



Influence of Ginger (*Zingiber officinale*) on Sperms Parameters, Spermatogenesis and Sexual Hormones of Male Mice

*Mohammed .S.Alessia¹; Amr .A, Shalaby^{1,3}; Hind Abd alkarim³, Nasir, A.Ibrahim¹, Sharif Hossain A.B.M¹ and Ghanim Stam¹

1. Department of Biology, Faculty of Science, Ha'il University, Kingdom of Saudi Arabia.
2. Department of Biology, Faculty of Science, Al imam Mohammed Bin Saud University, Kingdom of Saudi Arabia.
3. zoology department, faculty of science, ain shams university.

*Corresponding Author.dr.aleissaa@gmail.com

Abstract

The use of ginger (*Zingiber officinale*), and specifically its medicinally active rhizome, has gained popularity among modern physicians in recent years. The effect of an aqueous extract of ginger was investigated on morphological of seminiferous tubules of mice and sperm parameters of semen for 90 male mice that were divided into three groups of 30 mice for each group. The experimental groups split into two groups each included 30 mice. (G.1) received 50mg/k/body weight and (G.2) received 100 mg/k/ body weight of ginger were dissolved in drinking water for 21 respectively days. This study aimed to evaluate the effect of ginger on Semen characteristics, sex hormones and the histology of the testes to show the spermatogenesis affected by ginger.

In the present study we showed that, the average of body weight decreased in all experimental ginger-treated groups (G1) and (G2) The testes weight decreased in both experimental groups, compared with the control group (mice without treated only distilled water); however, this decrease was statistically significant in body weight ($p < 0.05$). There was no significant testes weight decreased in experimental groups (G1) compared with the control group. The sperm counts increased in all experimental groups relative to the control group, although this increase was not statistically significant in group (G1), and there was statistically significant in experimental group (G2). However, there were highly significant increases ($p < 0.05$) in sperm motility and viability in all treated groups (G1 and G2) compared with the control group. The effects of ginger, on Sex hormones and antioxidant, was significantly different ($p > 0.05$) in Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and testosterone hormone ,total antioxidant capacity (TAC), and malondialdehyde (MDA) in all animal experiment when compared with control. Furthermore the histological study of testes showed a significant increase in the lumina spermatozoa at the level of Spermatogonia and primary spermatocytes.

Key words: Ginger, Spermatogeisis, Testosterone, Luteinizing hormone (LH) Follicle-stimulating hormone (FSH) antioxidant, malondialdehyde

1. Introduction

Infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor (Carlsen *et al*, 1992) and (Isidoriet *et al*, 2006). Several conditions can effects 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 (Mosher, and Pratt, 1991). The pharmacological effects of ginger and its fresh and dried rhizome, including its antioxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxic and anti-arthritic activities, have been demonstrated (Kamtchouing *et al*, 2002). Ginger extracts have also been reported to have a potent androgenic activity in male rats. There is evidence in the literature on the beneficial effects of oral antioxidant supplementation of vitamins in male infertility (Agarwal *et al*, 2006). Ginger extract has recently been shown to have a variety of biological activities, including anticancer, antioxidation, anti-inflammation and antimicrobial properties (Fisher-Rasmussen *et al*, 1991) and (Kamtchouing *et a.*, 2002). Today, ginger root is broadly used to prevent or treat pregnancy and cancer chemotherapy (Sripramoteand L, 2003). Ginger was also found to possess a protective against DNA damage induced by H_2O_2 and enhanced sperm healthy parameters in rats (Gmzanna *et a.*, 2005) and (Khaki *et a.*, 2009). On the other hand it can improve sperm quality and consequently increase fertility ratio in men (Rajeev *et a.*, 2006) and (Yang *et a.*, 2006).

1. Material and method

2.1 Experimental animals

Adult male mice (n=90) Swiss albino strain included in the present study. The mice were 8 weeks old with average weight $28 \pm 3g$ each. Male mice housed in temperature controlled rooms ($25^\circ C$) with constant humidity (40-70%) and 12h/12h light/ dark cycle according experimental protocols. All animals treated in accordance to the Principles of Laboratory Animal Care. All mice fed a standard diet. The daily intake of animal water monitored at least one week before start of treatments to determine the amount of water needed per experimental animal. Thereafter, the mice were randomly divided into control (n=30) received daily 8 ml distilled water and experimental groups (n=60), split into two groups each included 30 mice. Gmoup-1 (G1), received 50 mg/L and gmoup-2 (G2), received 100 mg/L of ginger (*Zingiber officinale*) dissolved in water for 21 consequence days.



