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ORIGINAL RESEARCH

Effect of aqueous extract of *Allium sativum* (Garlic) on fertility in male Wistar rats

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Abstract

Background: Allium sativum (Garlic) is widely used as a food flavour and it has antimicrobial medicinal purposes. It has other beneficial effects such as improving the cardiovascular and immune systems. The likely effect of garlicon the testicular functions remains controversial.

Objective: To investigate the effects of fresh garlic on the fertility of male Wistar rats by quantitative determination of testosterone, follicle-stimulating hormone (FSH), and Luteinizing hormone (LH) levels, as well as the sperm properties.

Methods: The aqueous extracts of garlic were administered to the animals through the oral route. Thirty-five male rats were categorized into seven groups, with five rats each distributed as follows:high-dose, medium-dose, low-dose that were administered 750, 500, and 250 mg/kg body weight dosages of the aqueous extracts, respectively. Others were the control group, which received no extract, and the recovery groups received the same doses of the extract. The recovery groups were left for additional twenty-one days without receiving the extract.

Results: The results showed an increase in testosterone levels at medium to high-doses of the extract including sustained high levels in recovery groups. Also, there were increases in sperm motility and sperm viability.

Conclusions: These findings suggest garlic may play beneficial roles in the reproductive functions of male Wistar rats. The mechanisms for these effects may involve the interplay of many currently unknown factors.

Keywords: Allium sativum, Garlic, Male fertility, Sperm motility, Serum Testosterone

Introduction

Allium sativum, commonly known as garlic, is a species that belongs to the genus Allium. Some other plants of this genus include onions, shallot, leek, chive, and rakkyo, which are monocotyledonous flowering plants. The use of garlic dated back to several millennia when it

was native to the central Asian people but its uses for food and medicinal purposes have spread throughout the continent and to Africa and Europe. [1-3] Garlic is known for its flavours in food as a result of its sulfur-containing compounds and it is also used to prevent or treat infections and diseases. [4] Garlic has antimicrobial activities against bacteria, viruses,

fungi, and may also have anti-cancer and anti-coagulant activities. [4] Garlic has also been suggested to be useful as a food additive/adjuvant to prevent toxins or food poisoning. [5-7]

Garlic is presumably used for many conditions related to the functioning of the heart and blood systems. [8,9] It was thought that garlic may have ameliorating effects on cardiovascular diseases by lowering high blood pressure in patients suffering hypertension but certain studies have ruled out such benefits on a long-term basis. [10] One of the active ingredients in garlic is a thiosulfinate compound called Allicin. [11] Allicin which has a strong, pungent smell, is may be associated with the medicinal properties attributed to garlic.[11]The synthesis of allicinis triggered by the release of enzymes following the breaking of the cell wall of the garlic plant.[12] In addition to allicin, garlic contains over 100 beneficial nutrients; these include betacarotene, folate, beta-sitosterol, ferolic acid, geraniol, oleanolic acid, p-coumaric acid, rutin, quercetin, thiamine, niacin, vitamin C, cysteine, zinc, calcium, magnesium, manganese, and selenium. [12] Interestingly, the other important roles of garlic include its benefits on the reproductive system. It was reported that different preparations of garlic, which include raw garlic, heated garlic juice, dehydrated garlic powder, and aged garlic extract, to varying extent, are effective in the recovery of testicular functions after experimental testicular hypogonadism in rats although, no effect was observed on impotence. [13] Also, the aqueous extract of garlic or its metabolites has been shown to have a protective effect on cadmiuminduced damages to the testicles in rats. [14-16] In contrast, previous researches reported that garlic powder [17] or crushed garlic preparation impaired testicular and male reproductive functions. [18-20] Also, it was reported that the aqueous extract of garlic affects serum

testosterone level and have a spermicidal effect in studies involving ram epididymal cells and human spermatozoa. [21] Due to contradictions in outcomes of many studies as regards the importance of garlic on reproduction, this study aimed to investigate the effects of garlic on serum testosterone levels, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sperm parameters in adult male Wistar rats.

Methods

Handling of Experimental Animals

Thirty-five (35) male Wistar rats weighing 150 to 260 g were purchased from the Animal House of the Department of Physiology, Olabisi Onabanjo University, Ago-Iwoye. They were grouped into seven categories according to body weights and the doses of the Garlic extract administered. The rats were kept in cages built with wire gauze which ensures proper ventilation. The animals were left to acclimatize for three weeks but were fed with standard rat chows (procured from Animal Care Nigeria Limited) and given water ad libitum. They were maintained under ambient (12 hours darkness, and 12 hours light; temperature range: 24 - 28°C) and hygienic conditions that ensured their cages, water, and food troughs were properly cleaned daily. Their wastes (excreta, urine, and crumbs) were received with wood shavings and properly disposed of, thus, ensuring dryness. The experimental rats were carefully treated based on the guidelines of the National Institute of Health for Laboratory Animal Care and Use. [22] The approval for this study was obtained from the Department of Physiology's departmental committee on the use of animals for research.

