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Effects of Ingestion of Various Cassava (*Manihot utilissima*) Components on Serum Testosterone Level and Testicular Morphology in Male Wistar Rats

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ABSTRACT: The effect of consumption of cassava by products on the testosterone level and testicular morphology of Wistar rats was studied. The objective was to assess any changes in the testosterone level and histology of the testis. Male rats (n=28) weighing between 165g-260g were randomly assigned (n=7 each) into control group and experimental groups. Experimental groups were given the normal rat chow with inclusion of 50% cassava by product daily for 8weeks. The control group received equal amount of normal rat chow without inclusion of cassava by products for 8weeks after which rats were sacrificed. Blood samples were collected into plain bottles for serum testosterone levels estimation. The testes were dissected for histological assessment. Findings showed that rats in the experimental groups showed a statistically significant decrease in weights and serum testosterone level relative to those in the control group ($P < 0.05$) with altered testicular morphology. Therefore, inclusion of 50% cassava components in diet may have adverse effect on the weight, serum testosterone levels and altered testicular morphology.

Keywords: *Manihot utilissima*, Serum testosterone, Testicular morphology.

Introduction

Testosterone is a steroid hormone from the androgen group synthesized by the Leydig cells in the testes in males. It exerts a wide-ranging influence over sexual behaviour, muscle mass and strength, energy, cardiovascular health and bone integrity. Testosterone biosynthesis coincides with the spermatogenesis and fetal Leydig cell differentiation in the male rat. The capacity of Leydig cells to produce testosterone is higher in young than in old rats (Zirkin and Chen, 2000).

Cassava (*Manihot utilissima*) is a major food crop in Nigeria (Ogbe *et al.,* 2007) providing major source of calories to perhaps 200-300 million people (FAO, 2001; Coursey and Haynes, 1970). Cassava has been known for decades and strategically valued for its role in food security, poverty alleviation and as a source of raw materials for agro-allied industries in Nigeria with huge potential for the export market (Egesi *et al.,* 2007). It may be a powerful poverty fighter.

Different methods have been adopted over the years to reduce cyanogenic glucosides which constitute a major limitation to the use of cassava in both human and animal foods. These have been known to affect the proper functioning of the human systems depending on its concentration (Gonzalez-Reimers *et al.,* 1994). It has also been implicated in the aetiology of various disease conditions (Tylleskar *et al.,* 1992; Banea *et al.,* 1992; Rosling, 1987).

Some abnormalities that border on pathological changes as well as metabolic integrity of the organism under repeated intake of cassava with high cyanide content do occur which may be due to either the direct effects of hydrocyanic acid or its product of bio transformation, has been demonstrated to draw on various nutrients such as protein in the human or animal metabolic pool for accomplishment of the processes which they undergo (Maner and Gomez, 1973). The objective of the study was to investigate possible effects of ingestion of various cassava by products on serum testosterone level and testicular morphology of male Wistar rats.

Materials and Methods

Animals: The rats were obtained and maintained in the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin. They were fed with rat chow obtained from Edo Feeds and Flour Mill Limited, Edo State, Nigeria.

Cassava and administration of cassava components: Twenty-eight adult male Wistar rats weighing between 160-265 g were randomly selected for the study and assigned into a control group and three experimental groups. There were seven (7) rats per group. The cassava components used were the cassava fiber (*popo gari*), grated, roasted cassava tuber (*gari*) and cassava starch obtained from fresh cassava tubers from the local market in Benin-City, Nigeria. The rats in the experimental groups were given normal rat chow with the inclusion of 50% cassava components respectively in their 20 g/day feed. The different cassava components were thoroughly mixed with the feeds and given on a daily basis for 8weeks. The control group received equal amount (20 g) of feed daily without any cassava components added for the same period. All the rats were given water ad libitum. The rats were sacrificed by anaesthetizing them using chloroform after the 8weeks of the experiment. Blood was drawn out through the aorta and collected in plain bottles for the estimation of serum testosterone levels. The testes were removed carefully and fixed in formalin for routine histological analysis.

Serum testosterone level estimation and histology of the testis: The serum testosterone level was estimated using the enzyme-linked immunosorbent assay, a one-step immunoassay based on the principle of competitive binding while the testicular morphology was studied using Carleton's method (Carleton, 1967).

