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Research Article

EFFECT OF GINGER SUPPLEMENTS ON SOME REPRODUCTIVE PARAMETERS AND SPERMATOGENESIS OF MICE

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ABSTRACT
The effect of an aqueous extract of ginger *(Zingiberofficinale*) was investigated on morphometric of seminiferous tubulestestis of mice and sperm parameters of semen for 60 male mice. They were divided into three groups of 22 mouse each, Group served as the control and was administered distilled water while experimental groups G1, and G2 were treated with 50mg/kg and 100mg/kg body weight of aqueous extract of Ginger respectively for 22 days. There was no significant difference in the body weight between control group and experimental group (G2), (100gm/L), but with decreased in experimental group, including 50gm/L (G1), however, this decrease was statistically significant in body weight ($p<0.01$). There was no significant difference in testes weights between the groups. The results of analyzing the changes in weighing the testes of the control group was (0.20±0.081gr), in the group(G1), 50mg/L was (0.20±0.019 gr), in the group (G2), 100mg/L was (0.19±0.088gr).Administration of 50mg/kg/mouse and 100mg/kg/mouse ginger for 22 consecutive days significantly increased Sperm motility and viability in both experimental groups as compared with the control group. The testicular histology shows no abnormality. Spermatogenic cell progeny is normal containing all cell in the progeny. This follow hypothesis that ginger have potent androgenic and antioxidant effects on the testis. the present study has demonstrated that, ginger have an antioxidant and androgenic activity in doses of 50 mg/kg/mouse and 100mg/L mouse and have a useful effects on spermatogenesis and sperm parameters in mouse and have positive effect on reproductive behavior.

Keywords: *Zingiberofficinale, Ginger, Sperm, Spermatogenesis, Semen*

INTRODUCTION
Ginger is common is name for plant belongs to family Zingiberaceae, which contains about 1300 species in 50 genera, along with four other families is placed in the order *Zingiberales* (Garner *et al.,* 2006). Nowadays ginger rhizome (Zingiber officinale R., family: Zingiberaceae), is used worldwide as a spice. Many studies were carried out on ginger and its pungent constituents, fresh and dried rhizome. The plant is completely sterile (produce no seed) and only propagated by rhizomes (Fnimh, 2001). Ginger is a plant that comes from south-east Asia, and also in cultivated in Africa, China, India and Jamaica (Ravindran *et al.,* 2004; Altman *et al.,* 2001).
Chemical constituents of ginger classified to volatile oils (including borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine and zingiberolpenols (gingerol, zingerone and shogaol) and resin) which constitutes (1-3%) mainly of zingeberene nonvolatile pungent compounds oleo-resin constitutes (4-7.5%) mainly gigerols and other constituents with more than 50% of starch (Fnimh, 2001; Ravindran, 2004). The nutritional contents of ginger includes protein, lipids, carbohydrates, and its good source from sources Fe, Mg, Ca and vitamins especially vitamin C (Sekiwa *et al.,* 2000).
Ginger extract has recently been shown to have a variety of biological activities, including anticancer, ant oxidation, anti-inflammation and antimicrobial properties (Fisher-Rasmussen *et al.,* 1991; Sharma *et al.,* 1994; Kamtchouing *et al.,* 2002).
Today, ginger root is broadly used to prevent or treat pregnancy and cancer chemotherapy (Sripramote and Lekhyananda, 2003).
Ginger was also found to possess a protective against DNA damage induced by H_2O_2 and enhanced sperm healthy parameters in rats (Grzanna *et al.,* 2005; Khaki *et al.,* 2009). On the other hand can improve sperm quality and consequently increase fertility rate in men (Rajeev *et al.,* 2006; Yang *et al.,* 2006).

