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The effects of Ginger on spermatogenesis and sperm parameters of rat

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Abstract

Background: Ginger rhizome (Zingiber officinale R., family: Zingiberaceae) is used medicinally and as a culinary spice.

Objective: Medicinal use of ginger dates back to ancient China and India. Ginger and its constituents are stated to have antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory, stimulant, cholagogue and antioxidant. It has been used since ancient time as medicinal and food origins it contain antioxidative and androgenic activities and have well effect in diseases treatment in more countries world-wide. As an antioxidant's ginger has a useful effect on spermatogenesis and sperm parameters.

Materials and Methods: Wistar male rat (n=30) were allocated into three groups, control (n=10) and test groups (n=20), that subdivided into groups of 2 that received ginger rhizome powder (50 and 100mg/kg/day) for 20 consequence day. Animals were kept in standard conditions. In twentieth day the testes tissue of Rats in whole groups were removed and sperm was collected from epididymis and prepared for analysis.

Results: Serum total testosterones significantly increased in experimental group that has received 100 mg/kg/day Ginger ($p<0.05$) in comparison to control group. Besides, the percentage of sperm viability and motility in both test groups significantly increased ($p<0.05$) in comparison to control group, Whereas, LH, FSH hormones, sperm concentration, morphology and testes weights in both experimental and control group were similar.

Conclusion: Results revealed that administration of 100 mg/kg/day of ginger significantly increased sperm percentage, viability, motility and serum total testosterones. This suggested that ginger may be promising in enhancing sperm healthy parameters.

Key words: Ginger rhizome, Sperm, Spermatogenesis, Rat, Testis, Testosterone.

Introduction

Infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor (1, 2). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production (3). Several studies have reported that antioxidants and vitamin A, B, C, and E in diet can protect

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sperm DNA from free radicals and increase blood testis barrier stability (4, 5). Nowadays ginger rhizome (Zingiber officinale R., family: Zingiberaceae), is used worldwide as a spice. Both antioxidative (6) and androgenic activity (27) of Z. officinale were reported in animal models. All major active ingredients of Z. officinale, such as Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols, have antioxidant activity (7). Besides, other researches showed that ginger oil has dominative protective effect on DNA damage induced by H_2O_2 and might act as a scavenger of oxygen radical and might be used as an antioxidant (8).

Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men (9, 10).

Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important. The present study was planned to asses the ability of ginger to promote sperm parameters and modulate follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone concentration, spermatogenesis and oxidative stress. The results obtained will provide further insights into appropriate treatment of male patients by improving spermatogenesis and sperm parameters.

Materials and methods

Experimental animals

Adult Wistar albino male rats (n=30) were included in the present study. The rats were 8 weeks old and weighing 250±10g each. They were obtained from animal facility of Pasture Institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly divided into control (n=10) and experimental (n=20) groups. The control group just received 4CC distilled water daily. However, the experimental groups split into two groups each included ten rates. (G.1) received 50mg/kg/rat and (G.2) received 100mg/kg/rat of ginger for 20 consequence days. Body weight daily intake of food and water were determined several times per week throughout the study (11).

Surgical procedure

In twentieth day, the Pentobarbital sodium (40 mg/kg) was administered intra peritoneal for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. Thereafter testis in control and experimental groups were immediately removed. The weights of testis in each group were registered. The animals were decapitated between 9:00 AM and 11:00 AM, and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250Xg and the serum obtained was stored at −20°C until assayed.

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (11).After 5 min incubation at 37°C (with 5% CO2), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method (12).

Serum FSH, LH total testosterone hormone measurements

Serum concentration of FSH and LH were determined in duplicated samples using radioimmunoassay (RIA). Rat FSH / LH kits obtained from Biocode Company-Belgium, according to the protocol provided with each kit. The sensitivities of hormone detected per assay tube were 0.2ng/ml and 0.14ng/ml for FSH and LH respectively. Serum concentration of total testosterone was measured by using a double antibody RIA kit from immunotech Beckman Coulter Company-USA. The sensitivities of hormone detected per assay tube were 0.025ng/ml (13, 14)

Total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentration measurement in serum

A TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute-China. According to this method, the antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe3+ to Fe2+. TAC was measured by the reaction of phenanthroline and Fe2+ using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of serum (15). Free radical damage was determined by

specifically measuring malondialdehyde (MDA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm (MDA detecting kit from Nanjing Jiancheng Bioengineering Institute-China) (16).

Histopathology and Light microscopy

The testis was fixed in 10% formalin and embedded in paraffin. Five-micron thick sections were prepared and stained with Hematoxylin and Eosin (H&E). The specimens were examined under Olympus/3H light microscope-Japan.

Statistical analysis

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). Significant difference is written in parentheses.

