Effects Of Aqueous Extract Of Allium Sativum (Garlic) On Semen Parameters In Wistar Rats.

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Citation

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Abstract

Garlic has been in use worldwide since ages, especially as food and for its health benefits. However, concern has been raised on its untoward effects on male reproductive functions. The present study examined the effects of aqueous garlic extract on some semen parameters and erythrocyte superoxide dismutase in Wistar rats. Twenty-four male Wistar rats were grouped into 3, and aqueous extract of garlic was administered orally at different doses (Group B: 500 mg/kg/d; Group C: 1000 mg/kg/d) to the 2 treated groups, and distilled water given to the control group (Group A), for 28 days. Sperm concentration, motility and morphology were studied, and the activity of superoxide dismutase (SOD) was measured. The results of the semen analysis revealed reduction in all the parameters, which was dose-dependent. The percentage of morphologically normal spermatozoa was significantly reduced, as well as sperm concentration, compared with findings in the control animals. Garlic also caused a significant reduction in SOD activity in the blood, and this was dose-dependent, as the least activity was recorded among the high dose group. As people desire to enjoy the maximum beneficial health effects of garlic, its potential to adversely affect the reproductive functions, especially at higher doses, should be borne in mind.

INTRODUCTION

Garlic (Allium sativum) is one of the most researched plants, with a long history of medicinal use[1]. Many of its constituents contribute to its medicinal properties and potential to lower the risk of diseases[2]. Various formulations of garlic exist, which include raw garlic homogenate[3], garlic powder[4], aged garlic extract[2], steam-distilled garlic[5], etc. Aqueous extract of garlic is a form of raw garlic homogenate[6]. The important components, among others, include allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) which is thought to be the chief bioactive compound present in aqueous garlic extract; allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate and -L-glutamyl-S-alkyl-L-cysteine[6]. Garlic preparations are known for their cardioprotective effects[7,8], ability to lower blood pressure[9] in people with high blood pressure, lower lipid levels, reduce the risk of atherosclerosis[6], increase blood levels of antioxidant enzymes, and also offer some benefits in conditions like diabetes mellitus and cancer[10]. Although many positive health effects have been attributed to the use of garlic, the effects tend to be undesirable on testicular functions, as many studies have observed some adverse effects on reproductive functions. Although the mechanisms of this action of garlic are not very clear, apoptosis has been suggested to be a likelihood[11]. Allium sativum is an antioxidant and a detoxifying agent, which scavenges the reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes, (such as superoxide dismutase, catalase, glutathione peroxidase, etc) thereby protecting the cells against disease-causing oxidative damage[2]. Administration of garlic can alter the activities of endogenous antioxidants, depending on the dosage as well as the type of antioxidants[12]. Studies by Banerjee et al[12] showed that use of garlic at low doses could significantly increase superoxide dismutase (SOD), whereas at higher doses, the activity of SOD is reduced. Garlic causes a dose-dependent increase in the percentage of empty seminiferous tubules, thereby altering spermatogenesis and reducing testosterone secretion[13,11]. Meanwhile, studies by Oi et al[14] found an increased testicular testosterone with garlic supplementation. Also associated with garlic use is the inhibition of Leydig steroidogenic enzyme expression and Sertoli cell markers, which are capable of inducing apoptosis in testicular germ cells (spermatocytes and spermatids), characterised by increased levels of active Caspase 3 (CASP3)[11].

In the present study, the effect of orally administered aqueous extract of Allium sativum was studied on some semen parameters in wistar rats and its effect on erythrocyte

superoxide dismutase (SOD).

MATERIALS AND METHODS

PREPARATION OF GARLIC AQUEOUS EXTRACT

The raw garlic plants were collected from Ilorin, Kwara State and authenticated at the Department of Plant Biology, University of Ilorin, Nigeria. The raw garlic cloves were peeled, chopped into small pieces and blended. It was then dissolved in distilled water and kept in the Refrigerator for 12 hours. The solution was thereafter filtered, and the filtrate was concentrated in a water bath at a temperature of 40ºC, into the paste form, from which the required dosages (500 mg/kg/d and 1000 mg/kg/d) were prepared.

EXPERIMENTAL ANIMALS

The experiments were performed in conformity with the Rules and Guidelines of the Animal Ethics Committee of the University of Ilorin. The rats were purchased from Home-made Research Institute in Ilorin, Nigeria. They were housed within the Animal House of the Department of Anatomy, College of Health 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 two weeks before the commencement of the experiment.

EXPERIMENTAL PHARMACOLOGICAL PROTOCOL

Twenty-four (24) rats with an average weight of about 116 g were randomly grouped into 3 classes as follows:

Group A:- Control Group given distilled water.

Group B:- Treated Group with aqueous extract of garlic in a dose of 500 mg/kg/d.

Group C:- Treated Group with aqueous extract of garlic in a dose of 1000 mg/kg/d.