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Therefore, the role of nutritional and biochemical factors in reproduction and sub- fertility treatment is very important. To evaluate protective effect of ginger against cisplatin-induced reproductive toxicity, ethanolic extract of ginger (1 g/kg/day) were given to male albino rats for 26 days. Result showed that ginger treatment increased the activities of testicular antioxidant enzymes and restored sperm motility of cisplatin-treated rats which was in contrary with present results. Also the aqueous extract of ginger significantly increased weight of the testis, the serum testosterone level and epididymal a-glucosidase activity *in vivo* (Kamtchouing *et al.,* 2002). A clinical trial study showed that high concentrations of inhibited motility at 24 and 48 h (Mascolo *et al.,* 1989; Srivastava and Mustafa, 1992).
Ginger extracts have also been reported to have a potent androgenic activity in male rats. Human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D3 (Kametani and Furuyama, 1987).
Ginger is example of plants which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally and wlinary useful part (Mascolo *et al.,* 1989). Khaki *et al.,* (2009) reported that ginger extract possess a protective effect against DNA damage induced by H2O2 and enhancedsperm healthy parameters in rats. The effect of ginger on male reproduction was studied by some investigators (Hafez, 2010; Zahedi *et al.,* 2010; Sakrand, 2011). The current study was designed to investigate the effects of ginger supplements on sperm parameters and histomorphological changes of semini-ferous tubule of testis.

MATERIALS AND METHODS

Experimental Animals

This study was conducted at the reproductive and embryology laboratory, Department Biology, Hail University. Ginger (*Z. officinale*R.) rhizome was purchased from the local market. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered.
Sixty Adult mice (n=60) were included in the present study. They were 8 weeks old and weighing 28±3g each. They were obtained from animal house at college of Science, king Saud University and 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 mice were fed a standard diet. 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 mice were randomly divided into control (n=20) and experimental (n=40) groups. The control group just received 8ml distilled water daily. However, the experimental groups split into two groups each included twenty mouse. (G.1) received 50mg of ginger /L/mouse and (G.2) received 100ml of ginger /L/mouse of ginger, ginger dissolved in drinking water for 22 consequence days.

Sperm Concentration, Motility and Viability

The epididymis of each mouse was used for the determination of epididymal sperm concentration using the Neubauerhaemocytometer, while percentage of sperm motility was determined as described by (Sönmez *et al.,* 2005; Aleissa *et al.,* 2009). Fluid was obtained from the epididymis with a pipette and diluted to 2ml with tris-buffersolution. The percentage of motility was evaluated at 37 °C by using Olympus IX70 microscopeat×400 magnification on wormed stage (Aleissa *et al.,* 2011a).
Evaluated at least 200 spermatozoa in a total of at least five fields in each replicate, calculated the average percentage and difference between the two percentages for the most frequent motility grade in the replicate wet preparations. Reported the average percentage for each motility grade .

Sperm Vitality

Sperm vitality was assessed as soon as possible after liquefaction of the semen sample, preferably at 30 minutes, to prevent observation of deleterious effects of dehydration or of changes in temperature on vitality. Test was carried out by using eosin Y, 0.5%.Slides was Examined preferably with negative-phase-contrast optics (positive phase-Contrast makes faint pink heads difficult to discern) at ×400

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magnification. Then the number of stained (dead) and unstained (vital) cells was recorded at least 200 spermatozoa in each replicate were evaluated as described by (Aleissa *et al.,* 2011b)

Histological Studies

The testis were dissected apart, cut into small pieces and were immediately fixed in alcoholicBouins solution for 24 hours, then dehydrated and finally embedded in paraffin and weresectioned serially in 5 mm thick sections. The sections were stained with hematoxylin and eosin(H&E) and studied with 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). The p values < 0.05 was considered as significant throughout this study.

RESULTS

Weight of Individual Male Body and Testis

The obtained results in this study are illustrated in Table 1, There was no significant difference in the body weight between control group and experimental group (G1), (100gm/L), but with decreased in experimental group, including 50gm/L (G1), however, this decrease was statistically significant in body weight ($p<0.01$). There was no significant difference in testes weights between the groups. The results of analyzing the changes in weighing the testes of the control group was (0.20±0.081gr), in the group(G1), 50mg/L was (0.20±0.019 gr), in the group (G2),100mg/L was (0.19±0.088gr).

In addition, the results indicate that the sperm concentration increased in all experimental groups relative to the control group, although this increase was statistically significant in groups(G1,G2), 50gm/Land 100gm/Lat level ($p<0.01$) as seen in table1 respectively.