Results

Weight of individual male testis

The obtained results in this study are illustrated in tables I. There was no significant difference in testes weights between the groups.

Results of sperm motility, viability and count

Administration of 50mg/kg/rat and 100mg/kg/rat ginger for twenty consecutive days significantly increased Sperm motility and viability in both experimental groups as compared with the control group. The motility and vitality were (73±4.35% and 95.80±1.68%) in G.1 and the corresponding value in G.2 were (81±5.33%; 98.80±80%). However, the motility and vitality in control group were significantly lower in comparison to the values in G.1 and G.2 (33.75±6.88%; and 66.25±4.73%) (Table I). In addition, sperm concentrations were similar in control and both experimental groups. The results were as follow: Control group, 48.68±7.70mill/ml; G.1= 51.90±5.36mill /ml and 61.60±2.34 mill/ml in G.2) (Table I).

Results of serum total testosterone, LH and FSH hormones measurement

Administration of 50mg/kg/rat and 100mg/kg/rat ginger for twenty consecutive days hadn't significant effect on LH and FSH concentration in the serum between the control and G.1 and G.2 groups. The concentration of LH and FSH were (1.66±0.316 and 21.59±2.69) in G.1 and the corresponding value in G.2 were (2.23±0.453 and 21.68±2.11) However, the LH and FSH in control group were (1.51±0.138and 20.37±1.788) (Table I). In addition, serum total testosterone level increased significantly ($p<0.05$) in animals received 100mg/kg/rat ginger (G.2) in comparison to control group. The concentration of serum total testosterone level was (2.91±0.349, 3.71±0.387and 1.60±0.091 ngr/ml, respectively) in G.1, G.2 and in control group (Table I).

Results of total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentration measurement in Serum

The mean concentration of Malondialdehyde (MDA) level was significantly ($p<0.05$) lower in G.1 (2.64±0.193) and G.2 (0.81±0.192) in comparison to control group (4.80±0.212).Total antioxidant capacity (TAC) was significantly higher ($p<0.05$) in G.1 (0.92±0.016) and G.2 (0.88±0.341) as compared with control group (0.53±0.77) (Table I).

Table I. The effect of the 50mg/kg/rat and 100mg/kg/rat ginger on sperm parameters, serum FSH, LH, total Testosterone and testis weight of control and experimental groups in the rats.

	Control(n=10)	G. 1 Ginger rhizome (50mg/kg-perday) (n=10)	G.2 Ginger rhizome (100mg/kg-perday) (n=10)
Testis (gr)	1.40±0.821	1.47±0.373	1.41±0.479
Sperm concentration (total count) (No of sperm/rat $\times 10^6$)	48.68±7.70	51.90±5.36	61.60±2.34*
Motility (%)	33.75±6.88	73±4. 35*	81±5.33*
Viability(%)	66.25±4.73	95.80±1.68*	98.80±80*
Serum Testosterone levels (ngr/ml)	1.60±0.091	2.91±0.349	3.71±0.387*
LH levels (ngr/ml)	1.51±0.138	1.66±0.316	2.23±0.453
FSH levels (ngr/ml)	20.37±1.788	21.59±2.69	21.68±2.11
Total Antioxidant capacity (TAC)	0.53±0.777	0.92±0.016*	0.88±0.341*
Malondialdehyde (MDA)	4.80±0.212	2.64±0.193*	0.81±0.192*

Data are presented as mean ± SE.
*Significant different at $p< 0.05$ level, (compared with the control group).

Result of light microscopic study

Histopathological study showed the cycle of spermatogenesis was regular in all experimental and control group. However, in all animals exposed to 50mg/kg/rat and 100mg/kg/rat ginger accumulations of sperm, in lumen of seminiferous tubules were seen (Figure 1).

