Journal of Infertility and Reproductive Biology

J Infertil Reprod Biol, 2021, Volume 9, Issue 3, Pages: 130-135. https://doi.org/10.47277/JIRB/9(3)/130

Dose-dependent Effect of Garlic (*Allium Sativum*) Extract feeding on Semen Characteristics and Testes in Wistar Rats

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Received: 27/06/2021 Accepted: 11/09/2021 Published: 20/09/2021

Abstract

Allium sativum (garlic) is widely used all over the world for the prevention and treatment of several diseases. However, the dose-dependent effect of garlic on the male reproductive system in apparently healthy subjects has not been sufficiently evaluated. The objective of the study was to determine the dose-dependent impact of aqueous extract of garlic on semen characteristics and testicular morphology in male Wistar rats. Twenty adult male Wistar rats were assigned into four groups of five rats in each group.. Group 1 (control) was given feed and water only, group 2 was given 500mg/kg body weight (BW) of *Allium sativum* extract, group 3 was given 750mg/kg BW of *Allium sativum* extract and group 4 received 1000mg/kg BW of *Allium sativum* extract. All groups were given feed and water ad libitum. The experiment lasted for 30 days after which the animals were sacrificed; the testis and epididymis were collected for laboratory analysis. The epididymis was homogenized in buffer, diluted, and analyzed microscopically. The data generated were compared using analysis of variance (ANOVA). Data showed that the administration of high concentrations of garlic caused teratozoospermia characterized by a poorly formed head, tailless sperm cells, and bent body with premature sperm cells. Histological findings also showed mild distortions in the shape of seminiferous tubules, congestions in interstitial blood vessels (suggestive of inflammation), slight reductions in size, and mildly thickened outlines of seminiferous tubules. Sperm progressive motility was significantly higher in the high dose group while no significant difference was observed in sperm count, morphology, viability, and pH. The consumption of high concentrations of *Allium sativum* may lead to adverse consequences on teratozoospermia and inflammation of the interstitial blood vessels in Wistar rats. Therefore, raw garlic should be consumed with caution as high concentrations may be deleterious to male reproductive health.

Keywords: Garlic, Semen, Testes, Wistar rats

Introduction

Garlic (*Allium sativum*) has been considered to possess cleansing properties that remove impurities from arteries to improve blood flow to the testes which may improve fertility potentials. It contains some essential elements such as zinc which is known to increase testosterone, sperm motility, and sperm count (1). Garlic (*Allium sativum*) has a long history of medicinal use with numerous medicinal properties with the potential to lower the risk of several diseases (2). Even though garlic has been attributed to have useful health benefits, its dose-dependent effects on testicular functions have not been sufficiently reported. The consumption of garlic as a medicinal herb and food additive in our setting is increasingly popular. We hypothesized that the consumption may improve the semen characteristics in a dose-dependent manner. Fertility in men depends largely on the quality and quantity of the spermatozoa and the disruption of each of these sperm indices may adversely impact fertility in men (3).

The incidence of male infertility has been on the increase in the sub-Saharan region of Africa including Nigeria. Anything that will improve the characteristic of semen of men will be worthwhile especially now when the use of herbal medicine is Increasingly popular. Also, the high cost of medical interventions has encouraged people to turn to complementary medicine (4). Plants are more affordable and accessible than invasive and chemical treatments (5). Medicinal herbs with high antioxidant properties are used to treat sperm abnormalities, sexual dysfunction, erectile and ejaculatory disorders (6). Some plants change the number and motility of the spermatozoa by regulating sex hormones while others with androgenic properties affect the hypothalamus-pituitary axis and increase sex hormones (7). Improvement of sperm characteristics in apparently healthy and fertile subjects is the focus of this study. Any lifestyle behavior or diet that could prevent male infertility as a major public health change is very important. Therefore, this study was designed to investigate the impact of garlic consumption in a dose-dependent manner on the quality and quantity of spermatozoa and testicular morphology in Wistar rats.

