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Beneficial Effect of Ginger Aqueous Extract on Some Reproductive Functions in Male Rabbits

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Abstract: The aim of this study was to assess the effect of ginger on the fertility of male rabbits, in order to get benefit of its useful effect on semen quality and the reproductive performances. Twenty adult male New Zealand white rabbits (5-6 months old) and weighing (3-3.5 kg) were used. The bucks were divided into two equal groups; group A that kept as control (n = 10) and group B (n = 25) where bucks subjected to oral administration of ginger aqueous extract in a dose of 200 mg kg^{-1} daily for 4 weeks. Both groups were kept under standard conditions and allowed free access to pelleted ration and water ad libitum. At the end of the experiment, semen was collected for analysis of; ejaculate volume, sperm motility, count and abnormalities. In addition, sera were used for determination of testosterone level, Total Antioxidant Capacity (TAC) and Malondialdehyde concentration (MDA). Histological examination of testes and epididymis from both groups was performed. The obtained results revealed that ginger treated bucks showed; no change in ejaculate volume, a significant increase in the individual sperm motility, sperm count with a significant decrease in the percentage of abnormal spermatozoa, a significant increase in testosterone level, TAC with a significant reduction in MDA concentration in ginger as compared with the control. The histological examination of testes and epididymis showed no abnormalities in both groups with accumulation of the spermatozoa in the lumen of the seminiferous tubules, increased epithelial cells height of the epididymis with overcrowded lumens with sperms in ginger treated bucks. It could be concluded that ginger has a positive effect on the male fertility which could be attributed to the antioxidant and androgenic activity of ginger.

INTRODUCTION

Infertility is considered one of the important problems of human society where it is influenced by many environmental, behavioral, genotoxic and genetic factors causing impaired spermatogenesis at various stages and male infertility[1].

Several chemical drugs were used to treat infertility but some had a side effect, so, the researchers are looking for using drugs with less adverse effects and toxicity[2].

Recently, the herbal medicine and the medicinal plants are used for the treatment of various diseases as it is effective, inexpensive, safe and available. Moreover, they have a great antioxidant activity that can scavenge free radicals and have appositive effect on spermatogenesis[3].

Ginger (*Zingiber officinale*) is a plant belongs to family Zingiberaceae where it is considered one of the most widely used spices for food, in addition, it has a long history of medicinal use in Chinese traditional medicine[4]. The plant is cultivated in South-East Asia, West Africa and Caribean where China and India are considered the main sources of it.

The most important constituents of ginger are gingerols, zingibrene, protodioscin, schogaols, saponins and gingerdiol[5]. The major therapeutic effects and uses of ginger include relieve of nausea and vomiting accompanied pregnancy, surgery and motion sickness[6], anti-inflammatory, anti-hepatotoxic, antithrombotic, antiemetic, cholagogue and antioxidant[7]. The anti-oxidant content of the herbal medicine is important for enhancing the anti-oxidant defense and reducing the oxidative state similar to other natural anti-oxidant as vitamins A, C, E which can protect DNA damage and other important molecules from oxidation and damage causing improvement of sperm quality and the fertility rate in men[8]. The favorable effect of ginger on the male fertility could be attributed to the anti-oxidant[9] and androgenic activity[10]. The main anti-oxidant components in ginger are; gingerol which is responsible for its taste[11], shogaols and some phenolic ketone derivatives[12].

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Several researches suggested that ginger improved the reproductive performance through increasing sperm motility, viability, testosterone concentration with a decrease in the Malondialdehyde (MDA) and so, decrease lipid per-oxidation[13-15]. Moreover[16], suggested that the protodioscin and saponins of ginger could enhance testosterone and Luteinizing Hormone (LH) level and libido which was important for treatment of sexual dysfunction in the traditional medicine. In addition, Sabik *et al*.[17] found in men that ginger could increase sexual potency, the levels of estrogen, pregnenolone and testosterone. The present study was planned to study the effect of ginger on some reproductive functions in male rabbits as represented by its effect on semen parameters, testosterone, MDA concentration and Total Anti-oxidant Capacity (TAC) in addition to histological examination of testes and epididymis.

