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EFFECT OF MICROWAVE TREATED PELLET IN THE KIDNEY AND SOME IMMUNOGLOBULIN LEVELS IN SERUM OF FEMALE RAT

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ABSTRACT

Microwave food consumption is becoming a cause for concern due to health hazard often associated with exposure to radiation. The purpose of this research is to validate the bio-safety of special microwave dish used in microwave oven, in line with other non-microwave utensils. Thirteen female albino rats (N=13) were used for the study and were divided into five groups; aluminium group (A), ceramic group (B), plastic group (C), special microwave dish group (D) and control group (E). The animals were fed with microwave treated rat pellets using different utensils, apart from the control group. The experiment lasted for 60 days. The animals were sacrificed and blood samples were collected by cardiac puncture for biochemical and histopathological analyses. Creatinine, urea, uric acid, chloride test, sodium and potassium ions, total protein, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) concentrations were evaluated in the kidney homogenate, including total protein using analytical kits. immunoglobulins G and E were evaluated in the serum using ELISA kits. Comparative analyses of the control and experimental groups showed that intake of microwave treated pellets caused significant ($P<0.05$) alterations in the biochemical parameters evaluated. The result of the histopathology shows that there were lesions in the kidney tissues of the experimental animals as compared to control which could lead to kidney failure. The findings of the histopathology test further authenticate the biochemical imbalances in the parameters investigated. The study therefore discourages the use of non-microwave and microwave dishes in cooking and heating of food

Keywords: Microwave oven; kidney; electrolyte; immunoglobulin; lipid profile; histopathology; rats.

INTRODUCTION

A microwave oven is an electric oven that heats and cooks food by exposing it to electromagnetic radiation in the microwave frequency range. Quite a number of people now make use of the microwave either for thawing or warming of food or even for fast cooking. As such, virtually every comfortable home now makes use of it (Sumnu, 2001). Advantages of microwave heating include savings in time and energy and easy to use. The advantages make it one of the most attractive cooking methods. Mechanism of microwave heating is different from the conventional heating. It represents the conversion of electromagnetic field energy to thermal energy, which arises in the field by polar water molecules in food (Sumnu, 2001). Bipolar water molecules are rotated in accordance with changes in the alternating electromagnetic field, which leads to the intermolecular friction and overcoming intermolecular forces, thus creating heat and leads to rapid heating of material. Microwave can penetrate to the depth of material, according to the dielectric parameters, thereby heating the material at the same time, both inside and on the surface.

Microwave radiationis a type of non-ionizing electromagnetic radiation and considered as an environmental pollutant (Paulraj and Behari, 2004). Radiation is also known as environmental pollution (Parker et al., 2010). It also results in the destruction and deformation of food molecules, most especially proteins, enzymes, vitamins and phyto-chemicals, plus the formation of new radiolytic compound (Bohr et al., 2000). The radiation leakage from improperly maintained oven is a source of environmental pollution and this may pose risk on human health (Parker et al., 2010). The exposure to microwave radiation is said to cause biological effects in living organisms. The hard electromagnetic radiations produced from microwave oven are forced to reverse polarity one to one hundred billion times a second. This causes the body system to lose the ability to fight these radicals, thus resulting in many diseases like cancer, Parkinson's disease and stroke.

The disorders in the digestive system results from eating microwave treated food as well as nutritional quality of food decreased by 60% to 90 % (lee Lita, 2001). The oven door is the most dangerous place in case of microwave leakage and magnetic fields can be present around the oven. (IEEE, 2002).The microwave radiation affects the integrity of the kidney and hemoglobin macromolecule (Moussa, 2009). The continuous use of equipment at home and industry that produce microwave radiation has led to concerns about its effect on several organs in the body. The enormous amount of energy going into the food molecules from microwave radiation is sufficient to break protein molecules and produce other strange molecules. Amongst them are radiolytic compound, which are substance formed from deform macromolecule as result of radiation.

Microwaves transform more than our food. The radiation itself directly influences all bodily systems and functions: circulatory, digestive, metabolic, lymphatic, immume, hormonal and nervous systems (Ajayi et al., 2014). People suffer from: hormone imbalances, damage to the electrical functioning of the nervous system, a disruption in the cellular membrane electrical potentials, significant loss of vital energy, and a higher-than-normal percentage of cancerous cells in their blood serum. In short, microwave oven radiation disrupts the electrical field of the body, which in turn affects functioning on biological, chemical and physiological levels. Being within just a three foot range of a microwave oven will expose you to these dangers.

