

Effects of *Zingiber officinale* on Reproductive Functions in New-Zealand Male Rabbits

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Abstract: Infertility is one of the major health problems, approximately 30% of infertilities are due to a male gene. *Zingiber officinale* has been reported to be an important antioxidant. This study was aimed to investigate the effects of *Zingiber officinale* on male reproductive functions and study the mechanisms underlying these effects. The experiment was planned to examine the effects of *Zingiber officinale* on semen characteristics, testosterone levels, testicular lipid peroxidation, testicular antioxidants and histological study in male New-Zealand white rabbits for 12 work weeks. Results obtained indicated that *Zingiber officinale* significantly increased in ejaculate volume (EV), sperm concentration (SC), total sperm output (TSO), semen initial fructose (IF), percentage motile of sperm (SM), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF) and seminal plasma thiobarbituric acid-reactive substances (TBARS). Likewise, testosterone levels and relative weights of the testes were increased. Body weight, reaction time (RT), glutathione s-transferase (GSH), superoxide dismutase (SOD) and lactate dehydrogenase (LDH) were decreased in seminal plasma of rabbits treated with *Zingiber officinale* compared to the control. Our results suggested that an extract of *Zingiber officinale* possesses pro-fertility properties in male rabbits which might be a product of both its potent antioxidant properties.

Keywords: *Zingiber officinale*, New-Zealand white rabbits, male and reproductive functions.

INTRODUCTION

From 1990s, consumers started to view food not only as a means to gratify hunger, prevent diet-deficiency diseases or to provide essential nutrition (e.g., water, protein, sugar, fat, vitamins and minerals), but also as an important vehicle to keep us healthy [1]. Antioxidant applications are important for protecting the human body from various sources of oxidative damage and are practiced extensively for prevention of a diversity of diseases. It takes in many bio-functions including anti-allergenic, anti-inflammatory, anti-bacterial and anti-viral activities, and the prevention of carcinogenesis, diabetes and heart disease [2]. Previous studies revealed that oxidative stress has deleterious effects on mesenchymal progenitor cells in terms of decreasing cell proliferation, increasing apoptosis and inhibiting their differentiation [3]. The high absorption of free radicals inside the human physical structure can cause oxidative stress and cellular damage by changing the biological activities of lipids, proteins, DNA and carbohydrates, even to cellular death [4]. Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is one of the most commonly used spices around the universe, particularly in the Southeastern Asian nations. Ginger is

also a medicinal plant that has been widely used in Chinese, Ayurvedic and Unani-Tibb medicines, since antiquity, for a broad array of complaints that include arthritis, rheumatoid arthritis, sprains, muscular throats, muscle spasms, constipation, upset stomach, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis [5]. It has been eaten since antiquity and is recognized to take on diverse biological functions including anti oxidation, anti-inflammation, hyperlipidemia, anti-carcinogenesis, anti-nausea, anti-thrombosis, and antibacterial process [6]. It has been also reported that nephron-protective and hepato-protective activity of an aqueous ethanol extract of *Zingiber*. Its beginnings and the obtained extracts contain polyphenolic compounds (6-general and its derivatives), which possess a high antioxidant activity [7].

The intake of *Zingiber officinale* Roscoe significantly decreased the concentration of thiobarbituric acid-reactive substances (TBARS), lipid peroxidation and the formation of malonaldehyde in rats [8]. [9] reported that ginger improves the histological alterations and reduce apoptosis in testis of

mice treated with metiram fungicide. *Zingiber officinale* extract was reduced the extent of cisplatin-induced sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants [10]. Another study reported that intake of ginger roots as a beverage may be good for diabetic patients who suffer from sexual impotency as their extracts induce antidiabetic activity and enhance male fertility in diabetic rats [11]. Ginger was attenuated the testicular damage and decreased apoptotic damage both in testes and sperms [12]. Different studies reported that co-governing body of the aqueous ginger extract with arsenic was found to protect against adverse changes in the reproductive organ weight, attenuate the decrease in sperm functions, enhance the plasma reproductive hormone level along with increased antioxidant activities and reduced peroxidation [13]. Aqueous extract of *Zingiber officinale* was found to increase weight of testes, the serum testosterone level and epididymal α -glucosidase activity in male rats [14]. Another researchers reported that administration of ginger significantly increased sperm percentage, viability, motility and serum total testosterone in rats [15]. The effect of ginger and its extracts were attributed to the antioxidant activity of its major ingredients, namely Zingerone, gingerdiol, Zingiberene, gingerols and shogaols [16]. Infertility is one of the major health troubles in life, and about 30 % of infertilities are due to a male factor [17]. Various conditions can interfere with spermatogenesis and reduce sperm quality and yield. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamin intake have harmful effects on spermatogenesis and sperm normal production [18]. Various surveys have reported that antioxidants and vitamin A, B, C, and E in the diet can protect sperm DNA from free radicals and increase blood testis barrier stability [19].