MATERIALS AND METHODS

The ethical approval was taken from the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Experimental animals: A total twenty adult male New-Zealand white rabbits (5-6 months old) and weighing (3-3.5 kg) were selected for this study. The bucks were individually housed in galvanized wire batteries and maintained under identical hygienic conditions throughout the experiment. The study was performed at the experimental building unite, Faculty of Veterinary Medicine, Zagazig University. Fresh water was available ad libitum and the animals were fed a commercial balance pelleted ration containing 18.43% crude protein, 12.7% crude fiber, 2% fat and 2502 kcal ME/kg diet according to NRC[18].

Experimental design: The bucks were divided into two equal groups; group A that kept as control (n = 10) and group B (n = 10) where bucks subjected to oral administration of ginger aqueous extract in a dose of 200 mg kg^{-1} daily for 4 weeks using a stomach tube.

Preparation of ginger aqueous extract: The ginger rhizomes were obtained from the local markets, cleaned, cut into small pieces, sun dried and crushed into powder. About 1 g of the dried powder was macerated in 50 mL distilled water where the final concentration of the extract was 20 mg mL^{-1}. The extract was then kept throughout the experiment in air-tight container in the refrigerator[15].

Sampling

Semen: Semen was collected from the animals by using artificial vagina after training of the bucks during a preliminary period of 3 weeks using a female rabbit as a teaser according to Breederman *et al*.[19]. The semen was immediately evaluated after collection.

Semen analysis: Ejaculate volume was assessed visually using graduated collecting tubes after removal of the gel mass.

Individual motility was expressed by the percentage of spermatozoa showing progressive forward motility; one

drop of semen was diluted two folds by sodium citrate 2.9% on a clean warm slide with a cover slip placed over it, then examined under a high power (X_{40}) according to Bearden and Fuquay[20].

Sperm cell concentration: sperm cells were counted by using a hemocytometer slide and expressed in million mL^{-1} according to the method described by Hafez[21].

Sperm abnormalities: The percentage of abnormal spermatozoa was determined in eosin-nigrosin stained smears according to Bearden and Fuquay[20].

Serum preparation for measurement of testosterone, TAC and MDA concentrations: Blood samples were collected from the ear vein into clean tubes without anticoagulant. The blood was allowed to clot at room temperature for 20-30 min and then centrifuged at 3000 rpm for 15 min. The sera were kept at 20°C untilused.

Measurement of serum testosterone level: Serum testosterone concentration was carried out by using; testosterone Enzyme Immunoassay (EIA) DSL-10-4000 kit obtained from Diagnostic Systems Laboratories Inc. According to Burtis and Ashwood[22].

Determination of total antioxidant capacity level: Serum TAC which is defined as the amount of anti-oxidants required to make absorbance increase 0.01 mL of serum was measured by the reaction of phenanthroline and (Fe^{2+}) at 37°C by using a spectrophotometer at 520 nm according to Feng *et al*.[23]. The principle of this test depends on antioxidant defense system can reduce Fe^{3+} to Fe^{2+}.

Determination of lipid Peroxidation (Malondialdehyde level): Serum MDA concentration was assessed by using a commercial kit obtained from Diagnostic Systems Laboratories Inc. It is based on the colorimetric reaction with Thiobarbituric Acid (TBA) to form pink colored product in acidic medium (pH 2-3) and at temperature 90-100°C for 15 min. The pink colored product can be measured by spectrophotometer at 532 nm, according to Satoh[24-26].

Histological examination: The testis with the epididymis were fixed in 10% formalin, then processed by standard paraffin methods, dehydrated in a series of graded concentration of ethyl alcohol, cleared in xylol, embedded in melted paraffin at 55-60°C, then sectioned into five-microns thick sections and stained with Hematoxylin and Eosin (H&E), then examined under light microscope[27].

