International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Histological and Weight Changes in Testes of Male Albino Rats Fed with Diets Containing Yaji (A Local Meat Sauce)

Ukoha U. U[1], Egwu O.A[2], Dimkpa U[3], Maduka S.O[3], Okafor J.I[4], Ndukwe G.U[5], Ogonnadi K[1]

[1]Anatomy Department, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[2]Anatomy Department, Federal University of Nigeria, Ndufu-Alike Ikwo, Ebonyi State, Nigeria.
[3]Physiology Department, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[4]Anatomy Department, Faculty of Basic Medical Sciences, Anambra State University, Uli, Anambra State, Nigeria.
[5]Anatomy Department, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.

Corresponding Author: Dimkpa U

Received: 01/06//2014 *Revised: 17/06/2014* *Accepted: 09/07/2014*

ABSTRACT

Aims: To determine the effect of yaji on the histology and weight of testes of male Wistar rats.
Material and Method: Twenty young Wistar rats of weights 183-207g were divided into four groups. The control group A rats were administered with feed and water while the experimental groups B, C and D rats were fed with a mixture of feed and 5g, 10g and 15g doses of yaji respectively and water for 6 weeks. The testicular and body weights were measured and used in calculating the gonadosomatic index of the rats. Tissue processing techniques were applied to determine the histological effects of yaji on the testes.
Result: Weight gain was observed in control group A (11.4 g) compared to group B rats administered with 5g of yaji, which indicated relatively smaller weight gain (5.2 g). Weight losses were observed in group C (-5.2 g) and D (-9.8 g) administered with 10 g and15g of yaji respectively. The stained micrograph of group B indicated normal but slightly separated seminiferous tubules; group C indicated slightly separated seminiferous tubules, reduced number of interstitial leydig cells and reduced sperm cells in the central lumen of the tubules; group D micrograph indicated atrophied seminiferous tubules with mild fluid accumulation within the stroma.
Conclusion: The present findings suggest that excessive and uncontrolled consumption of yaji may cause damage to the testes, thus impairing testicular functions. On the other hand, yaji may be useful in weight management in so far as its production and consumption is regulated.

Keywords: Testes, seminiferous tubule, Leydig cell, stroma.

INTRODUCTION

The male reproductive system is a complex process that involves the testes, epididymis, sex glands and associated hormones. [1] Almost all healthy male vertebrates have two testes, [2] which are housed in a sac known as the scrotum and separated by an incomplete septum. The testes perform two highly organized and intricate functions called spermatogenesis

and steroidogenesis, which are crucial for the perpetuation of life. [1] Spermatogenesis is a highly dynamic and synchronized process which takes place in the seminiferous tubules of the testis with the support of somatic sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells. [1] The interstitial compartment which comprises of the leydig cells is the site for steroidogenesis in the testis. [3]

Historically, yaji was named after a 14th century Hausa ruler called Yaji (meaning the 'hot one'). [4] Yaji is typically used as a sauce for a local meat '*suya*', (a thinly sliced skewered beef delicacy) and generally believed among the male population in West African regions to stimulate sexual appetite and stamina. [5] Yaji is a complex mixture of groundnut cake powder, spices, additives and salt. [6] The spices in yaji include ginger, cloves, red pepper and black pepper. [7] These spices in turn contain active principles, gingerol, [8] eugenol, [9] capsaicin, [10] and piperine [11] respectively. The additive, white magi, contains monosodium glutamate, [12] the salt contains sodium chloride [13] and groundnut cake powder contains oil. [14] Existing reports indicate that some of the active principles in yaji such as capsaicin, piperine and monosodium glutamate are excitotoxic. [15-18] Furthermore, some of the histological findings on the pancreas, [7] liver, [19] and kidney, [20] suggest that an excessive consumption yaji can induce pancreatic, liver and kidney damage. The above facts therefore suggest that yaji is a complex combination of ingredients with active principles that are potentially harmful when consumed in excess. [21]

Despite the reported neurotoxic, excitotoxic and apoptotic potentials of yaji, a large proportion of the male population in Nigeria and many African societies consume large quantities of yaji daily, basically because of the lovely taste it adds to the 'suya' meat and mostly, because of its 'widely believed' aphrodisiac potentials. However, it is not certain if this uncontrolled consumption of yaji has harmful effects on the testes. The present study therefore is aimed at investigating the effect of yaji on the histology and weight of testes of male Wistar rats.