Using a feeding tube (size-6), distilled water and aqueous garlic extracts were administered to the control and treated animals respectively for a period of 28 days.

ANIMAL SACRIFICE AND SAMPLE COLLECTION

Twenty-four hours (24 h) after the last day of administration, the animals were weighed and thereafter sacrificed by cervical dislocation. Blood samples of animals were collected into lithium heparinized bottles. Using a midline abdominal incision, the abdominal cavity was opened to access the reproductive organs. The testes were excised and weighed using an electronic sensitive analytical balance (Gallenkomp FA 2104A).

ASSAY OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Estimation of superoxide oxidase (SOD) in the blood was based on the method described by Mistra and Fridovish[15], using chemical reagents kit produced by Randox Laboratories Limited (UK). Samples were measured spectrophotometrically at 30 sec interval for 3 min, and the data expressed as IU/ml.

EPIDIDYMAL SPERM CONCENTRATION

A modified method of Yokoi and Mayi[16] was adopted in counting the spermatozoa from the right epididymis. The epididymis was minced with anatomic scizzors 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 Neuber's counting chamber (haemocytometer) was used in counting the total number of spermatozoa. About 10 ml of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min, and thereafter observed under a binocular light microscope.

SPERM MOTILITY

The fluid from the caudal epididymis was diluted with Tris buffer solution[17] to 0.5 ml. An aliquot of this solution was observed under the light microscope at a magnification of x400. The mean motility estimation was reported as the final motility score for each sample.

SPERM MORPHOLOGY

The morphology of the spermatozoa was determined using the original dilution for motility, diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). The sperm cells were categorised based on the presence of one or more abnormal features such as tail defects (short, irregular, coiled or multiple tails); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (round head, small or large size, double or detached head). Findings were expressed as percentage of morphologically normal sperm[18].

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 mean (SEM), and values of p <0.05 were considered to be statistically significant.

RESULTS

There were no statistically significant changes in the body weight difference of the experimental animals in all the groups, and the ratio of the testis weight to that of the body weight of each animal (Table 1:p>0.05). Semen parameters in all the treatment groups (Groups B and C) decreased when compared with the control group. Only a minimal decrease was observed in the percentage sperm motility of the treated groups, and in the percentage of morphologically normal spermatozoa of the low dose group (Table 2). The percentage of normal spermatozoa was markedly reduced in the animals exposed to 1000 mg/kg/d (high dose) of garlic (69.00 ± 1.22) compared with animals that received a lower dose of 500 mg/kg/d (85.50 ±1.66) and the control animals (86.25 ± 1.75) given distilled water. There was a decrease in sperm motility in the treatment groups (Control: 92.17±0.66; Low dose group: 90.87±0.76; High dose group:90.27±1.13), but this was not statistically significant (p>0.05). Sperm concentration decreased in the treated groups with the animals treated with 1000 mg/kg/d aqueous garlic extract having the lowest value (63.85 ± 3.03) compared with those that received 500 mg/kg/d of the extract (65.35 ± 3.13) and the control group (72.15 ±0.74) that received distilled water. Superoxide dismutase enzyme was markedly reduced in the low dose group (279.75 ± 05.02 IU/ml; p<0.05) compared with the control animals (490.00 ± 20.10 IU/ml), with a much more decreased activity in the group that received a higher dose of 1000 mg/kg/d (188.00 ± 09.82 IU/ml; p<0.05).

Figure 1

Table 1: Weight Of Testes Following Administration Of Extract

Treatment groups	Initial body weight (g)	Final body weight (g)	Body weight difference (g)	Testis weight (g)	Testis-Body weight ratio
A:Control	104.25 ±1.93	155.75 ±2.56	51.50	1.3850 ±0.08	0.0089
B:Garlic (500 mg/kg/d)	117.75 ±5.57	161.25 ±1.31	43.50	1.5700 ±0.09	0.0097
C: Garlic (1000 mg/kg/d)	124.25 ±3.33	176.75 ±2.84	52.50	1.7200 ±0.07	0.0097

Mean ±SEM;*p>0.05

Figure 2

Table 2: Semen Parameters and SOD activity

Parameters	Groups		
	A: Control	**B:** 500 mg/kg/d	**C:** 1000 mg/kg/d
Sperm concentration (x10^6/ml)	72.15 ± 0.74	65.35 ±3.13	63.85 ± 3.03
Sperm motility (%)	92.17 ± 0.66	90.87 ± 0.76	90.27 ±1.13
Morphology (% normal)	86.25 ± 1.75	85.50 ± 1.66	69.00 ± 1.22
Superoxide dismutase (IU/ml)	490.00 ± 20.10	279.75 ± 05.02**	188.00 ± 09.82**

Mean ±SEM;**p<0.05

DISCUSSION

The effects of garlic on male gonads, and by extension, the male reproductive function, may appear somewhat different from the usual cytoprotective activities observed by various workers in other tissues and organs of the body[19,3]; although, the amount of garlic administered, to some extent, could be a factor[12]. Administration of garlic augments the activities of SOD and also protects the cells against the damaging effects of free radicals, as given by some biochemical and histopathological evidences[3]. However, on the testes, use of garlic has been noted to compromise some male reproductive functions, as it affects spermatogenesis and testosterone levels, which are vital to reproduction[11].