Statistical analysis: The obtained data were statistically analyzed by using (t-test) according to Tamhane and Dunlop[28]. The results were expressed as means±SEM (Standard Error of Means). Significant difference was expressed as parentheses. p-values 0.05 were considered significant.

RESULTS AND DISCUSSION

The obtained results are illustrated in Table 1 and 2 where there was no change in the ejaculate volume between both groups.

Concerning, the individual sperm motility and sperm count group B showed a significant increase in sperm motility and sperm count (83.4±1.28%; 246.8±6.79 million/mL, respectively) as compared with group A (70.6±2.24%; 198.2±2.43 million mL^{-1}, respectively), in addition, the percentage of abnormal spermatozoa showed a significant decrease (12.4±0.50%) as compared with group A (21.2±0.73%) as mentioned in Table 1.

Regarding, the serum parameters mentioned in Table 2; group B (ginger treated bucks) showed a significant increase in testosterone level (6.29±0.63 ng mL^{-1}) as compared with group A (3.45±0.68 ng mL^{-1}), a significant increase in TAC (3.79±0.29 mmol mL^{-1}) and a significant reduction in MDA concentration (0.71±0.02 mmol mL^{-1}) as compared with group A (5.04±0.39 mmol mL^{-1}; 0.56±0.57 mmol mL^{-1}, respectively).

Results of Histological examination of testes and epididymis: The obtained results of this study suggested that gingerhas a favorable and beneficial effect on the male fertility in adult rabbits. This effect is confirmed by the increase in the sperm functions (motility and count), serum testosterone and total antioxidant capacity with the reduction in the percentage of abnormal spermatozoa and malondialdehyde concentration. This effect could be attributed to the potent androgenic, protective and antioxidant effects of ginger[29]. Concerning, the effect of ginger on semen parameters, the results showed no change in the ejaculate volume between both groups but there was a significant increase in the sperm motility and count with a significant reduction in sperm abnormalities in ginger treated group (group B) as compared with group A (Table 1 and Fig. 1). The results are supported by the finding of Morakinyo *et al*.[16], who showed that ginger extract increased the sperm motility and count in a dose and duration dependent manner. Furthermore, Khaki *et al*.[14] found that ginger increased sperm motility, count, viability, serum testosterone with a decrease in MDA concentration in a dose 50-100 mg kg^{-1} rat for twenty days. Moreover, Hafez[30], revealed that ginger extract could increase the sperm functions (motility, viability and count) with a decrease in the sperm

Table 1: The overall means of semen parameters of adult male New-Zealand white rabbits supplemented with ginger aqueous extract 200 mg/kg

Sperm parameters	Group A (n = 10)	Group B (n = 10)	p-value (t-test)
Ejaculate volume (mL)	0.47±0.01	0.49±0.004	0.13
Sperm motility (%)	70.6±2.24	83.4±1.28**	0.001
Sperm count (million/mL)	198.2±2.43	246.8±6.79**	0.00
Sperm abnormalities (%)	21.2±0.73	12.4±0.50**	0.00

Data is expressed as mean±SEM *p<0.05; ** p<0.01

Table 2: The effects of ginger aqueous extract 200 mg/kg on serum testosterone- MDA and TAC in adult male New-Zealand white rabbits

Criteria	Group A (n = 10)	Group B (n = 10)	p-value (t-test)
Serum testosterone (ng/mL)	3.45±0.68	6.29±0.63*	0.016
Serum TAC (mmol/mL)	0.56±0.57	0.71±0.02*	0.031
Serum MDA (mmol/mL)	5.04±0.39	3.79±0.29*	0.036

