

# THE EFFECT OF ZINGIBER OFFICINALE (GINGER) ROOT ETHANOLIC EXTRACT ON THE SEMEN CHARACTERISTICS OF ADULT MALE WISTAR RATS

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## ABSTRACT

**Background:** Ginger has been shown to have a positive effect on the function of the male reproductive organ. Studies have shown that ginger possess antidiabetic, antimicrobial, antioxidant, anticholestereolemic, anticancerous, antiemetic, anti-rhinoviral, anti-inflammatory and anti-insecticidal properties. Dietary patterns in Africa especially Ghana are characterized by a high consumption of ginger. However, there is very little information on the effects of ginger on semen characteristics.

**Aims:** The study was designed to investigate the effects of ginger on the semen parameters of the male rats using quantitative and qualitative methods.

**Materials and Methods:** Forty-eight male wistar rats were divided into four groups designated as control, A, B and C and administered daily by gavage with 1 ml distilled water, 100 mg/kg, 300 mg/kg and 500 mg/kg of ethanolic ginger extract respectively for 30 days.

**Results and Discussion:** The present study showed that there was a numerical increase in sperm count, sperm morphology, sperm viability and sperm motility in the extract-treated rats in a dose dependent manner which was statistically significant ( $p < 0.05$ ).

**Conclusion:** We concluded that ginger extract may be potentially useful in the management of male infertility especially those with low sperm count.

**KEY WORDS:** Ginger, Semen, Sperm count, Rats, Infertility.

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## INTRODUCTION

Plant-based traditional medicines are still of vast significance to people living in the developed and developing countries to prevent and cure diseases [1,2]. According to the World Health Organization (WHO) and other research works,

80% of most Asian and African countries use herbal medicine for primary health care [3-5]. This is because herbal medicine offers the advantages of easy accessibility, availability and affordability as compared to the conventional drugs.

Child bearing is of great value and respect in most African communities hence, there is yet to be identified a condition more demoralizing to man's personality than infertility. It overpowers his very essence of masculinity [6].

Infertility is a major health issue worldwide and has been estimated that about 30% of infertility cases may be due to a male factor [7].

There is increasing evidence to suggest that sperm counts have declined over the last 50 years resulting in a consistent increase in male infertility [8] and therefore there is the need to research in order to reverse this trend.

One medicinal plant commonly used for herbal preparations in Africa is ginger (*Zingiber officinale* Roscoe). It is mostly used as a dietary spice condiment and natural medicine in most parts of the world [9,10]. In Ghana, ginger roots are used in the preparation of drinks such as ginger tea, "Soobolo", a local drink, and "Amuduro" also a local drink, "Hausa Koko", a local porridge and also used in almost all staple foods especially in spicing fresh fish and meat. In Nigeria, ginger is used commonly in traditional medicine preparation, and used as spice in most of their local delicacy including tea, and serves as a key ingredient in "Zobo" a local drink. Ginger has been demonstrated to have beneficial therapeutic effect in the treatment and management of several diseases such as male infertility, cancer, ulcer, common cold, headaches, flu-like symptoms, rheumatism, menstrual pains, toothache, osteoarthritis, orchiditis, bronchitis and diarrhoea [11].

Several studies have speculated that ethanolic ginger extract can increase testicular weight and also influence the growth and function of the accessory reproductive organs [12].

In addition, other investigators have reported that ginger causes an increase in testosterone levels [13-15]. Furthermore, ginger has been shown to affect spermatogenic activity in the testes of male rat. However, there is very little information on the effect of ginger on semen characteristic analysis of the male testes.

Based on the broad usage of ginger in the local dishes, beverages and herbal preparations in Ghana, the present study was designed to investigate the possible effects of ginger on the

structure of the semen parameters of male rats (*Rattus norvegicus*) using qualitative and quantitative methods. Specific objective was to determine the effects of ethanolic ginger extracts on the sperm morphology, motility, viability and sperm count in the male rats.

## MATERIALS AND METHODS

The study was conducted from February 2015 to May 2016 at the Kwame Nkrumah University of Science and Technology on Wistar male rats (*Rattus norvegicus*).