Table 1: Effects of ginger on the average of body, testis weight (g), sperm count (10^6/ml), sperm motility (%) and sperm viability (%)

	Control(n=10)	G. 1 Ginger rhizome (50mg/kg-/day) (n=10)	G.2 Ginger rhizome (100mg/kg/day) (n=10)
Body (gr)	28.20±0.67	24.5±0.86*	28.5±0.96
Testis (gr)	0.20±0.081	0.20±0.019	0.19±0.088
Sperm concentration (sperm/rat x10^6)	65.30±3.06	79.33±2.83*	89.90±1.49*
Motility (%)	35.79±2.36	74.80±2. 93*	84.00±1.12*
Viability(%)	65.26±3.06	80.10±3.90*	93.19±1.16*

Administration of 50mg/kg/mouse and 100mg/kg/mouse ginger for 22 consecutive days significantly increased Sperm motility and viability in both experimental groups as compared with the control group. The motility and vitality were (74.80±2. 93% and 80.10±3.90%) in G.1 and the corresponding value in G.2 were (84.00±1.12 % and 93.19±1.16%) respectively. both experimental groups were significantly higher in comparison to the values of motility and vitality of control group (35.79±2.36 %; and 65.26±3.06 %) as showed in table 1. In case of male mice treated with ginger daily for 22 days the lumen of the seminiferous tubules showed a significant increase in the luminal spermatozoa (for both experimental animalG1,G2). microscopic examination showed that in the control group, testes had a normal testicular architecture with an orderly arranged spermatogenic cells. The spermatogonia and sertoli cells were rested on the basement membrane of the seminiferous tubules. The Leydig cells were located in the interstitial tissue among seminiferous tubules (Figure 1).In the experimental group showing many histomorphological changes. After 22 days, the seminiferous full up with cells. A large number of germ cells were found in the lumen of seminiferous tubules (Figure 2). The sertolicells seemed not to be clear in experimental group(G2), 100 mg/L compare to (G2), 50mg/L as showed in (Figure 2 and 3) respectively. The Leydig cells in the interstitial spaces had a normal morphology for all groups.

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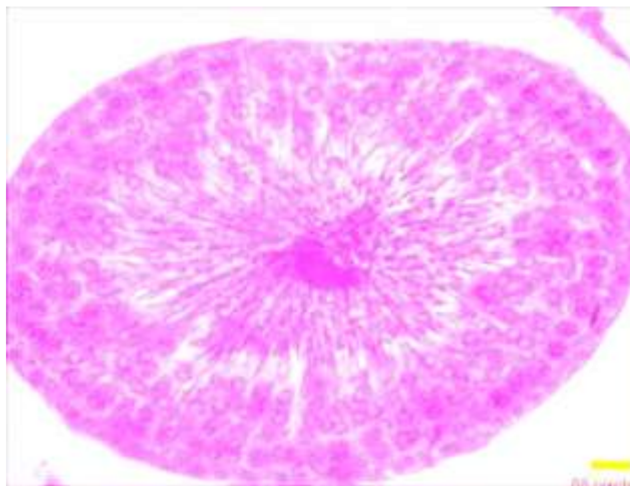

Figure 1: Light microscopic micrograph of seminiferous tubule with normal germinal epithelium morphology, (x640) in control group

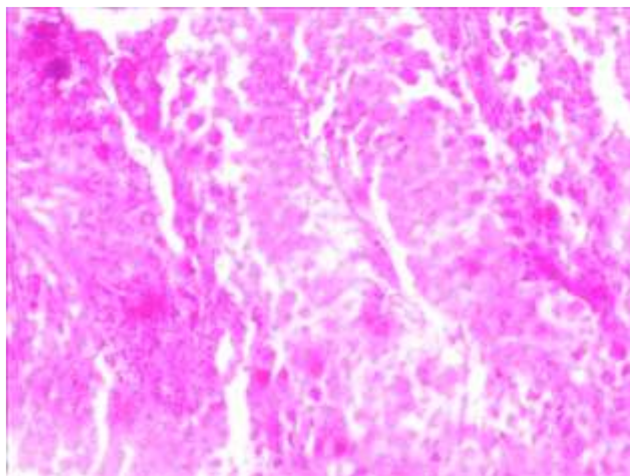

Figure 2: Regular seminiferous tubule with normal germinal epithelium morphology in 50mg/L/mouse of ginger (G.1) group (x640)