MATERIALS AND METHODS

Animals and treatments

Twenty adult male rats of weights 183-207g were used for the study. They were housed in the animal house of College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria under standard conditions (29 ± 2°C temperature, 40-55% humidity, good ventilation) and have free access to water and diet (normal rat chow). They were acclimatized for two weeks before the start of the experiment. The experiments were carried out following the ethical approval of the Faculty of Basic Medical Sciences Ethical Board which concurs with the NHI ethical approval for animal experiments. The animals were weighed on the first day of acclimatization and then divided into four groups, A-D which is made up of five rats each. Group A served as the control group while groups B-D served as the experimental groups.

Preparation of the yaji

The commercially produced yaji sauce is usually not standardized with respect to the quantities of its constituents. In this study however, we measured the quantities of the constituents of yaji sauce in order to determine their proportions in a given measure of yaji. This was to ensure standardization of the doses of the substance that could be administered to the different groups. The constituents of yaji were purchased at the Nkwo market, Nnewi, Anambra state, Nigeria, and subsequently

mixed together in powdery forms according to the method used by Nwaopara et al. [22] A hand-grinding machine was used to grind them into powdered form and then measured as appropriate with a compact analytical weighing balance of (model No. DP-B00481V4C0; manufactured by A & D Company, Japan). The measured quantities of yaji constituents were as follows: ajinomoto (150g), clove (39g), black Pepper (30g), ginger (78g) and groundnut cake powder (230g), red Pepper (22g) and salt (100g). The total weight of these constituents summed up to 649g.

Administration of yaji

The daily administration of yaji mixture to the animals were carried out as follows: the control group A received 71g of feed; group B, C and D received 5g, 10g and 15g of yaji respectively mixed with same quantity of feed. The mixture of the yaji and feed were orally administered to the animals using plate feeding method for the period of six weeks. All the groups also had access to equal volume of drinking water though out the study period.

Sample collection

The animals were sacrificed by cervical dislocation and were quickly dissected to remove the testes. After removal, the testes were placed immediately into a container containing normal saline fluid (to prevent tissue autolysis or putrefaction) and weighed.

Histological processing of the testicular tissues

After weighing the testes, the testicular tissues were immediately fixed in a fixative (Bouni's fluid) and transferred into specimen bottles and kept frozen for 48 hours before being used. The tissues were dehydrated using concentrated ethanol, cleared using xylene, embedded in paraffin wax, sectioned at 8 μm and stained by hematoxylin and eosin. The photomicrographs of the stained sections were observed using Nikkon research microscope (Novex, Holland) at a magnification of x200. The micrograph pictures were taken with digital camera (DCM 510.5M Pixels, CMOS chip) connected to the microscope.

Measurements

The body weights and the testicular weights of each of the rats were taken using a compact balance of 300g capacity (Model No: DP-B00481V4C0, manufactured by A & D Company, Japan). The mean testicular and body weights of the rats were obtained and these parameters were used to derive the gonadosomatic index for each rat in their respective groups using the formula:

$$\text{Gonadosomatic Index } (\sigma) = {}^{\text{Testicular weight}}/_{\text{Body Weight}} \times 100.$$

Data analysis

Descriptive data was expressed as mean ± standard deviation. Comparative analysis involving two continuous variables was done using independent sample t-test, while those involving more than two variables were done using one-way analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$. All statistics were done using IBM-SPSS statistical software (version 20.0).

RESULTS

Independent sample t-test indicated significant differences between the mean body weight of rats at baseline and at the end of experiment in groups A, C and D respectively. The mean weights at the end of experiment were significantly higher in group A ($p<0.01$), but lower in group C and D ($p<0.01$) respectively when compared to baseline values. However, there was no significant differences in weight of rats between baseline and end of experiment in group B. Data also indicated that the group A and B rats gained weight (11.40 ± 3.13 and 5.20 ± 5.07), while groups C and D lost weight (-5.20 ± 2.28 and -9.80 ± 3.96). The

mean weight gain in group A was insignificantly higher (p = 0.114) compared to group B. Similarly, though weight loss was higher in group C, however, it did not differ statistically from group D (Table 1).