*Zingiber officinale* (ginger) was classified and authenticated in the herbarium at the Department of Theoretical and Applied Biology at the Kwame Nkrumah University of Science and Technology. Ethical approval was sought from the Committee on Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences and the Komfo Anokye Teaching Hospital in Kumasi, Ghana. Fresh ginger rhizomes weighing 4.5 kg was prepared, sun-dried for two weeks, ground into powder (243.3g) and dissolved completely in 1500 ml of 70% ethanol and kept at room temperature for 48 hours. The filtered mixture was concentrated using a rotary vacuum water vapour (Rotavapor R-215, BUCHI Labortechnik AG, Fawil, Switzerland) at a temperature of 50°C. A syrup mass was collected in a porcelain bowl and kept in an electric hot air oven for five days to ensure complete dryness at 40 °C. The solid mass (20.0g: 8.22%) extract of ginger was used to prepare three different aqueous extracts (100 mg, 300 mg and 500 mg). These concentrations were prepared by dissolving the solid extract with distilled water: 2.5 g/1ml, 7.5 g/ml and 10 g/ml respectively and kept in a refrigerator at 4-8 °C.

A total of 48 healthy 12-week old adult male Wistar rats (*Rattus norvegicus*) with an average weight of  $254 \pm 31.05$ g (200g - 300g) were procured for this study. The rats were randomly divided into 4 groups with 12 rats per group. The experimental groups were designated as control, A, B and C. The rats were acclimatized for one week prior to the study.

The rats were accommodated in temperature regulated housings (25°C) with continuous humidity (40 to 70%) and 12 hour light and dark

cycle preceding the research practices at the animal house. All rats were fed with standard diet and water and were treated according to the Principles of National Institute of Health Guidelines for Care and Use of Laboratory animals.

The control group (CG) for the experiment were given 1 ml of distilled water. Treatment groups A, B and C received 100 mg/kg, 300 mg/kg and 500 mg/kg body weight of ethanolic ginger extract respectively. The animals were given a single oral daily dose of 0.6 ml/200g body weight by gavage for thirty (30) consecutive days.

After the four weeks, all the animals in groups Control, A, B and C were anaesthetized using chloroform and dissected to remove the epididymis after which the animals were euthanized. The covering fat, blood vessels and connective tissues were removed and the organ from each animal was placed in a beaker containing normal saline solution.

The epididymis was transferred onto a Petri dish and the sperms were ejected by making a longitudinal incision of the cauda epididymis with a pair of fine-pointed scissors and squeezing with forceps to release the sperms from the duct of the epididymis. The semen was diluted with 10  $\mu$ L of diluent. The diluent was prepared with 100 ml of distilled water, 50 g of sodium bicarbonate ( $\text{NaHCO}_3$ ) and 10 ml of 35% (v/v) formalin. Using a Pasteur pipette, 5  $\mu$ L of the homogenate was diluted with 95  $\mu$ L of diluent to give a 1 in 20 dilution.

Sperm cell count was carried out using the Bearden and Fuquay method (Bearden and Fuquay, 1980 [16]). Haemocytometer (Weber Scientific International Ltd., England) with improved double Neubauer ruling was used for the evaluation of spermatozoa under a light microscope (Leica DMD 500, Germany). An aliquot of the final solution was then dropped onto the improved Neubauer Haemocytometer. To ease counting, the loaded haemocytometer was positioned in a humid place for about 5 minutes so that the sperms head may relax to the same focal plane. The quantity of sperm heads were enumerated with the help of a light microscope (Leica DM EBasic, Germany). The average counts of four haemocytometer cham-

bers were recorded. The sperm count of each cauda epididymis was calculated using the formula:

$$\text{Sperm number} = \text{Cm} \times \text{F} \times \text{V}$$

Where Cm = mean count, F= dilution factor, V = Volume of counting chamber.

The data were expressed as the  $\times 10^6$  spermatozoa per ml ( $\times 10^6$  cell/ml) [17]. The results were recorded.