Statistical analysis: Statistical analysis was done using a one-way Analysis of Variance (ANOVA). P value of 0.05 or less was taken as significant and means difference was compared using a multiple comparison test (Duncan, 1955). The mean and standard error of mean for each of the groups of animals were calculated.

Results

Results are shown in Figures 1 & 2 and Plates 1-5. Figure 1 shows that there was a statistically significantly decreased in weight ($P<0.05$) of the experimental group fed with 50% inclusion of cassava fiber (*popo gari*) in their daily diet, from 272±12.1g to 234±4.0g after the study.

Figure 2 shows that the serum testosterone levels were significantly reduced in the experimental groups when compared with the control group ($P<0.05$). Serum testosterone level was least in the group with inclusion of 50% cassava starch in their diet (group 4).

After 8weeks of the ingestion cassava by products by various groups, the histological studies revealed as shown in Plates 1-5. The testicular morphology of the control group showed numerous seminiferous tubules bounded together by loose interlobar connective tissue containing fibroblasts, collagen fibers, blood vessels, abundance of germ cells at various stages of maturation from type A spermatogonia to type B spermatogonia, primary and secondary spermatocytes and spermatids. Interfering sertoli cells were also seen. In the interstitial tissue between the seminiferous tubules, normal appearing leydig cells were seen (Plates 1&2).

The testicular morphology of the experimental group with the inclusion of 50% *popo gari* in their diet (Plate 3) showed seminiferous tubules that are reduced in diameter, containing spermatogonia and with a relatively reduced number of the later developmental stages (spermatocytes and spermatids) and an increased proportion of sertoli cells.

The testicular morphology of the experimental group with the inclusion of 50% *gari* in their diet (Plate 4) showed numerous seminiferous tubules containing spermatogonia with a relatively reduced number of the later developmental stages (spermatocytes and spermatids) and an increased proportion of sertoli cells.

The testicular morphology of the experimental group with the inclusion of 50% starch in their diet (Plate 5) showed seminiferous tubules with highly reduced diameter, increased interstitial spaces and reduced number of the leydig cells. The seminiferous tubules contained spermatogonia with relatively reduced number of the later developmental stages (spermatocytes and spermatids) and an increased proportion of sertoli cells.

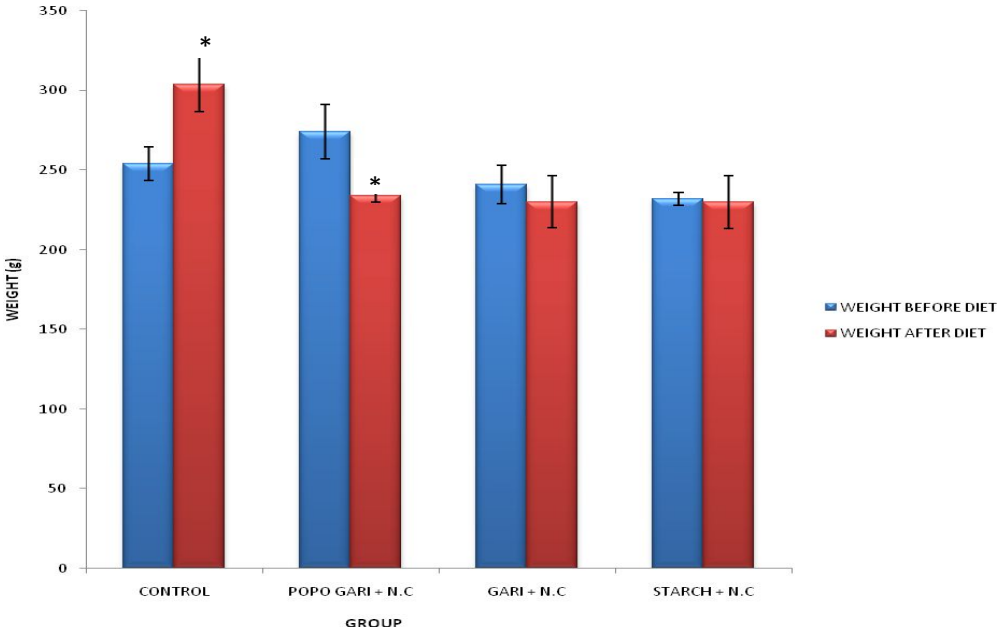

Figure 1: The weight (g) of the control and experimental animals in different groups before and after the experiment.

