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Full length Research paper

Serum testosterone and morphology of the testes in wistar rats following chronic garlic feeding

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Garlic (*Allium sativum*) is a popular spice usually eaten raw or cooked in various doses and its potential medical properties have been recognized for thousands of years (Agarwal, 1996). Testosterone is a steroid hormone from the androgen group. 21 adult male wistar albino rats were randomly divided into control, test 1 and test 2 groups. The test 1and test 2 groups received oral doses of 1.4 and 2.4 ml respectively of 100 mg/ml garlic extract for 31 days. Their body weights were measured before and after the study. The rats were sacrificed and blood samples collected by cardiac puncture while serum testosterone was evaluated by radioimmunoassay technique. The testes were also taken for histological analysis. The control group was compared with test 1 and with test 2 group and a significant decrease in serum testosterone levels was observed between the controls and test 2 group. The body weight of each group before the study was compared with their weight after the study and the decrease observed was not significant. There were morphological changes in the testes of the garlic – fed rats. The results are discussed.

Key words: Garlic, serum testosterone, testes.

INTRODUCTION

Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, spicy flavour that mellows and sweetens with cooking (Gernot, 2005). It displays therapeutic effects such as in the treatment of hypercholesterolemia (Mahmoodi et al., 2006), prevention of atherosclerosis (Koscielny et al., 1999), and some cancers (Galeone et al., 2006), and presents anticoagulant (Chan et al., 2007) and antihypertensive (Silagy and Neil, 1994) properties. Non-pharmacological treatment with garlic preparation is suggested to reduce blood pressure in hypertensive individuals (Ried et al., 2008). Not much has been reported on the side-effects, particularly on male reproduction, of such a garlic treatment. The few reports in the literature are conflicting. To date, it has been reported that heated garlic juice was effective in recovery of testicular function after experimental testicular hypogonadism (Kasuga et al., 2001), but other laboratories have reported that powder (Dixit and Joshi, 1986) or crude (Hammami et al., 2008) garlic preparations impaired testicular and male reproductive tract functions. Garlic metabolites such as diallyl trisulfide have been reported to have spermicidal effects (Chakrabarti et al., 2003). The mechanisms and effect of garlic action on serum testosterone level and testicular morphology, however, remain unclear.

Only a few researches have been conducted to investigate the effect of garlic on male reproductive function and so it is not quite clear what the effect of garlic on serum testosterone level and testicular morphology in males could be. Due to the conflicting views and findings as to the effect(s) of garlic on the male reproductive system, the researchers aimed to investigate the effect of garlic on serum testosterone level and testicular morphology in rats.

MATERIALS AND METHODS

Experimental design

21 adult male wistar albino rats weighing 250 – 350 g were used for the study. They were purchased from the Zoology department, University of Benin, Nigeria and housed in wooden cages in the

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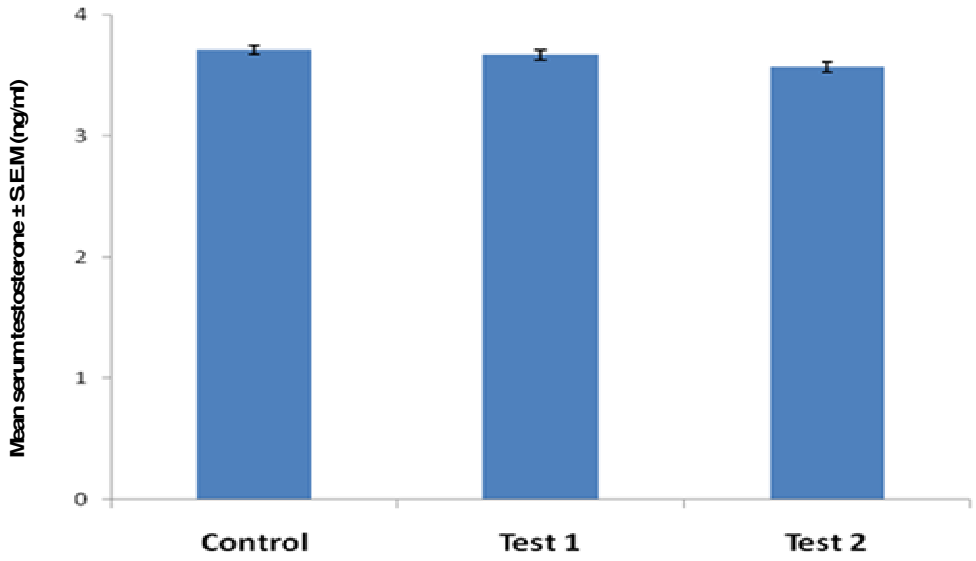

Figure 1. Serum testosterone levels in the control, test 1 and test 2 groups.

animal house of the Anatomy department, University of Benin. They were allowed to acclimatize for a period of two weeks prior to the study. Food and water was given to the animals *ad libitum* during the acclimatization period and throughout the study.

