

Full Length Research Paper

Investigation of the androgenic activity of ginger (*Zingiber officinale*) on the histology of the testis of adult sparague dawley rats

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Abstract

Ginger Rhizome (*Zingiber officinale*; Family: Zingiberaceae) is used medicinally and as a culinary spice. The objective is to investigate the androgenic activity of Ginger on testicular histology of adult Sparague Dawley rats. 30 adult male Sparague - Dawley rats weighing between 120g -150g were used. They were allotted into 3 groups. The control group (n=10) and treated groups for 28 days (n=10) and 56 days (n=10) respectively. The treated groups received 200mg/kg of the aqueous extract. Extract was given via gastric intubation. The controls as well as treated animals were allowed free access to rats' pelleted feds and water *ad libitum*. The animals were sacrificed. Testicular weight was taken and caudal epididymis excised for biochemical analysis. There was an increase in animal body weight gained throughout the duration of the experiment as compared with the control. There was also a significance increase in testicular weight. Semen analysis appears normal; no oligospermic or azoospermic condition detected. Sperm morphology is normal no teratozoospermic sperm found. The aqueous extract of *Zingiber officinale* at dose of 200mg/kg for 4 weeks and 8 weeks respectively had a positive impact on testosterone level. Results stipulate that it stimulates secretion from the testes (and other male accessory sex organs) that influences semen. Hence this study reveals no deleterious effects of aqueous extraction of dry fruits of *Zingiber officinale* on male reproductive indices, so if any deleterious effect should occur then it could be dose – dependent or duration dependent.

Keywords: Oligospermic, azoospermic, teratozoospermic, testosterone, *Eugenia Aromaticum*

INTRODUCTION

Infertility is one of the major health problems in life and approximately 30% of infertilities is due to male factor (Isidori et al., 2006 and Carlsen et al., 1992). Several conditions can interfere with spermatogenesis and reduce sperm production and quality. Most factors are traced to drug treatment. Chemotherapy, toxins, air pollutions, insufficient vitamins ingestion has harmful effects on spermatogenesis and normal sperm production and functions (Mosher and Pratt, 1991).

Ginger belongs to the family *Zingeraceae*. Local name in Yoruba is *Atale / Aje or Atale Fun –Fun* (Ogungbemi, 2006) in Hindi called *Aadrak* and in Gujarati called *Aadu* and *Cjitta* in Hausa. Its antioxidant and androgenic

activities have been reported in animal model (Sekiwa *et al.*, 2000 and Kamtchouing *et al.*, 2002). In India, ginger is applied as a paste to the temples to relieve headache and consumed when suffering from a cold, people use ginger for making tea (Kamtchouing *et al.*, 2002) (Figure 1)

China and India are the principal sources of Ginger. Since 1500s Chinese herbalist differentiates the used of dry and fresh ginger root in traditional medicinal roles. The ginger plant has a long history of cultivation known to originate in China and then spread to India, Southeast Asia, West Africa, and the Caribbean (Lister 2003).

It is made up of phytochemicals such as zingerone, gingerdiol, zingibrene, shogaols and *gingerol* (Zancan *et al.*, 2002). Its active component is *gingerol* which act as an anti- oxidant and is responsible for its taste (Witchl 2004).

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Figure 1. Ginger Rhizomes

Medicinal properties of ginger are numerous due to its active components. It is general known to be use as a culinary in making ginger tea, as well as a spice in food. Its pharmacologic properties allow it to act as a stimulant; stimulating the production of saliva, (Chaiyakunaparak *et al.*, 2006) carminative, anti - oxidant, analgesic, laxative and aphrodisiac (Ogungbemi, 2006). Helps treat diseases such as osteoarthritis, cancer, kidney disease toothache, ulcer, motion sickness, orchiditis, bronchitis, impotence, diarrhea etc. Japanese traditional medicine has suggested caution in pregnant women as regard consumption of dried ginger (Altman 2001 and Chaiyakunaparak *et al.*, 2006).