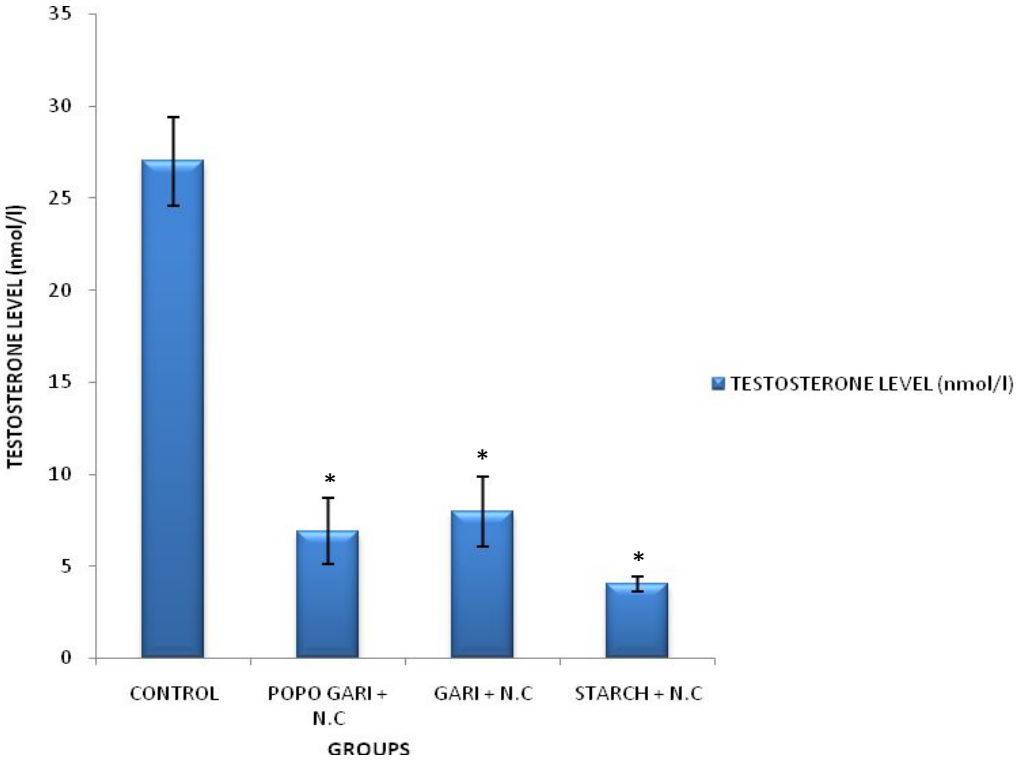

Figure 2: Testosterone Level (nmol/l) in mean ± SEM in the Control and Experimental Animals
*mean value significant at $p < 0.05$; N.C means Normal Chow

Testicular morphology

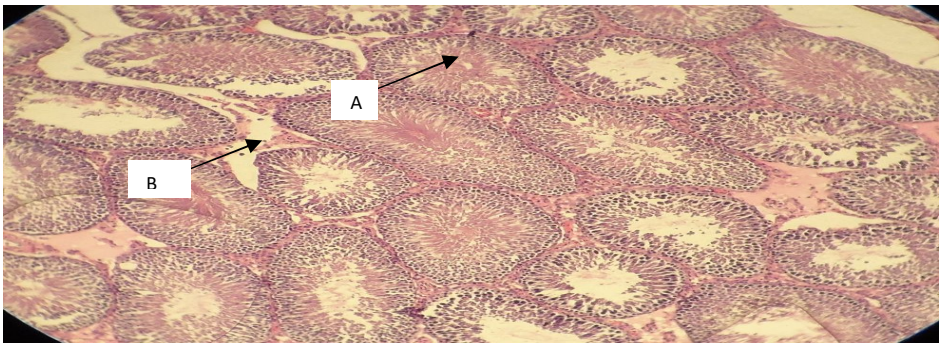

Plate 1: Section of the testis of the control group (x10) with arrows showing stages I-VIII of spermatogenesis