Other researchers demonstrated that ginger oil has the dominative protective effect on DNA damage induced by H_2O_2 and might pretend as a scavenger of oxygen radical and might be used as an antioxidant [20]. Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men [21, 15] reported that ginger extract has a protective effect against DNA damage induced by H_2O_2 and enhanced sperm healthy parameters in rats. This work was therefore carried out in view of the action of *Zingiber officinale* extract on reproductive function's antioxidant activities and histological effects in male rabbits.

MATERIALS AND METHODS

In this study ginger was obtained from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, USA. All other chemicals used in the experiment were of analytical grade. Mature male New Zealand White rabbits (age of 7 months and initial

weight of $(2.917 \pm 28.9 \text{ Kg})$ were used. Ten mature male rabbits were randomly divided into couple equal groups (each five rabbits): Group I: Rabbits were used as control and received an equivalent volume of the vehicle (corn oil) alone by oral gavage daily for 12 successive weeks. Group II: Rabbits were treated with ginger. Ginger was given ginger daily by gavage at a dose of 100 mg/kg B.W [22], which dissolved in corn oil for 12 successive weeks.

Semen collection was done weekly and continued throughout the 12-week experimental period. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate (EV) was recorded (using a graduated collection tube) after removal of the gel mass. A weak eosin solution was used for evaluation of sperm concentration (SC) by the improved Neubauer hemocytometer slide (GmbH + Co., Brandstwierte 4, 2000 Hamburg 11, and Germany) [23]. Total sperm output (TSO) calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration (IF) in seminal plasma was determined immediately after semen collection [24]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture [25]. The percentages of motile sperm (SM) were estimated by visual examination under low-power magnification (10x) using light microscope. Total number of motile sperm (TMS) was calculated by multiplying the percentage of motile sperm and total sperm obtained. Reaction time (RT) was determined as the moment of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Initial hydrogen ion concentration (PH) was determined immediately after collection using PH cooperative paper (Universalindikator PH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) was calculated as the product of total sperm output, motility (%), and normal morphology (%) [26]. Seminal plasma thiobarbituric acid-reactive substances (TBARS) were measured in the seminal plasma using the method [27]. Seminal plasma glutathione content (GSH) was determined using commercial glutathione reductase kits according to the method of [28]. Superoxide dismutase (SOD) activity was assayed according to [29]. Lactate dehydrogenase (LDH) activity was measured. After incubation of the testes or Sertoli cells in the absence or presence of the glyphosate-Roundup at nominal concentrations ranging from 0.72 to 360 ppm for 30 min, the incubation medium was collected for determination of extracellular LDH activity by a spectrophotometric method. The estimation of LDH activity was carried out by measuring the oxidation of NADH and the results were expressed as U/L/mg of protein. Blood samples were spun at 2500 rpm for 10 minutes in a table top centrifuge. The serum samples obtained were analyzed to determine the concentration of testosterone. The

analysis was carried via the tube-based enzyme immunoassay (EIA) method. The protocol used for the hormone was according to the method described for the kit (Immunometrics Limited UK) and meet the WHO standards in research programme for human reproduction. Catalase activity was determined using the Luck method involving the decomposition of hydrogen peroxide [30]. In term of histological examination, specimens of testes were put in 10% buffered formalin for histopathological examinations. Histological preparation of testes was carried out according to [31].

RESULTS

Physiological observations

Table 1 was shown the overall means of the data of different parameters. Administration of *Zingiber officinale* caused decreases in the body weight, however, testicular weight was increased significantly compared to control. Treatment with *Zingiber officinale* significantly decreases ($P<0.05$) the RT, GSH, SOD and LDH in the experimental rabbits as compared with the control. This reduction was highest for GSH and SOD. In other hand, EV, SC, TSO, IF, SM, TMS, PSV, TFSF, TBARS and level of testosterone were significantly

increased ($P<0.05$) after treatment with *Zingiber officinale* compared with the controls. Only PH wasn't changed in couple groups.