Table 1: Mean body weights of rats at 'baseline' and 'end of experiment' and the corresponding mean changes in their body weights.

GROUPS	WEIGHT AT BASELINE (g)	WEIGHT AT END OF EXPERIMENT (g)	CHANGE IN WEIGHT (g)
A	196.2 ± 5.49	207.6 ± 8.41*	11.4 ± 3.13
B	197.0 ± 6.44	202.2 ± 9.95	5.2 ± 5.06
C	197.0 ± 7.68	191.8 ± 9.44*	-5.2 ± 2.28
D	195.8 ± 9.44	186.0 ± 6.28*	-9.8 ± 3.96

*Significant difference (p<0.01) between weight of rats at baseline and end of experiment

Analysis of variance (ANOVA) indicated lack of significant differences (right, F = 0.997; P = 0.419 and left, F = 0.611; P = 0.618) in testicular weights among the study groups at the right and left testicles respectively (Fig. 1). The same trend was observed in the mean gonadosomatic index (GI) among the study groups in the right (F = 1.12; P = 0.369) and left (F = 0.675; P = 0.580) testicles (Fig. 2). However, our data indicated a decline in GI with increasing dosage level of yaji from group B to D and in both testicles (Figure 2).

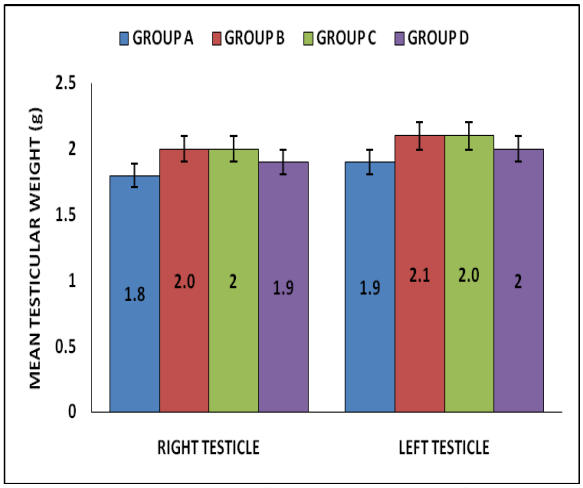

Figure 1. Mean right and left testicular weights of control and experimental rats.

The histological study indicated that the control group A, administered with feed and distilled water, maintained normal testicular histology at the end of the study (Figure 3).

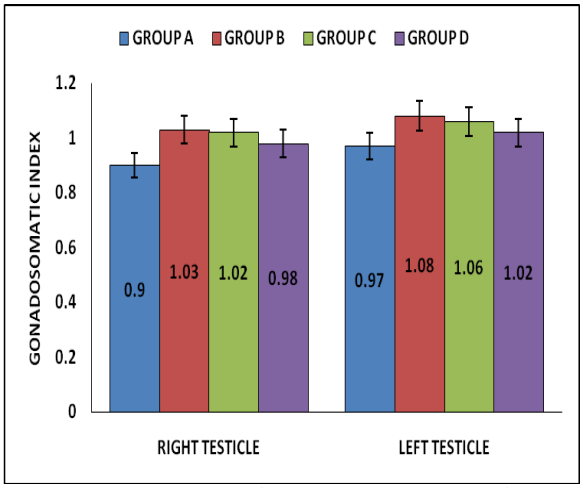

Figure 2. Mean right and left gonadosomatic indexes of control and experimental rats.

In group B administered with 5g of yaji, the testicular histology indicated normal but slightly separated interstitial spaces and seminiferous tubules (ST) and a few number of interstitial cells (IC) of leydig (Figure 4). Group C administered with 10g of yaji indicated sections of the testes with slightly separated seminiferous tubules and interstitial spaces; reduction of sperm cells in the central lumen of the tubules; slightly separated and reduced number of interstitial cells of leydig (Figure 5). The micrograph of group D administered with 15g of yaji presented a picture with atrophied seminiferous tubules with a mild accumulation of fluid within the stroma and destruction of the arrangement of sperm and leydig cells (Figure 6).