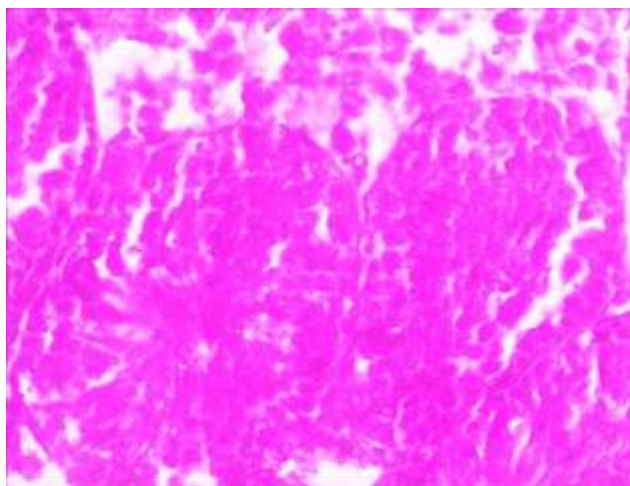

Figure 3: Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence In lumen in 100mg/ L/mouse of ginger (G.2) group (x640)

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DISCUSSION

Previous results were supported by the finding of (Arsh *et al.,* 2009; Ali *et al.,* 2008), who reported that administration of 50 mg/kg or 100 mg/kg/rat ginger for 20 days significantly increased sperm functions (sperm count, motility, viability), concentrations in rat, ginger have protective effects against oxidative stress in rat. This finding is in accordance with present results.

Free radicals are a group of highly reactive chemical molecules with one or more unpaired electrons that can oxidatively modify biomolecules they encounter. Reacting almost immediately with any substance in their surrounding area, they begin a chain reaction leading to cellular damage (Warren *et al.,* 1987). Superoxide anion, hydroxyl radical and hydrogen peroxide are major reactive oxygen species (ROS) present in seminal plasma. Cells living under aerobic conditions require oxygen to support life; however, metabolites, such as ROS, can modify cell functions and endanger cell survival (Agarwal *et al.,* 2003). Male germ cells at various stages of differentiation have the potential to generate ROS and low physiologic levels are needed to regulate sperm capacitation, acrosome reaction and sperm–oocyte fusion (Agarwal & Saleh, 2002; Agarwal *et al.,* 2004). To maintain normal cell function, excess ROS must be continuously inactivated by seminal plasma antioxidants. These block the formation of new ROS or act as scavengers and remove ROS already generated. In healthy men, a delicate balance exists between physiological ROS and antioxidants in the male reproductive tract (Sikka *et al.,* 1995). The study 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 (Sharma and Agarwal, 1996).

Smart choices for better foods might prevent body from many diseases (Reddy *et al.,* 2006; Suryavathi *et al.,* 2005). As all spermatogenesis stages occur in semini-ferous tubule of testis, it is possible to evaluate the extent of spermatogenesis by determination of number of spermatozoa produced per one gram of testicular parenchyma (Acharya *et al.,* 2008; Hew *et al.,* 1993). The sperm count is considered as important parameter assesses the effects of chemical on spermatogenesis (Yousef, 2005). It has also been reported that there is a direct correlation between the epididymal sperm count and motility with fertility in animals (Dawson *et al.,* 1992; Timmermans, 1989 and Yu *et al.,* 2005). The oxidative damage, elevated lipid peroxidation and the alteration of membrane properties can lead to germ cell death at different stages of development and the sperm count decrease (Bestas *et al.,* 2006). Accordingly, it is expected that antioxidant therapy acts as a protective defense against oxidative stress and improve fertility parameters (Zahedi and Khaki, 2014).

In the present study, administration of 50mg/kg/mouse and 100mg/kg/ mouse ginger for 22 consecutive days significantly increased sperm concentration, motility and viability in both experimental groups as compared with the control group as seen in table 1. The increase in sperm motility of experimental groups in comparison to control group could be due to the protective effect of ginger rhizoma administration this result Compatible with the result was reported by (Khaki *et al.,* 2009; Kamtchouing *et al.,* 2002). In consistency with these reports; the present study showed accumulations of sperm in the lumen of somniferous tubules in both experimental groups. On the other hand, The testicular histology shows no abnormality. Spermatogenic cell progeny is normal containing all cell in the progeny. This follow hypothesis that ginger have potent androgenic and antioxidant effects on the testis (Zancan.*et al.,* 2000; Sekiwa *et al.,* 2000; Amir and Hamza, 2006; Kamtchouing *et al.,* 2006).