Materials and Method

Collection and Preparation of Plant Materials

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Fresh garlic bulbs were obtained from Uselu market, Benin City and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin-City, Nigeria. The bulbs were screened of bad ones, washed and air-dried for 48hrs and thereafter, pulverized into smooth powder using the British Grinding Machine. The pulverized sample was weighed and suspended in 1L of distilled water with regular agitation for 24hrs. The solution obtained was filtered and the resulting filtrate was concentrated over a water bath at 40°C and yielded crude extract. The dried crude extract was stored in the refrigerator before use. All extraction and preparations of the aqueous extract of Allium sativum were performed at the Department of Pharmacognosy, University of Benin, Benin-City.

Animal Care and Management

Twenty (20) adult Wistar rats weighing approximately 220-300grams were used for the experiment. The animals were purchased and bred in well-ventilated conventional cages in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin-city. The animals were acclimatized for two weeks before the commencement of treatment. During this period, they were fed with growers mash livestock feed with clean water, and weighed. The animals were raised at room temperature with a reverse natural light/dark cycle in the animal house. The animals were housed and cared for following the guidelines of the Faculty of Pharmacy Research Ethics Committee (EC/FP/015/11).

Study Design

Twenty Wistar rats were assigned into four(4) groups using a completely randomized design with five(5) rats in each group (n=5). Group 1 (Control group): received normal feed mash and water ad libitum only without the extract; Group 2: received 500mg/kg body weight of the extract (low dose of *Allium sativum* extract); Group 3: received 750mg/kg body weight of the extract (medium dose of *Allium sativum* extract); Group 4: received 1000mg/kg body weight of the extract (high dose of *Allium sativum* extract).

The extract was freshly prepared on daily basis and administered orally using an orogastric tube. The rats were weighed at 24hour intervals and also subjected to thorough observation for mortality and behavioral pattern during the 30days experimental period. The administered doses are as shown below.

At the end of the experiment period, the animals were weighed and sacrificed under slight chloroform anesthesia. After anesthetizing the rat for about 2minutes, the rat was placed in a supine position on a dissection table and an abdominal incision was made with a sterilized surgical blade to expose the internal genital organs. The sperm were collected for semen analysis and testes were fixed for histological analysis using Bouin's fluid (Aqueous solution of picric acid, acetic acid, and formaldehyde) for 48hrs.

Macroscopic Examination

The animals were observed closely for behavioral patterns throughout the experiment. The harvested organ (testes) were examined macroscopically during grossing/cut-up. The description was done taking into cognizance the color, consistency (hard or soft), and weight. Then a representative of the organs not thicker than 3mm was taken for histopathological investigation.

Histopathological Examination

The testicular tissues were processed, sectioned, and stained using Haematoxylin and Eosin method. The sections were examined under a Leica DM750 research microscope with a digital camera Leica ICC50 attached. Digital photomicrographs of the tissue sections were taken at various magnifications.

Microscopic Examination

Epididymal Sperm Concentration

The epididymis was minced with anatomic scissors in 5 ml of normal saline, placed in a rocker for 10 min, and allowed to incubate at room temperature for 2 min. After incubation, the supernatant was diluted at 1:100 with a solution containing 5 g sodium bicarbonate and 1 ml formalin (35%). The new improved Neubauer counting chamber (hemocytometer) was used in counting the total number of spermatozoa (8). A drop of the diluted sperm suspension was transferred to charged Neubauer counting chamber with a plastic pipette and was allowed to settle for 5 min, and thereafter observed under a binocular light microscope. The sperm cells found at the appropriate sessions of the counting were counted.

Semen Analysis

Sperm cells were collected from the vas deferens of the sacrificed rats; the rats were sacrificed and the vas deferens located and ligated with a minimum of 36mm length, both extremities of the vas deferens was ligated, cut, and placed in a sterile petri dish. To the petri dish, 6µl of normal saline already adjusted to 37±2°C was added. The vas deferens were teased to allow the sperm cell to diffuse out of it. A drop of the semen from the petri dish was placed on a grease-free clean slide and covered with a transparent coverslip and examined to ascertain adequate collection was made. The semen analysis was done according to World Health Organization (2010) criteria (9).