The present study is designed to assess the bio-safety of microwave dish in line with non-microwave dish in the kidney and some immunoglobulin levels in the serum of female albino rats exposed to micro wave treated pellet.

Animal Sacrifice and blood collection:

All animals were sacrificed after overnight fast prior to the sacrifice. The weight of the rats was taken before the sacrifice. The rats were sacrificed by suffocation using diethyl ether in a desiccator and blood samples were collected by cardiac puncture into plain bottles. Some portion of the blood was centrifuged at 4000g for 10 minutes to separate the serum which was used for the hormonal analyses while the kidney homogenate was used for biochemical analysis. The samples were stored in a freezer at 5^{o}C for further analysis.

Homogenization

The kidney of the rats was obtained and 2% of the kidney homogenate was prepared with phosphate buffer, pH 7.4 using mortar and pestle. The homogenate was stored in eppendorf tube at -5^{o}C for further analysis.

2.5. **Biochemical Assays**: Kidney function test (sodium and potassium ion concentrations, uric acid, urea and creatinine levels, total cholesterol (TCHOL), total protein and total triglycerides (TAG), high density lipoprotein (HDL), cholesterol, (HDL-C) and low density lipoprotein (LDL) cholesterol (LDL-C) were determined using test kits (Linear Chemicals). The concentrations of immunoglobulin's G and E (IgG and IgE were determined by ELISA machine

Materials and Methods

Collection and Management of Animals

Female adult albino rats of the Wistar strain weighing between 153 to 203g were used for the study. They were obtained from the animal breeding unit of Institute for Advance Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health (NIH, 1985) and approved by Crawford University Ethical Committee on Animal Research (CUECAR), The animals were handled humanely, kept in plastic suspended cages, placed in a well-ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets (Mobat feeds) and served water ad libitum. The animals were allowed two weeks of acclimatization prior to the commencement of study.

Experimental Design:

Thirteen (13) female albino rats were randomly assigned into five (5) groups. Group A animals (control) were fed with pellets microwave with aluminum plate, groups B contains three (3) animals fed with pellet microwave with ceramic plate, group C contains two (2) animals fed with pellet microwave with plastic plates, group D (positive control) consist of two animals (2) fed with pellet microwave with microwave plate and group E (normal control) contains three (3) animals fed with pellet not microwave. The rat pellets spent one (1) minute in the microwave oven. The microwave oven used is a product of Hisense, China. It has a model number H20M0MM1/ H20M0MME, power input of 1050W and power output of 700W. All the animals were allowed equal access to their respective experiment pellets and water ad libitum throughout the study. The feeding lasted for four (4) weeks after acclimatization of two weeks.

Table1: The experimental design Key:

Experimental Group	Utensils used in microwave oven	Nomenclature	Number of Rat
A	Aluminum plate	Aluminum group	3
B	Ceramic plate	Ceramic group	3
C	Plastic plate	Plastic group	2
D	Microwave plate	Positive control	2
E	Rat pellet not microwave	Normal control	3

N= 13

3.0 Statistical Analysis

Data obtained from the different parameters studied were subjected to analysis of variance (ANOVA) to test for the level of homogeneity and the Duncan multiple range test was used to, separate means where heterogeneity occurred. P values at < 0.05 was considered significant using IBM SPSS version 20.The results were expressed as mean ± standard deviation

Results

Table 2: The body weights of the female albino rats exposed to microwave treated pellet for 60 days.

Experimental Group	Utensil used in the microwave oven	Initial body weight(g)	Final body weight(g)	Change in body weight
A	Aluminum plate	176.50±36.06	168.50±51.62	11.00±11.31
B	Ceramic plate	124.33±39.00	172.67±14.29	30.33±71.68
C	Plastic plate	157.50±20.50	163.00±9.90	5.50±71.68
D	Microwave plate	160.00±14.14	157.00±1.41	10.00±5.65
E	Rat pellet not microwave	75.50±0.71	120.00±7.81	44.00±8.48

Values are expressed as mean $\pm$ SD for two determinations.
Values with different alphabet within the same column are significantly different at P<0.05

Table 3: kidney weight of female albino rats exposed to microwave treated pellet for 60 days

Experimental Group	Utensil used in the microwave oven	Weight of kidney(g)	Relative body weight (%)
A	Aluminum plate	0.02±0.00	0.01±0.00
B	Ceramic plate	0.02±0.00	0.01±0.00
C	Plastic plate	0.02±0.00	0.01±0.00
D	Microwave plate	0.02±0.00	0.01±0.00
E	Rat pellet not microwave	0.01±0.01	0.01±0.01

Values are expressed as mean ± SD for two determinations.