In conclusion, the present study has demonstrated that, ginger have an antioxidant and androgenic activity in doses of 50 mg/kg/mouse and 100mg/L mouse and have a useful effects on spermatogenesis and sperm parameters in mouse and have positive effect on reproductive behavior.

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REFERENCES

Acharya UR, Mishra M, Patro J and Panda MK (2008). Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reproductive Toxicology* **25** 84-88.

Agarwal A & Said TM (2003). Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update* **9** 331–345.

Agarwal A & Saleh RA (2002). Role of oxidants in male infertility: rationale, significance, and treatment. *Urologic Clinics of North America* **29** 817–827.

Agarwal A, Nallella KP, Allamaneni SS & Said TM (2004). Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive Biomedicine Online* **8** 616–627.

Al-Eissa Mohammed Saad, Alhamidi AR , Mohamed Abd El Kader Sandouka, Ibraheem AS, Hamad Al-Yahya, AL-Farraj S, Saad Alkahtani and Al-Dahmash B (2011a). Scrotal, Testicular and Semen Characteristics of The Mountain Gazelle Males (*Gazellagazella*). *African Journal of Microbiology Research* **5**(28) 5137-5141.

Al-Eissa Mohammed, Alhamidi A, Saad Alkahtani and Mohamed Abd El Kader Sandouka (2011b). Determination of the Arabian Sand Gazelle Sperm and Acrosomes Defects by Using Spermac Staining Technique. *Asian Journal of Biological Science* **4**(2) 138-147.

Al-Eissa MS, Mohammed Abd El Kader S, Saad H and Ashraf AQ (2009). Reproductive characteristics of The Arabian Sand Gazelles *(Gazellasubgutturosamarica)*. *Journal of the Saudi Society of Agricultural Sciences* **9**(1a) 70-80.

Ali BH, Blunden G, Tanira MO and Nemmar A (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiberofficinale Roscoe): a review of recent research. *Food and Chemical Toxicology* **46** 409-420.

Altman RD and Marcussen KC (2001). Effects of a ginger extract on knee pain in patients with osteoarthritis. *Arthritis and Rheumatism* **44**(11) 2531-2538.

Amir Amin, AlaaEldin A and Hamzaa AA (2006). Effects of ginger and Roselle on Cisplatin induced reproductive toxicity in rats. *Asian Journal of Andrology* **5** 607 – 612.

Bestas A, Bayar MK, Akpolat N and Okuducu MN (2006). Effect of sevoflurane anesthesia on the severity of renal histopathologic changes in rabbits pretreated with gentamicin: A controlled, investigator-blinded, experimental study. *Current Therapeutic Research* **67** 386-395.

Dawson EB, Harris WA, Teter MC and Powell LC (1992). Effect of ascorbic acid supplementation on the sperm quality of smokers. *Fertility and Sterility* **58** 1034-1039.

Fisher–Rasmussen W, Kjaer S, Dahl C and Asping U (1991). Ginger treatment of Hyperemesis gravidarm. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **38** 19–24.

Fnimh AC (2001). *Encyclopedia of Medicinal Plants* (London, UK) 155-156.

Garner-Wizard M, Milot SHB, Neustadt EJ, Oliff HS and Oppel M (2006). Ginger, an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food* **8** 125-132.

Grzanna R, Lindmark L and Frondoza CG (2005). Ginger – An Herbal Medicinal Product with Broad Anti-Inflammatory Actions. *Journal of Medicinal Food* **8**(2) 125-132.

Hafez D (2010). Effect of extracts of ginger goots and cinnamon bark on fertility of male diabetic rats. *Journal of American Sciences* **6** 940–7.

Hew KW, Heath GL, Jiwa AH and Welsh MJ (1993). Cadmium in vivo causes disruption of tight junction-associated microfilaments in rat Sertoli cells. *Biology of Reproduction* **49** 840-849.