Statistical Analysis

All data collected was presented as graphs, tables, and figures also as mean ± standard deviation of the mean (SDM) of controls and experimental groups. The data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS), version 17. A value of $p < 0.05$ was considered significant.

Results

Table 3.1 shows the comparison of sperm characteristics in rats administered with different concentrations of Allium sativum. The results indicate that there were no significant differences in the means of total sperm count among the groups. Progressive motility was insignificantly lower in rats administered with 500mg/kg Allium sativum and insignificantly higher in those administered with 750mg/kg and significantly higher ($p<0.05$) in rats administered with 1000mg/kg Allium sativum. Immobile sperm cells were significantly higher ($p<0.002$) in rats administered with 500mg/kg, but significantly

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lower in those administered with 750mg/kg ($p<0.05$) and 1000mg/kg ($p<0.002$).

Sperm viability was significantly lower ($P<0.002$) in rats administered with 500mg/kg, higher ($p<0.05$) in rats administered with 750mg/kg and 1000mg/kg ($p<0.002$). More importantly, sperm normal morphology was significantly lower ($p<0.05$) in rats administered with 500mg/kg ($p<0.05$) in 750mg/kg and 1000mg/kg, while abnormal morphology was higher among rats in the treated groups but the difference was insignificant ($p>0.05$).

Figure 1A shows the spermatozoa in the control group with normal morphology, normal head, body, and tail. Figure 1B shows the morphology of rats administered with 500mg/kg Allium sativum (group 2), figure 1C shows the morphology of rats administered with 750mg/kg Allium sativum. It indicates mild to moderate teratozoospermia (tail-less sperm cell), bent neck and poorly formed head, and normal sperm cell count and motility. Finally, figure 1D indicates the morphology of sperm cells in rats administered with 1000mg/kg Allium sativum. There is evidence of marked teratozoospermia, (Poorly formed head), bent body with premature sperm cells. Figures 2-6 show the histograms of sperm count, motility, viability, morphology, and pH respectively among the different groups of rats.

Figure 7 shows the photomicrographs of the testis of adult male Wistar rats treated with distilled water, control (7A and 7B). The sections indicate the seminiferous tubules in circular outline and pyknotic Sertoli cells, Leydig cells, and other cells of the spermatogenic series. The photomicrograph of rats treated with 500mg/kg Allium sativum shows that the sections of the seminiferous tubules are circular in outline at various stages of maturation pyknotic Sertoli cells, Leydig cells, and other cells of spermatogenic series with congestion in the interstitial vessels (7C and 7D).

Figure 8 shows the photomicrograph of the testis of adult Wistar rats treated with 750 mg/kg (8A and 8B) and 1000mg/kg garlic extract (8C and 8D). Testis of rats given 750mg/kg show sections of the seminiferous tubules is circular in outline but with a slight reduction in size and mildly thickened outline. There are pyknotic reactive Sertoli cells and other cells of the spermatogenic series without notable Leydig cells. Testis of rat given 1000mg/kg Allium sativum show sections of the seminiferous tubules are fairly circular in outline with mild distortion in shape at various stages of maturation pyknotic Sertoli cells, Leydig cells, and other cells of spermatogenic series with congestion in the interstitial vessels.

