Original Research Article

Effect of Ingested foods preheated (using microwave) in plastic containers on the reproductive profile of male albino rats

Abstract

Recently, there has been a notably increased use of microwave energy for heating and processing foods particularly in catering services and in our homes owing to its speed, convenience and efficiency compared to the traditional heating method. Due to the availability and affordability of plastic containers, they are mostly used to contain foods for preheating in a microwave. However, these plastics pose great risk on humans' health if they get scratched or heated, and leach out chemicals into the contained foods. Also, the microwave energy has been speculated to have posed risks to the health of humans considering the electromagnetic radiations it emits. This study was aimed at evaluating the effect of ingested foods preheated (in microwave) using plastic containers on the reproductive profile of male albino rats. A total of twenty-four (24) male albino rats weighing between 120 to 200g were used for this study. The rats were divided into three (3) groups (group I, II and III) of eight (8) rats per group. Group I (Negative control) rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish daily for 40 days (not preheated in a microwave). Group II rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish put in a ceramic plate and preheated in a microwave daily for 2 minutes for 40 days. Group III rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish put in plastic containers (not labeled as ''microwave safe'') and preheated in a microwave daily for 2 minutes for 40 days. After an overnight fast, the rats were anaesthetized using chloroform, and blood samples obtained (via cardiac puncture) were used to analyze serum luteinizing hormone

(LH), follicle-stimulating hormone (FSH) and testosterone using the ELISA method. The epididymis was also excised and immediately macerated to obtain semen, which was used for semen analysis. The results obtained showed a significantly decreased ($p<0.05$) mean LH, FSH, testosterone and sperm count in group III (plastic-microwaved) compared to group I and II. Also noted, was a significantly decreased ($p<0.05$) sperm count in group II compared to group I. However, there was no significant difference in LH, FSH and testosterone between groups I and II. Conclusively, it was evident from this study that, foods contained in plastics, preheated in microwave and ingested overtime, may predispose male individuals to dysfunction in their reproductive system which may eventually lead to male infertility. It is therefore, highly recommended that preheating of foods (in microwave) using plastic containers should be highly prohibited, but should rather be preheated using ceramics.

Keywords: microwave, preheating, plastic containers, dysfunction, infertility.

1.0 INTRODUCTION

Over the years, there has been a tremendous increase in the use of microwave energy for heating and processing of foods in the food industry, in catering services and in domestic field owing to its speed, convenience and efficiency compared to the conventional heating method (Finot and Merabet, 1993). Microwave radiations are very short waves of electromagnetic energy that are a part of Mother Nature's energy spectrum, and a type of non-ionizing electromagnetic radiations considered as environmental pollutant (Paulraj and Behari, 2004).

Increased exposure to microwave radiation induces biological effects in living organisms, and the most commonly used frequency in domestic and industrial food preparation is 2.45 GHz.

However, the leakage of radiation from improperly maintained ovens is a source of environmental pollution and may pose a risk on human health (Parkar *et al*., 2010).

During heating of food using a microwave, the alternating microwave electric current generated by the magnetron in every microwave oven forces the food molecules to rotate at the frequency of 1 to 100 billion times per second (Schrumpf and Charley, 1985). The friction from this violent motion tears up the foods, enzymes and vitamins; thereby destroying protein molecules and thus resulting in the generation of strange new molecules which are unnatural in the body, and are being considered as carcinogenic substances (Schrumpf and Charley, 1985).

Plastic food containers are readily available for use due to their cheap nature; however they pose great risk on our health if they get scratched or heated, and leach out chemicals into the contained foods. A report from National Toxicology Program of the U.S department of the health and human service noted that two chemicals, formaldehyde and styrene, commonly found in these containers are known or suspected carcinogens (National Toxicology Service, U.S. Department of Health Human Services, 2014).

Plastic containers also leach toxic chemicals when heated; polycarbonate leaches bisphenol A, polystyrene leaches styrene or polyvinyl chloride (PVC) which break down in vinyl chloride, and sometimes pthalates can leach various chemicals. Bisphenol A (BPA) poses several health challenges such as the disruption of normal breast development and thus causes breast cancer in later life (Husain *et al*., 2015); it is also an endocrine disruptor by interfering with endocrine systems. Other chemicals in plastics such as pthalates, arsenic, benzene, trichloroethene (TEC), formaldehyde, and polycyclic aromatic hydrocarbons (PAHs) are also implicated in endocrine disruption (Husain *et al*., 2015). This study was however, aimed at determining the effects of

ingested foods preheated (using microwave) in plastic containers on the reproductive profile of male albino rats.

2.0 MATERIALS AND METHOD

2.1 The experimental animals/design

A total of twenty-four (24) male albino wistar rats weighing between 120 to 200 g were purchased from the animal house, in the department of Physiology, University of Port-Harcourt, Nigeria, and were used for this study. The rats were divided into three (3) groups (group I, II and III) of eight (8) rats each, and were allowed to acclimatize for two (2) weeks by maintaining 12-hour light and dark cycles daily, with access to standard feed and water *ad libitum*.