Kametani T and Furuyama H (1987). *Synthesis of vitamin D3 and related compounds*. *Medicinal Research Reviews* **7**(2) 147–171.

Kamtchouing P, Kahpui SM, Dzeufiet PD, T'edong L, Asongalem EA and Dimoa T (2006). Anti-diabetic activity of methanol/methylene chloride stem bark extracts of Terminaliasuperba and Canariumschweinfurthii on streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* **104** 306-309.

Kamtchouing P, Mbongue Fandio GY, Dimo T and Jatsa HB. (2002). Evaluation of angrogenic activity of Zingiberofficinale and pentadiplandrabrazzeana in male rats. *Asian Journal of Andrology* **4** 299-301.

Khaki A, Fathiazad F, Nouri M, Khaki AA, Chelar CO, Ghafari-Novin M and Hamadeh M (2009). The Effects of Ginger on Spermatogenesis and Sperm parameters of Rat. *Iranian Journal of Reproductive Medicine* **7**(1) 7 – 12.

Mascolo N, Jain R, Tain SC and Capasso FJ (1989). Ethnopharmacologic investigation of ginger (Zingiberofficinale). *Journal of Ethnopharmacology* **27**(1-2) 129-140.

Rajeev K, Gagan G and Narmada P (2006). Drug Therapy for Idiopathic Male Infertility: Rationale Versus Evidence. *Journal of Urology* **176** 1307-1312.

Ravindran PN and Babu KN (2004). *Ginger, the Genus Zingiber* (CRC Press, Boca Raton).

Reddy PS, Pushpalatha T and Reddy PS (2006). Reduction of spermatogenesis and steroidogenesis in mice after fentin and fenbutatin administration. *Toxicology Letters* **166** 53-59.

Sakr S and Badawy G (2011). Effect of ginger (*ZingiberofficinaleR.*) on metiram-inhibited spermatogenesis and induced apoptosis in albino mice. *Journal of Applied Pharmaceutical Science* **4** 131-136.

Sekiwa Y, Kubota K and Kobayashi A (2000). Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Journal of Agricultural and Food Chemistry* **48** 373-377.

Sharma J, Srivastava K and Gan E (1994). Suppressive effects of eugenol and ginger oil on arthritic rats. *Pharmacology* **49** 314–8.

Sharma PK and Agarwal AA (1996). Role of reactive oxygen species in male infertility. *Urology* **48** 835-850.

Sikka SC, Rajasekaran M and Hellstrom WJG (1995). Role of oxidative stress and antioxidants in male infertility. *Journal of Andrology* **16** 464-8.

Sönmez M, Türk G and Yüce A (2005). The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of Wistarrats. *Theriogenology* **63** 2063–72.

Sripramote M and Lekhyananda N (2003). A randomized comparison of ginger and vitamin B 6 in the treatment of nausea and vomiting of pregnancy. *Journal of the Medical Association of Thailand* **86** 846-853.

Srivastava K.C and Mustafa T. (1992). Medical.Ginger (Zingiberofficinale) in rheumatism and musculoskeletal disorders. *Hypotheses* **39**(4) 342-348.

Timmermans LM (1989). Modifications in spermatogenesis following antibiotic therapy. *Acta Urologica Belgica* **57** 35-46.

Warren JS, Johnson KJ & Ward PA (1987). Oxygen radicals in cell injury and cell death. *Pathology and Immunopathology Research* **6** 301–315.

Yang HS, Han DK, Kim JR and Sim JC (2006). Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. *Journal of Korean Medical Science* **21** 445-451.

Yousef MI (2005). Protective effect of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. *Toxicology* **207** 81-9.

Yu LL, Zhou KK, Parry J (2005). Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chemistry* **91** 723-729.

Zahedi A and Khaki A (2014). Recovery effect of *Zingiberofficinale*on testis tissue after treatment with gentamicin in rats. *Journal of Medicinal Plant Research* **8**(6) 288-291. DOI: 10.5897/JMPR11.312.

Zancan KC, Marques MO, Petenate A and Meireless MA (2000). Extraction of ginger (Z.O Roscoe) oleoresin with CO2 and co-solvent: a study of the antioxidant action of the extracts. *The Journal of Supercritical Fluids* **24** 57- 76.

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