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Assessment of the Influence of Aqueous *Allium sativum* Bulb (Garlic) Extract on Testicular Activity in Wistar Albino Rats

Kamenebali I[1], Aprioku JS[2*]

1.Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria.

2.Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, PMB 5323, East-West Road, Rivers State, Nigeria.

ABSTRACT: Garlic is a widely consumed medicinal plant and most of its biological effects have been established. But its effect on reproductive function and fertility in males is not conclusively studied. This study was conducted to investigate the influence of aqueous extract of garlic bulb on sperm characteristics and serum reproductive hormone concentrations in Wistar albino rats. Animals were grouped randomly (n=5/group) and gavaged with garlic extract (0, 50, 100, 200 or 300 mg/kg/day) for 28 days. Some other groups received garlic extract 24 h after they were injected alloxan (100 mg/kg, ip). Animals were sacrificed and serum levels of FSH, LH and testosterone were analysed as well as sperm collected from the epididymis. Sperm morphology, FSH and LH were not affected in all rats that received only garlic extract compared to control. In addition, sperm motility, sperm count and testosterone were reduced ($P < 0.05$) in rats that received 300 mg/kg extract, but the lower dose treatments failed to produce any significant effect. In alloxan+extract administered rats, sperm motility and count were reduced dose-dependently, but effect on sperm count was significant only in rats that received 200 and 300 mg/kg of extract. Furthermore, testosterone level was decreased, while the other hormones were unaltered. The results suggest that garlic alters sperm characteristics and decreases testosterone concentration in rats when chronically consumed at high doses.

KEYWORDS: Garlic, sperm parameters, testis, testosterone.

***Corresponding Author: Dr. Aprioku J S** Ph.D.

Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, PMB 5323, East-West Road, Rivers State, Nigeria.

* Email Address: sydaprio@yahoo.com

1.INTRODUCTION

The use of medicinal plants as herbal remedies for diseases has continued to gain relevance in many parts of the world. Currently, there is an extensive use of traditional phytomedicine with scientific and clinical importance in modern medicine, and among the plants with such medicinal importance is *Allium sativum* [1]. *Allium sativum*, commonly known as garlic, is a biennial medicinal plant that has a characteristic pungent smell. It is a species in the Onion Genus, Allium, and belongs to the Family, Liliaceae. Garlic is native to Central Asia and has been consumed by humans over several decades [2]. It is known to have high nutritional and medicinal properties and as such used widely by many [2]. Garlic contains about 0.5% of volatile oil and "Allicin" which is the main active ingredient of the plant and also the major source of garlic characteristic odour [3]. Garlic has been used to treat many disease conditions which include, cardiac diseases, diabetes, osteoarthritis, hay fever (allergic rhinitis), cold and flu, cough, headache, stomach ache, gout, rheumatism, haemorrhoids, asthma, bronchitis and warts [4-6]. It is equally used to treat athlete's foot [7], prostate cancer and bladder cancer, [8] and globally recognized as a potent wound healing agent [9]. Some biological studies have demonstrated that garlic has potential antioxidant, hypoglycaemic, hypotensive, hypothrombotic [10], nephroprotective and hepatoprotective properties [11]. Additionally, the plant extract has been shown to have antimicrobial properties [12], and its disease preventive property has been studied [13]. Aside from its wide medicinal application for the treatment of several ailments, garlic is used frequently for seasoning and as dishes [14]. Garlic supplements, which are readily available, are thus used daily and widely as nutritional supplement and for medicinal purposes. As such, the effect of garlic on reproductive health should be of concern in view of the increasing rate of infertility, including erectile dysfunction among couples, globally [15,16]. Some studies on garlic regarding reproductive function and fertility in males have been performed, however, results are widely inconsistent. It has been reported that garlic decreases spermatozoa quality as well as functionality in male rats [17]. Hammami et al. [18] reported that consumption of aqueous and ethanolic fractions of garlic impaired spermatogenesis, reduced serum testosterone level and induced testicular germ cell apoptosis. In contrast, Hammami and El May [19] suggested in their review that garlic improves male reproductive function. The objective of this study is therefore to evaluate the effect of aqueous *Allium sativum* bulb extract on sperm parameters and hormone levels in Wistar albino rats. The study is additionally intended to evaluate whether or not the extract would inhibit alloxan induced alteration of sperm parameters and hormone levels.