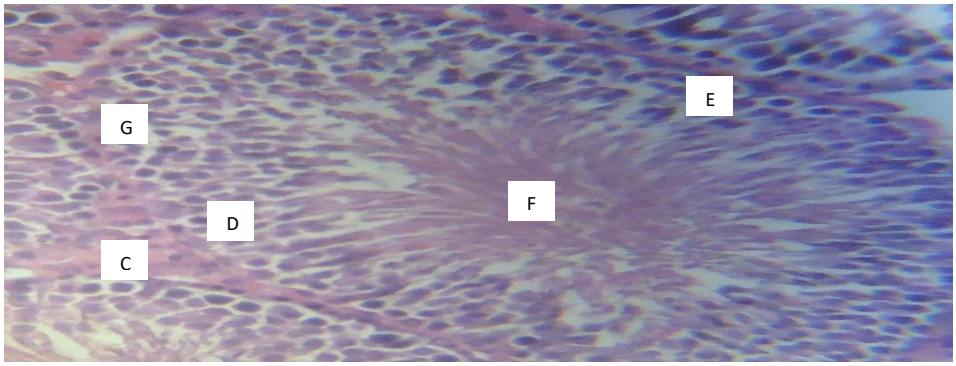

Plate 2: Section of the testis of the control group (x40)
A Seminiferous tubule
B Interstitial connective tissue
C Spermatogonia
D Primary spermatocyes
E Secondary spermatocytes
F Spermatids
G Leydig cells

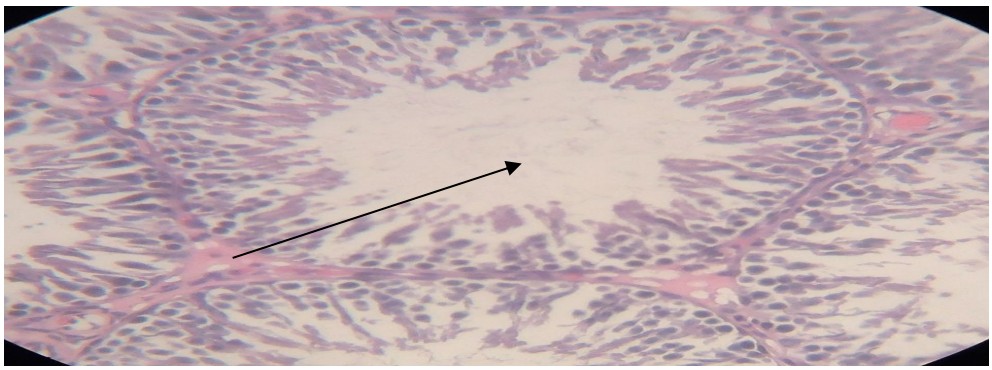

Plate 3: Section of the testis of the group with inclusion of 50% *popogari* in their diet (x40) with arrow showing direction of germ cell maturation from spermatogonia to the final stage (spermatids) (stage I-VII), hypospermatogenesis was observed, there was decrease in number of germ cells in different stages of spermatogenesis.

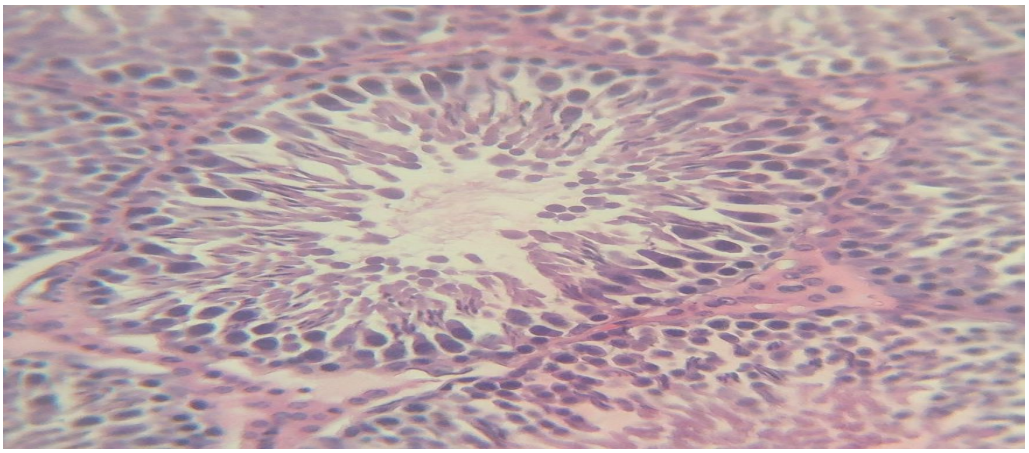

Plate 4: Section of the testis of the group with inclusion of 50% *gari* in their diet (x40) with arrow showing between stages VII-VIII where round spermatids are converted to elongated spermatids which were affected.