Table 1. Comparison of semen characteristics between rats administered with different concentrations of *Allium sativum*

	Groups				
	Control	**Group 2 (500mg/kg)**	**Group 3 (750mg/kg)**	**Group 4 (1000mg/kg)**	**P Value**
Total Sperm Count (x10^6cells/mm3)	404 ±18.6	430 ±18.97	428 ±41.76	400 ±26.46	0.814
Progressive Motility (%)	46 ±6.00	30 ±4.47	56 ±5.10	66 ±2.45^{c}	0.000
Non Progressive Motility(%)	22 ±2.00	18 ±2.00	18 ±2.00	18 ±2.00	0.418
Immotile Sperm Cells (%)	32 ±7.35	52 ±4.90^{b}	26 ±6.00^{a}	16 ±2.45^{b}	0.002
Sperm Cell viability (%)	68 ±7.35	48 ±4.90^{b}	74 ±6.00	84 ±2.45^{b}	0.002
Normal Morphology (%)	87 ±2.00	74 ±2.45^{a}	74 ±4.00^{a}	78 ±3.74^{a}	0.052
Abnormal Morphology (%)	13 ±2.00	26 ±2.45	26 ±7.75	22 ±3.74	0.095
pH	8.20 ±0.11	8.14 ±0.13	8.11 ±0.06	8.11 ±0.06	0.890

Values are in Mean ± SEM. a=0.05; b=0.02; c=0.001 indicates statistical significant difference compared with control group.

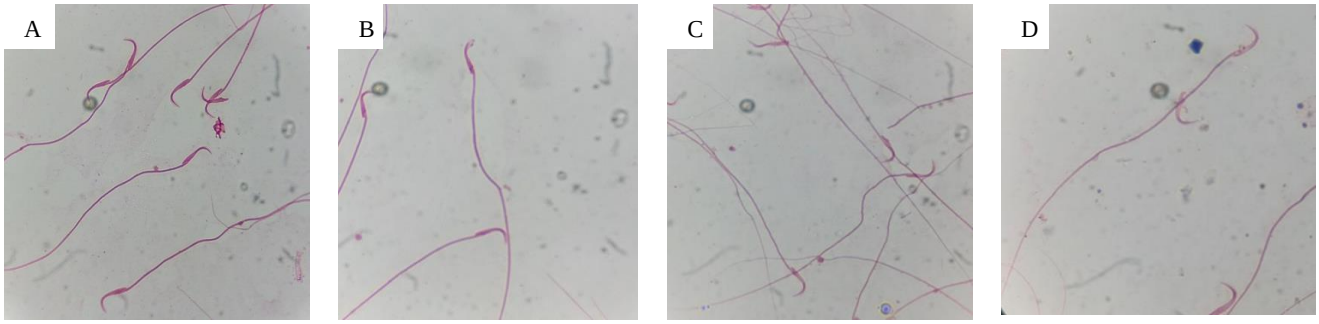

Figure 1. A-D Photomicrograph of sperm morphology. A is the control group with a normal head, body, and tail. B is from group 2 treated with 500mg/kg garlic extract. It shows normal morphology with a normal head, body, and tail. C from rats treated with 750mg/kg garlic extract, shows mild to moderate teratozoospermia (tailless sperm cell, bent neck) with poorly formed head and normal sperm cell count and motility. D is from rats treated with 1000mg/kg garlic extract. It shows marked teratozoospermia (poorly formed head, bent body with premature sperm cells

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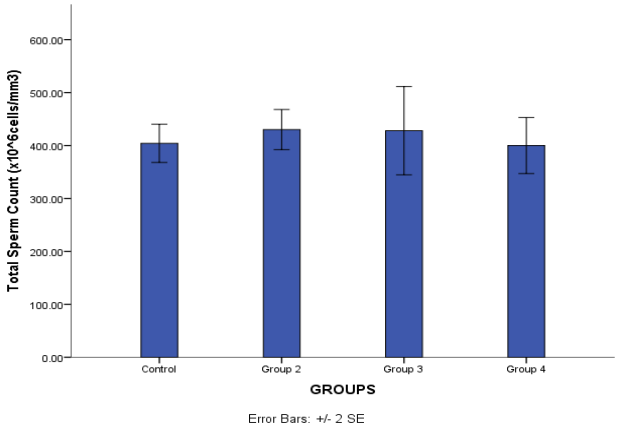

Figure 2. Chart showing Total Sperm Count of all groups. No statistically significant difference between the experimental group and the control group