2. MATERIALS AND METHODS

Chemicals

Alloxan monohydrate powder (Sigma-Aldrich, Germany), diethyl ether (Loba-Chemie PVT. Ltd., Mumbai, India), formaldehyde (May and Baker Ltd, Dagenham England), potassium chloride salt (Merck, Darmstadt), normal saline (Dana Pharmaceutical Ltd, Minna, Nigeria).

Identification and extraction of plant material

Fresh garlic bulbs were obtained and authenticated by a botanist. The outer skin was peeled and the seeds were pulverized. The powder was macerated in distilled water for 48 h with continuous agitation and filtered with a filter paper. The extract solution was evaporated to dry oily extract on a steam bath at 45^{o}C. The extract was stored in an amber coloured bottle and preserved in a refrigerator.

Experimental design

Fifty male Wistar rats weighing 250-300 g were used for the study. They were fed with standard rat feeds, given water *ad libitum* and maintained under natural lighting condition and temperature of 25±5^{o}C. The animals were handled carefully and experimental protocol was approved by Research Ethics Committee before commencement of study. The animals were randomly distributed into different groups (n=5 per group) and given extract (50, 100, 200 or 300 mg/kg) directly into the stomach by oral gavage once daily for 28 days. Some other groups were given similar extract treatment 24 h after they were injected single dose alloxan (100 mg/kg) intraperitoneally. The positive control group received only alloxan and expressed maximal adverse testicular effects of alloxan. The normal control group received only water, which was used as the vehicle. The doses of garlic used were based on previous works [20,21] and below its LD_{50}, 3034 mg/kg [22]. At the end of the administrations, the animals were anaesthetized with diethyl ether and sacrificed by cervical dislocation. Blood samples of animals were collected by cardiac puncture into specimen bottles and centrifuged to separate the cells from serum. Serum levels of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were quantified using enzyme-linked immunosorbent assay (ELISA) method with AccuBind ELISA Microwells (Monobind Inc. USA). The testis and epididymis were also removed and sperm was expressed from the epididymis by maceration into a petri-dish containing sperm fluid and analysed using standard laboratory methods [23]. Briefly, sperm motility was measured immediately after sperm collection. A drop of liquefied sperm was placed on a clean slide, coverslipped and viewed microscopically using 40x objective. The motility was determined by counting both motile and non-motile spermatozoa (at least 100) in at least 10 randomly selected fields. The percentage motile sperm was calculated from the mean percentage motility for the fields counted, and adjusted to the nearest 5%. Sperm concentration was performed with the Neubauer counting chamber. The Neubauer counting chamber was prepared and charged with the diluted seminal fluid (1:20) and allowed to stand in a moist chamber for 20 min. Morphologically matured sperm cells were then counted under light microscope using 40x objective. To analyse sperm morphology, sperm smear was air-dried and stained with two drops of Walls and Ewas. Slide was viewed under light microscope at a magnification of 40x.

Statistical analysis

Data are expressed as mean+SEM. Comparison between experimental and control groups was performed by one way analysis of variance (ANOVA), using GraphPad Prism 5 Software. Values

were considered significant at $P < 0.05$.

3.RESULTS AND DISCUSION

Effects of garlic extract treatment

Rats that were administered the highest dose (300 mg/kg) had lower ($P < 0.05$) values of sperm motility and count compared to control, whereas there was no change in the rats that received lower doses of extract (Figures 1a and 1b). Percentage of abnormal sperm cells (sperm morphology) in extract treated rats was not affected compared to control (Figure 1c). In addition, serum testosterone concentration was reduced in 300 mg/kg administered rats, but not in other groups when compared with control (Figure 2a). Serum FSH and LH levels were not altered in all extract administered rats (Figures 2b and 2c).

