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Short Communication

Effects of Unripe *Musa Paradisiaca* on the Histochemistry of the Testis and Testosterone Levels in Adult Albino Rats

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Summary: This study was aimed at determining the effects of the unripe fruit of *Musa paradisiaca* on the testis and testosterone levels in male Wistar rats. The animals were grouped into three, comprising a control, and 2 treatment groups administered with different doses (500 mg/kg and 1000 mg/kg) daily of the fruit flour over 28 days. Histochemical evaluation of the testes was done using Haematoxylin and Eosin, Periodic acid Schiff's (PAS) and Feulgen staining techniques, while the serum and homogenised testicular tissue were evaluated for testosterone levels using Accu-Bind ELISA Kit. The testis of the treated groups showed more rapidly dividing cells and more population of sperm cells compared to the control group, and also showed more positivity for Feulgen staining and PAS reaction. Both serum and testicular testosterone levels were however reduced. Serum testosterone was significantly lowered in the animals given the low dose (0.67 ± 0.03 ng/ml), compared to those given high dose (0.85 ± 0.02 ng/ml) and the control animals (1.88 ± 0.15 ng/ml) ($p < 0.05$). Changes in testicular testosterone were not statistically significant. The study suggests that *M. paradisiaca* fruit has reproductive enhancing potential when consumed moderately, but this benefit may not be related to testosterone levels.

Keywords*: Musa paradisiaca*, Testes, Testosterone, Histochemistry

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INTRODUCTION

The beneficial and medicinal effects of *Musa paradisiaca* (MP), or plantain, have been explored for many years, especially in the Tropical and Subtropical regions of the World where the crop is indigenous (Imam and Akter, 2011). The unripe plantain has a high dietary fibre content, surpassing those found in other fruits and vegetables, and enhances better food digestion (Osim and Ibu, 1991; Parmar and Kar, 2007). The plant has proved to be of benefit in cardiovascular and metabolic conditions, infectious diseases, allergy and in wound healing (Osim and Ibu, 1991; Parmar and Kar, 2007).

Phytochemical analysis of various parts of MP has yielded different carbohydrates, crystallisable and non-crystallisable sugars, amino acids such as arginine, aspartic acid, glutamic acid and leucine, vitamins, fats, mineral salts, and neurotransmitters such as serotonin, norepinephrine and tryptophan (Ketiku, 1973; Emaga *et al*., 2007). Various studies have been done to research into the beneficial effects of some parts of the plant in certain diseased conditions either for preventive or curative purpose, or to alleviate certain clinical conditions. According to Ojewole *et al*. (2003) the methanolic extract of MP fruit can be used in the management of diabetic-induced testicular damage. Similarly, both alcoholic and aqueous extracts of the stem of MP had significant beneficial effect on drug-induced hepatotoxicity (Nirmala *et al*., 2012). Yakubu *et al*. (2013) reported that aqueous extract of the root of MP enhanced the testosterone-dependent normal functioning of the rat's testes.

The current study investigated the effects of the fruit of *Musa paradisiaca* on the structure of the testis and testosterone levels in both the serum and testis of albino rats.

MATERIALS AND METHODS

The study was carried out in conformity with the Rules and Guidelines of the Animal Ethics Committee of the University of Ilorin. Eighteen (18) adult male albino rats of the Wistar strain, weighing between 160 g- 260 g, were obtained and maintained in the Animal House of the College of Health Sciences, University of Ilorin. The animals were grouped into three equal groups and kept at normal room temperature, with provision of feeds and water.

Experimental Design

The preparation of the unripe *Musa paradisiaca* (MP) flour was as earlier describe by Alabi *et al*. (2013). The fruits were obtained from Ipata market in Ilorin

and were cut longitudinally into chips of about 5 mm thick and air-dried till dry enough for grinding after which they were grinded and made into flour. Prior to oral administration, each dose of the flour was dissolved in 2 ml of double distilled water, and administered once daily as follows:

Group A: Control, received 2 ml/kg b wt double distilled water;
Group B: received 500 mg/kg b wt of MP flour; and,
Group C: received 1000 mg/kg b wt of MP flour.

Animal Sacrifice

Treatment of animals lasted for 28 days, and the rats were sacrificed by cervical dislocation. Blood samples were collected intracardially into plain bottles after centrifuging, for testosterone assay. The testes were identified and removed. Some of the testes of the rats in each group were fixed in freshly prepared 10% formalin and some fixed in Bouin's fluid, for histochemical studies, while the others were weighed and homogenised in 0.5 M cold sucrose solution and transferred into a deep freezer, for hormonal studies.

Histochemical Techniques

The fixed tissues were processed based on earlier staining techniques described for routine Haematoxylin and Eosin, demonstration of carbohydrates by Periodic acid-Schiff's reaction (for the testes fixed in Bouin's fluid) and Feulgen reaction for nucleic acids (for the testes fixed in 10% formalin) (Pearse, 1980; Bancroft and Stevens, 1990).