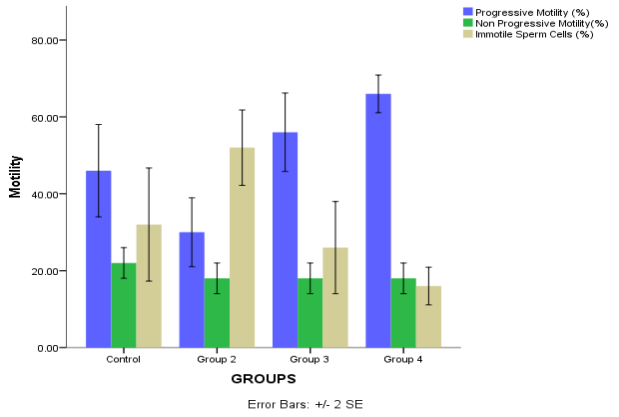

Figure 3. Chart showing the Sperm motility of all groups. *indicates statistically significant difference from the control. There was a statistically significant increase in Progressive motility in the high dose group.

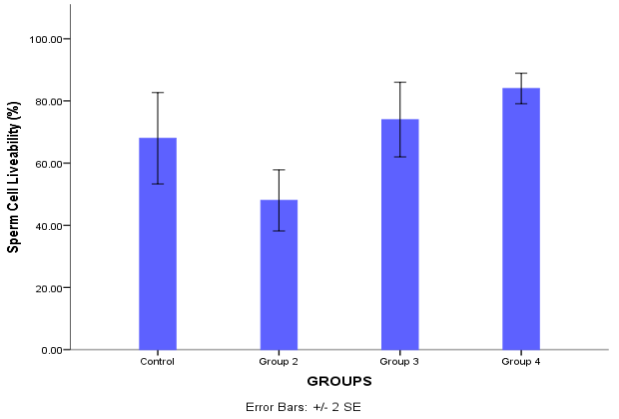

Figure 4. Chart showing Sperm cell liveability of all groups. No statistically significant difference between the experimental group and the control group

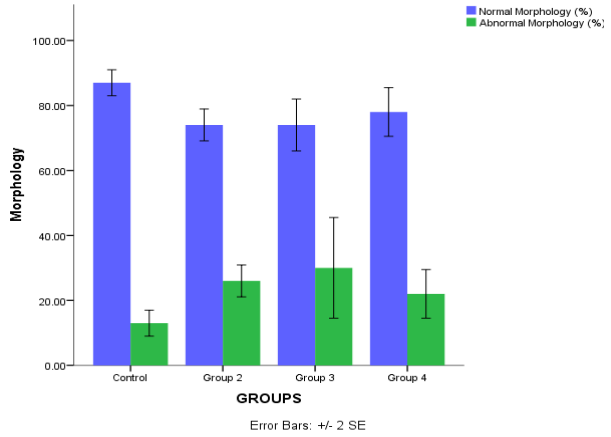

Figure 5. Chart showing sperm cell morphology of all groups. No statistically significant difference between the experimental group and the control group

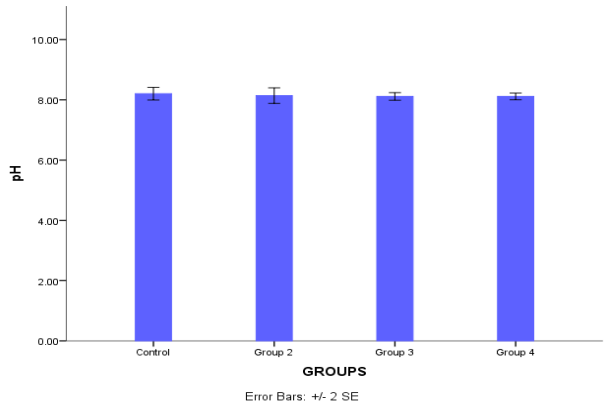

Figure 6. Chart showing pH of semen of all groups. No statistically significant difference between the experimental group and the control group

Discussion

There are several reported beneficial effects of *Allium sativum* in scientific literature, coupled with its nutritional uses as tenderizer and spices. Folklore medicine also refers to its use in the treatment of some diseases (10). This study was designed to evaluate the role of *Allium sativum* on the semen characteristics and testes of adult male Wistar rats without reproductive health abnormalities.