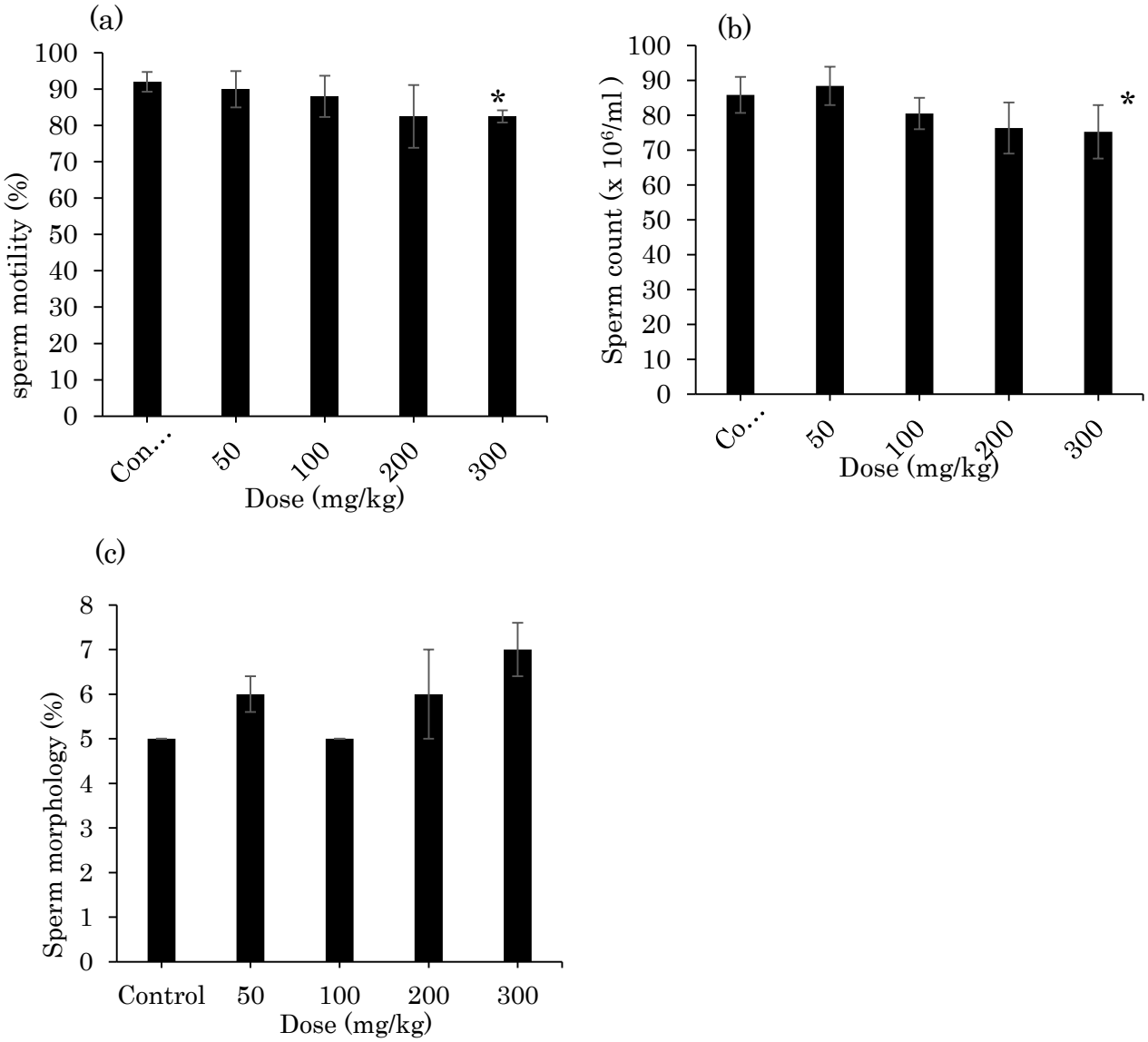

Figure 1: Effect of aqueous garlic extract treatment on sperm parameters in Wistar rats
Data expressed as mean ± SEM, n = 5 rats per group * Significant compared to control, p<0.05