Testosterone Assay

The homogenised testis was centrifuged at 5000 rpm for 5 minutes using a centrifuge (Model 90-1). The supernatants were immediately stored in the deep freezer (GC-B207WVQ) at -20^{0}C for hormonal studies. Both serum and tissue testosterone analysis was carried out using Accu-Bind ELISA Kit (Lashansky *et al*., 1991; Tietz, 1995).

Statistical Analysis

Data were analysed using SPSS version 15.0. Student-t test was carried out and data presented as mean and standard mean error (SEM). P-values < 0.05 were considered significant.

RESULTS

The treated animals tolerated the oral administration of the plantain flour, and there were no signs of regurgitation.

Testosterone Analysis

Animals exposed to MP had statistically significant decrease in serum testosterone when compared with the control group ($p< 0.05$), and the decrease was more in the low dose group (500 mg/kg). The difference between the treatment groups was also significant ($p < 0.05$). Although there was a slight decrease in the testosterone level in the homogenate of testicular tissue in animals exposed to 500 mg/kg MP, this was not statistically significant ($p > 0.05$), nor the slight increase observed in the high dose group (Tables 1).

Histological Observation

The seminiferous tubules were well-defined in all the groups, with the presence of spermatogenic cells at different stages of development. The sperm cells of the seminiferous tubules of the Control group were both positive for PAS and Feulgen stains, with the demonstration of the magenta-coloured stain (Figures 1A, 2A, 3A). There were more rapidly dividing cells

Table 1: Results of serum and testicular testosterone assay

Parameters/Groups	A	B	C
Serum testosterone (ng/ml)	1.88 ± 0.15	0.67 ± 0.03*	0.85 ± 0.02*†
Testicular testosterone (ng/ml)	12.25 ± 0.013	11.32 ± 0.034	12.50 ± 0.038

A= Control, B= 500mg/kg MP, C= 1000mg/kg MP. *****Significant statistical difference with the Control Group ($p < 0.05$) †Significant statistical difference between Treatment Groups ($p< 0.05$).

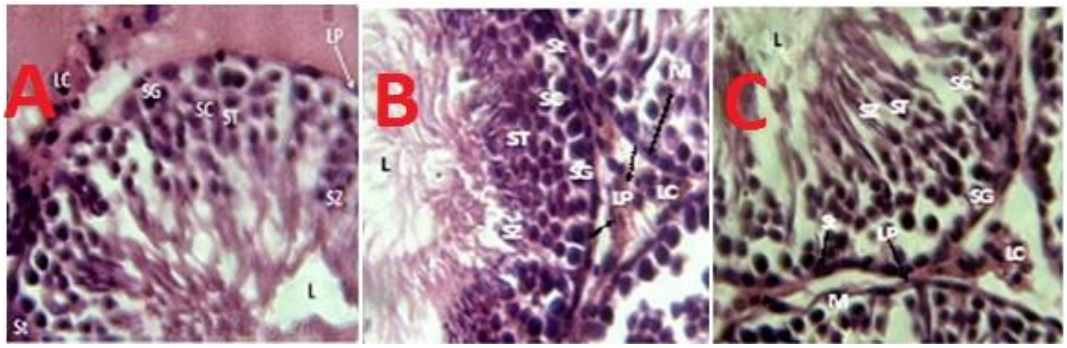
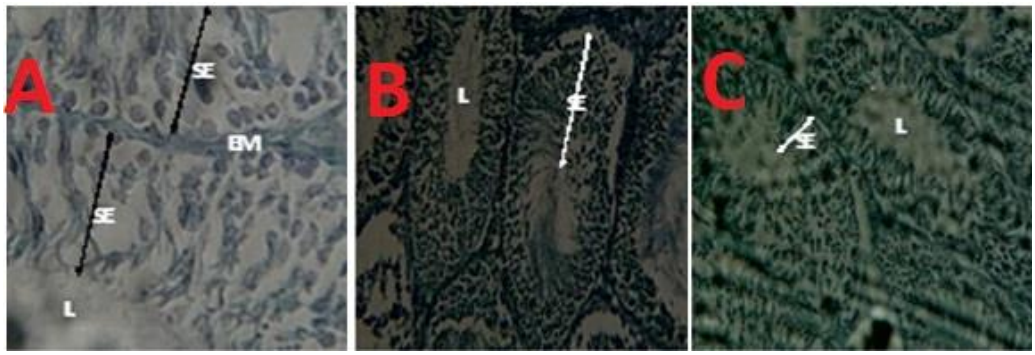


Figure 1: Photomicrographs of the testis of rats in the control group (A) showing normal cross section of the seminiferous tubule with the spermatogenic cells - spermatogonia (SG), spermatocytes (SC), spermatids (ST) and spermatozoa (SZ); the lumen (L), Leydig cells (LC) and lamina propria (LP); B (500 mg/kg MP): showed closely packed spermatogenic cells, more than in Control group; supporting Sertoli cells (St), and a well-delineated Interstitium with Leydig cells (LC); C(1000 mg/kg MP): showed less intensely stained seminiferous tubules and sperm cells; the Interstitium appeared scanty compared with Control. H&E x400.