Studies have shown that the administration of garlic has been noted to protect cells against the damaging effect of free radicals but the results from our studies have indicated that garlic may compromise some male reproductive functions. Data from this study showed that administration of different concentrations of garlic caused teratozoospermia (increase in concentrations of abnormal sperm cells) characterized by a poorly formed head, tailless sperm cells, bent neck, and bent body with premature sperm cells. This result is corroborated by previous studies (2, 10). The authors reported a decrease in normal sperm morphology in their findings. Conversely, an earlier study reported an increase in normal semen morphology (11).

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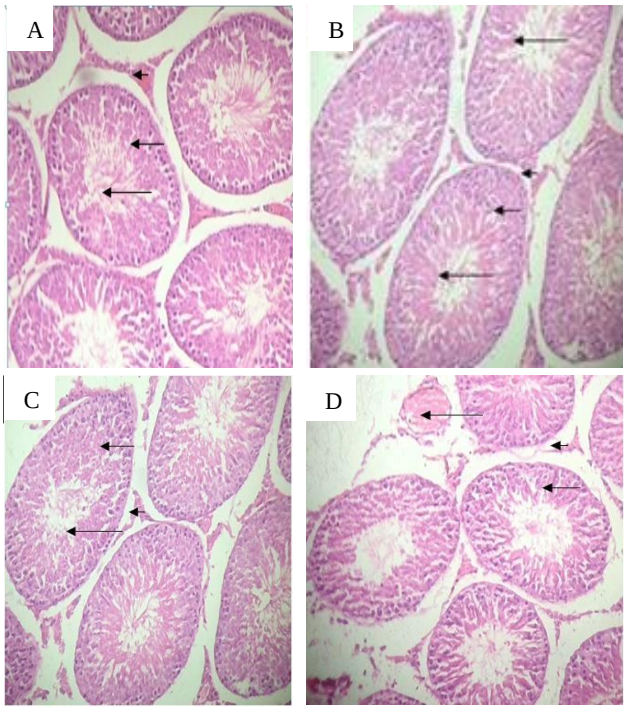

Figure 7. Photomicrographs of the testis of adult male Wistar rats treated with distilled water (A and B). Sections of the seminiferous tubules are circular in outline and pyknotic Sertoli cells (medium arrow), leydig cells (short arrow) and other cells of spermatogenic series (long arrow); and photomicrographs of the testis of adult male Wistar rats treated 500mg/kg garlic extract (C and D). Sections of the seminiferous tubules are circular in outline at various stages of maturation pyknotic Sertoli cells (medium arrow), leydig cells (short arrow) and other cells of spermatogenic series with congestion in the interstitial vessels in Group 2, (long arrow).

This discrepancy in the findings may be associated with differences in garlic preparations and the duration of treatments. Though the results from the analysis showed a statistically significant increase in progressive motility and no significant difference in sperm count, morphology, sperm viability, and pH, these microscopic findings cannot be overlooked.