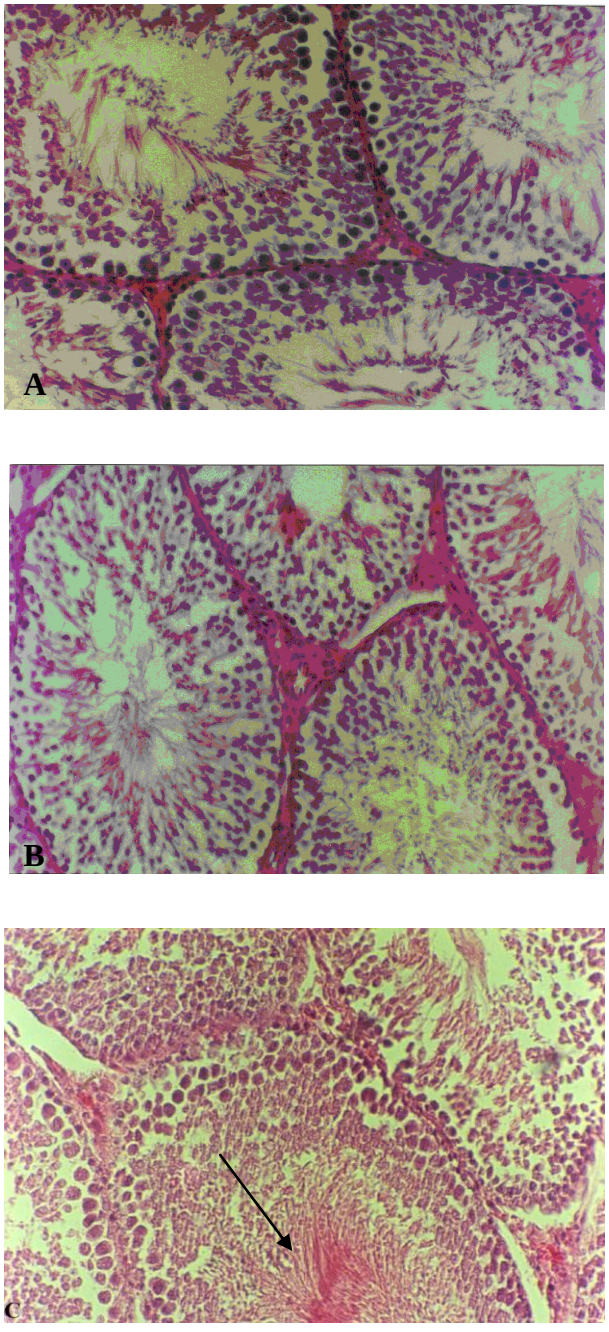

Figure 1. A) Regular seminiferous tubule with normal germinal epithelium morphology, (x640). **B)** Regular seminiferous tubule with normal germinal epithelium morphology in 50mg/kg/rat of ginger (G.1) group. (x640). **C)** Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence In lumen (arrow) in 100mg/kg/rat of ginger (G.2) group. (x640).

Discussion

The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (17).Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radicals, is an essential attribute of aerobic life (18). A disturbance in the pro-oxidant/antioxidant system has been defined as oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion (O^{-}_{2}), nitrogen oxide (NO) and hydroxyl radical (HO^{-}). Even though naturally present in the organism, they are mainly confined to cell compartments and counterbalanced by natural antioxidant molecules, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and vitamin C, acting as free radical scavengers (19,20).

Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities (21) 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. Cellular damage in the semen is the result of an improper balance between ROS generation and scavenging activities. Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress (22, 23).

Therefore, ROS production and total antioxidant capacity (TAC) can be used as a marker of oxidative stress in seminal fluid and is correlated with male infertility. Infertile men with male factor or idiopathic diagnoses had significantly lower ROS-TAC scores than controls (24).

Besides, Said *et al* (2005) suggested that abnormal sperm morphology combined with elevated ROS production may serve as a useful indicator of potential damage to sperm DNA. On the other hand, spermatozoa are highly susceptible to damage by excessive concentrations of ROS due

to the high content of polyunsaturated fatty acids within their plasma membrane. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and impairment of spermatogenesis (24).

In the present study, administration of 50mg/kg/rar and 100mg/kg/rat ginger for twenty consecutive days significantly increased sperm motility and viability in both experimental groups as compared with the control group (Table I). These results are supported by the finding of Aitken *et al* (1995), who reported that the conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen (25). This increase in sperm motility of experimental groups in comparison to control group could be due to the protective effect of ginger rhizoma administration. Besid, these productive effects is reflected by the decrease of malonaldehyde level and increase in total anti oxidants capacity (Table I).

In according with these results, Hamza *et al* (2006) have demonstrated that Z. officinale treatment increased the activities of testicular antioxidants enzyme and restore sperm motility of cisplatin-treated rats. Amr *et al* (26), reported in animal models that Z. officinale have protective effects against cisplatin-induced testicular damage and oxidative stress in rats. Ginger rhizome contains a wide variety of both antioxidative (6) and androgenic activity (27).

The major active phenolic ingredients isolated from Z. officinale (Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols) have antioxidant activity (7, 27, 28). Others reported that Z. officinale extracts have a potent androgenic activity in male rats (26). In agreement with these reports; the present study showed an increase in the testes weight, serum testosterone levels and accumulations of sperm in the lumen of seminiferous tubules (Figure 1).

In conclusion, the present study has demonstrated that, ginger possess an antioxidant and androgenic activity in doses of 50 mg/kg/rat and 100mg/kg/rat and have a useful effects on spermatogenesis and sperm parameters in rats.