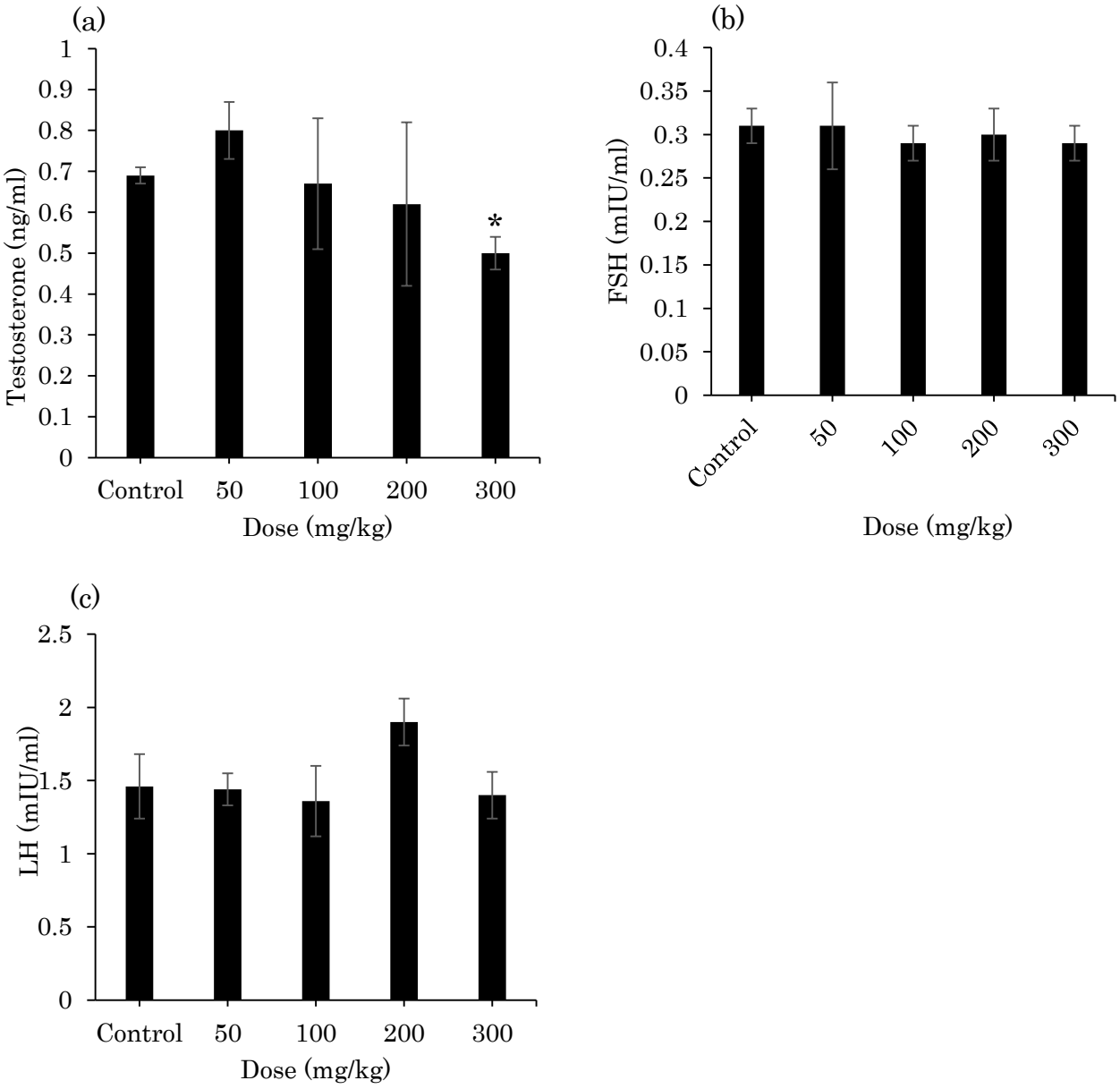

Figure 2: Effect of aqueous garlic extract treatment on serum levels of reproductive hormones in male Wistar rats

Data expressed as mean ± SEM, n = 5 rats per group * Significant compared to control, $p<0.05$