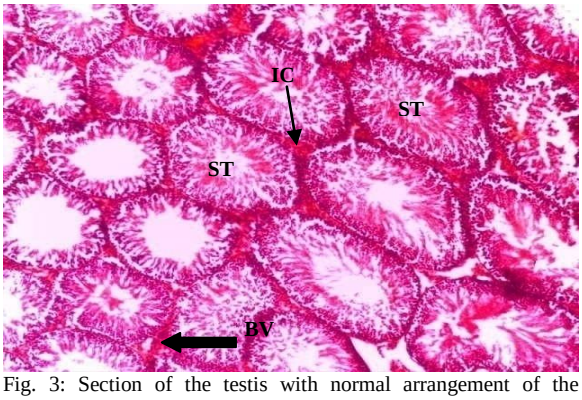

Fig. 3: Section of the testis with normal arrangement of the seminiferous tubules (ST), Interstitial cells of leydig (IC) and blood vessel (BV) H&E Stain Mag. X200 ('Control' group A)

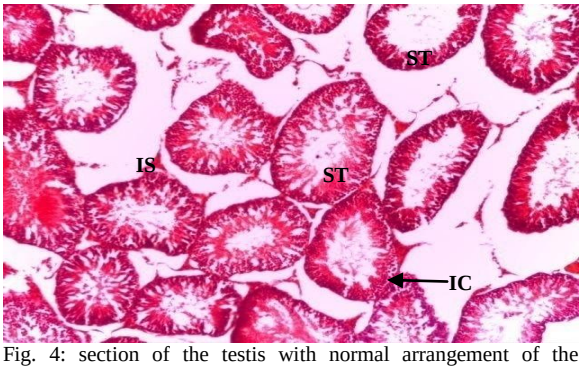

Fig. 4: section of the testis with normal arrangement of the seminiferous tubules (ST) which are slightly separated from each other, few numbers of interstitial cells of leydig (IC), and slightly separated interstitial spaces. H&E stain Mag. X200 ('Test' group B).

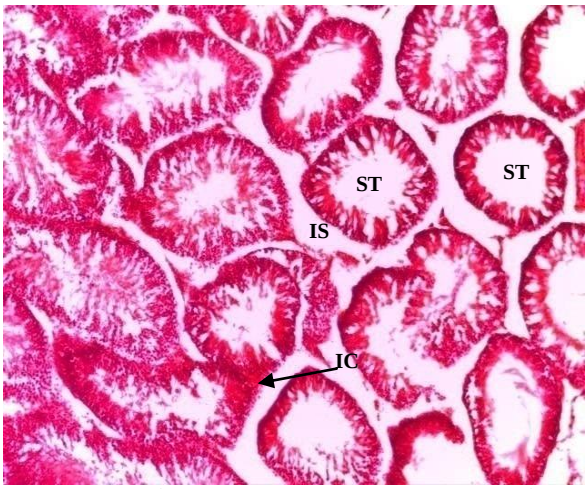

Fig. 5: Section of the testis with slightly separated seminiferous tubules (ST), reduction of sperms in the central lumen of the tubules and Interstitial cells of leydig (IC) H&E Stain Mag. X200 ('Test' Group C)

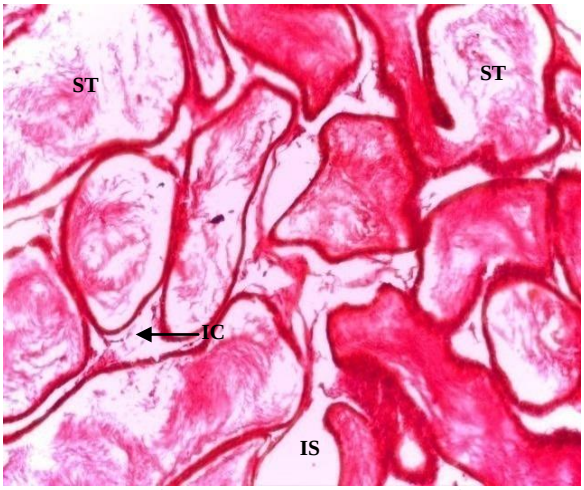

Fig. 6: Section of the testis with atrophied seminiferous tubules (ST) with a mild accumulation of fluid within the stroma, destruction of the arrangement of sperm cells and leydig cells (IC) H&E Stain Mag. X200 ('Test' group D).