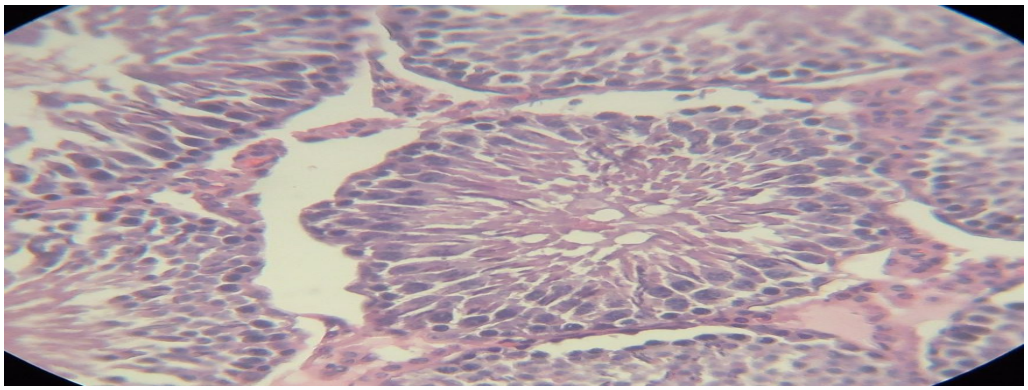

Plate 5: Section of the testis of the group with inclusion of 50% cassava starch in their diet (x40) with arrow showing reduction in the diameter of the seminiferous tubule with increased interstitial space, subsequently the germ cells present in the seminiferous tubule in their different maturation stages reduced in number.

Discussion

This study showed a drastic reduction in the level of testosterone in the groups fed various cassava byproducts compared to the control group, even though it was most severe in the group with the inclusion of 50 % cassava starch in their diet. This reduced beyond the low lower limit of the normal testosterone range. This can be ascribed to the deficiency of protein and fats in the diet which is in agreement with the report that the hypothalamo-hypophyseal gonadal axis is impaired when the consumption of protein and calorie is decreased (Herbert, 1980), seminiferous tubule of malnourished rats significantly decreased in diameter and in the stage of development of spermatogenesis which is associated with changes in circulating androgen and gonadotropin levels and subsequent disruption of spermatogenesis (Vawda and Mandalwana, 1990). Therefore, nutrition affects the endocrine function rather than the spermatogenic function of the testis (Gonzalez-Reimers *et al.,* 1987).

The serum luteinizing hormone, follicle stimulating hormone, prolactin and testosterone are lowered in malnourished rats at all ages (Herbert, 1980). However, the reduction in serum testosterone level may not be ascribed to the cyanide content as reported by Iyayi (1991) who also reported that non-significant correlation existed between daily cyanide intake and serum testosterone level.

Testosterone has been shown to be essential for normal spermatogenesis, because it stimulates the conversion of round spermatids into elongated spermatids between stage VII and stage VIII of the spermatogenetic cycle. Androgen deficiency disturbs the spermiation process (Saito *et al.,* 2000); it alters spermatid-sertoli cell junctions, which results in premature detachment of round spermatids from sertoli cells and seminal epithelium (Beardsley and O'Donnell, 2003). This was reflected in the testicular morphology which confirmed that the reduction in testosterone level caused hypospermatogenesis, which was observed in all the experimental groups but was severe in groups with inclusion of 50% cassava starch in their diet.

Conclusion

Inclusion of 50% cassava by products in diet may have adverse effect on the physiology and histology of living organisms in terms of alteration in body weight, reduced serum testosterone and disruption of spermatogenesis which may be ascribed to the deficiency of protein and fat or the increase in carbohydrate content of the diet. The definite cause of the observations is the subject of further studies.

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