Full Length Research Paper

# Effects of long and short term treatment of aqueous extract of ginger (*Zingibier Officinale*) on male reproductive function of adult Sprague dawley rats

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Abstract

The objective is to investigate the effects of the long and short term treatment of ginger (zingiber officinale) on male reproductive function of adult Sparague Dawley rats and study the mechanisms underlying these effects. 30 adult male Sparague - Dawley rats weighing between 120g -150g were used. They were divided into 3 groups. The control group and treated group. The treated groups received 200mg/kg of extracts for 4 and 8 weeks respectively. Extract was given via gastric intubation. The controls as well as treated animals were allowed free access to pelleted feed and water ad libitum. The animals were sacrificed. Testicular weight was taken and caudal epididymis excised for semen and biochemical analysis. There was a significant increase in the body as well as testicular weights in the treated animals as compared to the control (P<0.05). There was also a duration dependent increase in serum testosterone level as well as sperm count and motility (P<0.05). 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. These results stipulate that extract of ginger (Zingibier Officinale) has fertility properties; because it stimulates secretion from the testes (and other male accessory sex organs) that influences semen quality. It also has potent antioxidant properties. 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 eminent then it could be dose - dependent or duration dependent.

Keywords: Testosterone, azoospermic, teratozoospermic, androgenic, zingiber officinale.

# INTRODUCTION

Ginger belongs to the family *Zingeraceace*. A monocotyledon perennial plant, cultivated for is rhizome; the part used for its culinary and medicinal purposes. 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 the lvory Coast, ginger is ground and mixed with orange, pineapple and lemon to produce a juice called

Nyamanku. 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. Crystallized Ginger is imported from Australia. During the 15th century, gingerbread became a gift of love and respect. In the 1800's, Ginger was commonly sprinkled on top of beer or ale, and then stirred into the drink with a hot poker - thus the invention of gingerale (McCormick, 2005 McCormick Spice Encyclopedia). 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

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Figure 1. Ginger Rhizomes

Africa, and the Caribbean (Lister, 2003).

It is made up of phytocompounds such as zingerone, gingerdiol, zingibrene, shogoals and *gingerol* (Zancar *et al.,* 2002 and Sekiwa et al., 2000). It active component is *gingerol* which act as an anti- oxidant and is responsible for its taste (Witchl, 2004).

Medicinal properties of ginger are numerous due to its active component. It is general known to be use as a culinary in making ginger tea, as well as a spice in food. It acts as a stimulant i.e ginger has a sialagogue action; stimulating the production of saliva, (Chaiyakunaprak *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 Chaiyakunaprak *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  $\alpha$ -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 Amin *et al.*, 2006).

## MATERIALS AND METHODS

#### **Experimental Animals**

35 adult male Sparague - Dawley rats weighing between 120 g -150 g were used. The 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 (1g) and macerated in 50ml of distilled water. Final extract concentration obtained is 20mg/ml. Extract was stored in air- tight container and refrigerated throughout the cause of the experiment. 
 Table 1. Experimental design procedure

| Group N= 5  | Treatment                                    |  |  |  |
|-------------|--|--|--|--|
| A (control) | Given only feed and water ad libitum         |  |  |  |
| В           | Given 200 mg/kg of aqueous extract of Ginger |  |  |  |
| С           | Given 200 mg/kg of aqueous extract of Ginger |  |  |  |

#### **Experiment Duration**

This is a short- term (28days) 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 (Table 1). Administration was done once daily in the morning (between 7.30a.m - 9.00a.m)

## **Animal Sacrifice**

The animals were given last dose of extracts, weighed and serially 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.

#### Semen Analysis

The caudal part of the epididymis is excised and transferred into normal saline for about 3 minutes for spermatozoa to swim out after which a drop of saline is transferred to the Neubauer Counting Chamber (haemcytometer) for semen analysis under the light microscope. Semen analysis for each group was done as well as sperm count sperm count according to the recommended protocol of the WHO manual (WHO, 1999).

