver impairment. While AST is present in cardiac, skeletal muscle, erythrocytes, and liver tissue, making ALT is the most precise sign of liver injury. However, the results of the current study showed the effect of the livers of G3 compared to the control and other groups, especially the significant increase of ALT enzyme. This is not in agreement with a previous study indicating a significant increase in AST, ALT, and ALP in the microwave group compared to the control group. Another study revealed that polystyrene microplastic contact in fish livers prompts modifications in the metabolic profile, interferes with lipid then energy metabolism, as well as reveals polystyrene toxicity.²²

As for the lipid profile, the results of our study showed that all treated groups were unaffected com-

pared to the control group, except in LDL, where G2 was the most affected group. Polypropylene, polystyrene, and cellulose ethers, components of plastic containers, contain polyethylene with messy polymer chains. When those chains get touched with hot food, the weak bonds between the polymer chains break down and release massive free radicals.²³ Different diet substances have diverse temperatures in cooking, and polymers have glass change temperatures, which can break the bonds of polymers and discharge free radicals depending on the food kind. Free radical impairment influences various chronic diseases of cataracts, reproductive and cardiovascular systems and lastly, cancer. Histological analysis of liver sections from the control group in the current study showed normal anatomical structure. While continuous consumption of feed prepared in a microwave for four weeks in a glass, plastic, or oven tray resulted in several structural anomalies, These radiations dissolve the lipids from the hepatocytes, which causes the vacuolation of the cells. Lysosomal enzymes released by the impact of radiation on the lysosomal membrane of liver hepatocytes

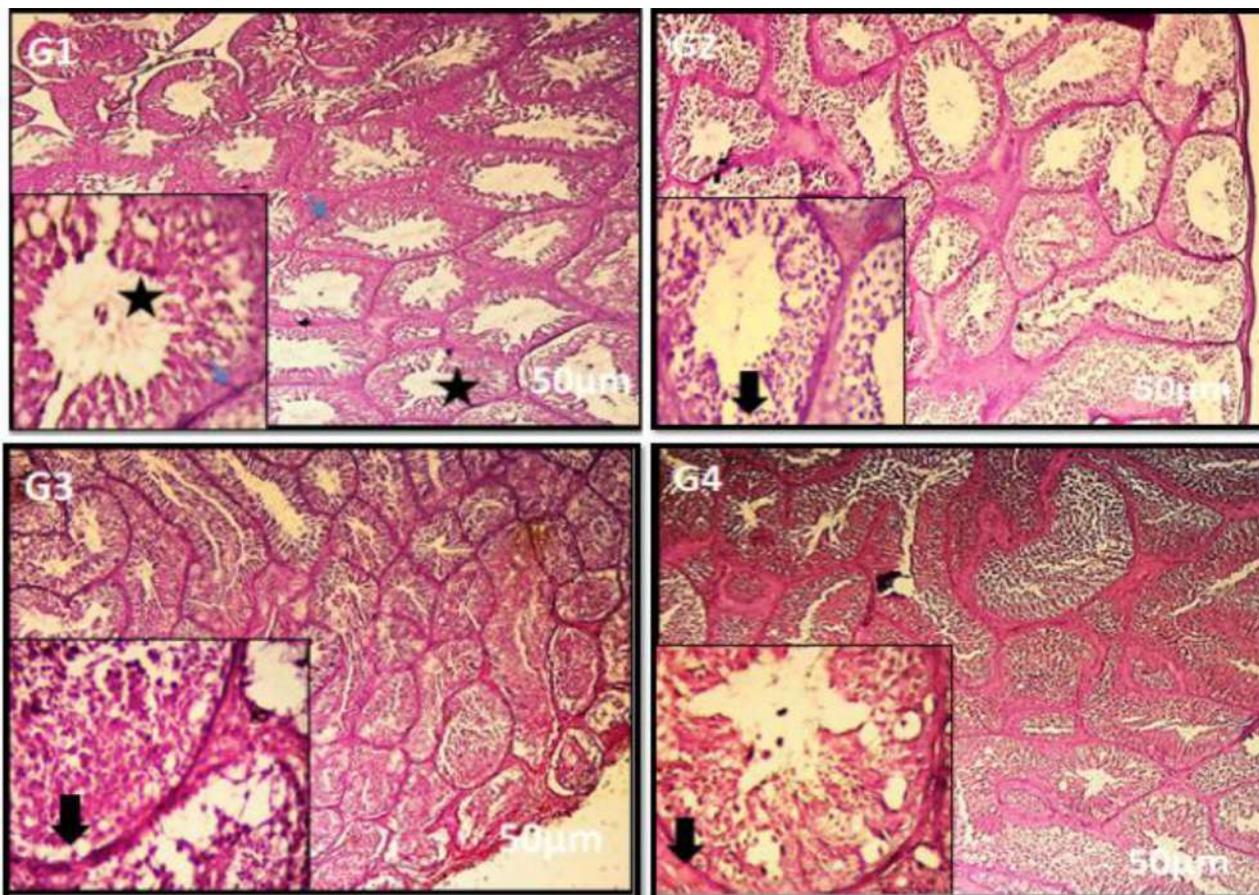


Fig. 3. (G1) Ordinary seminiferous tubule and sperm (asterisk) lined by germinal epithelium (blue arrow) and enclosed by interstitial connective tissue (black arrow) in the control group. Each group (3, 2 and 4) shows the same changes, such as the Separation of spermatogenic cells (double black arrow) and loss of interstitial connective by means of foci of the congestion in addition to degeneration in several seminiferous tubules, and clear vacuolization. Stain: Haematoxylin & eosin. Magnifications of main images: 100X; inset: 400X.