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Table 4: The electrolytes in the kidney homogenate of female albino rats exposed to microwave treated pellet for 60days.

Group	Utensil used in the microwave oven	K+(mmol/g tissue)	Na+(mmol/g tissue)	Uric acid(mg/g tissue)	Urea(mg/g tissue)	Creatinine(mg/gtissue)
A	Aluminum plate	6.794±0.292[c]	2.964±11.546[a,b]	0.030±0.000[a]	0.229±0.070[a]	0.337±0.464[b]
B	Ceramic plate	3..497±42.457[c]	9.225±6.089[a,b]	0.018±0.008[a]	0.117±0.100[a]	0.0412±0.031[a]
C	Plastic plate	5.424±0.016[b,c]	9.410±10.176[a,b]	0.016±0.007[a]	0.269±0.0211[a]	0.328±0.009[b]
D	Microwave plate	3.946±0.077[b]	5.757±2.036[a]	0.030±0.009[a]	0.087±0.053[a]	0.233±0.222[b]
E(control)	Rat pellet not microwave	1.239±0.866[a]	8.615±6.317[a]	0.034±0.005[b]	0.136±0.121[a]	0.896±0.765[b]

Values are expressed as mean ± SD for two determinations
Values with different alphabet within the same column are significantly different at P<0.05

Table 5: The lipid profile of the experimental animals exposed to microwave treated pellet for 60 days.

Group	Utensil used in the microwave oven	Total CHOL (mg/g tissue)	Total Protein (g/g tissue)	Total TRIG (mg/g tissue)	HDL (mg/g tissue)	LDL (mg/g tissue)
A	Aluminum plate	0.789±0.452[a]	0.061±0.052[a]	0.01±0.002[a]	0.09±0.02[a]	0.157±0.090[a]
B	Ceramic plate	0.554±0.056[a]	0.122±0.069[a]	0.02±0.007[a]	0.07±0.07[a]	0.110±0.011[a]
C	Plastic plate	0.606±0.216[a]	0.039±0.001[a]	0.009±0.007[a]	0.06±0.01[a]	0.121±0.043[a]
D	Microwave plate	0.722±0.008[a]	0.046±0.010[a]	0.009±0.007[a]	0.21±0.13[a]	0.144±0.002[a]
E	Rat pellet not microwave	0.669±0.501[a]	0.037±0.017[a]	0.01±0.005[a]	0.19±0.16[a]	0.133±0.100[a]

Values are expressed as mean ± SD for two determinations.
Values with different alphabet within the same column are significantly different at P<0.05

Table 6: Serum immunoglobulins (IgG and IgE) levels of the experimental animals exposed to microwave treated pellet for 60 days.

Group	Utensil used in the microwave oven	IgG (ng/ml)	IgE (ng/ml)
A	Aluminum plate	65.32±0.68[a]	29.73±1.49[b]
B	Ceramic plate	95.24±30.20[a]	28.13±0.25[b]
C	Plastic plate	65.08±1.20[b]	33.54±15.06[a]
D	Microwave plate	108.69±34.17[a]	50.57±0.70[c]
E	Rat pellet not microwave	107.62±6.48[a]	65.68±5.89[c]

Values are expressed as mean ± SD for two determinations.
Values with different alphabet within the same column are significantly different at P<0.05

Histopathological Results:

