EVALUATION OF SEX HORMONES OF MALE RATS TREATED WITH GARLIC AQUEOUS EXTRACT AND HIGH FATTY DIET

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ABSTRACT

Aim

The effects of garlic and high fatty diet on reproductive hormones [testosterone, prolactin, follicle-stimulating hormone (FSH) and luitenising hormone (LH)] were determined in this study.

Methods

Sixteen adult male Wistar rats were divided into four groups of four rats per group. Group A (Control) was given distilled water; Group B was given 1000 mg/kg/day of aqueous garlic extract; Group C received high fatty diet; and Group D was given high fatty diet and 1000 mg/kg/day of aqueous garlic extract. The administration lasted for a period of 28 days, after which the animals were sacrificed by cervical dislocation and the levels of the sex hormones assessed in the serum.

Results and Conclusion

Administration of garlic and high fatty diet independently and in combination, led to increased levels of serum testosterone, suggesting a possible enhancement of male reproductive functions.

Keywords: fatty diet, garlic, sex hormones

INTRODUCTION

Testosterone, prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are four very important hormones involved in human reproduction and development. These are very vital in the production and maturation of sperm cells, regulation of lactation, and promotion of secondary sexual characteristics [1].

In the male, LH acts on the Leydig cells of the testis, which is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intra-testicular activity on spermatogenesis [2].

The level of prolactin can be an indicator for the amount of sexual satisfaction and relaxation, and unusually high amounts are suspected to be responsible for impotence and loss of libido. The level rises after exercise, meals, sexual intercourse, minor surgical procedures, or following epileptic seizures [3].

*Allium sativum,*is known to have diverse medicinal potentials, that have made it useful as hypoglycemic, anticoagulative, antihypertensive and hypolipidemic supplements, among others [4]. It has also been investigated to confer some protective effects on the male gonads [5,6]. However, the effects of garlic supplementation on protein metabolism have not been fully clarified [7]. It was reported that the supplementation of garlic powder at 0.8 g/100 g to a high fat diet and the administration of diallyldisulfide, a major volatile sulfur-containing compound in garlic, enhanced triglyceride catabolism and growth of interscapular brown adipose tissue (IBAT) by increasing noradrenaline secretion in rats [8].

In animals fed with high protein diet, administration of garlic was found to enhance the production of testosterone [7]. Dietary supplementation of garlic has been suggested to enhance hormone-regulated protein anabolism [7]. While testosterone is related to protein anabolism, corticosterone affects protein catabolism in rats [9].

MATERIALS AND METHODS

Preparation of Garlic Aqueous Extract

The raw garlic were collected from an area in Ilorin, Nigeria, and authenticated at the Department of Plant Biology, University of Ilorin, Nigeria. The raw garlic cloves were peeled, chopped into small pieces, weighed and blended. It weighed 2,147g. The blended garlic was dissolved in 4 litres of distilled water for about 4 hours. The solution was then filtered with the use of filter paper. The filtrate was evaporated at 40°C. The fatty diet was prepared by mixing together 700 g of coconut cake, 600 g of groundnut cake, butter and the yolk of 6 pieces of eggs.

Experimental Animals

The experiments conformed to the Rules guiding the Animal Ethics committee of University of Ilorin. The rats were purchased from the Research Institute Ilorin, Nigeria. They were housed within the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin, in different cages, at room temperature, and maintained under a 12 h light/ 12 h dark cycle, with feeds and water available *ad libitum*. They were allowed to acclimatize for 2 weeks before commencement of the experiment.

Experimental Pharmacological Protocol

Sixteen rats with an average weight of about 202 g were randomly grouped into 4 groups as follows:

- Group A (Control): (n = 4) - fed on rat pellets and normal saline.
- Group B: (n = 4) - these animals were given 1000 mg/kg/day aqueous extracts of garlic
- Group C: (n = 4) - these animals were fed with high fatty diet
- Group D: (n = 4) - given fed with high fatty diet and 1000 mg/kg/day aqueous extracts of garlic

Administration of Extracts

The experiment lasted for 28 days (4 weeks), during which Aqueous extract of garlic was administered orally by means of feeding tube, while the high the high fatty diet served as feeds for Groups C and D for the 28-day period.

Animal Sacrifice and Sample Collection

Twenty-four hours after the last administration, the animals were sacrificed by cervical dislocation. Using a median plane incision blood was drawn from the heart and blood samples of the animals were collected into EDTA bottles. The testis was also retrieved with forceps, weighed using an electronic sensitive analytical balance (Gallenkomp FA 2104A) and put into a specimen bottle containing 10% formal saline.

