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Effect of Ginger Aqueous Extract on Some Reproductive and Antioxidant Parameters in Male Rabbits

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ABSTRACT

The objective of the current study was to investigate 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 groups; the control group (n= 10) and ginger treated group (n=10). The treated group received 200mg/Kg of ginger aqueous extract orally using stomach tube daily for 4 weeks. At the end of the experiment, semen was collected for analysis of; ejaculate volume, sperm motility, count and abnormalities. Sera were used for determination of testosterone level, Total antioxidant capacity (TAC) and malondialdehyde concentration (MDA). Histological examination of testes and epididymis was performed. The obtained results revealed; no change in ejaculate volume, significant increase in the individual sperm motility, sperm count with a significant decrease in the percentage of abnormal spermatozoa in ginger treated group. A significant increase in testosterone level, TAC with a significant reduction in MDA concentration in ginger treated group. The histological examination of testes and epididymis showed 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 group. 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.

1. 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 (Toshimori et al., 2004). 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 (Austin, 1991). 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 (Palipoch, 2013). 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 (Afshin et al.,2012) . The plant is cultivated in south-east Asia, West Africa and Caribean, where China and India are considered the main sources of it (Lister, 2003).

The most important constituents of ginger are gingerols, zingibrene, protodioscin, schogaols, saponins and gingerdiol (Sakr and Badawy, 2011) . The major therapeutic effects and uses of ginger include relieve of nausea and vomiting accompanied pregnancy, surgery and motion sickness (Gilani and Rahman, 2005), anti-inflammatory, anti-hepatotoxic,

antithrombotic, antiemetic, cholagogue and antioxidant (El-Morsy Ibrahim and Al-Shathly, 2015). 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 (Rajeev et al., 2006). The favorable effect of ginger on the male fertility could be attributed to the anti-oxidant (Sekiwa et al., 2000) and androgenic activity (Kamtchouing et al., 2002). The main anti-oxidant components in ginger are; gingerol, which is responsible for its taste (Bahmanpour et al., 2012), shogaols and some phenolic ketone derivatives (Witchl, 2004)

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 (Ippoushi et al., 2005; Khaki et al., 2009; Memudu et al., 2012).More over Morakinyo et al. (2008) 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. (2009) 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.

2. MATERIALS AND METHODS

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

2.1. Experimental Animals:

A total twenty adult male New-Zealand white rabbits (5-6 months old) and weighing (3-3.5kg) 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 (1984).

2.2. Experimental Design:

The animals were divided into two groups; one used as control and the other as experimental group. The animals were orally administered with aqueous ginger extract 200 mg/kg daily for 4 weeks using a stomach tube.

2.2.1. 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.(1) gm of the dried powder was macerated in 50 ml distilled water, where the final concentration of the extract was 20mg/ml . The extract was then kept throughout the experiment in air-tight container in the refrigerator (Memudu et al., 2012).

2.2.2 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 Bredderman et al. (1964). The semen was immediately evaluated after collection.

2.2.2.1 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. (1980).

Sperm cell concentration: sperm cells were counted by using a hemocytometer slide and expressed in million /ml according to the method described by Hafez (1976).

Sperm abnormalities: the percentage of abnormal spermatozoa was determined in eosin-nigrosin stained smears according to Bearden and Fuquay. (1980).

2.2.2.2 Sera for testosterone, malondialdehyde (MDA) concentration and total anti-oxidant capacity (TAC) measurements:

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 minutes, and then centrifuged at 3000rpm for 15 minutes. The sera were kept at-20^0c until used.

-Serum concentration of testosterone was measured by using; Testosterone enzyme immunoassay (EIA) DSL-10-4000 kit obtained from Diagnostic Systems Laboratories Inc. According to Burtis and Ashwood (1994).

-Serum MDA measurement was carried out 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^{o}c for 15 minutes. The pink colored product can be measured by spectrophotometer at 532nm, according to Satoh**,** (1978); Ohkawa et al., (1979); Janero, (1990).

-Serum TAC, which is defined as the amount of anti-oxidants required to make absorbance increase 0.01 in ml of serum. It is measured by the reaction of phenanthroline and (Fe^{2+}) at 37^{o}c by using a spectrophotometer at 520nm according to Feng et al., (2001). The principle of this test depends on antioxidant defense system can reduce (Fe^{3+}) to (Fe^{2+}).