Data is expressed as mean±SEM *p<0.05

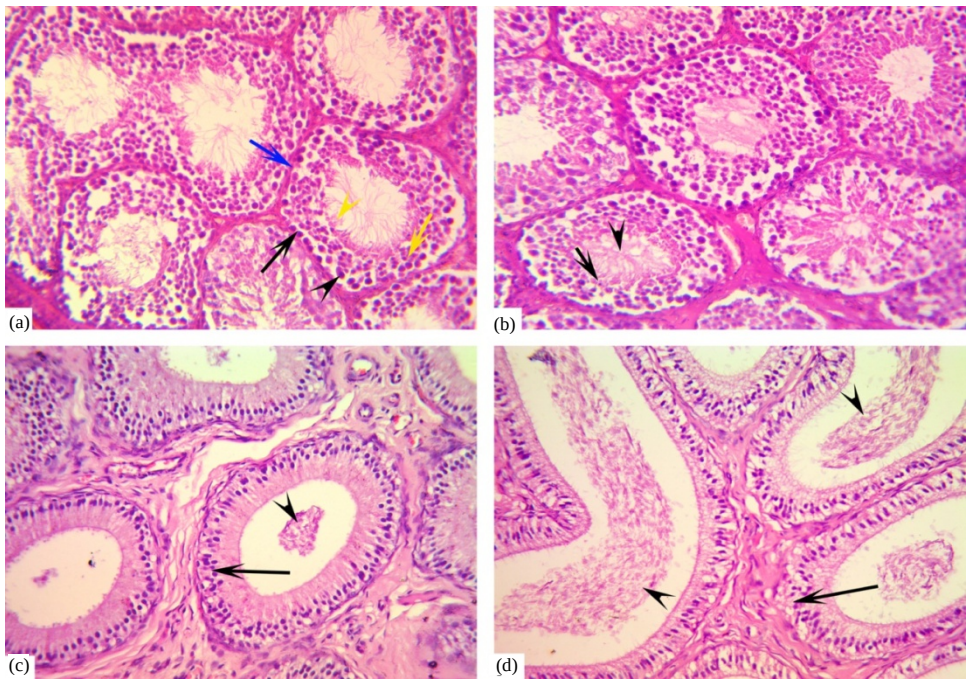

Fig. 1(a-d): (a) Histological section of control rabbit testis; seminiferous tubules showing a normal stratified epithelial arrangement of spematogonia (black arrow), primary spermatocytes (black arrowhead), secondary spermatocytes (yellow arrow) and spermatids (yellow arrowhead) that rested on clear basement membranes (blue arrow). H&E stain. X400, (b) Histological section of the ginger-treated rabbit testis; seminiferous tubules showing increased numbers of spermatozoa (arrow and arrowhead, respectively) that mostly engorged the adluminal parts of the tubules. H&E stain. X400, (c) Histological section of the control rabbit epididymis coiled tubes showed pseudostratified columnar ciliated epithelium (arrow) with stored sperms at their lumens (arrowhead) H&E stain. X400 and (d) Histological section of the ginger-treated rabbit epididymis showing increased epithelial cells height (arrow) with overcrowded lumens with sperms (arrowheads) H&E stain. X400

abnormalities in diabetic rats. In addition, Dawson *et al*.[31] reported that there is a direct correlation between epididymal sperm count and motility with animal fertility. Also El-Speiy and El-Hanoun[32] showed that ginger could improve semen quality and the reproductive performance in V-line rabbits. In the same respect Khaki *et al*.[33] conclude that combined ginger and cinnamon had abeneficial effects on sperm viability, motility, testosterone and LH, FSH and antioxidant level in Streptozotocin (STZ)-induced diabetes in rats. Moreover, El-Speiy *et al*.[34] suggested that ginger enhanced the semen quality and antioxidant status of New Zeland rabbits and 1% is adequate concentration. In addition, Afzali and Ghalehkandi[35] observed a dose dependent increase in the spermatozoa forward movement and sperm viability in male rats.