Both at a lower and higher doses of garlic extract, the activity of SOD was noted in this study to decrease with increasing dosage. The endogenous antioxidative functions of the enzyme was compromised, thereby predisposing the cells of the testes to the detrimental effects of various oxygen radicals and other chemically-induced oxidative stress. In this study, both the percentage morphology and sperm count were significantly reduced, although sperm motility was only slightly reduced. Irrespective of the motility, in the event of increasing number of abnormal spermatozoa morphologically, spermatogenesis and other reproductive capability of an intact spermatozoon, could still be compromised. This is because previous studies have shown that use of Allium sativum is associated with increased percentage of empty seminiferous tubules and consequent reduction in testosterone secretion and altered spermatogenesis. The effects of garlic on semen parameters may not be unconnected to the suggested mechanism of apoptosis[11].

References

1. Block, E. The chemistry of garlic and onion. Sci. Am 1985;252:114-119.
2. Borek, C. Antioxidant health effects of aged garlic extract. Journal of Nutrition 2001;131:1010S-1015S.

3. Banerjee SK, Dinda AK, Manchanda SC and Maulik SK. Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury. BMC Pharmacology 2002;2:16.
4. Isaacsohn JL, Moser M, Stein EA, Dudley K, Davey JA, Liskov E, Black HR. Garlic powder and plasma lipids and lipoproteins: a multicenter, randomized, placebo-controlled trial. Arch Intern Med 1998;158:1189-1194.
5. Lawson LD. Garlic: a review of its medicinal effects and indicated active compounds. In: Phytomedicines of Europe. Chemistry and Biological Activity. Series 691 (Edited by Lawson LD & Bauer R). Washington, DC., 1998;176-209.
6. Banerjee SK and Maulik K. Effect of garlic on cardiovascular disorders: a review. Nutritional Journal 2002;1:4.
7. Aqel MB, Gharaibah MN, Salhab AS. Direct relaxant effects of garlic juice on smooth and cardiac muscles. J Ethnopharmacol 1991;33:13-19.
8. Martin N, Bardisa L, Pantojaa C, Vargas M, Quezaada P, Valenzuela J: Antiarrhythmic profile of a garlic dialysate assayed in dogs and isolated atrial preparations. J Ethanophrmacol 1994; 43:1-8.
9. Ried K, Frank OR, Stocks NP, Fakler P,Sullivan T. Effect of garlic on blood pressure: A systematic review and meta-analysis BMC Cardiovascular Disorders 2008;8:13.
10. Milner, JA. "Mechanisms by which garlic and allyl sulfur compounds suppress carcinogen bioactivation. Garlic and carcinogenesis." Adv. Exp. Med. Biol 2001;492: 69-81.
11. Hammami I, Amara S, Benahmed M, El May MV, Mauduit C. Chronic crude garlic-feeding modified adult male rat testicular markers: mechanisms of action. Reprod Biol Endocrinol 2009;7: 65.
12. Banerjee SK, Maulik M, Manchanda SC, Dinda AK, Das TK and Maulik SK. Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food and Chemical Toxicology 2001;39(8):793-797.
13. Hammami I , Nahdi A , Mauduit C, Benahmed M, Amri M ,Amar AB, Zekri S, El May A, El May MV. The inhibitory effects on adult male reproductive functions of crude garlic (Allium sativum) feeding. Asian Journal of Andrology 2008;10(4): 593 – 601.
14. Oi Y, Imafuku M, Shishido C, Kominato Y, Nishimura S, Iwai K. Garlic Supplementation Increases Testicular Testosterone and Decreases Plasma Corticosterone in Rats Fed a High Protein Diet. Journal of Nutrition 2001;131:2150-2156.
15. Mistra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247(10):3170–3175.
16. Yokoi K, Mayi ZK. Organ apoptosis with cytotoxic drugs. Toxicology 2004;290:78-85.
17. Sonmez M, Turk G, Yuce A. The effects of ascorbic supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology 2005;63:2063-2072.
18. Saalu LC, Osinubi AA, Jewo PI, Oyewopo AO and Ajayi GO. An evaluation of influence of Citrus paradise seed extract on doxorubicin-induced testicular oxidative stress and impaired spermatogenesis. Asian Journal of Scientific Research 2010;3(1):51-61.
19. Pal R, Vaiphei K, Sikander A, Singh K, Rana SV. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. World J Gastroenterol 2006;12(4):636-639.

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