2.2 Epididymis sperm count, viability and motility

Sperms from the cauda epididymis released by cutting into 2 ml of medium containing 0.5% bovine serum albumin. After 5 min. incubation at 37°C (with 5% CO₂), the cauda epididymis sperm concentration determined using the standard hemocytometric method and sperm viability using eosin stain and sperm motility analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according. (WHO, 1992).

Total antioxidant capacity (TAC) and Malondialdehyde (MDA) concentrations measurement in serum of mice. (TAC) detecting commercial kit, (Randox Laboratories, Crumlin, UK). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) with a peroxidase (methmyoglobin) and H₂O₂ to produce the radical cation ABTS, which has a relatively stable blue-green color measured spectrophotometrically at 600 nm. The suppression of the color is compared with that of Trolox, which is widely used as a standard for TAC measurements and the assay results are expressed as Trolox equivalents (in nmol/mL) (Quintanilha et al., 1982).

On the other hand the Malondialdehyde (MDA) concentrations, the free radical damage were determined by specifically measuring of (MDA). Serum MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with a spectrophotometer. A calibration curve was prepared using 1,1',3,3'-tetramethoxypropane as the standard (Randox Laboratories Crumlin, UK). (Quintanilha et al., 1982).

2.3 Serum FSH, LH total testosterone hormone measurements

Serum concentration of follicle stimulating hormone FSH, luteinizing hormone (LH), and testosterone were determined in duplicated samples using kit is based on the principle of a solid phase enzyme-linked immunosorbent assay, according to the protocol provided with each kit. (ELISA kit), which is based on competitive binding of hormones on immobilized antibody. Horse radish peroxidase was used for color development and absorbance at 450nm measured on a plate reader for (FSH) and (LH) while testosterone was measured at 420nm. Values are reported as ng/ml of serum.

2.4 Histology and Light microscopy

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

2.5 Statistical analysis

After 21 days, the mice were anesthetized and blood samples were collected directly from the heart and blood serum was separated. By using ELISA method, the LH, FSH, and testosterone Concentrations were measured and the data were entered into a computer. Data were analyzed using SPSS for Windows (version 17, SPSS Inc., Chicago, IL, USA), and one-way ANOVA statistical test and Duncan's post-hoc test. All P values of less than 0.05 were considered significant. The results expressed as mean ± S.E.M (standard error of means). Significant difference is written in parentheses.

2. Results

As shown in Table.1. , the average of body weight decreased in experimental groups, including (G1) and (G2) ginger-treated groups compared with the control group; however, this decrease was statistically significant in body weight ($p < 0.05$). There was no significant testes weight decreased in experimental groups, including (G2) compared with the control group. In addition, the results indicate that the sperm concentration increased in all experimental groups relative to the control group, although this increase was not statistically significant in groups, including (G1) and there was statistically significant in experimental groups, including (G2) at level ($p < 0.05$). However, there were highly significant increases ($p < 0.05$) in sperm motility and viability in all treated groups compared with the control group. Moreover the viability was determined for control and both treated groups (G1 and G2) the viability, concentration and viability in treated groups were statistically significant at level ($p > 0.01$). The effects of ginger on sex hormones the study showed that there was significant different ($p > 0.05$) in FSH, LH and Testosterone hormone ,Total Antioxidant capacity (TAC) and Malondialdehyde (MDA) in two experiments groups when compared with control (Table 1&2). The histological study illustrated. Figure 1 illustrated the regular seminiferous (A) tubule with normal germinal epithelium morphology (B), in the control group magnification at (X400). While figure 2 explain the regular seminiferous tubule (A) with normal germinal epithelium morphology and sperm presence in lumen and a significant increase in the luminal spermatozoa in (G1) and (G2) magnification at (X400). On the other hand figure 3 showed regular seminiferous tubule (A) with normal germinal epithelium morphology and sperm presence in lumen and a significant increase in the luminal spermatozoa in (G1) and (G2) magnification at (X400).

Table 1. Effects of ginger on the average of body, testis weight (g), sperm count (10⁶/ml), sperm motility (%) and sperm viability (%).