They were randomly divided into 3 groups of 7 rats each. The control group was fed rat feed; Test 1 group was fed rat feed and 1.4 ml/day of 100 mg/ml garlic extract; Test 2 group was fed rat feed and 2.4 ml/day of 100 mg/ml garlic extract. They were all fed for 31 days, after which blood samples were collected from the rats and serum testosterone levels evaluated. The testes of the rats were also taken for histological analysis.

Garlic source and extraction

Local cultivar of garlic (Allium sativum) was purchased from the local market. The garlic was hand-peeled and dried for 6 h. The dried garlic cloves, weighing about 320 g, were crushed with mortar and pestle and then weighed. The crushed garlic was exposed to the environment for 10 to 15 min so that there was conversion of alliin to allicin by the enzyme allinase. It was then mixed with distilled water and left for 24 h to macerate. The resulting mixture was filtered and the filtrate was concentrated by placing it in a water bath to concentrate the mixture by evaporation, leaving behind a chocolate-coloured syrup extract, weighing 180 g. The whole process of aqueous garlic extraction was the maceration method (Lachance, 1997). The extract was weighed again, collected in a glass jar and administered using an orogastric tube at 4.00pm daily.

Specimen collection

After 31 days, the animals were weighed again after which they were sacrificed. Blood samples were collected directly from the heart by cardiac puncture. All samples were collected in the morning following an overnight fast. The serum testosterone levels were assayed by radioimmunoassay technique using the kit by Adaltis Inc., 2007. The abdominal cavity was also opened and the testes remove. Formaldehyde was used in fixing the tissue. All data were expressed as means ± standard error of mean. The student t-test was used to compare the control group with the test 1 and with the test 2 groups, and p values less than 0.05 ($p<0.05$) were taken as statistically significant. All analyses were done with SPSS 17.0.

RESULTS

As shown in Figure 1, after 31 days of garlic ingestion, the mean serum testosterone level of the control group was 3.71 ± 0.03 ng/ml, that of the test 1 group was 3.67 ± 0.03 ng/ml, while that of the test 2 group was 3.57 ± 0.04 ng/ml. Comparison of the serum testosterone level of the control group with that of the test 1 group did not show any significant difference. However, the mean serum testosterone level of the control group was significantly greater than that of the test 2 group. There was also no significant difference between the serum testosterone level of the test 1 group and that of the test 2 group.

The mean body weights of the control, test 1 and test 2 groups before the study was 296.43, 280.0 and 320.0 g respectively, while at the end of the study, their mean body weights were 292.86, 277.14 and 312.86 g respectively (Table 1). The changes in body weights after the study were not significant (Figure 2). The histological studies revealed that in the control group (Figures 3 and 4), the seminiferous tubules were bounded together by loose intertubular connective tissue which contained fibroblasts, collagen fibers, blood vessels and groups of interstitial cells or leydig cells. These cells were large and polyhedral with euchromatic nucleus, containing nucleoli. The cytoplasm was scanty and poorly stained. The capillaries were infiltrated among the clumps of leydig cells.

In the test 1 group (Figures 5 and 6), there was marked degeneration of most of the seminiferous tubules. The architecture of the testis was maintained but the germinal

Table 1. Weights of the control and test groups before and after the study.

Groups	Control before study	Control after study	Test 1 before study	Test 1 after study	Test 2 before study	Test 2 after study
Weights (g)	350.00	350.00	250.00	250.00	350.00	350.00
	350.00	350.00	300.00	300.00	350.00	340.00
	275.00	280.00	300.00	305.00	300.00	300.00
	300.00	220.00	300.00	240.00	325.00	300.00
	250.00	300.00	250.00	250.00	275.00	270.00
	300.00	250.00	260.00	295.00	340.00	330.00
	250.00	300.00	300.00	300.00	300.00	300.00
Mean weights (g)	296.43	292.86	280.00	277.14	320.00	312.86
S.E.M (g)	15.84	18.22	9.51	10.90	10.97	10.63