Histopathological observations

Histopathological examination of rabbit testicular tissue of couple studied groups showed the next modifications. In the control group, the light microscopic examination of the testes showed that complete active spermatogenic cycle was regular in all male rabbits of the control group. The structural components of the testes are the seminiferous tubules and interstitial tissues (Leydig cells). Two types of cells were identified in rabbit seminiferous tubules, the Sertoli cells and the spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatides and sperms). The Sertoli cells, reside along the thin basal lamina, while the spermatogenic cells are set in many layers, namely, the spermatogonia, primary and secondary spermatocytes, spermatoids and finally mature spermatozoa, (Figure 1 A-C). In the treated group, the light microscopic examination of the testes of ginger treated rabbits showed normal testicular morphology similar to that of control group (Figure 2 A-C).

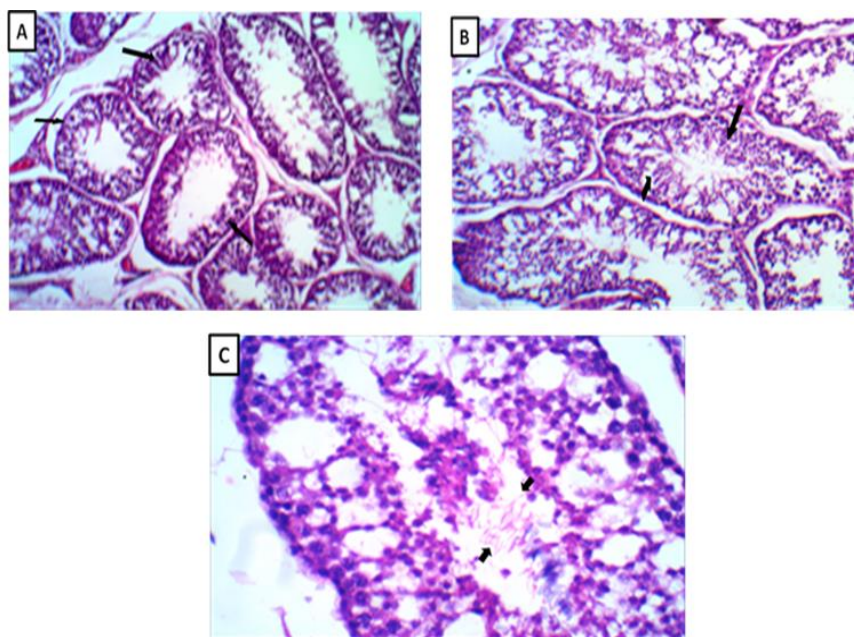


Fig-1 (A): A section in the testes of the control cases showing seminiferous tubules lined by germinal epithelium at various stages of aturation till the mature sperm stage; **(B):** A similar case showing the lining germinal epithelium where the spermatogenic maturation is full and complete; **(C):** High power view in the seminiferous tubules in a control case demonstrating the presence of mature sperms within the tubular lumen (H&E stain).

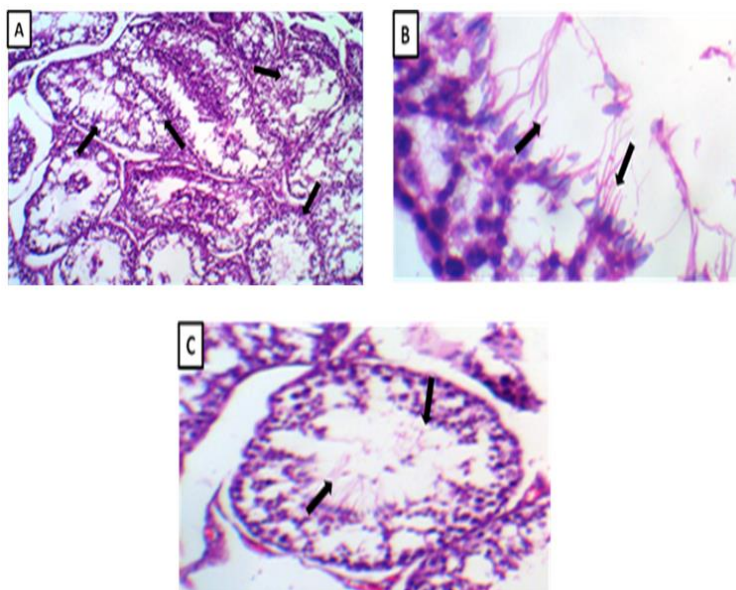


Fig-2 (A): A section in the testes of the cases receiving ginger alone. Note the crowding of the seminiferous tubules and the full range of germinal epithelium till the mature sperm stage. (B&C): A high power view of the tubules showing high number of well-formed sperms (H&E stain)

Table-1: The overall means (\pm SEM) of different parameters during treatment of male rabbits with ginger

