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# Effect of ginger, *Zingiber officinale* on sex hormones and certain biochemical parameters of male Wistar rats

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## ABSTRACT

It is well demonstrated that phytoestrogens play an important role in the male reproductive system. So, the aim of the current study was to determine effect of ginger on sex hormones and blood biochemical levels in rat. A 40 male Wistar rats (200–250 g) kept as folk and fed basal chew pellet for a week, then randomly divided into 4 experimental groups. Group 1 was kept as control and fed basal diet (commercial chew pellet) and orally gavage with distilled water. Groups 2-4, provided basal diet and orally gavage of the 100, 200 and 300 mg ginger powder in distilled water, respectively for 4 weeks. At the end of the study, Serum glucose, cholesterol, triglyceride, LDL, HDL, albumin, total protein, urea as well as LH, FSH and testosterone determined. Then animals sacrificed and sperm was collected from epididymis and prepared for spermatozoa characteristics, semen testosterone, LH and FSH levels. According to the results, dose dependent increase observed on spermatozoa forward movement (P=0.0001). A dose dependent increase observed on sperm viability (P=0.0001). Orally administration of the 100, 200 and 300 mg of the ginger significantly increased serum total protein (P=0.0008) and decreased glucose (P=0.014) compared to the control group. Administration of the ginger in a dose dependent manner increased serum triglyceride (P=0.01) and HDL (P=0.0006) levels in rat compared to the control group. Orally gavage of the ginger significantly increased semen testosterone levels in comparison to the control group (P=0.009) while had no significant effect on LH and FSH levels (P>0.05). No significant effect observed on serum LH, FSH and testosterone levels compared to the control group (P>0.05). These results suggested ginger improves spermatozoa characteristics and semen hormone level.

**KEY WORDS:** GINGER, LH, FSH, TESTIS, BLOOD BIOCHEMICAL, RAT

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## INTRODUCTION

Infertility is one of the major health problems in the world (Ghalehkandi, 2014). Infertility is a multi-parametric phenomenon which more than 30 % of infertilities are related to a male factor (Vincent et al. 2012). Several factors affect spermatogenesis and sperm quality such as drug treatment, chemotherapy, toxins (Adeeyo et al. 2011), air pollutions and insufficient vitamins intake (Barkhordari et al. 2013). It is reported administration of 100 mg/kg of ginger increased sperm percentage, motility viability, and serum testosterones in rat (Khaki et al. 2009). Based on the literature has a long history in order to fertility regulation (Kooti et al. 2015). Also, there are increasing interests on plant-derived chemicals on the endocrine system and the activity of sexual organs (Kooti et al. 2015).

Ginger (*Zingiber officinale R.*) is a medicinal plant which is gaining popularity amongst modern physicians (Sakr and Badawy, 2011). The major isolated bioactive ingredients of the ginger are gingerdiol, zingibrene, gingerols, protodioscin, saponins and shogaols (Sakr and Badawy, 2011). Ginger has many therapeutic effects such as antioxidant, antiemetic, antithrombotic, anti-hepatotoxic, anti-inflammatory and cholagogue (El-Morsy Ibrahim and Al-Shathly, 2015). Ginger relieves nausea and vomiting associated with motion sickness, pregnancy and surgery (Gilani and Rahman, 2005). Ginger has protective role in reproductive toxicities such as cyclophosphamide, cisplatin, malathion and diabetes (Riaz et al. 2017).

It is reported that aqueous extract of ginger (24 mg/ml) has a positive effect on metiram-inhibited spermatogenesis and induced apoptosis in mice (Sakr and Badawy, 2011).Ginger decreases body weight, serum glucose, cholesterol and serum alkaline phosphatase in adult male rats (Bhandari et al. 2005). Many positive effects of ginger on male reproductive system have been reported. It is reported that co administration of ginger (0.5-1 g/kg) with lead resumed the plasma testosterone level to near normal levels (Riaz et al. 2011). Since years ago, there are increasing interests focus on verification of pharmacological and physiological actions in ginger as a therapeutic agent (James et al. 2015).

The protodioscin and saponins of ginger increase testosterone and luteinizing hormone (LH) hormone levels as well as libido which can be used in traditional medicine to treat sexual dysfunctions (Morakinyo et al. 2008). Tribestan (patented extract of ginger) increases libido, infertility and menopausal disorders (Imani and Ainehchi, 2014). Also, ginger increases estrogen, pregnenolone and testosterone levels and sexual potency in men (Sabik et al. 2009). Despite researches have been done on effects of the ginger on male reproductive system, its role on sex hormones in blood and testicular levels is not fully elicited. So, the aim of the current study was to determine effect of the ginger on blood biochemical follicle stimulating hormone (FSH), LH, testosterone levels as well as semen FSH, LH, testosterone hormones in rat.