DISCUSSION

The present study indicated a dose-dependent decline in mean change in weight of rats administered with yaji sauce. Weight gain was observed in control group A administered with feed and water and in group B rats administered with 5g of yaji. Weight losses were observed to be dose dependent in group C (-5.2 g) and D (-9.8 g) administered with 10 g and15g of yaji respectively. Our study agrees with previous studies, [23, 24] which indicated weight loss in rats fed with the combination of all the yaji spices. Akpamu et al [24] also demonstrated that individual yaji spices such as clove, ginger, red pepper and black pepper can induce weight loss. Other studies have also shown that ginger can cause a decrease in body weight, [25] while oral and gastrointestinal exposure to capsaicin (a constituent of red pepper) increases satiety and reduces energy and fat intake. [26] In contrast, weight gain has been reported in rats fed with ginger, clove and red pepper [23] and those fed with black pepper, [27,28] while absence of any effects of yaji on body weights of rats has been found in rats fed with black pepper. [23] It is believed that the weight-reducing effect of yaji observed in

this study may be due to the combined influence of the active ingredients with potentials for body weight reduction. The present finding therefore suggests that yaji may be useful in weight management in so far as its production and consumption is regulated.

To the best of our knowledge, no previous study has investigated the effect of yaji sauce on the testicular weight of animals or humans. The present findings indicated no significant differences in the testicular weights of rats administered with yaji sauce compared with the controls. However, it is noteworthy that a previous study [29] has demonstrated that a 10 mg dose of piperine treatment caused a significant reduction in the weights of testes and accessory sex organs.

The histological findings of the present study revealed that higher doses of yaji caused damage to seminiferous tubules; reduction of viable sperm and leydig cells; and destruction of the structural arrangement of the sperm and leydig cells. A previous study [30] has associated monosodium glutamate of white magi (one of the constituents of yaji) with oligozoospermia and increased abnormal sperm morphology. This could be confirmed by the findings of an experiment that the structural changes of testes of the MSG-treated rats were found to be dosage-duration-dependent and ranged from slight to moderate damage in case of the short-term treatment; however severe damage was recorded in the case of long-term treatment. [31] The above facts therefore suggest that monosodium glutamate may be a major contributor to the moderate to adverse effects observed in the groups B and C and the severe destruction of the arrangement of the testicular architecture as observed in the group D in the present study.

Further evidences have also implicated clove, one of the major constituents of yaji containing eugenol, as a possible contributor to the reduced sperm cells observed in testicular tissues of rats administered with higher doses of yaji. Previous studies [32, 33] have demonstrated the spermicidal effects of clove oil and eugenol. In another study, it was observed that rats treated with alcoholic extract of cloves contained fewer sperms than those of the control animals. [34] Similarly, it has been established that high dose of clove extract is toxic to spermatogenesis. [35]

The noticeable adverse histological changes in the seminiferous tubules agrees with the work of Malini et al, [36] which indicated that the seminiferous tubules regressed showing heavy loss of germinal elements, particularly, the elongating and mature spermatids in piperine treated rats. Black pepper contains piperine and has been identified to be cytotoxic and that this cytotoxicity is enhanced by the presence of tocopherol suggesting a mechanism of lipid peroxidation. [37] Also piperine has been implicated for DNA damage; [38] germ cell and seminiferous tubules damage; [36] spermatogenic arrest; [29] impairment of reproductive function, induction of oxidative stress and triggering of apoptosis. [39]

The histological findings of the present study is further explained using the mean values of the gonadosomatic indexes (σ) of the test groups of rats administered with varying doses of yaji. The gonadosomatic index (σ) is a tool for measuring the sexual maturity of animals in correlation to testicular development. [40] Though no significant differences were observed in σ between the experimental groups and the control as well as within the experimental groups themselves, however, we observed a decline in σ with increasing dosage level of yaji from group B to D. This dose-dependent effect of yaji on the gonadosomatic indices of the experimental groups suggests that the testicular development, hence the sexual maturity of

the young rats may decline with increasing dosage of yaji.