2.2.2.3 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^{o}c, then sectioned into five-microns thick sections and stained with Hematoxylin and eosin(H&E), then examined under light microscope (Carleton,1967).

2.3. Statistical analysis:

The obtained data were statistically analyzed by using (t-test) according to Tamhane and Dunlop (2000). The results were expressed as means± S.E.M (standard error of means). Significant difference was expressed as parentheses. P-values <0.05 were considered significant.

3. RESULTS

The obtained results are illustrated in table (1) and table (2), where there was no change in the ejaculate volume between control and ginger treated group.

Concerning, the individual sperm motility and sperm count there was a significant increase in sperm motility and sperm count (83.4±1.28 %; 246.8±6.79 million/ml respectively) in ginger treated group as compared with control (70.6±2.24%; 198.2±2.43 million /ml respectively).

It is obvious from table (1) that the sperm abnormalities showed a significant decrease in ginger treated group (12.4±0.50 %) as compared with control group (21.2±0.73%).

Regarding, the serum testosterone level (table2), the ginger treated group showed a significant increase in testosterone level (6.29±0.63 ng/ml) as compared with control group (3.45±0.68 ng/ml).

The data shown in table (2) revealed a significant reduction in MDA concentration, with a significant increase in TAC in ginger treated group (3.79±0.29 mmol/ml; 0.71±0.02 mmol/ml respectively) as compared with the control group (5.04±0.39 mmol/ml; 0.56±0.57mmol/ml respectively)

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

Sperm Parameters	Control (n=10)	Ginger treated group (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	Control (n=10)	Ginger treated group (n=10)	P-value (t-test)
Serum testosterone (ng/ml)	3.45±0.68	6.29±0.63*	0.016
Serum MDA(mmol/ml)	5.04±0.39	3.79±0.29*	0.036
Serum TAC (mmol/ml)	0.56±0.57	0.71±0.02*	0.031