After the acclimatization period, Group I (Negative control) rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish daily for 40 days (not preheated in a microwave). Group II rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish put in a ceramic plate and preheated in a microwave daily for 2 minutes for 40 days. Group III rats were fed *ad libitum* with porridge beans and jellof rice with meat and fish put in plastic containers (not labeled as ‘‘microwave safe’’) and preheated in a microwave daily for 2 minutes for 40 days.

2.2 Collection of whole blood and preparation of serum/Biochemical analysis

After an overnight fast, the rats were anaesthetized in a jar containing cotton wool soaked in chloroform to render them unconscious. They were quickly removed from the jar, and 5ml of whole blood specimen was collected (using a sterile syringe and needle) into sterile sample

containers (plain bottles) via cardiac puncture. After blood collection, the testes were surgically excised and the epididymis was also surgically removed and used immediately for semen analysis. The collected whole blood specimen was allowed to clot in an upright position for 20 minutes at room temperature, and then spun using a centrifuge for 20 minutes at 2500 rpm to obtain the serum, which was transferred into other plain bottles and stored in the freezing compartment (maintained at -20^{o}C) of a refrigerator until the time for analysis.

The serum was used to analyse testosterone, FSH and LH using rat-specific enzyme-linked immunosorbent assay (ELISA) kits manufactured at the Bioassay Technology Laboratory in Shanghai, China. The semen was diluted with semen diluting fluid (5 g of sodium bicarbonate, 1 ml of formalin and 99 ml of distilled water) in a ratio of 1:20, and was applied on the haemocytometer, which was then examined under the microscope, followed by counting of the sperm cells in 5 small squares (at the central square subdivided into 25 smaller squares) using the x40 objective lens, and then calculation of the sperm count using the formula below:

Number of sperm cell/ml = (number of counted sperm cells x dilution factor/volume) x 1000. Where, the dilution factor is 20, and the volume = (0.1/5 = 0.02) mm^3 (depth of the each square = 0.1mm, and counting was made in 5 squares).

2.3 STATISTICAL ANALYSIS

The data generated from the analysis were expressed as Mean ± Standard deviation, and analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Comparisons of mean and standard deviation values were made for the various parameters for tests and

control using the one-way ANOVA and Tukey tests. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

3.0 RESULTS AND DISCUSSION

Table 1 Comparison of the LH, FSH, Testosterone and Sperm Counts (SC) of Group I (Negative Control), II and III

	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/mL)	SC (Cells x 10^6)/mL
Group I (n=7)	1.00 ± 0.20^{c}	1.44 ± 0.35^{c}	99.60 ± 2.71^{c}	350.00 ± 37.80^{bc}
Group II (n=7)	1.04 ± 0.19^{c}	1.50 ± 0.33^{c}	99.88 ± 2.79^{c}	281.25 ± 65.12^{ac}
Group III (n=7)	0.55 ± 0.15^{ab}	0.72 ± 0.11^{ab}	87.55 ± 6.20^{ab}	202.50 ± 35.76^{ab}
F-value	18.073	18.435	22.20	18.816
P-value	<0.001*	<0.001*	<0.001*	<0.001*

KEY

*= statistically significant

n= number of samples

[a] = significantly different from group I (negative control)

[b]= significantly different from group II

[c]= significantly different from group III

From the results above, there was a significant difference in LH between groups I, II and III. The mean LH level in Group III (plastic-microwaved) was statistically lower compared to those of groups I (not microwaved) and II (ceramic-microwaved). However, no statistical difference was noted between groups I and II. Similarly, a significant difference in FSH between groups I, II and III was noted, such that the mean FSH level in Group III was statistically lower compared to those of groups I and II. However, no statistical difference was also noted between groups I and II. The report from this study agrees with that from a similar study that accessed the effect of bisphenol F, an analog of bisphenol A, on the reproductive functions of male rats, which reported a reduced concentration of LH and FSH in albino rats exposed to bispenol F when compared to the control (Ullah *et al*., 2019). The report also agrees with that from a study that accessed the efficiency of naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats (Alboghobeish *et al*., 2018); the similarity between these studies may be attributed to the fact that plastic containers contain bisphenol (Husain *et al*., 2015), and during heating in a microwave, these chemicals may leach out of the plastics into foods, and ingestion of such foods overtime may result in bioaccumulation of the bisphenol. LH and FSH are gonadotrophic hormones released from the anterior pituitary in response to gonadotrophic-releasing hormone (GnRH) secreted in pulsatile manner from the hypothalamus (Fraietta *et al*., 2013). Therefore, a decrease in these gonadotrophic hormones may be due to insufficient secretion of GnRH, or due to decreased response of the anterior pituitary gland to the GnRH.