Effects of alloxan and garlic extract combined treatment

There was reduction ($P < 0.05$) in sperm motility and count in rats that were injected with only alloxan when compared with control (Figures 3a and 3b), but sperm morphology was not affected (Figure 3c). In addition, as shown in Figure 3a, sperm motility was reduced ($P < 0.05$) in all alloxan injected rats that received extract, compared to control. Sperm counts in extract treated rats were also decreased but only those that received 200 or 300 mg/kg were significant compared to control (Figure 3b). Furthermore, the sperm motility and count values in 300 mg/kg extract administered group were significantly lower when compared to the alloxan alone treated group (Figures 3a and 3b). Sperm morphology was not affected in alloxan alone treated rats, but it was increased in rats that

received alloxan and extract (200 or 300 mg/kg) compared to the control (Figure 3c). Serum testosterone concentration was decreased in alloxan alone injected rats, whereas FSH and LH concentrations were not changed compared to control (Figures 4a-c). Testosterone was decreased in the rats that received 200 or 300 mg/kg extract, but androgen levels obtained in other extract treated rats were not significantly different compared to control (Figure 4a). When compared, the testosterone level in alloxan alone treated rats was greater ($P < 0.05$) that those that received alloxan and garlic extract (Figure 4a). FSH and LH were not affected in the rats that received alloxan and extract (Figures 4b and 4c).