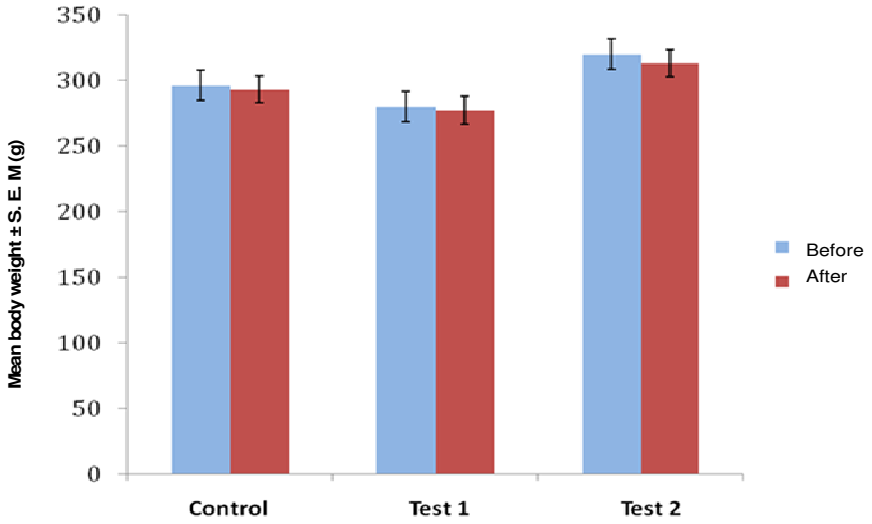

Figure 2. Body weights of the control, test 1 and test 2 groups before and after the study.

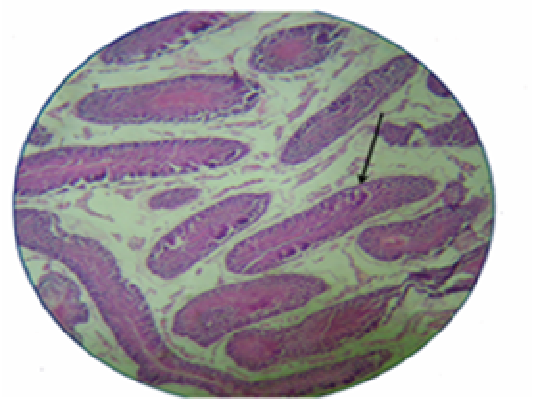

Figure 3. Section of the testis of the control group with the black arrow showing a normal seminiferous Tubule (×10).

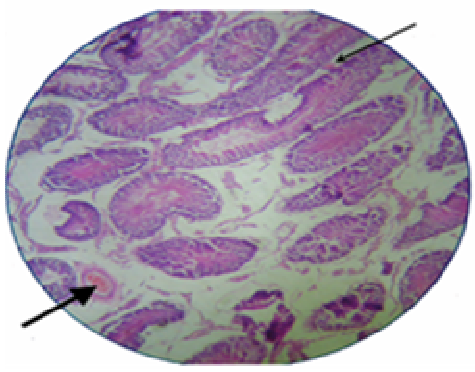

Figure 4. Section of the testis of the control group with the thick arrow showing blood vessels and the thin arrow showing the interstitial cells of leydig (×10).

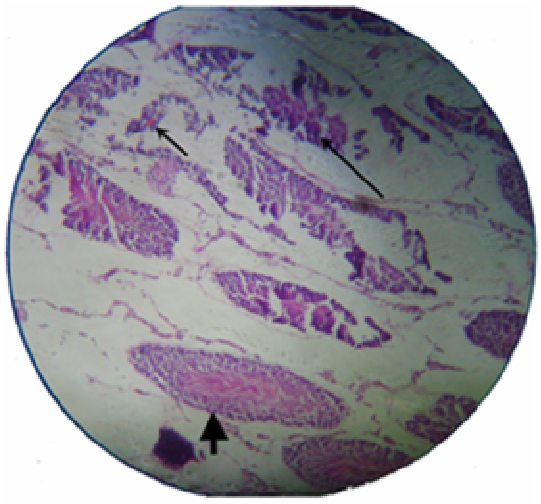

Figure 5. Section of the testis of test 1 group with the thick black arrow showing a normal seminiferous tubule, the small thin arrow showing a blood vessel and the long thin arrow showing a degenerated seminiferous tubule (×10).

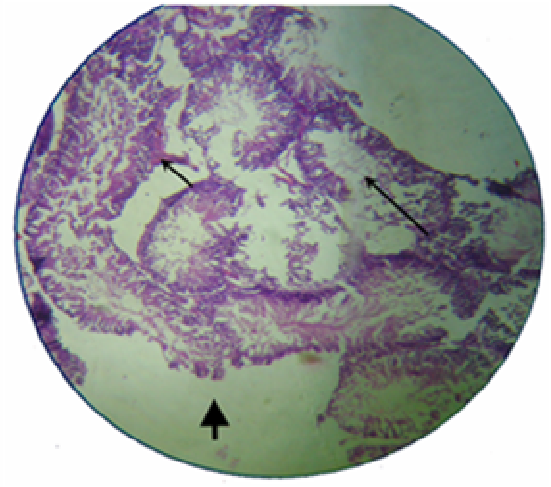

Figure 6. Section of the testis of the test 1 group with the longer thin black arrow showing a degenerated seminiferous tubule, the small thin arrow showing leydig cells and the thick black arrow showing a large interstitial space (×10).