vacuolate and then die.²⁴ All animals that received microwaved processed feed showed a variety of histological changes in their livers, including a decrease in the number of hepatocytes with distorted and big nuclei and a loosening of the hepatic tissues with an uneven arrangement of hepatocytes Cv enlargement was seen in conjunction with cellular infiltration. Spaces in sine waves were shrunk.²⁵

Additionally, there was a decrease in the average number of kupffer and oval cells per unit area. The histological investigation of all treated groups compared to the control group indicated the kidney's natural anatomical structure loss. Finally, the whole treatment group revealed significant changes in the structure of the Testis. These results agreed with what was stated in the previous study.²⁶

Conclusion

We conclude from the current study that there is a significant overlap between the effect of microplas-

tic containers, aluminum foil and hot water with or without microwave effect. Increasing the temperature accelerates the leaching of harmful chemicals found in food containers or aluminum foil; thus, these materials affect some physiological parameters. Therefore, we must be careful when using these materials, especially when heating them.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been

included with the necessary permission for republication, which is attached to the manuscript.

- No human studies are present in the manuscript.
- The author(s) has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at University of Basrah/college of pharmacy. An approval number is (04SA).

Authors' contributions statement

M. N. performed the conceptualization and corresponding. N. F. N. did the formal analysis, writing - review & editing. F. S. M. performed data curation.

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التأثيرات الضارة للتفاعل بين الحاوية البلاستيكية الدقيقة, ورقائق الألمنيوم و الماء الساخن على بعض المعايير الفسيولوجية باستخدام نموذج الحمام

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المستخلص

نظرا لشيوع استخدام حافظات الطعام البلاستيكية حاليا. اقيمت هذه الدراسة لتقييم السمية الناتجة عن التفاعل بين ماء الصنبور المغلي وحافطة الطعام البلاستيكية ورقائق الألمنيوم مع او بدون اشعاع المايكرويف . تم تصنيف اربعين حمامة الى اربع مجاميع . تشرب الطيور في المجموعة الاولى ماء الصنبور العادي دون ان يغلي ويوضع في وعاء الشرب المعتاد . تلقت الطيور في المجموعة الثانية ماء الصنبور في حافطة بلاستيكية حرارية سبق تسخين الماء فيها لمدة 5 دقائق، بينما في المجموعة الثالثة، تشرب الطيور ماء الصنبور المغلي الذي يوضع في الحافطة البلاستيكية الحرارية لمدة 5 دقائق والمجموعة الرابعة تشرب الطيور ماء الصنبور المغلي الموضوع في اناء مغطاة بالكامل برقائق الألمنيوم ولمدة 5 دقائق، في المجاميع الثلاثة الاخيرة عندما تنخفض درجة الماء المغلي الى 27 درجة مئوية , يوضع الماء في الاوعية المعتادة للشرب . استمرت التجربة لمدة 30 يوم . اظهرت نتائج اختبار الدم انخفاض في مستويات الهيموجلوبين بشكل كبير في كل المجاميع العاملة مقارنة بمجموعة السيطرة . انخفضت النسبة المئوية للخلايا الحبيبية بشكل كبير في جميع المجاميع العاملة، ومع ذلك زادت خلايا الدم البيضاء والخلايا الليمفاوية والخلايا الاحادية بشكل ملحوظ في كل المجاميع العاملة مقارنة بالمجموعة الاولى، اما في نتائج تحليل الدهون اظهرت ان مستويات الدهون واطئة الكثافة ازدادت بشكل معنوي في المجموعة الثانية، بينما في تحليل كفاءة الكبد فقط قيمة انزيم ALT ازداد في المجموعة الثالثة مقارنة بالمجموعة الاولى . اظهرت المقاطع النسيجية المرضية لأنسجة الكبد والكلى والخصى تغييرات مرضية في كل المجاميع. يمكن الاستنتاج ان استخدام الحافظات البلاستيكية ورقائق الألمنيوم يمكن ان يكون ساما عند ازدياد درجة الحرارة مع او بدون المايكرويف.

الكلمات المفتاحية: رقائق الألمنيوم، معايير الدم، الموجات الكهرومغناطيسية، ملف الدهون، وظائف الكبد، حمامة، الحافظات البلاستيكية.