Parameter	Control(n=30)	G. 1 (n=30)		G.2 (n=30)	
	(mice without treated only distilled water)	Ginger (50mg/kg-/day)	rhizome	Ginger (100mg/kg/day)	rhizome
Body (gm)	25.20±0.67	26.5±0.86*		28.5±0.96*	
Testes (gm)	0.20±0.081	0.20±0.019		0.28±0.088*	
Sperm concentration 10 ⁶ /ml)	66.30±3.06	80.33±2.83*		91.90±1.49**	
Motility (%)	36.79±2.36	75.80±2.93**		85.00±1.12**	
Viability %	63.26±3.06	78.10±3.90*		89.19±1.16**	
(TAC)	0.43±0.80	0.82±0.02*		0.78±0.211*	
(MDA)	3.70±0.110	1.54±0.183*		0.71±0.163**	

*significant at P<0.05. **significant at P<0.01. mean ± SD

Table 2. Effects of ginger on the concentration of serum FSH, LH & testosterone in serum of male mice.

Parameter	FSH (ng/ml)	LH(ng/ml)	Testosterone (ng/ml)
Control	0.11±0.07	0.14±0.02	0.33±0.09
Ginger	0.21±0.05*	0.15±0.04*	1.16±0.068**

*significant at P<0.05. **significant at P<0.01. mean ± SD

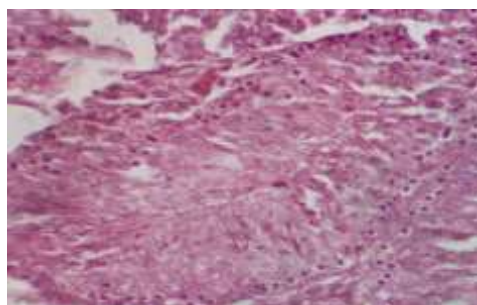


Figure 1. Regular seminiferous and tubules with normal germinal epithelium morphology in the control group at (X400).

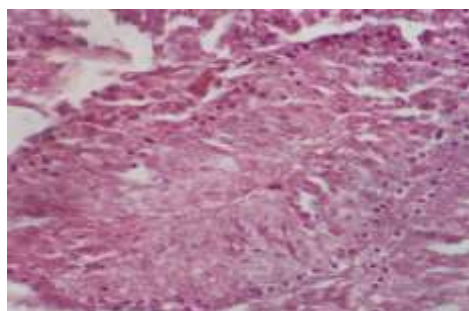


Figure 2. Regular seminiferous tubules of (G1) at (X400), with normal germinal epithelium and sperm presence in lumen.

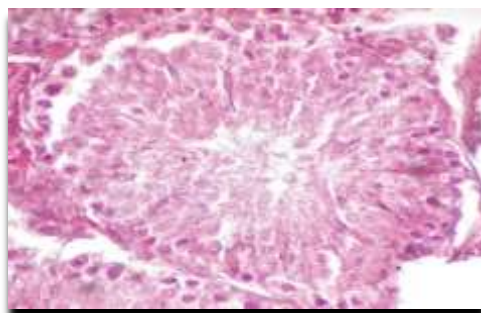


Figure 3. Regular seminiferous tubule (A) with normal germinal epithelium morphology and sperm presence in lumen and a significant increase in the luminal spermatozoa in (G2) at (X400).

3. Discussion

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 (Ali BH *et al* 2008). 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 (Sharma PK and Agarwal A 1996). In the present study, administration of 50 mg/L and 100 mg/L the results were supported by the finding of some researcher who reported that the conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen. This increase in sperm motility of experimental groups in comparison to control group could be due to the protective effect of ginger administration and this result appropriate to the study carried out by (Dalia A. 2010), who find an optimistic effect of oral administration of extracts of ginger on sperm cell characteristics of male diabetic rats.

In according with these results have demonstrated that ginger treatment increased the activities and sperm motility of treated mice. (Amr and Hamza A 2006), reported that, the ginger have protective effects against cisplatin-induced testicular damage and oxidative stress in rats. Ginger rhizome contains a wide variety of both antioxidative and androgenic activity (Sekiwa Y *et al* 2000), (Kamtchouing P *et al* 2002). The major active phenolic ingredients isolated from *Z. officinale* are (Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols) have antioxidant activity (Zancan KC, *et al.* 2002), (Kamtchouing P *et al* 2002). Others reported that *Z. officinale* extracts have a potent androgenic activity in male rats (Amr and Hamza A 2006) and (Dalia A. 2010).

In the present study sex hormones FSH, LH and testosterone was significant different. This result was constant with result obtained by

(Waleed A *et al*, 2012), whom reported that serum levels of follicle stimulating hormone (FSH), Letelizing hormone (LH) and testosterone in men were increased signicantly for FSH at $p < 0.05$ and highly significant at $p < 0.01$ for LH and Testosterone.