At a large dose it can result in CNS depression and cardiac arrhythmia. It interacts with drugs such as warfarin, digoxin, heparin and herbal supplements such as garlic and *Ginkgo biloba* (Altman, 2001). It also prevents blood clotting by preventing clumping of platelets; it inhibits thromboxane in vitro (Backen, 1991).

Various studies have been done in the field of male reproductive biology to make inference if it has any influence on fertility. Androgenic activity of ginger have been tested in Cisplatin induced testicular toxicity in adult male rats; in which both *Zingiber Officinale* and *Pentadiplandra brazzeana* aqueous extracts were given by gavage at dose 600mg/kg for 8 days after 21 days of Cisplatin administration. Result showed that both extracts increase relative weight of the testis, serum testosterone level, testicular cholesterol and epididymal α -glucosidase activity. But a similar result in ginger treated rats was more potent as compared to *P.brazzeana* (Kamtchouing *et al.*, 2002). Similar study done to compare the effects of ethanolic extracts of ginger and Roselle on Cisplatin induced testicular toxicity, showed that both plants increase the activity of testicular anti - oxidants enzymes and restored sperm motility to normal in Cisplatin treated rats (Amr *et al.*, 2007).

Both antioxidant (Zancan *et al.*, 2002) and androgenic activity (Kamtchouing *et al.*, 2002) of *Z. Officinale* has been reported in animal models. Ginger oil has a strong protective effect on DNA damage induced by H₂O₂ and acts as scavenger of oxygen radical and might be used as an antioxidant (Grzann *et al.*, 2005). Antioxidants protect DNA damage and other important molecules from oxidation and damage, and can improve

sperm quality and hence increase fertility rate in men (Rajeev *et al.*, 2006 and Yang *et al.*, 2006).

Therefore, the role of nutritional and biochemical factors in reproduction and subfertility is very vital. This present investigation is done to assess the ability of ginger to promote sperm paramaters, testosterone concentration and spermatogenesis based short and long term treatment. The result obtained will give an insight on how to combat male infertility by improving spermatogenesis and semen quality in a way that is cost deficient and other beneficial role in general body metabolism.

MATERIALS AND METHODS

Experimental Animals

30 adult male Sparague - Dawley rats weighing between 120g -150g were used. The Male rats were authenticated in the Department of Zoology and kept in well ventilated metal cages in the Rats control room of the Department of Anatomy, College of Medicine, Idi – Araba, Lagos.

They were kept at room temperature of 28°C-38°C and exposed to 12:12 hr natural light – dark cycle. They fed with animal fed and given water *ad libitum*; and allowed to acclimatize for three weeks before commencement of experiment.

Plant material

The rhizomes of ginger were procured from Lawanson market; Surulere, Lagos. They were taken to The Botany Department in University of Lagos, Akoka; for identification and authentication, where a voucher specimen was deposited.

Preparation of the aqueous extracts of each spice

The rhizomes of ginger were sun-dried and crushed into powdered form using a blender. The dried powder was weighed (1 g) and macerated in 50 ml of distilled water. Final extract concentration obtained is 20 mg/ml. Extract

Table 1. Experimental design procedure

Group N= 5	Treatment
A (control)	Given only feed and water <i>ad libitum</i>
B	Given 200 mg/kg of aqueous extract of Ginger
C	Given 200 mg/kg of aqueous extract of Ginger

was stored in air- tight container and refrigerated throughout the cause of the experiment.

Experiment Duration

This is a short- term (28 days) and long – term experiment for eight weeks (56 days).

Experimental Design

30 animals were randomly divided into 3 groups. Group A serves as the Control Group, Group B; the short termed group and Group C, the long term group.

Category A: given distilled water served as control

Category B: was given 200mg/kg of the extract for four weeks. (28 days)

Category C: was given 200mg/kg of the extract for eight weeks (56 days)

Administration was done once daily in the morning (between 7.30a.m – 9.00 a.m) (Table 1).

Animal Sacrifice

The animals were given last dose of extracts, weighed and sacrificed using the humane method; in which the animals were anaesthetized by intraperitoneal injection of 50mg/ml of ketamine injection, manufactured by Claris lifesciences Limited Chacharwadi- Vasana, India. Animals were dissected and testes collected, weighed and fixed in Bouin's fluid.