#### Serum testosterone assay

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.

# Sperm Motility

Sperm motility was access using the WHO classification system. Each sample was assessed twice. For consistency all readings were carried out at 37°C (WHO, 1999).

# **Organ Weight**

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

# **Statistical Analysis**

The data obtained were complied and 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).

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

| Group n=5          | Initial<br>weight(g) | Final weight(g) | Weight<br>difference(g) | %<br>increase | Testicular weight<br>(g) |
|--------------------|----------------------|-----------------|-------------------------|---------------|--------------------------|
| Control            | 123.55±1.30          | 1.62±2.05       | 38.90                   | 31.49         | 0.90±0.10                |
| Ginger (200 mg/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 56 days

| Group n=5          | Initial<br>weight(g) | Final<br>weight(g) | Weight<br>difference(g) | %<br>increase | Testicular<br>weight (g) |
|--------------------|----------------------|--------------------|-------------------------|---------------|--------------------------|
| Control            | 122.95±6.25          | 204.00±4.30        | 81.05                   | 65.92         | 0.90±0.10                |
| Ginger (200 mg/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<br>(days) | Serum testosterone<br>level(ng/ml) | Treatment (days) | Serum testosterone<br>level(ng/ml) |
|--------------------|---------------------|------------------------------------|------------------|------------------------------------|
| Control            | 28                  | 1.49±0.01                          | 56               | 1.56±0.02                          |
| Ginger (200 mg/kg) | 28                  | 1.87±0.01                          | 56               | 1.98±0.02                          |

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

Table 5. Sperm count and motility after treatment (semen analysis)

| Group n=5          | Treatment<br>(days) | Sperm counts<br>(millions/ml) | Sperm<br>motility (%) | Treatment<br>(days) | Sperm counts<br>(millions/ml) | Sperm motility<br>(%) |
|--------------------|---------------------|-------------------------------|-----------------------|---------------------|-------------------------------|-----------------------|
| Control            | 28                  | 192.00±5.01                   | 59.00±2.89            | 56                  | 216±0.01                      | 66.20±2.50            |
| Ginger (200 mg/kg) | 28                  | 196±1.22                      | 64.98±2.20            | 56                  | 223±0.01                      | 70.01±2.67            |

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

The caudal epidiymal sperm count and motility were significantly increased (P<0.05) after the short and long term treatment as compared with the controls. (Table 5).

#### DISCUSSION

The result of this investigation suggested that Zingiber Officinale has beneficial effects on male reproductive functions/ parameter in adult rats. This inference is confirmed by the increased sperm count, serum testosterone level, sperm motility and normal sperm morphology seen.

The increase testicular weight is traced to increase in androgen biosynthesis. Androgen have been shown to be important for the development, growth and normal functioning of the testis, studied have shown that androgenic plant have increased testis weight and accessory reproductive organs(Prins et al., 1991). The general increase in weight in the treated groups is due to their androgenic properties since androgens possess anabolic activities (Johnson and Everitt, 1988).

Increase in testicular weight as well as serum testosterone level follows report made by Kamtchouing et al., who observed an increase in testicular weight after 8days 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 spermatogenic activity is also evident in the production of morphologically normal spermatozoa due to the increase in the testosterone level. Since testosterone is important in the production of spermatozoa (Zhang et al., 2001). According to Morakinyo *et al.*, 2008, extracts of ginger has pro- fertility potential in male rats. The increase in sperm count and fertility following treatment with 200mg/kg of aqueous extract of ginger improves semen quality and enhances fertility potential of semen. Low sperm count, motility and high percentage of abnormal spermatozoa level have been associated with reduced fertility (Raji *et al.*, Adeeko and Dada, 1998).

# CONCLUSION

In conclusion, the aqueous extract of ginger at 200 mg/kg body weight given for short time and long term basis has beneficial effects based on this study. The fertility properties showcased is due to its potent androgenic and antioxidant activities. This investigation however is subject to further research; if it possesses any effects to male reproduction parameter which might probably be dose- dependent.

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