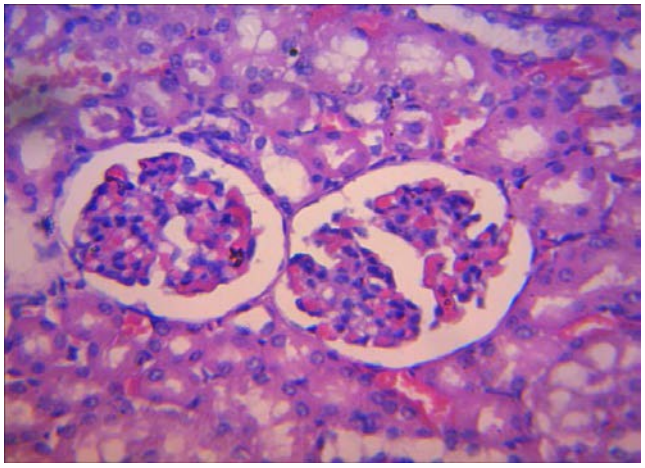

Figure 1: The photomicrograph of the kidney of female albino rat exposed to microwave radiation in aluminium plate (Group A) ×400. There is a moderate to severe diffuse tubular degeneration (arrows)

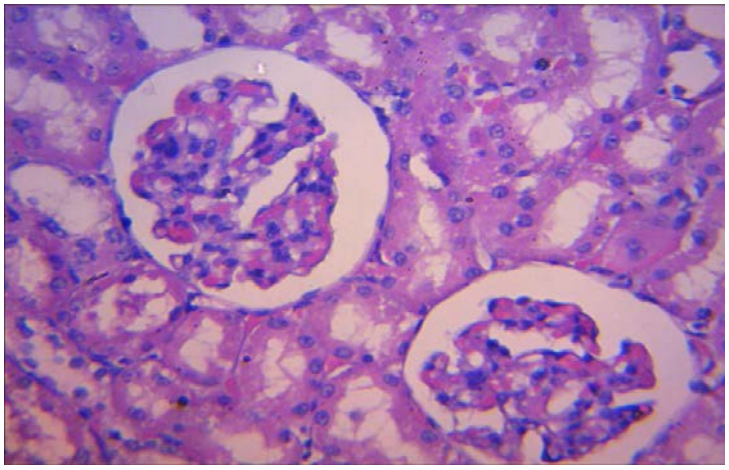

Figure 2: The photomicrograph of the kidney of female albino rat exposed to microwave radiation in ceramic plate (Group B) ×400. There is a moderate to severe diffuse tubular degeneration (arrows), few tubules have proteinaceous material in the lumen (arrowhead)

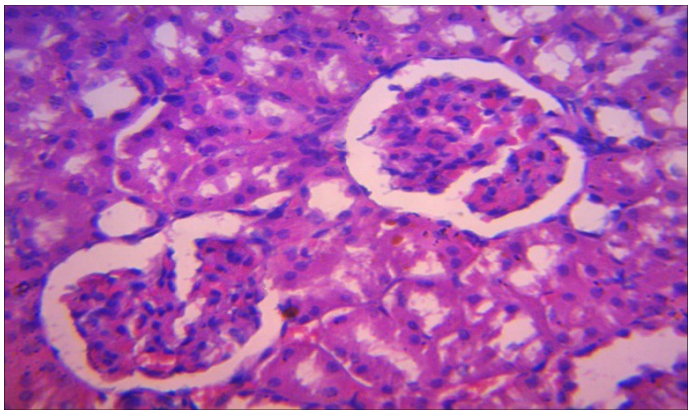

Figure 3.The photomicrograph of the kidney of female albino rat exposed to microwave radiation in plastic plate (Group C) ×400. There is a severe tubular degeneration, with protein casts in renal tubules (arrow heads)

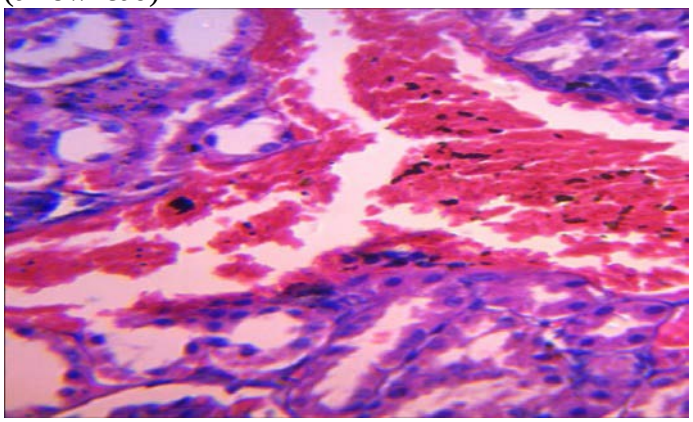

Figure 4: The photomicrograph of the kidney of female albino rat exposed to microwave radiation in microwave plate (Group D) ×400. There is a severe congestion of the interstitium (arrowheads) with mild degeneration of the tubules (arrows).