Serum Biochemical Analysis

Blood was obtained by cardiac puncture from the rats in each study group after anasthesize with Ketamine. Each blood samples was spun at 2500 rpm for 10minutes in a desktop centrifuge, to obtain the serum from the whole blood. The serum was then frozen for serum biochemical analysis. The serum sample was assay for testosterone level using the Enzyme linked immunoassay (ELISA) technique. Test kit used was made by the BIOTEC Laboratories Ltd, 32 Ansons Road Martlesham Heath, UK. The test kit gave a reference value for serum testosterone level in man to range from 1.8ng/ml –

9.0ng/ml.

Organ Weight

The testes were weighed using light – sensitive weighing balance obtained from the Department of physiology before fixing in Bouin's fluid.

Statistics

The data obtained from the groups were complied and stastically analyzed using ANOVA method. The results (mean, standard error of mean and the test of significance [p-value was done using the student t- test]) were shown. Results of the data were expressed as mean \pm SEM (Standard error of mean). Test of significance was compared using ANOVA where (p< 0.05) was taken as significant.

RESULTS

Effects of ginger on body and organ weight

There was a significant increase in body weight of animals treated for both short and long term as compared with the control. Testicular weights were also significantly increased (Table 2 and Table 3).

Data is express as mean \pm SEM. Statistical analysis involve the use of the paired t-test (p<0.05)

There was a significant increase in the serum testosterone level in the short term and long term group as compared with the control. The increase was statistically significant (Table 4).

The caudal epididymal sperm count and motility were significantly increased (P<0.05) after the short and long term treatment as compared with the controls.

Results of light microscopy study

Histopathological investigation showed the cycle of spermatogenesis was regular in both control and treated groups. All cells of spermatogenic lineage were present (Figures 3 and 4).

Seminiferous tubules: presence of longitudinal to

Table 2. Changes in body and testicular weight after treatment for 28days

Group n=5	Initial weight(g)	Final weight(g)	Weight difference(g)	% increase	Testicular weight (g)
Control	123.55±1.30	1.62±2.05	38.90	31.49	0.90±0.10
Ginger (200mg/kg)	123.15±1.56	182.15±2.27	59.00	47.91	0.90±0.00

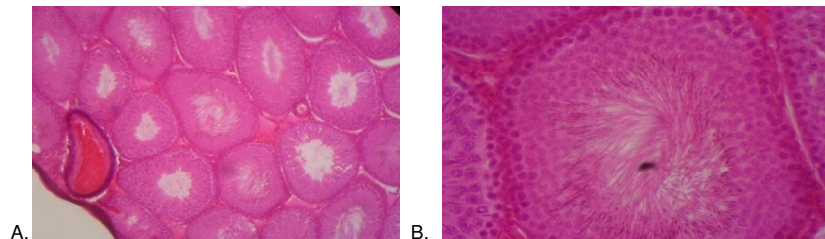
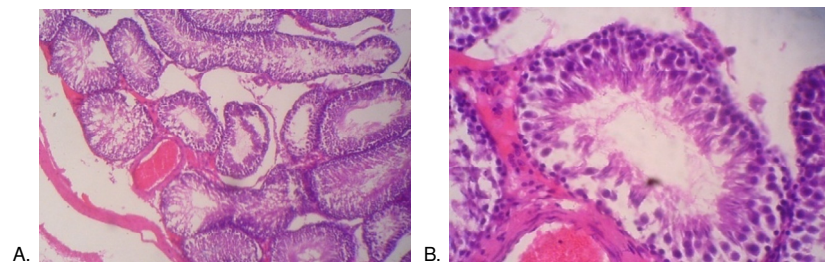
Table 3.Changes in body and testicular weight after treatment for 56days

Group n=5	Initial weight(g)	Final weight(g)	Weight difference(g)	% increase	Testicular weight (g)
Control	122.95±6.25	204.00±4.30	81.05	65.92	0.90±0.10
Ginger (200mg/kg)	121.45±1.07	204.05±4.38	82.60	68.01	0.92±0.00

Table 4. Biochemical changes in the testis (serum testosterone level) after treatment

Group n=5	Treatment (days)	Serum testosterone level(ng/ml)	Treatment (days)	Serum testosterone level(ng/ml)
Control	28	1.49±0.01	56	1.56±0.02
Ginger (200mg/kg)	28	1.87±0.01	56	1.98±0.02

Data is express as mean \pm SEM. Statistical analysis involve the use of the paired t-test ($p < 0.05$).