The histological findings also show mild distortions in the shape of seminiferous tubules, congestions in interstitial blood vessels (suggestive of inflammation), slight reductions in size, and mildly thickened outlines of seminiferous tubules. This result shows that histologically, consumption of garlic may have some negative effect on the testis. These changes were more pronounced in the high dose group, which suggests that garlic consumption may have a dose-dependent effect on the histology of the testis. The consumption of garlic may have caused a reduction in the size of the seminiferous tubules. A previous study had shown that garlic may cause congestion in the interstitial blood vessels (12). The deleterious effect of crude garlic consumption in rats could be attributed either to the inhibitory effect on steroidogenesis resulting in a decrease in testosterone level or to its phytoestrogens activity. Garlic possibly has direct estrogen-like actions on adult male rat testes by inducing disruption in spermatogenesis (12, 13). Other studies have reported conflicting findings; garlic or its metabolites have been reported as a protective adjuvant to different types of toxins (14-17). Indeed, induction of testicular hypogonadism by heat is prevented in part by different types of garlic preparations (garlic juice, heated garlic juice, garlic powder, or the more potent aged garlic extract (18). Others have reported that aqueous extract of garlic (16) or its metabolites diallyl sulfide (17) and diallyl tetrasulfide (15) was reported to offer protection against cadmium-induced testicular damages on adult rats. Crude garlic, at 5 mg/ kg dose was also reported to have an ameliorative effect by restoring the testicular histology altered by EDTA on the rat (16-18).

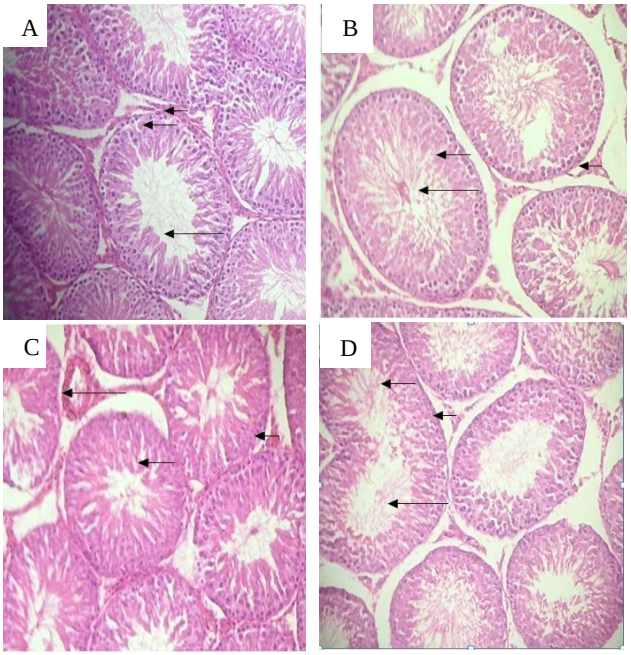

Figure 8. Photomicrographs of the testis of adult male Wistar rats treated with 750mg/kg garlic extract (A and B), and from rats treated with 1000mg/kg garlic extract (C and D). The A and B sections of the seminiferous tubules are circular in outline but with slight reduction in size and mild thickened outline. There are pyknotic reactive sertoli cells (medium arrow) and other cells of spermatogenic series (long arrow) without notable leydig cells (short arrow). C and D sections of the seminiferous tubules are fairly circular in outline with mild distortion in shape in D at various stages of maturation pyknotic Sertoli cells (medium arrow), leydig cells (short arrow) and other cells of spermatogenic series with congestion in the interstitial vessels (long arrow).

These discrepancies in results could be related to three main factors (i) the type of preparations, (ii) the route of administration, and (iii) the dose. Moreover, the concentration of bioactive components of garlic is highly variable from one preparation to another.

Conclusion

The data from this study provides evidence to suggest that extract of Allium sativum has a dose-dependent adverse effect on the reproductive functions of rats by altering the histological architecture of the testis and sperm morphology. Raw garlic may

be consumed with caution as high concentrations may be deleterious to male reproductive health.

Acknowledgments

The authors appreciate the contributions of staff of the Faculty of Pharmacy and Medical Laboratory Scientists at the Department of Medical Laboratory Science, University of Benin, Benin City toward the completion of this study.

Funding sources

None

Conflict of interests

The authors declare that there is no conflict of interest.

Authors contributions

This work was conducted and approved in collaboration between all the authors. MA designed the study; OO sourced for funding; MA, OO wrote the protocol; OO contributed in literature search; OO did the experiments; MA, OO did statistical analysis; OO drafted the manuscript; MA supervised the study; MA Wrote the final manuscript; MA proofread the manuscript.

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