Parameter	Groups	
	Control	Ginger (100mg/kg)
Body weight (gm)	3402 \pm 28.2	3648 \pm 48.8* \uparrow
Testes weight (g/100 g body weight)	0.207 \pm 0.013	0.374 \pm 0.015* \uparrow
EV (ml)	0.74 \pm 0.017	0.82 \pm 0.018* \uparrow
SC	263 \pm 4.5	319 \pm 7.0* \uparrow
TSO ($\times 10^6$)	195 \pm 5.1	265 \pm 9.5* \uparrow
IF (mg/dl)	257 \pm 3.9	276 \pm 3.7* \uparrow
SM (%)	68.2 \pm 0.7	73.3 \pm 0.9* \uparrow
TMS ($\times 10^6$)	133 \pm 4.1	197 \pm 8.4* \uparrow
RT (sec.)	4.05 \pm 0.099	3.23 \pm 0.145** \downarrow
PH	7.83 \pm 0.022	7.69 \pm 0.038
PSV (%)	15.3 \pm 0.16	17.6 \pm 0.38* \uparrow
TFSF ($\times 10^6$)	108 \pm 3.4	168 \pm 7.9* \uparrow
TBARS (nmol/ml)	60.05 \pm 0.99	40.54 \pm 0.52* \downarrow
GSH (g/dl)	4.48 \pm 0.99	5.92 \pm 0.52* \uparrow
SOD (%)	12.28 \pm 0.99	17.94 \pm 0.52* \uparrow
LDH (U/L)	1268 \pm 14.0	1172 \pm 16.4** \downarrow
Testosterone (ng/mL)	1.59 \pm 0.034	2.53 \pm 0.130* \uparrow

*P<0.05, values expressed as mean \pm SEM, n= 5

DISCUSSION AND CONCLUSION

The results of the present study suggested that *Zingiber Officinale* have a beneficial effect on male reproductive functions in rabbits. These data are confirmed by our observation on the increased in weight of testis, EV, SC, TSO, IF, SM, TMS, PSV, TFSF, TBARS and level of testosterone, and decrease in the RT, GSH, SOD and LDH in the experimental rabbits as compared with the control. The significant increase in the absolute weight of the testis could therefore be due to increased androgen biosynthesis as evidenced by a significant increase in serum

testosterone levels in the experimental rabbits. This increased reproductive organ weight is equally consistent with the previous reports that observed an increase in the testicular weight of rats treated with *Zingiber Officinale* with a concomitant increase in testosterone level [13-14-32]. Moreover, an effect due to testosterone changes alone should have led to an increase in the weight of all accessory organs; it is therefore plausible that the increased weight of the testis and epididymis reflects a dual effect of increased testosterone levels and sperm contained in these organs. This observation equally consistent with study found

that increase in the sperm functions in the *Zingiber Officinale* administered rabbit [33, 34]. And that could be attributed to favourable and increased spermatogenic activities as results of high testosterone levels. Testosterone is known to be critically involved in the development of sperm cells and derangement results widely in leydig cell dysfunction and testicular steroidogenic disorder [33]. Histological sections were confirmed these observations, the crowding of the seminiferous tubules and the full range of germinal epithelium till the mature sperm stage were observed. The histopathology of our study agreed with the work, where testis showed marked necrosis of spermatogoneal cells lining seminiferous tubules, degeneration and desquamation of germ cells lining seminiferous tubules [35, 36]. Previous study was found the administration of 100mg/kg ginger reveals that there is a significant increase in sperm motility and viability [37]; this is similar to our result where there were increases in parameters EV, SC, TSO, IF, SM, TMS, PSV, TFSF that treatment with *Zingiber. Officinale*. These parameters improves and enhances the fertilizing capacity of the semen. Ginger has been indicated to improve testicular function, sperm quality and quantity, sex hormones levels (Testosterone, LH and FSH), and serum antioxidants level [38, 39, 40]. These qualities were often used as a measure of sperm production, testicular function and/ or male fertility. Low sperm count and motility and high percentage abnormal spermatozoa level each have been associated with reduced fertility [13-32-41]. Other observations for testicular/sperm function in this study were the reduced level of RT, GSH, SOD and LDH. Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities. These studies found that ginger significantly lowered lipid per oxidation by maintaining the activities of the antioxidant enzymes –super oxide dismutase, catalase and glutathione peroxides in rats [32, 42, 43].

CONCLUSION

These results concluded that ginger has significant beneficial effects on the sperm viability, motility, and serum total testosterone and serum antioxidants' level and could be effective for maintaining healthy sperm parameters and male reproductive function.

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