- Figure 2: Photomicrographs of the testis of rats in the control group (A) showing normal cross section of the seminiferous epithelium (SE), with DNA positive cells; B (500 mg/kg MP): showed increased staining intensity for DNA; while C (1000 mg/kg MP): showed decreased staining intensity for DNA compared with A. Feulgen DNA x100.

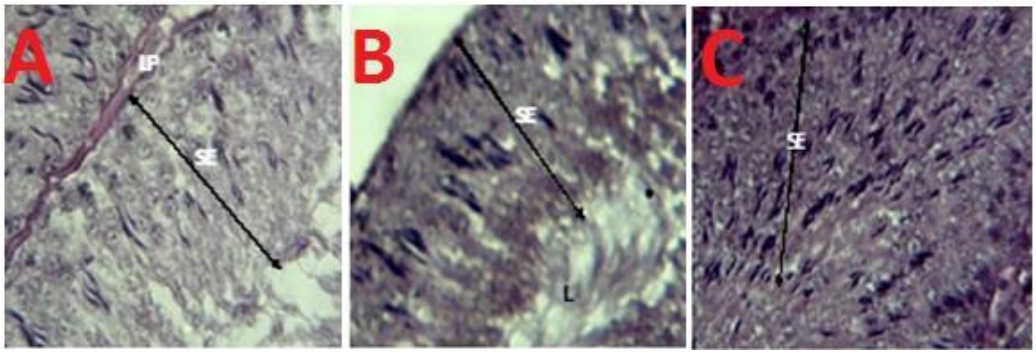


Figure 3: Photomicrographs of the testis of the control (A) and treated groups (B:500 mg/kg MP; C:1000 mg/kg MP) showed positivity for Periodic acid Schiff (PAS) staining inspermatogenic cells. LP: Lamina propria, SE: seminiferous epithelium. PAS x400.

and more spermatozoa in the low dose group B, and these showed increased staining intensity for PAS reaction, and were also more Feulgen positive than the control group A and the high dose group C (Figures 1B, 2B, 3B). The Interstitium of the low dose group appeared to be better defined than others, with the demonstration of Interstitial cells (Leydig cells) in the Interstitium (Figure 1B). Positive demonstration of Feulgen staining and PAS reaction were more in the high dose group than the Control, but less stained when compared with the low dose group (Figures 3B, 3C).

DISCUSSION

Previous studies have shown that consumption of plantain is both beneficial and therapeutic (Ojewole and Adewumi, 2003). However, in this study, animals treated with MP showed consistent low levels of testosterone which was particularly significant in the serum. Conversely, low dose of MP exerted more lowering effects than high dose of the plantain flour.

Rapidly dividing cells were observed especially in animals treated with low dose MP, compared to those of the Control and high dose groups. Furthermore, the population of the sperm cells was also more in the low dose group. This observation is in consonance with our earlier findings of improved semen quality in animals administered with moderate dose of *M paradisiaca* (Alabi *et al*., 2013). However, this fertility or sperm-enhancing effect of *M paradisiaca* might not be directly related to testosterone activity, since the level of testosterone both in testes and serum was reduced following MP administration. A review by Yakubu *et al*. (2013) on the aqueous extract of the root of *M paradisiaca* reported that the plant has both androgenic and anabolic properties. Although our study has a contrary view, this could be due to the fact that the fruit, and not the root of the plant, was used in the current study; and the concentration adopted could also be implicated.

The greater positivity of the testes of animals treated with low dose MP to Feulgen reaction, showed the presence of more nucleic acids, and hence the occurrence of rapid cell division. This enhances spermatogenesis and the basis for the good outcome of the semen analysis (Alabi *et al*., 2013).

Similarly, carbohydrates, which are demonstrable using PAS reaction, are essential for effective processes of spermiogenesis and spermatogenesis. This reaction was well demonstrated in the treated animals unlike in the Control, and was supportive of the rapidly dividing sperm cells, especially in animals that received moderated dose of *M paradisiaca*.

In conclusion, it is becoming more proven that *M paradisiaca* has beneficial effects. However, this study showed that MP is not beneficial with respect to testosterone as it enhances its low level both in the

serum and in the tissue. Therefore, the mechanism of action of *M paradisiaca* in the reproductive system and function should also be comprehensively studied.

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