Preparation of Aqueous Plant Extract

Fresh garlic cloves (purchased from the Ikenne market, Ikenne-Remo, Ogun State, Nigeria) were peeled and sliced into small pieces and ground to paste with a mortar and a pestle. The working solution is then prepared by dissolving 5g of the paste in 100 ml distilled water, whereby 1 ml of the extract contains 50 mg of crushed garlic. Fresh garlic extract was orally administered to rats using oral cannula daily in doses of 750mg/kg, 500mg/kg, and 250mg/kg body weight for three weeks. [23]

Experimental Design

The rats were categorized into seven groups; each group consisted of five rats (n=5). There were high-dose group, medium-dose group, low-dose group which received 750mg/kg, 500mg/kg, and 250 mg/kg body weight doses of the garlic extracts respectively, for 21 days. Also, three recovery groups were given the same dosages as the test groups but were left for an additional 21 days without receiving the garlic extract before they were sacrificed. The rats in the control group did not receive the extract but were only fed food and water throughout the experiment.

Sample Collections

The rats were anaesthetized with diethyl ether and were sacrificed by exsanguinations. Blood samples were collected via cardiac puncture. Blood sera were obtained by centrifugation at 5000 rpm for five minutes and were then stored in specimen bottles inside a refrigerator at -4°C for later use.

Hormone Assay Determinations

The serum levels of reproductive hormones: testosterone; and luteinizing hormone (LH) were assayed using the Enzyme Immunoassay (EIA), and Enzyme-linked Immunosorbent Assay (ELISA), respectively as previously described by Raji and co-workers. [24] The level of follicle-stimulating hormone (FSH) was determined based on the principles described in the Clinical Guide to Laboratory Tests. [25]

Determination of Sperm Parameters

Sperm motility: A simple classification system proposed by the World Health Organization, [26] which provides the best possible assessment of sperm motility, was used. The new improved Naubauers counting chamber was used for sperm counts. Drops of semen were placed in a chamber with a coverslip placed on top; this was then examined under a light microscope and the spermatozoa were counted on each of the five squares. Sperm viability was determined using the Eosin/Nigrosin stain method. Vital sperm cells had intact cell membranes, were not stained and remained white in the smear, whereas eosin diffused through the disrupted cell membranes of sperm cells which were not viable.

Statistical Analysis

Statistical analyses were done using the SPSS 5 statistical software. [27] The data were presented as mean values (with standard deviations) and statistical comparisons were carried out using the One-way Analysis of Variance (ANOVA) test. The level of significance was tested at p < 0.05 with Games-Howell posthoc test.

Results

Three hormones; testosterone, FSH, and LH, were quantified in the serum of all the groups, including the control group, test groups, and the dose-recovery groups. Only the testosterone levels were affected by administration of aqueous garlic extract while the extract had no effects on the serum FSH and LH levels as the serum levels of these hormones in the test groups and dose-recovery groups were not significantly different from the levels in the control group. However, the effect on serum testosterone levels appeared to be nondependent on the dosage of the extract used. Rather, the effect became remarkable when the dosage was in the medium category (500 mg/kg body weight). The mean serum testosterone in the medium-dose test group (45.1 ± 0.2 nmol/L), and the high-dose test group (57.6 ± 0.1 nmol/L) and the corresponding medium-dose recovery group (41.4 ± 0.2 nmol/L) and high-dose recovery group (50.1 ± 0.02 nmol/L) were significantly different (p=0.03) from the values for the control group (33.2 ± 0.3 nmol/L).

It was also observed that the mean serum testosterone levels, showed an increase in both

the significantly affected test groups and the recovery groups; even after 21 days of recovery when no extract was administered to the rats, the serum testosterone levels never returned to the baseline levels. There was no significant boost in the serum levels of FSH and LH in the test groups when compared to the control group. Therefore, only the serum testosterone showed significant responses to aqueous garlic extract administration.

Table I: Mean serum levels of testosterone, follicle-stimulating hormone and luteinizing hormone in the various test and recovery groups and the control group

| Groups | Testosterone (nmol/L) | FSH (nmol/L) | LH (nmol/L) |
|----------------------|------------------------|--------------|-------------|
| Control | 33.2±0.3a | 9.9±0.02 | 11.1±0.02 |
| Low-dose Test | 38.3±0.4a | 10.±0.01 | 11.3±0.02 |
| Medium-dose Test | 45.1±0.2 ^b | 10.3±0.01 | 11.4±0.02 |
| High-dose Test | 57.6±0.1 ^b | 10.4±0.02 | 11.5±0.02 |
| Low-dose Recovery | 35.6±0.1 ^a | 10.0±0.02 | 11.2±0.01 |
| Medium-dose Recovery | 41.4±0.2 ^b | 10.1±0.01 | 11.3±0.02 |
| High-dose Recovery | 50.1±0.02 ^b | 10.1±0.03 | 11.4±0.01 |

Values in the same column with the same superscripts are not significantly different at p<0.05 from each other. The number of replicates, n= 5.