The liquefied well mixed semen was smeared on a glass slide and air dried overnight at room temperature. The slide was fixed in 4% formalin to clear any mucus detected, stained with Aniline Blue (pH 3.5) and Eosin [18], and then counted under the light microscope using a magnification of X40. The preparation was then examined for abnormal and normal spermatozoa. Under the light microscope the spermatozoa were classified as normal and abnormal. Normal spermatozoa had an oval-shaped head, a short middle piece, and a long thin tail whereas the abnormal spermatozoa had either no head, middle piece, and/or tail.

One drop of a suspension of epididymal semen was fixed on a glass slide (ASI™ Frosted Glass Microscope Slides, USA) and covered with a cover slip (MS-SLIDCV, USA). Using X40 objective lens of a light microscope (Leica DMD 500, Germany), several fields were scanned for sperm motility. A total of 200 spermatozoa were counted and the percentage; sperm motility was reported as either linear progressive motility, sluggish progressive motility or immotile sperm [7].

A drop of diluted semen was stained with 0.5% eosin-nigrosin using the Barth and Oko method [19]. The seminal smears were evaluated for sperm viability by determining the relative proportion of live and dead sperms. The proportion of live sperms were expressed as a percentage. Viable spermatozoa were unstained whereas non-viable spermatozoa stained red [20].

#### Statistical Analysis:

The data were analysed with Microsoft excel version 2013 and IBM Statistical Package for Social Sciences (SPSS) version 20.0. A p-value < 0.05 was considered significant at a confidence interval of 95%.

**Table 1:** The effect of the ethanolic extracts of *Z. officinale* on semen characteristics of control and experimental groups 100mg/kg, 300mg/kg and 500mg/kg in the rats for 30 days.

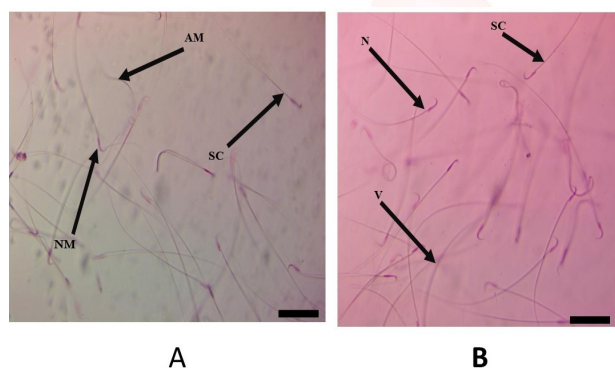
CONCENTRATION OF <i>Z. OFFICINALE</i>	CONTROL (0 mg/kg)	GROUP A (100 mg/kg)	GROUP B (300 mg/kg)	GROUP C (500 mg/kg)	P VALUE
SPERM COUNT ( $\times 10^7$ cell/ml)	9.04 $\pm$ 1.95	8.54 $\pm$ 0.78	10.02 $\pm$ 1.24	13.4 $\pm$ 1.34	0.001 <sup>c,e,f</sup>
SPERM VIABILITY (%)					
Viable	65 $\pm$ 5.48	68 $\pm$ 7.48	74 $\pm$ 8.60	83 $\pm$ 6.00	0.01 <sup>c,e</sup>
Non-Viable	35 $\pm$ 5.48	32 $\pm$ 7.48	26 $\pm$ 8.60	17 $\pm$ 6.00	0.01 <sup>c,e</sup>
SPERM MORPHOLOGY (%)					
Normal Morphology	65 $\pm$ 4.47	71 $\pm$ 6.63	75 $\pm$ 6.23	80 $\pm$ 6.32	0.02 <sup>b,c,e</sup>
Abnormal Morphology	35 $\pm$ 4.47	29 $\pm$ 6.63	25 $\pm$ 6.32	20 $\pm$ 6.32	0.02 <sup>b,c,e</sup>
SPERM MOTILITY (%)					
Linear Progressive Motility	59 $\pm$ 6.63	64 $\pm$ 7.35	69 $\pm$ 6.63	76 $\pm$ 5.83	0.01 <sup>b,c,e</sup>
Sluggish Motility	25 $\pm$ 4.47	15 $\pm$ 4.47	18 $\pm$ 7.48	15 $\pm$ 4.47	0.06 <sup>a,c</sup>
Non-Motile	16 $\pm$ 8.00	21 $\pm$ 4.90	13 $\pm$ 4.00	9 $\pm$ 2.00	0.03 <sup>d,e</sup>

Data are presented as group means  $\pm$  SD (standard deviation) Experimental groups significantly different from control:

P < 0.05, One-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test) a = control versus A; b= control versus B;

c = control versus C; d=A versus B; e= A versus C; f= B versus C. There were 12 animals in each group.