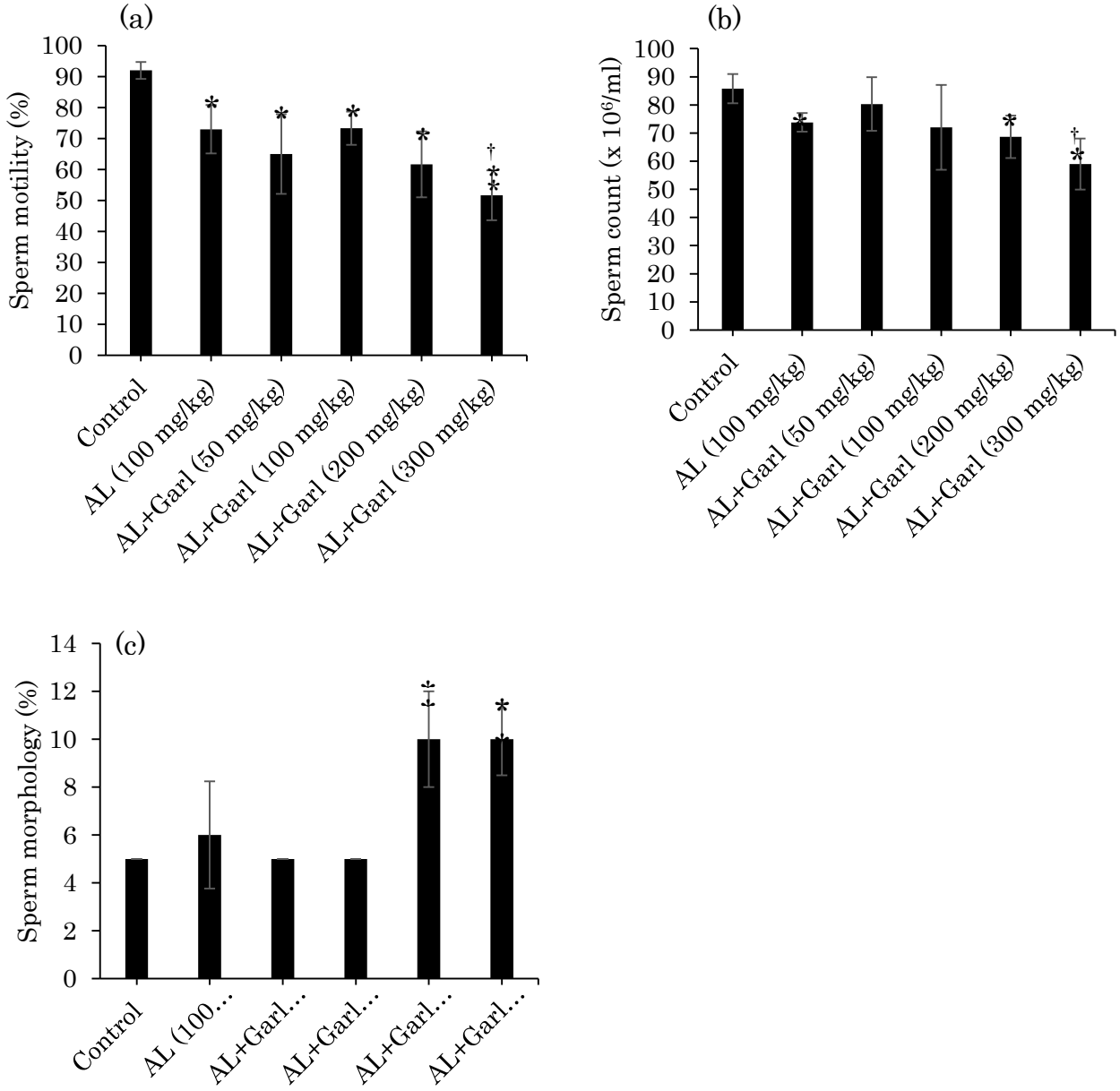

Figure 3: Aqueous garlic extract (Garl) increases inhibitory effect of alloxan (AL) on sperm indices in male Wistar rats