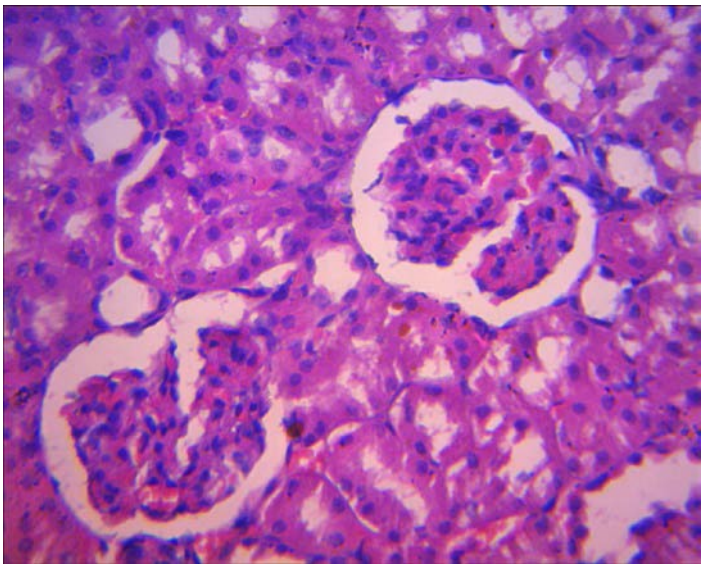

Figure 5: The photomicrograph of the kidney of female albino rat not exposed to microwave radiation (Group E) ×400. No visible lesions seen.

Discussion

The use of microwave radiation equipment at home and industry raises adverse concerns about the effect of microwave leakage on biological systems. Radiation adversely affects biomolecules present in food and this lead to the formation of radiolytic compounds that are carcinogenic and mutagenic in nature and when consumed invariably affect the metabolic system of the body (Benedict *et al*., 2017). Microwave technology has been widely used in different fields in our lives (microwave oven, WIFI and cell phones) [Mathur*et al*., 2013]. It is also good for domestic purpose but it has several drawbacks on the nutritional contents of food (Kushwaha, 2012). The present study evaluates the biochemical parameters and histopathology study of the kidney of female albino rat fed with rat pellet exposed to microwave radiation using different utensils. The biochemical parameters evaluated in the kidney homogenate are creatinine, urea, uric acid, sodium and potassium ion concentrations, including total protein level using research kits. Immunoglobulins G and E (IgG and IgE) were also evaluated in the serum using ELISA machine.

During the experimental period, it was observed that there was significant increase ($P<0.05$) in the body weight of the animals in experimental group compared to the control animals. This may be attributed to the fact that rat pellet exposed directly to the microwave radiation enhances palatability which created an irresistible aroma that trigger the consumption of the pellets

voraciously leading to increased metabolic rate and consequently increase in body weight of the exposed animals. This was closely followed by the groups (aluminum, ceramic, plastic and microwave plate) compared to the control animals. This observation was similar to the reports of Eke *et al*., (2007) and Ikanone, *et al*., (2021).