Data is expressed as mean ± SEM * p< 0.05

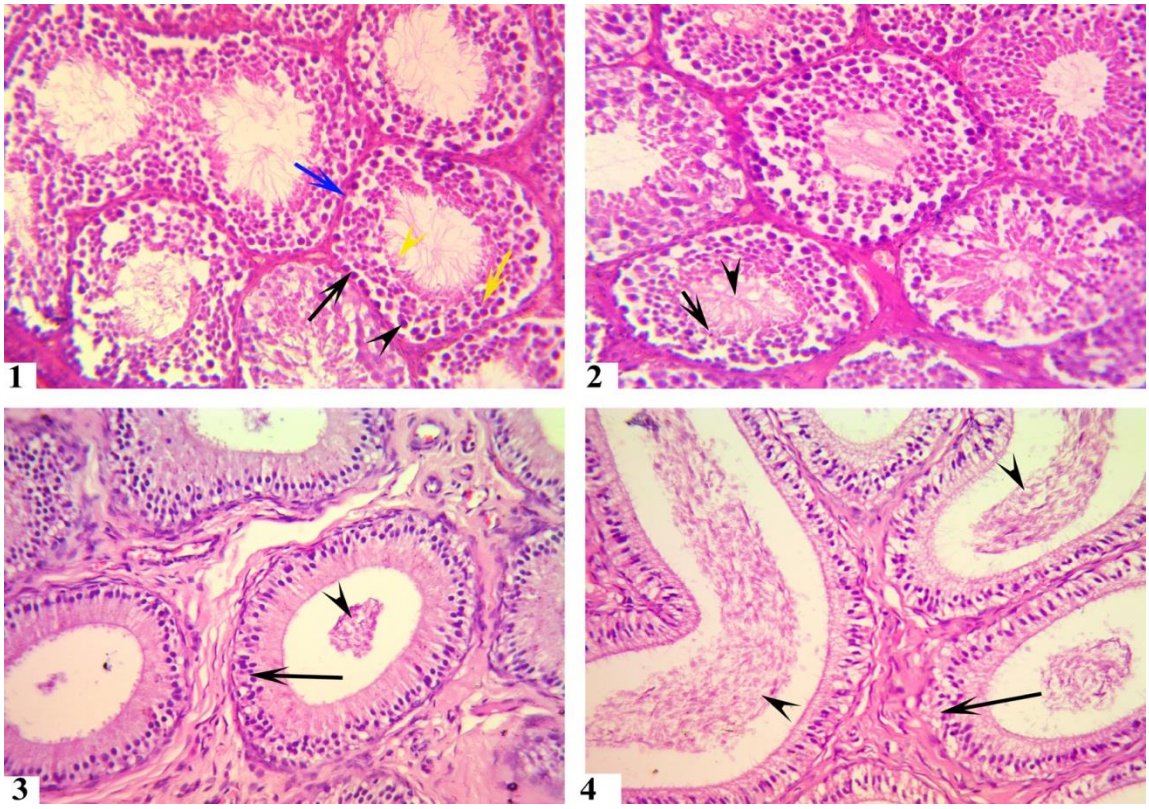

Fig (1): Histological section of control rabbit testis; seminiferous tubules showing a normal stratified epithelial arrangement of spematogonia (black arrow), primary spermatocytes (black arrowhead), secondary spermatocytes (yellow arrow) and Spermatids (yellow arrowhead) that rested on clear basement membranes (blue arrow). H&E stain. X400.
Fig (2): 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.
Fig (3): 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.
Fig (4): Histological section of the ginger-treated rabbit epididymis showing increased epithelial cells height (arrow) with overcrowded lumens with sperms (arrowheads) H&E stain. X400.

4. DISCUSSION

The obtained results of this study suggested that ginger has 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 (Aitken et al., 1995). Concerning, the effect of ginger on semen parameters, the results showed no change in the ejaculate volume between control and ginger treated group, but there was a significant increase in the sperm motility and count with a significant reduction in sperm abnormalities in ginger treated group as compared with control (Table 1). The results are supported by the finding of Morakinyo et al. (2008), who showed that ginger extract increased the sperm motility and count in a dose and duration dependent manner. Furthermore, Khaki et al. (2009) found that ginger increased sperm motility, count, viability, serum testosterone with a decrease in MDA concentration in a dose 50-100 mg/kg rat for twenty days. Moreover, Hafez, (2010) revealed that ginger extract could increase the sperm functions (motility, viability and count) with a decrease in the sperm abnormalities in diabetic rats. In addition, Dawson et al.(1992) reported that there is a direct correlation between epididymal sperm count and motility with animal fertility.Also El-Speiy and El-Hanoun (2013) showed that ginger could improve semen quality and the reproductive performance in V-line rabbits. In the same respect Khaki et al. (2014) 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. (2017) suggested that ginger enhanced the semen quality and antioxidant status of New Zeland rabbits and 1% is adequate concentration. In addition, Afzali and Ghalehkandi, (2018) 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. (2009) who revealed that ginger rhizome powder increased testosterone without effect on LH and FSH hormones. In the same respect, Khaki et al. (2012) 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 Amr and Hamza et al .(2006) 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, (2018) 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.(2017) found that ginger at a dose of 1.5 gm/kg decreased plasma testosterone and LH levels in male rats after toxicity with lead.

The increase in testosterone level in this study could be responsible for the effect of ginger on semen quality and the accumulation of sperms in the lumen of seminiferous tubules, where, testosterone have been shown to be important for the development, growth and normal function of the testis and production of normal spermatozoa, where androgens possess anabolic activities (Johnson and Everitt, 1988).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 (Osman et al., 2015).

Gual-Frau et al. (2015) 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 (Bestas et al.,2006). 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 (Zahedi and Khaki, 2014).Furthermore, these compounds prevent DNA damage and destruction of genome induced by H_2O_2 (Micinski et al., 2011). 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 Ippoushi et al. (2005) who found that ginger decreased the concentration of MDA in rats and so decrease the lipid-peroxidation. Moreover, Khaki et al. (2009) found that ginger produced lower concentration of MDA and higher concentration of TAC as compared with control

Aitken and Baker (2004) 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. (2012) 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. (2012) 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 (Bordbar et al., 2013) In the same respect, Jalil et al. (2016) 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 (Aitken et al., 1995). Moreover, Zahedi et al. (2010) showed that some plant extract as ginger had a protective and increasing effect on spermatogenic cells and the diameter of seminiferous tubules.

In 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.

5. Conflict of interest

None of the authors have any conflict of interest to declare

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