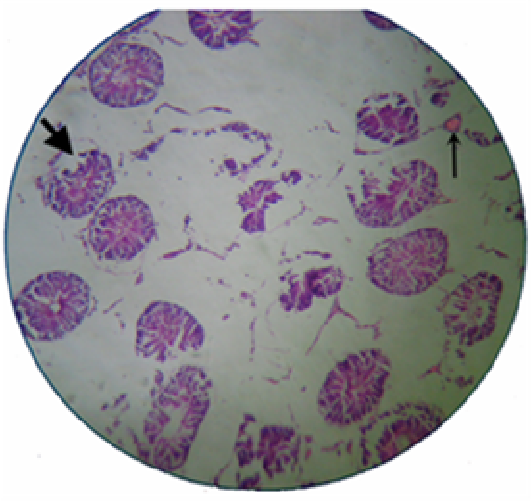

Figure 7. Section of the testis of test 2 group with the thick arrow showing a degenerated seminiferous tubule and the thin arrow showing blood vessels (×10).

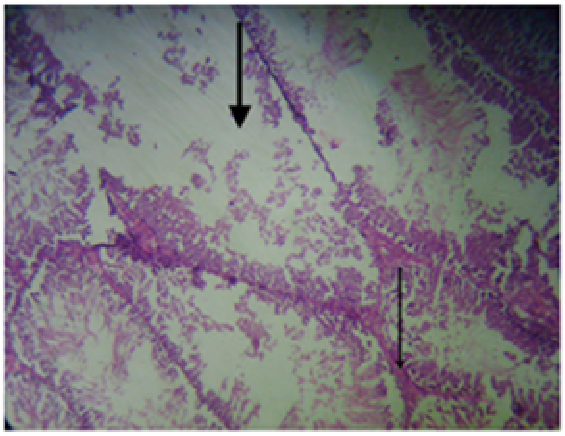

Figure 8. Section of testis of test 2 group with thick arrow showing a degenerated seminiferous tubule and thin arrow showing the interstitial space containing few leydig cells (×10).

epithelium showed disorganization as well as marked degenerative changes. As the seminiferous tubules were reduced in diameter, the interstitial spaces were increased. The number of fibroblasts was also increased. The leydig cells were reduced in number.

In the test 2 group (Figures 7 and 8), many parts of the tissue were distorted and there were structural changes in the seminiferous tubules and their content, which had fewer numbers of spermatogenic epithelium (Sertoli cells), and spermatids. The seminiferous tubules were reduced in diameter and the interstitial spaces increased grossly. Leydig cells in the interstitial spaces were grossly reduced in number.

DISCUSSION

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) by altering spermatid-Sertoli cell junctions, which results in premature detachment of round spermatids from Sertoli cells and seminal epithelium (Beardsley and O'Donnell, 2003), along with apoptosis and activation of caspases (Tesarik et al., 2002).

In this study, the observation that chronic ingestion of garlic significantly reduced serum testosterone levels in test 2 group was in accordance with the study of Imen et al. (2009), which reported a decrease in serum and intratesticular testosterone levels following treatment with crude garlic. This decrease in testosterone levels led to a decrease in spermatogenesis. Hammami et al. (2009), in their study, hypothesized that garlic inhibits steroidogesnesis in three different ways other than a decrease in its substrate income, which include: it might affect free cholesterol mobilization towards Leydig cell mitochondria, disrupt cholesterol mitochondria translocation, which is an important step in steroidogenesis with the steroidogenic acute regulatory (STAR) protein as an effector, and that garlic might prevent cholesterol conversion into testosterone by impairing activities of key regulatory enzymes of steroidogenesis. Perhaps the decrease in serum testosterone level observed in this study may have been caused by one of these hypothesized mechanisms, but this is the subject of future research.

The degenerative changes observed from the histology of the testes of test 1 and test 2 groups probably caused the observed decrease in the serum testosterone levels of test 1 and test 2 groups, because the interstitial cells of leydig, which produce and secrete testosterone, were markedly degenerated and reduced in number, especially in the test 2 group.

It was concluded that garlic consumption, especially at high doses or concentrations, caused a decrease in serum testosterone level and marked degenerative changes in the testicular morphology.

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