Hormonal Assay Procedures

Blood samples were spun at 3000 revolution per minute for 10 minutes in an angle head centrifuge at 25°C and serum LH, FSH and testosterone was assayed. Hormone profiles were done using a non-competitive (sandwich) ELIZA kit, and read using a microplate reader (Model RT-2100C). The microwell kit used was from Syntron ELIZA kit. With 10 µl of the standard, the specimens and control were dispensed into the number of coated wells to be used. 50 µl testosterone, 25 µl LH/FSH substrate reagent was added, swirled gently for 20-30 seconds to mix and then 50 µl of Biotin reagent to the wells, swirled for 20-30 seconds. The contents of the microwell were thoroughly mixed and incubated for 60 minutes for LH/ FSH, 90 minutes testosterone at room temperature. The mixture was decanted, washed in distilled water and blotted the plate dry with absorbent paper. Enzyme conjugates A, B were then added, mixed and incubated for 15 minutes for LH/ FSH, but 20 minutes for testosterone. The reaction was stopped with 50 µl of stopping solution (1M HCl). Absorbance was measured with an automatic spectrophotometer at 450 nm.

Statistical Analysis

Data were analysed statistically by application of student's t-test, using the SPSS version 15 software, presented as mean and standard error of mean (SEM), and values of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 shows a very high increase in testosterone secretion in each of the experimental groups compared with the Control group and this was statistically significant in all the experimental groups.

Changes in the serum prolactin levels were not statistically significant. Group B animals treated with garlic extract showed a reduction in the level of prolactin ($p>0.05$), while both Groups C and D had increased levels of prolactin ($p>0.05$) compared with the Control. Group D which was given both garlic extract and high fatty diet had a higher increased level of prolactin when compared with Group C given only the fatty diet.

The levels of FSH in the serum were also higher in all the treated groups compared with the Control ($p>0.05$).

Animals fed with high fatty diet (Group C) had a slight reduction in LH level compared with the Control, while those animals that were given garlic extract in addition to the high fatty diet had the higher levels of LH (Group D). All these differences were not significant when subjected to statistical analysis.

Aqueous Garlic Extract and high fatty diet, as used in the current study, led to increased serum levels of male sex hormone (testosterone), which was comparable with earlier studies by Oi et al [7]. The use of garlic extract is capable of enhancing testosterone secretion and, although high fatty diet could achieve the same at a lesser rate. However, in animals given both garlic extract and fatty diet, the increased in testosterone secretion seen was not as high as that of the animals given the garlic extract only. This suggests that both garlic and fatty diets could enhance the male reproductive functions.

The low serum prolactin level in the animals treated with garlic only ($p>0.05$) was inversely proportionate to the increased level of testosterone ($p<0.05$), when both are compared with the Control animals. However, the levels of the gonadotropins (FSH and LH) increased in almost all the groups in a pattern that is not inversely related to the rise in testosterone levels, although these were slight increases, and not of statistical significance ($p>0.05$). this unusual relationship could be due to the duration of exposure of the animals; other factors however might be responsible.

Table 1: Effect of garlic and high fatty diet on Male Sex Hormones (Mean±SEM)

Parameters	**Groups**			
	A	B	C	D
Prolactin (ng/ml)	**1.76±0.282**	**0.99±0.375**	**2.12±0.799**	**2.26±0.271**
Testosterone (ng/ml)	5.84**±0.365**	8.55**±0.838***	7.76**±0.530***	7.97**±0.445***
FSH (MIU/ml)	**9.96±0.539**	**10.41±0.302**	**10.10±0.211**	**10.11±0.147**
LH (MIU/ml)	**11.17±0.494**	**11.53±0.297**	**10.94±0.123**	**11.70±0.337**

*Statistically significant difference compared with the Control Group.