A significant difference in the level of testosterone between groups I, II and III was also noted, such that the mean testosterone level in Group III was statistically lower compared to those of

groups I and II. However, no statistical difference was noted between groups I and II. This report also agrees with that from two similar studies by Ullah *et al*., (2019) and Alboghobeish *et al*., (2018), which both reported a decrease in the mean testosterone level when compared to the control. The testes require stimulation by the pituitary gonadotrophins (LH and FSH), which are secreted in response to GnRH from the hypothalamus. LH and FSH induce the development of germ cell; the induction of which is mediated by the androgen receptors (present on Leydig cells) and FSH receptors (present on Sertoli cells). FSH acts directly on the germinal epithelium, while LH stimulates the secretion of testosterone by Leydig cell, with the implication that a low amount of these gonadotrophins results in low secretion of testosterone. Therefore, the decreased amount of serum testosterone reported from this study may be attributed to the reduced amount of LH and FSH (Fraietta *et al*., 2013).

Furthermore, a significant difference in sperm count between groups I, II and III was noted, with the mean sperm count in Group III being statistically lower compared to those of groups I and II. Also, a significant difference was noted between groups I and II, with the mean sperm count of group II being statistically lower compared to that of group I. Testosterone stimulates sperm production and virilization, in addition to providing feedback to the hypothalamus and pituitary to regulate GnRH secretion. Therefore, the reduced sperm count report obtained from this study may be attributed to the low amount of testosterone (Layman, 2007).

The hypothalamus, the pituitary, and the testes form an integrated system (called the hypothalamic-pituitary-gonadal axis) that is responsible for the adequate secretion of male hormones and normal spermatogenesis, and the endocrine components of the male reproductive system are integrated in a classic endocrine feedback loop (Fraietta *et al*., 2013). Male

hypogonadism is characterized by impaired testicular function which can affect spermatogenesis and/or testosterone synthesis. It can result from a primary testicular disorder or may occur secondary to hypothalamic-pituitary dysfunction. Low levels of serum LH and FSH, combined with low levels of serum testosterone is referred to as hypogonadotrophic hypogonadism, which is primary hypogonadism and is the most frequent form of hypogonadism found in adult men.

4.0 CONCLUSION

The decreased amount of the gonadotrophic hormones (LH and FSH), gonadal hormone (Testosterone) and sperm count is indicative of dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis. This may imply that ingested foods usually preheated (using microwave) in plastics overtime, may pose a serious health risk by inducing a dysfunction of the male reproductive system, a condition known as hypogonadotrophic hypogonadism, which may result in male infertility, whereas ingested foods preheated (using microwave) in ceramics are relatively safe. Therefore, it is highly recommended that preheating of foods (in microwave) using plastic containers should be highly prohibited, and should rather be preheated using ceramics which happens to be relatively safe.

REFERENCES

Alboghobeish, S., Mahdavinia, M., Zeidooni, L., Samimi, A., Oroojan, A. A., Alizadeh, S., Dehghani, M. A., Ahangarpour, A. & Khorsandi, L. (2018). Efficiency of naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats. *Iranian Journal of Basic Medical Sciences*, 22 (1), 315-323.

Finot, P. A. & Merabet, M. (1993). Nutritional safety aspects of microwaves. History and critical evaluation of reported studies. *International Journal of Food Science and Nutrition*, 44 (1), 65-75.

Fraietta, R., Zylberstejn, D. S. & Esteves, S. C. (2013). Hypogonadotropic Hypogonadism Revisited. *Clinics*, 68 (1), 81-88.

Husain, I., Alalyani, M. & Hanga, A. H. (2015). Disposable Plastic Food Container and Its Impacts on Health. *The Journal of Energy and Environmental Science*, Photon 130 (1), 618-623.

Layman, L. C. (2007). Hypogonadotropic hypogonadism. *Endocrinology and Metabolism Clinics of North America*, 36 (2), 283-296.

National Toxicology Service, U.S. Department of Health Human Services. (2014). 13th Report on Carcinogens (RoC). Retrieved from http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html.

Parkar, M. A., Ahmed, R., Abdullah, B. B., Patil, B. S. & Das, K. K. (2010). Effect of cell phone exposure on physiologic and hematologic parameters of male medical students in Bijapur (Karnataka) with Reference to Serum Lipid Profile. *Journal of Basic and Clinical Physiology and Pharmacology*, 21 (2), 201-210.

Paulraj, R. & Behari, J. (2004). Radiofrequency radiation effect on protein kinase C activity in rats' brain," *Mutation Research*, 545 (1), 127-130.

Schrumpf, E. & Charley, H. (1985). Texture of broccoli and carrots cooked by microwave energy. *Journal of Food Science*, 4 (1), 1025-1029.

Ullah, A., Pirzada, M., Afsar, T., Razak, S., Almajwal, A. & Jahan, S. (2019). Effect of bisphenol F, an analog of bisphenol A, on the reproductive functions of male rats. *Environmental Health and Preventive Medicine*, 24 (41), 1-11.