Data expressed as mean ± SEM, n = 5 rats per group

* Significant compared to control, p<0.05

** Significant compared to control, p<0.01

† Significant compared to alloxan, p<0.05

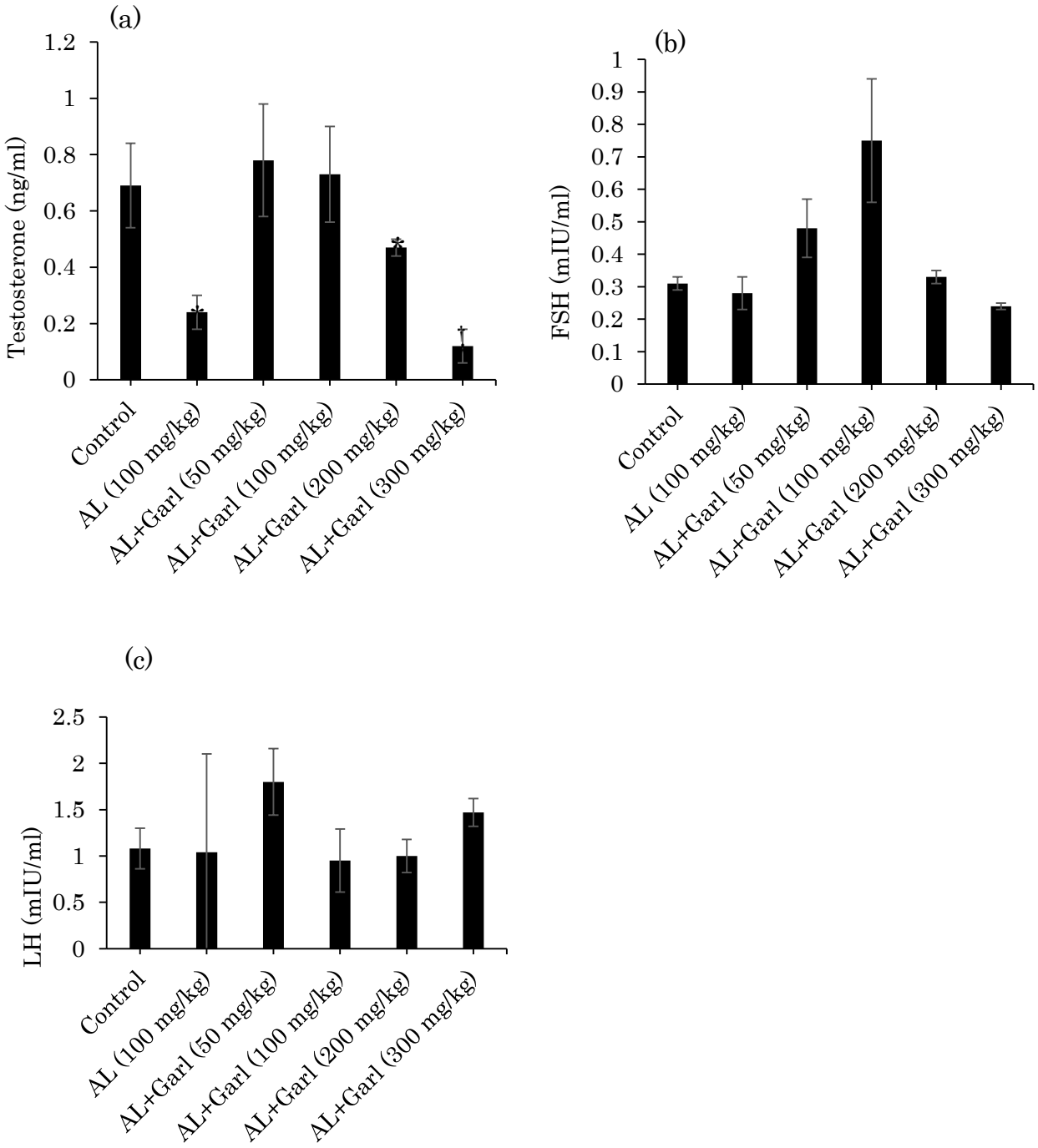

Figure 4: Aqueous garlic extract (Garl) increases alloxan (AL)-induced reduction of serum testosterone concentration in male Wistar rats

Data expressed as mean ± SEM, n = 5 rats per group

* Significant compared to control, p<0.05

† Significant compared to alloxan, p<0.05

The potential influence (positive or negative) of *Allium sativum* (garlic) bulb extract on sperm parameters and hormone levels was investigated and reported herein. The influence of garlic treatment (50-300 mg/kg) on the testis, alone or in the presence of a testicular toxicant (alloxan) was studied. From the results, garlic treatment caused reduction in sperm motility and count at the highest dose (300 mg/kg), but the lower doses failed to produce an effect. Also, garlic produced no effect on sperm morphology (percentage of abnormal sperm cells) at all the treatment levels. Although, garlic did not alter blood levels of LH or FSH, testosterone was decreased, but only at 300 mg/kg. Testosterone is essential for normal spermatogenesis [24] and interestingly, the reduction in androgen level by garlic correlated positively with the reduction in the sperm indices observed. This indicates that consumption of high concentrations of garlic may impact negatively on fertility in males. This finding supports some earlier studies which have reported that garlic caused impairment of spermatogenesis with reduction in sperm motility and count [25,26]. Garlic has equally been reported to induce germ cell apoptosis [27,28], and degenerative changes of testicular structures [20]. Furthermore, the reduction in serum testosterone level by garlic in our study is consistent with the findings of Ebomoyi and Ahumibe [20] who reported that garlic caused inhibition of Leydig cell function and reduction in testosterone level. However, there are contrary reports indicating that garlic increases serum testosterone level in experimental animals [29]. Obidike *et al*. [21] had also reported that garlic enhances testicular function and could be used to enhance fertility. The reason for the contrary observations is not very clear, but it may be related to extract, dosage and species of the garlic used. LH and FSH are gonadotropic hormones of the anterior pituitary gland, while testosterone is produced and secreted by Leydig cell. The steroid hormone expression is dependent on LH, and the gonadotropic hormones are directly involved in the regulation of spermatogenesis [30]. The lack of effect on LH and FSH in our study thus suggests that gonadotropic hormones regulatory role on testis function may not be altered by garlic. Alloxan is a known pancreatic toxicant [31], and capable of adversely affecting sperm and androgen secretion [32]. In this study, alloxan decreased sperm motility and count but did not alter sperm morphology. In addition, alloxan reduced testosterone level but caused no change in gonadotropic hormone levels. The negative effects on sperm indices as well as the inhibitory effect on hormone secretion by alloxan were not inhibited by garlic. Rather, the effects on sperm motility, sperm count and testosterone level were increased in the rats that received 300 mg/kg dose of garlic. This indicates that garlic at high concentrations may potentiate the alloxan-induced negative testicular effects that were observed. Putting the results together, it shows that administration of garlic may inhibit spermatogenesis and increase adverse spermatic and androgenic effects of alloxan. Garlic should therefore be used with caution in males particularly at high dose levels over long period of time.

4. CONCLUSION

This study demonstrates that subacute administration of high dose levels of aqueous garlic extract alters sperm characteristics and reduces serum testosterone levels in rats.

CONFLICT OF INTEREST

Authors have no conflicts of interest.

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