The sperm motility, viability and, count in the test groups showed varying degrees of differences in comparison to the control group (Table II). In particular, sperm motility showed similar responses as observed with serum testosterone levels. Also, only the parameters for the medium-dose test group, high-dose test group, medium-dose recovery group, and highdose recovery group were significantly different from the parameters for the control group whereby they showed improved motility. The recovery groups maintained elevated sperm motility even for days after the cessation of treatment with garlic extract. However, the sperm viability was not significantly different in all the groups except in both the high-dose test group and the high-dose recovery group which showed significant increases in viability (p =

0.04). Sperm counts in all the other groups showed a significant increase in the sperm count in all the groups except the control group and the low-dose recovery group.

Discussion

The findings in this study showed that only the sperm parameters such as sperm motility, sperm count, sperm viability, and serum testosterone significantly increased in rats treated with aqueous extract of garlic. The garlic extract had no effects on serum levels of follicle-stimulating hormone and luteinizing hormone. Steroidal hormones are synthesized from cholesterol in

mammals. Serum testosterone is derived from the *de novo* synthesis of cholesterol in the body. Testosterone is vital for testicular growth and spermatogenesis in most animals.

Garlic supplementation, as observed in the present study, may enhance testosterone production in the testes. In particular, garlic extract has antioxidant activities due to the presence or formation of certain compounds such as allyl cysteine, alliin, allicin, and allyldisulfide which have different protective activities against hydroxyl radicals and superoxide anions. Allyldisulfide prevents microsomal lipid peroxidation. [28]

Table II: Mean values of sperm motility and viability in the various test and recovery groups and the control group

| Groups | Sperm | Sperm viability (%) | Sperm count (×106/ml) |
|--------------------|-----------------------|---------------------|-----------------------|
| | motility (%) | | |
| Control | 71.9±0.5a | 73.2±0.4 | 69.7±0.1a |
| Low-dose Test | 77.1±0.1a | 79.4±0.2 | 75.2±0.2 |
| Medium-dose Test | 83.2±0.3b | 81.0±0.6 | 80.2±0.2 |
| High-dose Test | 88.5±0.1 ^b | 86.4±0.2a | 86.4±0.1 |
| Low-dose Recovery | 73.4±0.6a | 74.2±0.2 | 71.3±0.2a |
| Medium-dose | 80.3±0.2b | 77.2±0.4 | 76.2±0.4 |
| Recovery | | | |
| High-dose Recovery | 84.5±0.1 ^b | 81.0±0.2a | 81.0±0.2 |

Values in the same column with the same superscripts are not significantly different from each other. The number of replicates, n= 5.

The actions of these radicals are detrimental to gonadal functions. As such, it is thought that garlic extract may have direct or indirect consequences which result maintenance or improvement in the production of testosterone and improvement in sperm parameters. These changes may be attributed to the reduction of lipid peroxidation and increased antioxidant activities against free radicals. [14-16] In contrast, the antioxidant garlic against erythrocyte activities of superoxide anion, as measured by superoxide dismutase activity, have been shown to reduce significantly with a decrease in epididymal sperm concentration and sperm counts, [29] as opposed to what was observed in the present study.

Despite the disparity in the findings in the previous study, [29] and the present study, a

significant percentage increase in sperm viability was recorded in the present study and this agreed with another study which tested the effects of garlic extract on testis structure and sperm quality in rats treated with lead acetate. [30] LH and FSH are gonadotropins secreted by basophilic cells of the anterior pituitary gland. The LH stimulates the Levdig cells in the interstitial space of the testes whereas the FSH targets the Sertoli cells of the seminiferous tubules. By a combination of actions, the hormones enhance the production testosterone and other regulatory molecules deployed for maintaining sperm cell production. [31] The present study revealed no effects of the aqueous garlic extracts on FSH and LH. Perhaps, the LH and FSH levels reported were already optimal for the synergistic mechanisms required for testicular growth spermatogenesis in rats and that increment of

extract doses resulted in no significant change in their secretions. However, previous reports that excessive action of FSH has no significant influence on testicular growth and functions may not be surprising. [31] On the other hand,

the study by Salah and co-workers reported increases in the amounts of LH and FSH following garlic administration but this was in rats that were previously exposed to lead acetate. [32]

Conclusion

This study has demonstrated that aqueous garlic extract improved the production of testosterone and increased sperm cell motility, viability and counts in experimental rats, though at relatively high-doses of the extracts. The levels of anterior pituitary hormones LH and FSH were not significantly affected. As such, it is suggested that the active chemical compounds of garlic

Contributions of Authors: OIO conceived and designed the study and participated in data analysis. FAS conducted the literature review, participated in data analysis and drafted the manuscript. EEN participated in data collection and literature review, ATK participated in study design while AAA participated in data collection. All the authors approved the final version of the manuscript.

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may have positive effects only on testicular functions and spermatogenesis. However, this study could not unravel the physiological or biochemical interactions underlying the absence of direct influences on the productions of LH and FSH, even as the secretion of testosterone is largely dependent on their activities. Therefore, a more detailed study is needed to provide these insights.

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