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References

1. Isidori AM, Pozza C, Gianfrilli D, Isidori A. Medical treatment to improve sperm quality. *J Reprod Biomed* 2006;12:704 -714.
2. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; 305:609-613.
3. Mosher WD, Pratt WF. Fecundity and infertility in the United States: incidence and trends. *J Fertil Steril* 1991; 56:192-193.
4. Lu P, Lai BS, Liang P, Chen ZT, Shun SQ. Antioxidation activity and protective effection of ginger oil on DNA damage in vitro. *J Zhongguo Zhong Yao Za Zhi* 2003; 28:873-875.
5. Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B, Penkowski A. Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. *J Reprod Dev* 2006;52:203-209.
6. Sekiwa Y, Kubota K, Kobayashi A. Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *J Agric Food Chem* 2000; 48: 373-377.
7. Zancan KC, Marques MO, Petenate AJ, Meireles MA. Extraction of ginger (Zingiber officinale Roscoe) oleoresin with CO2 and co-solvents: a study of the antioxidant action of the extracts. *J Supercrit Flu* 2002; 24: 57-76.
8. Grzanna R, Lindmark L, Frondoza CG. Ginger--an herbal medicinal product with broad anti-inflammatory actions. *J Med Food* 2005; 8:125-132.
9. Rajeev K, Gagan G, Narmada P.Drug Therapy for Idiopathic Male Infertility: Rationale Versus Evidence. *J of Urology* 2006; 176: 1307-1312.
10. Yang HS, Han DK, Kim JR, Sim JC. Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. *J Korean Med Sci* 2006; 21:445-451.
11. Khaki A, Ghaffari Novin M, Khaki AA,Nouri M, Sanati E, Nikmanesh M. Comparative study of the effects of gentamicin, neomycin, streptomycin and ofloxacin antibiotics on sperm parameters and testis apoptosis in rats. *Pakistan journal of biological science* 2008; 11:1683-1689.
12. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 4th ed. Cambridge University Press, New York.1999.
13. Khaki A, Fathiazad F, Nouri M, Khaki AA, Jabarikh H, Hammadeh M. Evaluation of Androgenic Activity of Allium cepa on Spermatogenesis in Rat. *Folia Morphol* (Warsz).(in press) 2009; 68: 45-51.
14. Huang HFS, Linsenmeyer TA, Li MT, Giglio W, Anesetti R, von Hagen J,et al. Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study. *J Androl* 1995;16:148-157.
15. Feng R, He W, Ochi H. A new murine oxidative stress model associated with Senescence. *Mech Ageing Dev* 2001;122: 547–559.
16. Quintanilha AT, Packer L, Davies JM, Racanelli TL, Davies KJ. Membrane effects of vitamin E deficiency: bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. *Ann NY Acad Sci* 1982; 393: 32–47.
17. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. *Food Chem Toxicol* 2008; 46:409-420.

18. Sies, H. (ed.) Oxidants and antioxidants. In: Oxidative Stress. Academic Press, London. pp.1991; 15-22.
19. Aruoma A, Bolognini L, Macciantelli D, Carratelli M. The radical Cation of N, N Diethyl-para-phenilendiamine : a possible indicator of oxidative stress in biological samples. *Res Chem Intermed* 1994; 26: 253-267.
20. Miller JK; Brzezinska-Slebodzinska E, Madsen FC. Oxidative stress, antioxidant and animals function. *J Dairy Sci* 1993; 76:2812-2823.
21. Ahmed RS, Seth V, Banerjee BD. Influence of dietary ginger (Zingiber officinales Rosc) on antioxidant defense system in rat: comparison with ascorbic acid. *Indian J Exp Biol* 2000; 38:604-606.
22. De Lamminardi E, Leclerc P, Gagnon C . Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Mo Hum Reprod* 1997; 3: 175-193.
23. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci* 1996; 1: 78-86.
24. Sharma PK, Agarwal A. A Role of reactive oxygen species in male infertility. *Urology* 1996*;* 48: 835-850.
25. Aitken RJ, Buckingham DW, Brindle J, Gomez E, Baker HW, Irvine DS. Analysis of sperm movement in relation to the oxidative stress created by leukocytes in washed sperm preparations and seminal plasma. *Hum Reprod*1995; 10: 2061-2071.
26. Amr A, Hamza AEA. Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. *Asian J Androl* 2006; 8: 607-612.
27. Kamtchouing P, Mbongue Fandio GY, Dimo T, Jatsa HB. Evaluation of angrogenic activity of Zingiber officinale and pentadiplandra brazzeana in male rats. *Asian J Androl* 2002; 4: 299-301.
28. Jorsaraei SG, Yousefnia YR, Zainalzadeh M, Moghadamnia AA, Beiky AA, Damavandi MR. .The effects of methanolic extracts of ginger (Zingiber officinale) on human sperm parameters; an in vitro study. *Pak J Biol Sci* 2008 ; 11: 1723-1727.