**Figure 2.** shows control testis, A= magx100 and b= magx400**Figure 3.** treated for 28 days A=magx100 and B= magx400

transversely sectioned tubules and slightly space. Interstitial spaces are smaller. Stratified and well organized Semiferous tubule-epithelium. Spermatogenic cells and spermatogenesis: presence of a

stratified spermatogonic cell lineage. Normal spermatogenesis and progressive spermiogenesis. Stroma: normal leydig cells and presence of few congested blood vessels.

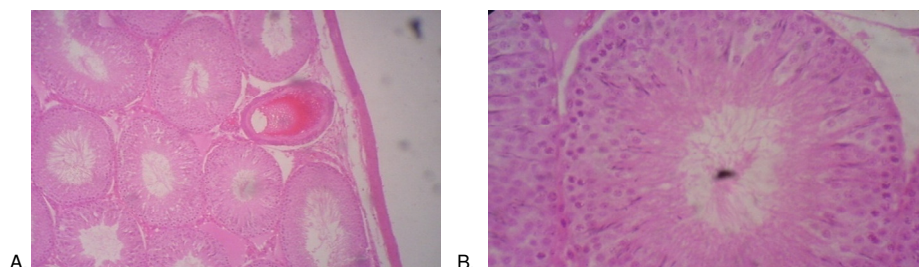


Figure 4. Tunica: fine tunica A= magx100 and b=magx400

Figure 4: treated group for 56 days

Seminiferous tubules: more transversely sectioned tubules that are uniformly arranged and spaced. Interstitial spaces are smaller. More Seminiferous tubules that are well stratified and thick.

Spermatogenesis and spermatogenic cells: all cells of spermatogenic lineage are present and finely stratified. Spermatogenesis and spermiogenesis appears normal.

DISCUSSION

The general increase in weight in the treated groups is probably due to their androgenic properties since androgens possess anabolic activities (Johnson and Everitt, 1988).

Testicular weights of the treated groups increase significantly when compared with the control. This increase is not too far from the value of the control.

Those fed with *ginger* has increased serum testosterone level due to gingerol contained in it and follows claim made by Kamtchouing et al., 2007 and Amir Amin et al., 2007 that both ethanolic and aqueous extracts of ginger increases serum testosterone level, testicular cholesterol level and activity of testicular antioxidant enzymes

The *ginger* treated group histology of the testis appears normal with the presence of spermatids and spermatozoa. Ginger extracts have been extensively studied for a broad range of biological activities especially antioxidant activities (Ahmed et al., 2000) found in ginger; that significantly lowers lipid peroxidation by maintaining the activities of the antioxidant enzymes- super oxide mutase, catalase and glutathione peroxidases in rats. Testicular damage is as a result of improper balance between ROS generation and scavenging activities. Excessive production that exceeds critical level overpowers all antioxidants defense strategies in the testis causing oxidative stress (Amr and Hamza, 2006).

In this investigation, administration of 200mg/kg of aqueous ginger for 28 and 56 days respectively significantly increase serum testosterone level as

compared with the control. The testicular histology shows no abnormality. Spermatogenic cell lineage is normal containing all cell in the lineage. This follow postulates that ginger have potent androgenic and antioxidant effects on the testis (Zancan et al., 2002; Sekiwa et al., 2006; Amr and Hamza, 2006 and Kamtchouing et al., 2006). In conclusion, this study has demonstrated that ginger has a strong antioxidant and androgenic potentials at dose of 200mg/kg. It also has no deleterious effects on spermatogenesis in the testis. Hence has strong fertility property.

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