The concentration of the different electrolytes in the kidney homogenates are as presented in table 4. The level of potassium ion in the different experimental groups increases significantly ($P<0.05$) when compared to the control group. Potassium ion maintains the intracellular osmotic pressure, water balance and acid base balance (Pankaja, 2005). It is needed by several glycolytic enzymes for their formation. The level of potassium ion in group A is the highest when compared with group C (plastic plate), group D (microwave plate) and group B (ceramic plate) respectively. The upsurge in potassium concentration of group A may be as a result of possible renal failure caused by the consumption of radiolytic molecules generated as a result of radiations from the microwave oven. In this condition, the kidney may not be able to excrete potassium load when the glomerular filtration rate (GFR) is very low (Pankaja, 2005). The utensils utilized in the experiment are mostly used by microwave oven users and these encourage hyperkalemia that is excess concentration of potassium ion in the blood. Conversely, for sodium ion concentration, the reverse is the case. The levels of sodium ion in the experimental groups showed no significant differences ($P>0.05$) when compared to the control animals. However, for group A (aluminium plate) and group D (microwave plate), there was significant decrease ($P>0.05$) when compared to the control animals. The metabolic implication of this fact is that the radiolytic molecules consumed by the treated groups irrespective of the utensil used encourage hyponatremia in groups A and D as compared to the control group. There was no significant difference ($P>0.05$) in the sodium ion concentration in groups B and C of experimental animals compared to the control group. The possible cause of hyponatremia could be linked to the consumption of radiolytic molecules by the experimental animals and by extension factors like retention of water, can dilutes the constituents of the extracellular space and loss of pathological sodium ions. The concentration of uric acid in the experimental animals did not showed any significant differences ($P<0.05$) when compared to the control animals. Similar trend was observed in the urea and creatinine levels.

The effect of microwave radiation on the lipid profile of rats was also studied in the current investigation. Lipid profile is the term that

collectively describes the amounts of total cholesterol, total triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol in milligram per decilitre. It may also involve phospholipids and other lipids. This profile is used to access the risk of cardiovascular disorders (CVDs) and is altered in the serum of various disease states (Betteridge, 1994)

In the study, microwave radiation exposure (irrespective of the utensils used) was observed to cause no significant differences ($P<0.05$) in the lipid parameters (total cholesterol, total triglyceride, HDL cholesterol and LDL-cholesterol) when compared with the control animal in the kidney homogenate. This observation implies that lipogenesis in the kidney was not affected by the radiolytic molecules (damaged nutrients) when consumed by the experimental animals and by extension the lipid portion of the kidney cell membrane was intact within the experimental period. However, this is not in agreement with Ikanone, *et al,* (2021). They recorded increase in lipid profile parameters in the serum of albino rats that were exposed directly and indirectly to microwave treated pellets.

Proteins are essential and indispensable nutrients to the functional and structural integrity of body cells and tissues. They are primarily synthesized in the liver, found in organs, tissues and membrane, and a compromise in protein system is the basis for several disease conditions or pathologies (Ighodaro and Akinloye, 2018). There is no significant increase in the protein concentration of the experimental animals when compared to the control group. Group B has the highest protein concentration compared to other experimental groups. Protein biosynthesis is not affected by the damaged done to the nutrients by microwave treated pellets when consumed by the animals within the period of study. This by implication suggest that the protein portion of the cell membrane was not compromised. This observation is not in agreement with Ikanone *et al.,* (2021) though the experimental design was different.

The result of immunoglobulins (IgG and IgE) concentration in the serum are as presented in table 5. There is significant differences ($P<0.05$) in immunoglobulins level (IgG) in the experimental group when compared to the control group. Radiation affects immunoglobulin G level adversely as observed from the study. These could lead to compromise in the functionality of the immune system. In the case of immunoglobulin E (IgE) level, similar trend was observed. The group B (aluminum plate) has the lowest concentration of IgE when compared to other experimental groups and this was closely followed by group A (aluminum plate) and group C (plastic plate) respectively. Animals fed with

pellets in microwave plate treated in microwave oven did not show significant differences ($P<0.05$) in IgE levels when compared to the control group. This has not been reported in literature.

The results of the histopathological evaluation from kidney portions of the animals are as depicted from figures 1 to 5. The photomicrographs from the experimental and control animals reveal the following information: for group A, there is a moderate to severe diffuse tubular degeneration. Group B shows that there is a moderate to severe diffuse tubular degeneration, few tubules have proteinaceous material in the lumen and for group C there is a severe tubular degeneration, with protein casts in renal tubules while for group D there is a severe congestion of the interstitium with mild degeneration of the tubules and for group E (control) there is no visible lesions seen. The structural integrity of the kidney has been compromised within the experimental groups when compared to the control animal.

Conclusion

The study shows that 60 days of exposure to microwave treated pellet in different utensils fed to albino rats had adverse effect on the biochemical parameters evaluated in the kidney. This is justified by the results of the photomicrographs of the experimental animals as compared to control group. This therefore discourages the use of microwave oven to treat food since it could impair kidney functions and possibly lead to kidney failure with time irrespective of the type dishes used.

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