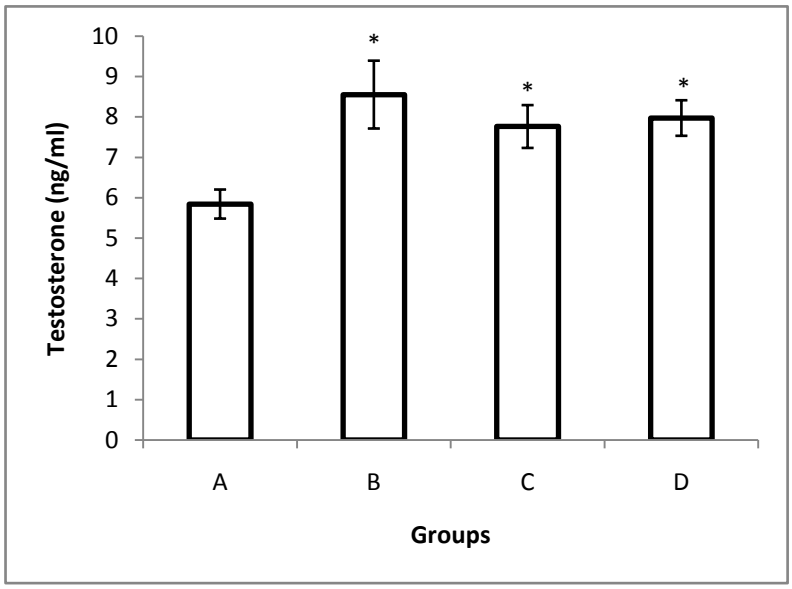

Figure I: Chart representing the concentration of Testosterone: *Statistically significant difference compared with the Control Group.

