

Garlic Consumption Alters Testicular Histology and Anti-Oxidant Status In Wistar Rats.

G.O.Omotoso, A.O Oyewopo, I.O Onanuga, B.U Enaike.

Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences,
University of Ilorin, Ilorin, Nigeria.

Abstract

Garlic is known for its cell and tissue-protective functions. This study examined the effects of aqueous garlic extract on the histology of the testes of Wistar rats and erythrocyte superoxide dismutase (SOD) activity.

Twenty-four (24) rats with an average weight of 116 g were used. They were randomly grouped into three: Group A Control: was given distilled water; Group B: was given 500 mg/kg/d aqueous garlic extract; and Group C: received 1000 mg/kg/d aqueous garlic extract; all for 28 days.

Findings revealed progressive depletion in sperm cell population, reduction in size of seminiferous tubules, and widening interstitial space. SOD activity was significantly reduced in both treated groups in a dose-dependent fashion.

These histological observations and the depletion in endogenous anti-oxidant status associated with the administration of garlic could result in significant affectation of male reproductive functions.

Keywords: garlic, superoxide dismutase, testis, histology

Introduction

Garlic also known as *Allium sativum*, is a popular spice usually eaten both raw and cooked in various doses, and its potential health benefits have been utilised for several years¹. It has been found useful in the treatment of many clinical conditions, such as, hypercholesterolemia, prevention of arteriosclerosis, cancers, and also possesses some anticoagulant and antihypertensive properties¹.

The important components of garlic, among others, include allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) which is thought to be the chief bioactive compound present in the aqueous garlic extract; allyl methyl thiosulfinate, 1-propenyl allyl thiosulfinate, and γ -L-glutamyl-S-alkyl-L-cysteine². Although many positive health effects have been attributed to garlic use, this is difficult to establish in the gonads, as the effects appear to be undesirable on testicular functions. It has been suggested that the process of apoptosis is a likely mechanism by which garlic exerts such adverse effects on the testis¹.

Aside the earlier mentioned properties, garlic also has antioxidant and detoxifying properties, and its consumption alters the activities of endogenous antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase, etc, depending on the dosage as well as the type of antioxidants^{3,4}. Banerjee et al⁴ 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^{1,5}. Meanwhile, Oi et al⁶ found an increased testicular testosterone with garlic supplementation. The current study examines the effects of aqueous garlic extract on the histology of the testes of Wistar rats and erythrocyte superoxide dismutase (SOD) activity.

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 40°C, 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

Correspondence to:

G O Omotoso,
Department of Anatomy,
Faculty of Basic Medical Sciences,
College of Health Sciences,
University of Ilorin, Ilorin, Nigeria.
Email: gabrielolaiya@yahoo.com.
Cellphone: 07030505707.

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 Design

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 Specimen Collection

The animals were sacrificed by cervical dislocation 24 hrs after the last administration. The testes and epididymis were delivered via an abdominal section. The testes were fixed in 10% formal saline solution, and appropriately processed for histological section using the Haematoxylin and Eosin staining technique⁷. Blood samples of animals were collected into lithium heparinized bottles.

Assay of Superoxide Dismutase (SOD) Activity

Estimation of superoxide oxidase (SOD) in the blood was based on the method described by Mistra and Fridovich⁸, 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.

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

Histological Observation

The seminiferous tubules of the testes of Wistar rats in the Control Group appeared to have a normal testicular architecture, with the presence of numerous spermatogenic cells in the seminiferous tubules at different stages of development, and lumina that were filled with many spermatozoa (Figure 1). Animals that were treated with the aqueous garlic extract showed a progressive decline in the concentration of sperm cells in the lumen, reduced diameter of seminiferous tubules compared with the Control Group, and wider and scanty interstitial spaces (Figures 2, 3).

Superoxide dismutase enzyme activity was

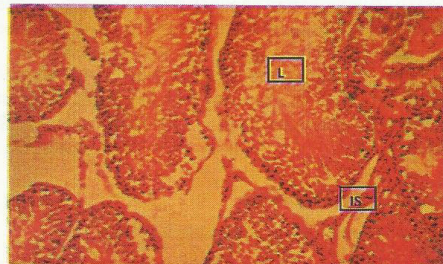


Figure 1: Photomicrograph of a transverse section of the testis of Control rat showing normal testicular architecture, seminiferous tubules with a good population of normal spermatogenic cells and spermatozoa within the lumen (L); also normal interstitial space (IS) (H&E method $\times 100$).



Figure 2: Photomicrograph of a transverse section of the testis of Wistar rat treated with 500 mg/kg/d aqueous garlic extract (Group B) showing a reduced population of spermatogenic cells, reduced population of spermatozoa within the lumen (L); reduced size of the tubules, and widening interstitial space (IS) compared to the Control (H&E method $\times 100$).



Figure 3: Photomicrograph of a transverse section through the testis of rat treated with 1000 mg/kg/d aqueous garlic extract (Group C) showing a distorted microarchitecture, poor staining intensity, reduced size of tubules and widening of interstitial space (IS) (H&E method $\times 100$).

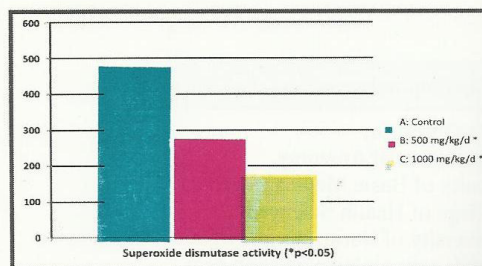


Figure 4: Superoxide dismutase activity (* statistical significant difference compared with Control: $p < 0.05$)

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; $p < 0.05$), and these differences were statistically significant (Figure 4).

Discussion

Whenever garlic is used for any of its numerous health benefits, it should be borne in mind that some other organs or systems in the body do not stand to benefit. This is particularly so with the gonads. Omotoso et al.⁹ performed semen analysis of animal models that received garlic extracts and found a significant reduction in the percentage of morphologically normal sperm cells and a reduction also in the sperm population. This could probably account for the histological observation in the current study, such as the reduction in the population of spermatogenic series, reduced population of spermatozoa within the lumen and reduced size of the tubules in animals treated with the low dose of 500 mg/kg/d compared to the Control. These effects were however seen to be more in animals that received a double dose of 1000 mg/kg/d aqueous garlic extract, which showed distorted microarchitecture of the testis, reduced size of tubules, further reduction in sperm cells, and widening of the interstitial space, compared with the low dose Group B animals.

Reasons for the histological findings in the current study could be related to earlier observation by other researchers, that the use of garlic is associated with 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)¹.

It has been noted in certain conditions that 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 evidences¹⁰. 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 reproduction¹. Results from the current study showed that both at lower and higher doses of garlic extract, the activity of SOD decreased significantly with increasing dosage. The endogenous anti-oxidative 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. This could also account for the histological findings.

With a decreasing population of sperm cells in the lumen of seminiferous tubules and an increasing

percentage of empty seminiferous tubules, coupled with various degrees of distortions in the normal microarchitecture of the testis, male reproductive functions are prone to be adversely affected with the use of aqueous garlic extract, and this is worse with increasing dosage.

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