CONCLUSIONS

The histological findings of the present study revealed that higher doses of yaji caused damage to seminiferous tubules, reduced viable sperm cells and destroyed the arrangement of sperm and leydig cells. These observed changes induced by yaji consumption were dose dependent and may be attributed to the combined adverse effects of individual constituents of yaji. Our findings also indicated lack of significant effects of yaji on the testicular weights and gonadosomatic indexes of the experimental rats. However, the observed decline in Ơ with increasing dosage level of yaji may suggest a possible decline in testicular development and sexual maturity of the young rats with excessive consumption of yaji. Furthermore, the present finding which indicated weight reducing effect of yaji suggests that yaji may be useful in weight management in so far as its production and consumption is regulated. On the other hand, excessive and uncontrolled consumption of yaji may cause damage to the testes and impair testicular functions and should be avoided.

REFERENCES

1. Ogbuewu I P, Unamba-Oparah IC, Odoemenam VU, et al. The potentiality of medicinal plants as the source of new contraceptive principles in males. North. Am. J. Med. Sci. 2011; 3 (6): 255-263.
2. Romer AS & Parsons TS. The vertebrate body. Philadelphia, Holt-Saunders International PA, 1997; p385-86.
3. Osinowo OA. Introduction to animal reproduction. Sophic Academic Services Limited, Abeokuta, Nigeria. 1st Edition, 2006: p91.
4. Betumi B. The African culinary network publications, 2006: KuliKuli is calling me to come and buy. Available from www.betumi.com. Assessed on 27th May, 2014.
5. Ali M. N. Could Yaji be the next Chiloptle. New Yorker, 2011; 87 (37): p87.
6. Okonkwo T M. About yaji and suya. J. Food Agric 1987; 1(1); 51.
7. Nwaopara AO, Anyanwu L C, Oyinbo CA, et al. The histological changes in pancreas of wistar rats fed with diets containing Yaji (Local Meat Sauce). J. Expt. Clin. Anat. 2004; 3(2): 44-47.
8. Witchl M. Herbal drugs and phytopharmaceuticals. Broca Raton FL: CRC Press. Third edition, 2004; p.653-56.
9. Krishnawamy K and Raghuramulu N. Bioactive phytochemicals with emphasis on dietary practices. Indian J. Med. Res.1998; 108: 167-81.
10. Collier HO, McDonald-Gibson and Saeed SA. Letter: Stimulation of prostaglandins biosynthesis by capsaicin, ethanol and tyramine. J. Physiol. (Lond) 1965; 179 (2): 248-62.
11. McGee H. Food and cooking: The science and lore of kitchen. New York, Scribner, 2004; p427-29.
12. Omojola AB. Yield and organoleptic characteristics of suya (an intermediate moisture meat product meat) prepared from three different muscles of a matured bull. Afr. J. Biotechnol 2008; 7 (13): 2254-2257.
13. Carson SH, Osborn JW, Wyss JM. Hepatic innervations chronically elevate arterial pressure in wistar-Kyoto rats. Hypertension, 1998; 32: 46-51.
14. Nwaopara AO, Anibeze CIP, Akpuaka FC. Histological signs of oligodendroglioma in the brain of rats fed with diet containing yaji: the complex Nigerian Suya meat sauce. Clin. Rev. Opinions, 2009; 1 (2): 21-25.
15. Choi, D. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1998; 1: 623-34.
16. Blaylock LR. Excitotoxins: the taste that kills-Neurodegeneration and