**Fig. 1:** Light micrographs of the semen showing the sperm cell (SC), normal sperm morphology (NM), abnormal sperm morphology (AM) in A and viable sperm (V) non-viable sperm (NV) in B; (Staining: Eosin-nigrosin. Bar represents 10 $\mu$ m).



**Effect of ethanolic ginger extracts on sperm morphology:** For each group, the morphology of two hundred sperms were counted and classified as either normal sperm morphology or abnormal sperm morphology based on the presence or absence of a head, middle piece and tail (figure 1A). The mean number of sperms with normal morphology observed in the male rats were 65  $\pm$  4.47, 71  $\pm$  6.63, 75  $\pm$  6.32 and 80  $\pm$  6.32 for the control and treatment groups A, B, C respectively. Administration of ginger extract significantly increased normal sperm morphology (p = 0.02) in a dose-dependent manner in the treated rats compared with the control group (Table 1).

The mean number of sperms with abnormal morphology observed in the male rats were 35  $\pm$  4.47, 29  $\pm$  6.63, 25  $\pm$  6.32 and 20  $\pm$  6.32 for the control and treatment groups A, B, C respectively (Table 1). The treated animals also demonstrated a significant decrease in abnormal sperm morphology (p = 0.02) compared with the control group in a dose-dependent manner.

**Effect of ethanolic ginger extracts on sperm viability:** Out of the two hundred sperms counted, viability was classified as either viable sperm or non-viable sperm based on the presence of their ability to pick the eosin-nigrosin stain or vice versa (viable remain colourless whereas non-viable are stained) Figure 1A. The mean viable sperms in the rats were 65  $\pm$  5.48, 68  $\pm$  7.48, 74  $\pm$  8.60 and 83  $\pm$  6.00 for the control and treatment groups A, B, C respectively. Administration of the ginger extract significantly increased viable sperm count (p = 0.01) in a dose-dependent manner in the treated rats compared with the control group (Table 1).

With respect to non-viable sperms, the mean observed in the male rats were 35  $\pm$  5.48, 32  $\pm$  7.48, 26  $\pm$  8.60 and 17  $\pm$  6.00 for the control and treatment groups A, B, C respectively. Indicating a significant decrease in the number of non-viable sperms (p = 0.01) compared with



the control group (Table 1).

**Effect of ethanolic ginger extracts on sperm counts:** In table 1, administration of the ginger extract significantly increased epididymal sperm count ( $p = 0.001$ ) in a dose-dependent manner in treatment rats B and C but decreased in treatment group A compared with the control group. The mean sperm count were  $9.04 \pm 1.95$ ,  $8.54 \pm 0.78$ ,  $10.02 \pm 1.24$ ,  $13.4 \pm 1.34$  for the control, group A, B and C respectively.

**Effect of ethanolic ginger extracts on sperm motility:** Out of the two hundred sperms counted for each group, sperm motility was classified as linear progressive motility, sluggish progressive motility and non-motile sperm. As shown in Table 1, the extracts significantly increased ( $p = 0.01$ ) linear progressive motility in a dose-dependent manner as compared to the control group.

In contrast, the extract-treated animals exhibited no significant decrease in sluggish motility ( $P > 0.05$ ) compared to the control group (Table 1). The mean number of non-motile sperms in the male rats were  $16 \pm 8.00$ ,  $21 \pm 4.90$ ,  $13 \pm 4.00$  and  $9 \pm 2.00$  for control and treatment groups A, B, C respectively. Compared to the controls, there was an increase in the number of non-motile sperms in treatment group A and a significant decrease ( $p = 0.03$ ) in treatment groups B and C (Table 1).