Regarding, testosterone level, oral administration of ginger significantly increased testosterone concentration, this in agree with Khaki *et al*.[14] who revealed that ginger rhizome powder increased testosterone without effect on LH and FSH hormones. In the same respect, Khaki *et al*.[33] found that administration of ginger and freshly prepared onion juice lowered the adverse effects of lamotrigine and can have a beneficial effect on sexual behavior in male rats. In the same respect Amin and Hamza[36] reported that ginger had androgenic activity that increase testosterone and accumulate the spermatozoa in the lumen of seminiferous tubules in male rats. Moreover, Afzali and Ghalehkandi[35] demonstrated that oral administration of ginger in a dose dependent manner increased testosterone level without significant effect on LH and FSH levels. On the other hand, Riaz *et al*.[37] found that ginger at a dose of 1.5 g kg^{-1} decreased plasma testosterone and LH levels in male rats after toxicity with lead. Sperm cell plasma membrane is different from most of other cell membranes in lipid composition where it contains high amount of Poly Unsaturated Fatty Acids (PUFA), this structure of the sperm resulting in greater sensitivity to the environmental hazards compared with other cells[38].

Gual-Frau *et al*.[39] reported that the harmful effect of ROS is due to its ability to reduce axonemal protein phosphorylation which associated with a decrease in membrane fluidity through propagating PUFA hydroperoxidation, in addition, it can diffuse into the cells and inhibit the activity of Glucose-6-Phosphate Dehydrogenase (G6PD) which considered as a key enzyme in controlling the intracellular viability of NADPH-dependent antioxidant enzymes.

Lipid per-oxidation lead to damage of the lipid matrix in the sperm cell membrane which resulted in germ cell death at the different stages of development loss of motility and impairment of spermatogenesis, so, antioxidant therapy act as a protective defense against oxidative stress and so, improve the fertility parameters[40]. The major important antioxidant components that isolated from ginger root are shogoal which is a pungent component of ginger; zingerone which produced when ginger is dried or cooked and gingerol. These antioxidants are associated with the protective effects of ginger against lipid peroxidation and amplified the level of antioxidant enzymes[41]. Furthermore, these compounds prevent DNA damage and destruction of genome induced by H_2O_2[42]. As observed in our results, showed that ginger produced significant reduction in MDA concentration with a significant increase in TAC. This agree with the previous findings of Ippouchi *et al*.[13] who found that ginger decreased the concentration of MDA in rats and so, decrease the lipid-peroxidation. Moreover, Khaki *et al*.[14] found that ginger produced lower concentration of MDA and higher concentration of TAC as compared with control.

Aitken and Baker[29] indicated that oxidative stress could be harmful to sperm viability and fertility where defective sperm function is considered the most common cause of infertility.

In the same respect, Zahedi *et al*.[4] found that ginger was able to counter the negative effect of gentamicin on sperm count and overcome its toxicity on testis tissue. Moreover, Yosef *et al*. concluded that ginger was effective in protection against Di-(2-ethylhexyl) phthalate (DEHP)-induced reproductive toxicity and oxidative stress in rabbits. In addition, ginger extract can increase the testicular volume and reduce the side effect of busulfan in rats in a dose dependent manner[43]. In the same respect, Hosseini *et al*.[44] found that ginger was effective in decreasing sperm DNA fragmentation in infertile men.

Histological sections in the testis and epididymis showed accumulation of the lumen with sperms in ginger treated group. This effect could be attributed to the potent androgenic, protective and antioxidant effects of ginger[45]. Moreover, Zahedi *et al*.[46] showed that some plant extract as ginger had a protective and increasing effect on spermatogenic cells and the diameter of seminiferous tubules.

CONCLUSION

The present study suggested that ginger has a positive effect on the male fertility in rabbits which could be attributed to the antioxidant and androgenic activity of ginger, so, it could be recommended the use of ginger for improving the semen quality, fertility and reproductive performance of male rabbits.

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