neurodevelopment. Health Press, Santa FE, NM 1997; 10: 248-54.
17. Whetsell W and Shapira N. Biology of disease, neuroexcitation, excitoxicity and human neurological disease. Lab. Invest 1993; 68: 372-87.
18. Ankarcrona M, Dypbukt JM, Bonfoco EB, et al. Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on the mitochondrial function. Neuron 1998; 15: 961-63.
19. Nwaopara AO, Odike MAC, Inegbenenor U, et al. The combined effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the liver. Pak. J. Nutr 2007; 6 (6): 524-27.
20. Nwaopara AO, Odike MAC, Inegbenebor U, et al. Comparative study on the effect of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the kidney. Pak. J. Nutr 2008; 7 (2): 287-91.
21. Southgate DAT. Spices and beverages in essential human nutrition and dietetics. Edinburg, Churchill Livingstone, 1993; p325-34.
22. Nwaopara AO, Anibeze CIP, Akpuaka FC. Induced histological feature of hypoxia-ischaemia in the brain of rats fed with diet containing Yaji: the complex Nigerian meat sauce. Res. J. Appl. Sci. Eng. Technol. 2010; 2 (1): 67-72.
23. Nwaopara AO, Odike MAC, Inegbenebor U, et al. Comparative study on the effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the Heart. Electronic Journal of Biomedicine, 2008; 3: 61-64.
24. Akpamu U, Nwaopara AO, Izunya AM, et al. A comparative study on weight changes in rats fed with a diet containing yaji, yaji additives and yaji spices. Biology and Medicine, 2011; 3 (5): 6-15.
25. Micheal O, Folaranmi OM, Ajoke OD, et al. Effects of ginger (Zingiberofficinale Roscoe) and garlic (Allium sativum L.) on rats infected with Kleibsiellapneumoniae. The Internet Journal of Alternative Medicine, 2009; 7 (1).
26. Westerterp-Plantenga MS, Smeets A, Lejeune MPG. Sensory and gastrointestinal satiety effects of capsaicin on food intake. International Journal of Obesity 2005; 29: 682–688.
27. Hassan DI, Ogah DM, Yusuf ND, et al. The effect of acute and chronic (short and long term) oral administrations of black pepper (Piper guineense) aqueous extract on the body weight and haematological values of albino-Wistar rat. Journal of Medicinal Plants Research, 2010; 4 (12): 1122–1125.
28. Mbongue FGY, Kamtchouing P, Essame OJL, et al. Effect of the extract of dry fruits of Piper guineense on the reproductive function of adult male rats. Indian Journal of Pharmacology, 2005; 37: 30-32.
29. Malini T, Manimaran RR, Arunakaran J, et al. Effect of piperine on testes of albino rats. Journal of Ethnopharmacology 1999; 64 (3): 219-225.
30. Nwaopara AO, Odike MAC, Ikhuoriah TA, et al. Potential health hazards of Yaji, the complex 'suya meat' sauce. Medi-link Journal 2007; 8(74): 34-38.
31. Ihab Mohamed. The effects of the oral dosage of monosodium glutamate applied for short- and long-terms on the histology and ultrastructure of testes of the adult rats. Journal of Animal and Veterinary Advances 2012; 11(1): 124-133.
32. Buch JG, Dikshit RK, Mansuri SM. Effect of certain volatile oils on ejaculated human spermatozoa. Indian J Med Res 1998; 87: 361-3.
33. Mishra R K, Singh S K. Safety assessment of Syzygium aromaticum flower bud (clove) extract with respect to testicular function in mice. Food chem. Toxicol 2008; 46 (10): 3333-8.

34. Dehghani F, Heshmatpour A, Panjeshanin M, et al. Toxic effects of water/ alcoholic extract of Syzygium aromaticum on sperm quality, sex hormones and reproductive tissues in male mouse. IUFS Journal of Biology 2012; 71 (2): 95-102.
35. Chaieb K, Hajlaoui H, Zamantar T, et al. The chemical composition and biological activity of clove essential oil, Eugenia Caryllophllata (Syzygium aromaticum L. Myrtaceae). Phytotherapy Research 2007; 26 (6): 501-6.
36. Malini T, Arunakaran J, Aruldhas MM, et al. Effects of piperine on the lipid composition and enzymes of the pyruvate-malate cycle in the testes of the rat in vivo. Biochem Mol Biol Int 1999; 47 (1): 537-45.
37. Unchern S, Saito H, Nishiyama N. Death of cerebellar granule neurons induced by piperine is distinct from that induced by low potassium medium. Neurochem Res. 1998; 23 (1): 97-102.
38. Piychaturawat P, Kingkaeohoi S, Toskulkao C. Potentiation of carbon tetrachloride hepatotoxicity by piperine. Drug chem Toxicol 1995; 18 (4): 333-34.
39. D'Cruz SC, Vaithinathan S, Saradha B, et al. Piperine activates testicular apoptosis in adult rats. J Biochem Mol Toxicol 2008; 22(6); 382-8.
40. Barber BJ and Blake NJ. Reproductive physiology. In Biology and aquaculture. Shumway SE and Parsons G J (Eds.). Scallops Amsterdam: Elsvier 2006; 35:133-223.

How to cite this article: Ukoha UU, Egwu O.A, Dimkpa U et al. Histological and weight changes in testes of male albino rats fed with diets containing yaji (a local meat sauce). Int J Health Sci Res. 2014;4(8):118-126.

****