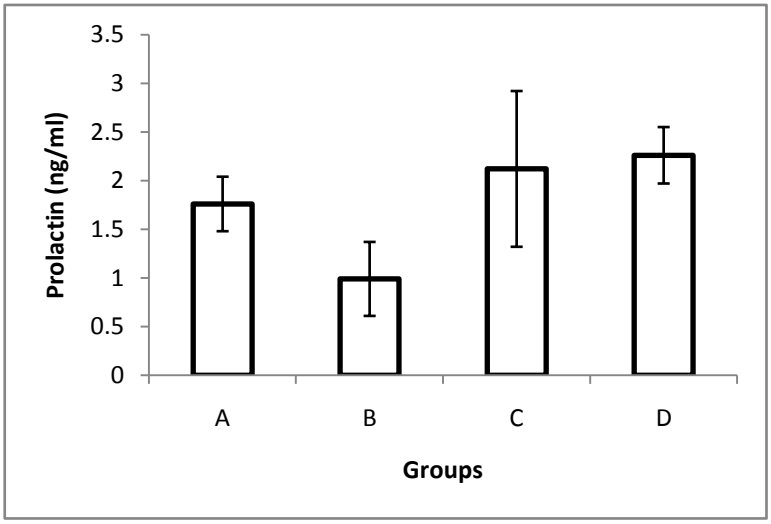

Figure II: Chart representing the concentration of Prolactin

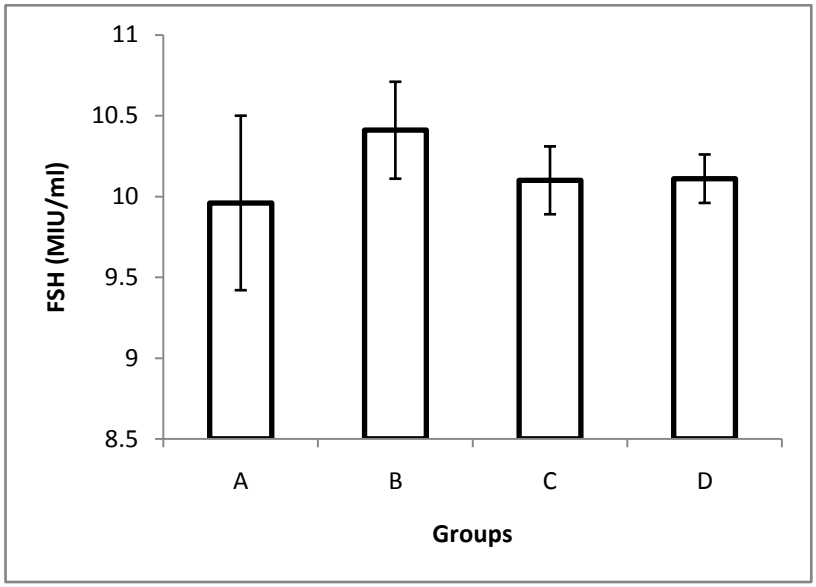

Figure III: Chart representing the serum levels of FSH

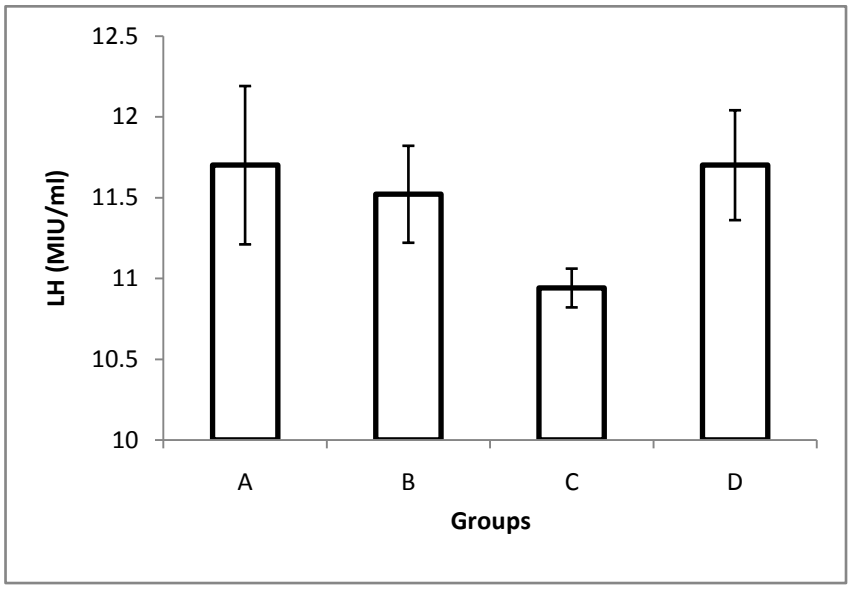

Figure IV: Chart representing the concentration of LH in all groups

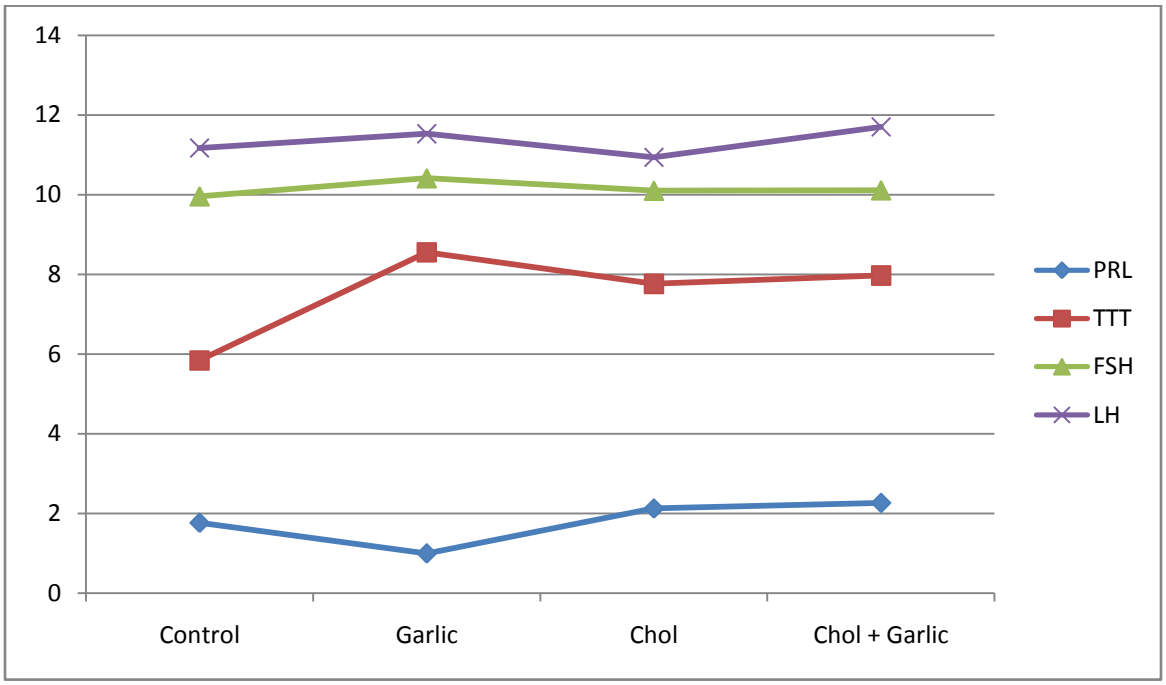

Figure V: Graphical representation of the actions of garlic and high fatty diet on sex hormones

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