Histological study of testes showed that significant increase of spermatogenesis in lumen of seminiferous tubules in both experimental group (G1 and G2) when compared with control group and the increase was clear at the level of primary spermatocytes and spermatids and free sperm as shown in figure 2 and figure 3, This result was supported by (Khaki A., *et al*, 2009) and (Aleissa M, 2014), who report that the ginger has good effect to accumulations of sperm in the lumen of seminiferous tubules. The present work conclude also that, ginger have an antioxidant and androgenic activity in doses of 50 mg/L/ and 100mg/L and have a useful effects on spermatogenesis and sex hormones encourages in mouse and have positive effect on reproductive parameter and our results with study carried out by (Sadeghi A *et al*, 2013), they find that , ginger supplementation, at and over 5.0 g/kg, caused improvement in plasma of broiler chicks by decreasing MDA, furthermore (Waleed A *et al*, 2012), reported that, ginger administration caused a significant reduction in serum MDA. Increasing of TAC in experimental group was significant at $P < 0.05$ compared to control group and our result was seemly with study by (Ismail I *et al* 2012) this elevation caused by a synergistic effect of ginger on these enzymes.

4. Acknowledgement

This work was kindly sponsored by University of Hail –Research Deanship NO. SCBI-15 .The authors would like to express their gratitude to the Biology Department, Faculty of Science University of Hail

References

- Aleissa M. 2014., Effect of Ginger Supplements on Some Reproductive Parameters and Spermatogenesis of Mice. *Indian Journal of Fundamental and Applied Life Sciences*. Vol. 4 (1), pp.271-277.
- Ali BH, Blunden G, Tanira MO, Nemmar A., 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiberofficinale* Roscoe): a review of recent research. *Food Chem Toxicol*; 46:409-420.



- Amr, A. and Hamza, A. E. A., 2006. Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. *Asian J. Androl*, 8: 607-612.
- Carlsen E, Giwercman A, Keiding N, Skakkebaek NE., 1992. Evidence for decreasing quality of semen during past 50 years. *British Medical Journal*; 305:609-613.
- Dalia A. Hafez. 2010., Effect of Extracts of Ginger Goots and Cinnamon Bark on Fertility of Male Diabetic Rats. *Journal of American Science*; (6)10. 940-947.
- Gmzanna R, Lindmark L, Frondoza CG., 2005. Ginger--an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*; 8:125-132.
- Ismail I. Abo-Ghanema, El-Nasharty M.A., El-Far A. H., and Hanan A.Ghonium. 2012., Effect of Ginger and L-Carnitine on the Reproductive Performance of Male Rats. *World Academy of Science, Engineering and Technology*.Vol:64 .04-22
- Kamtchouing P, MbongueFandio GY, Dimo T, Jatsa HB., 2002. Evaluation of anngmogenic activity of Zingiberofficinale and pentadiplandrabrazeana in male rats. *Asian Journal of Androl*; 4: 299-301.
- Khaki, A., Fathiazad, F., Nouri, M., Khaki, A.A., Ozanci, C.C., 2009. The effects of ginger on spermatogenesis and sperm parameters of rat. *Iran Journal of Reprod Med*; 7(1):7-12.
- Rajeev K, Gagan G, Narmada P., 2006. Drug Therapy for Idiopathic Male Infertility: Rationale Versus Evidence. *Journal of Urology*; 176: 1307-1312.
- Sadeghi, A.A., Izadi W, Shawrang P, Chaman M.and Aminafshar M. 2013., Total Antioxidant Capacity and Malondialdehyde Level in Plasma of Broiler Chicks Fed Diet Containing Different Levels of Ginger (*Zingiber officinale*), *Animal Science Applied of Iranian Journal*.287, 283. 3(2).
- Sekiwa Y, Kubota K, Kobayashi A., 2000. Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Journal of Agmic Food Chem*; 48: 373- 377.
- Sharma PK, Agarwal A. A., 1996. Role of reactive oxygen species in male infertility. *Journal of Urology*; 48: 835-850.
- World Health Organization WHO., 1999. Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 4th ed. Cambridge University Press, NewYork.
- Waleed Abid Al-Kadir Mares, Wisam S. Najam., 2012. The effect of Ginger on semen parameters and serum FSH, LH & testosterone of infertile men. *Tikrit Medical Journal*;18(2):322-329.
- Yang HS, Han DK, Kim JR, Sim JC., 2006. Effects of alphantocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. *Journal of Korean Med Sci*; 21:445-451.
- Zancan KC, Marques MO, Petenate AJ, Meireles MA., 2002. Extraction of ginger (*Zingiberofficinale* Roscoe) oleoresin with CO2 and co-solvents: a study of the antioxidant action of the extracts. *Journal of Supercrit Flu*; 24: 57-76.