## DISCUSSION

**Effect of ethanolic ginger extract on sperm characteristics of the rats:** The results of the present study showed a statistically significant increase in sperm count, motility, viability and normal morphology of the sperm in the extract-treated animals compared to the control group. These might be due to improved spermatogenesis by the ginger extract which is believed to have biologically active agents including androgenic, antioxidant, anticancer, anti-inflammatory and antimicrobial effects [21]. It has also been reported that ginger exhibits protective effects against oxidative stress in rats [22].

A positive feedback effect results in a higher release of Gonadotropin releasing hormone (GnRH) by the hypothalamus, and a subsequent release of Interstitial cell stimulating hormone

(ICSH) by the anterior pituitary [23]. The resultant effects cause an increase in testosterone levels and secretion by the gonads resulting in a rise in sperm count [13]. However, Ochsenkühn and De Kretser [24] reported that lower levels of testosterone may result in a decrease in sperm count. In addition, high levels of testosterone stimulate myoid cells to increase peristaltic movement resulting in the release of spermatozoa [25]. Even though the blood serum testosterone levels were not estimated in this study, it has been well documented that an increase in blood testosterone level results in an increase in spermatogenesis [26].

The significant increase in sperm count in the treated groups observed in the present study is in agreement with the findings of previous studies [17-20]. Waleed and Wisam [27], reported that there was a significant increase in sperm count by 16.2% in infertile men after treatment with ginger. Similarly, in the present study, sperm count increased from 12.8% to 48.2% when 300 mg/kg and 500 mg/kg of ginger extract were given. It is possible that the ginger extract regulates the production of Reactive Oxygen Species (ROS) and improves sperm parameters such as sperm morphology, viability, motility and thereby increase reproductive efficiency of the rats.

A significant increase ( $p = 0.018$ ) in normal sperm morphology was observed in the treated male rats. This finding is in line with the observations of previous studies which reported a significant ( $p < 0.01$ ) rise of 18.4% in normal sperm morphology of infertile men after treatment with ginger [27,28]. With respect to abnormal sperm morphology, the present study observed a significant decrease in the treated groups in a dose-dependent manner. Abnormal sperm morphology could be due to oxidative stress imposed by higher levels of free radicals of ROS in the absence of ginger. This observation is also in line with the findings of Dalia [20] who reported a significant decrease in abnormal sperm morphology in ginger-treated rats. The increase in progressive sperm motility of treated groups observed in the present study could be due to the counter oxidative stress of ginger on sperm production. This is in agreement with the findings of earlier studies

[20,22,28,29]. Sperm motility is as a result of ATP, produced by breakdown of fructose secreted by the seminal vesicle [30]. Ginger might play a role in the normal functioning of the seminal vesicle in increasing ATP production [34]. Sperm motility also depends on the coordinated propagation of flagella wave under acetyl cholinesterase control during sperm maturation [31,32]. In addition, increase in sperm motility may be due to the positive feedback from the functions of the seminal vesicle, epididymis and sperm acetyl cholinesterase activity by the ginger extract [33]. Arash *et al* [28] reported that on the administration of ginger extract, there was a significant increase in sperm motility in male rats. According to a study by Dalia [20] oral administration of ginger extract at 250 mg/kg and 500 mg/kg body weight to diabetic induced rats for 65 days increased sperm motility suggesting that ginger may have antidiabetic activity. Amr and Hamza [22] also established that *Z. officinale* treatment caused an increase in the activities of testicular antioxidant enzymes and restored sperm motility of cisplatin-treated rats.

In the present study, there was a significant increase in sperm viability in the extract-treated male rats. This finding is consistent with the reports of previous studies in which administration of ethanolic ginger extract to normal and diabetic rats for 20 consecutive days caused an increase in sperm viability [15,20,27,28]. In humans, it has been reported that low sperm count, malformed spermatozoa, and/or reduced or deficient motility are the main causes of the increase in male infertility [34,35]. Therefore, the use of ethanolic ginger extract may offer an accessible, affordable alternative for the treatment and management of infertility.

## CONCLUSION

In this study, administration of ethanolic ginger extract for 30 consecutive days to male albino rats resulted in a significant increase in sperm count, motility, viability and morphology. We therefore believe that, ginger extract may be potentially useful in enhancing healthy sperm characteristics and the management of male infertility especially in those with low sperm count.

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## Conflicts of Interests: None

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