## MATERIAL AND METHODS

*Animals:* A 40 male Wister rats (200–250 g) kept as folk and fed basal chew pellet for a week, then randomly divided into 4 experimental groups (n=10). Animals were kept in groups of 8–10 per cage (45 cm × 30 cm × 15 cm) at a controlled room temperature (23 ± 1 °C), relative humidity of 55–65% and were maintained on a light-controlled regime (12-h light cycle, lights on at 07:00 h) according to European Union recommendations for laboratory animals. During the study, all animals had *ad libitum* access to fresh water. Animals were acclimatized to laboratory conditions for one week prior to experiments. All experimental procedures were carried out during the light phase (10:00–17:00 h) and executed in accordance with the Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals (Zimmermann 1983). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (USA) and the current laws of the Iranian government.

*Study design:* All animals had free access to basal diet (commercial chew pellet) for a week prior the study. Then animals randomly allocated into 5 experimental groups. Group 1 kept as control and fed basal diet (commercial chew pellet) and orally gavage with distilled water. Groups 2-4, provided basal diet and orally gavage of the 100, 200 and 300 mg ginger powder in distilled water, respectively. All animals provided *ad libitum* access to the experimental diets based on their groups for 4 weeks. At the end of the sixth week, animals were food deprived for 12, blood samples were taken, centrifuged at 4°C for 10 minutes at 250×g and the serum stored at -20°C until assayed. Serum glucose, cholesterol, triglyceride, LDL, HDL, albumin, total protein, and urea were obtained using colorimetric assay using commercial kit (Pars Azmoon Co., Tehran, Iran). Serum concentration of LH and FSH were determined in duplicated samples using radioimmunoassay. Rat FSH and 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 (Khaki et al. 2009). Serum concentration of total testosterone was measured by using a double antibody radioimmunoassay kit

Table 1. Effect of the ginger on spermatozoa characteristics in rat

| Groups | Forward movement | Motility without movement | Without movement | Dead sperm (%) | Viability (%) |
|---|---|---|---|---|---|
| Control | 21[b] | 22.56[a] | 60.54[a] | 70.50[a] | 29.50[c] |
| Ginger (100 mg) | 20.70[b] | 20.80[b] | 58.30[a] | 51.95[b] | 48.05[b] |
| Ginger (200 mg) | 20.20[b] | 22.20[b] | 57.60[a] | 44.90[c] | 55.10[a] |
| Ginger (300 mg) | 37.45[a] | 31.45[a] | 30.80[b] | 44.80[c] | 55.20[a] |
| P value | 0.0001 | 0.0001 | 0.0024 | 0.0001 | 0.0001 |
| SEM | 2.99 | 1.99 | 4.30 | 2.15 | 2.15 |

SEM standard error of mean. Different letters (a, b and c) indicate significant differences between treatments at each time ($p< 0.05$).

(immunotech Beckman Coulter Company-USA). The sensitivities of hormone detected per assay tube were 0.025ng/ml (Huang et al. 1995).

*Surgical procedure:* At the end of the study, rats fasted overnight and were intraperitoneally (i.p) injected with pentobarbital (40 mg/kg). Peritoneum on each animal was opened by an incision and testes were taken out. Semen samples were collected from the Cauda epididymis and homogenized in 10% (W/V) ice-cold buffer (0.1 M phosphate buffer, pH 7.4 + 150 mM KCl). The homogenate was centrifuged at 9000 rpm for 20 min to obtain a supernatant which was used to determine semen testosterone, LH and FSH levels using radioimmunoassay kits (Biocode Company-Belgium) and (immunotech Beckman Coulter Company-USA) (Huang et al. 1995).

*Spermatozoa characteristics:* At the end of the study, semen samples were collected from the Cauda epididymis carefully separated from the testis and placed in a Petri dish containing Ham's F10. Epididymal caudal was minced with scissors to release sperm and then was placed in the incubator for 15min (Padmanabhan et al. 2008). Approximately, 10µL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and allowed to stand for 5 min (Wyrobek et al. 1983). The cells which settled during this time were counted by a light microscope at 200X magnification (Seed et al. 1996).

*Statistical analysis:* Data were prepared in excel, analysed with analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA) followed by Tukey's post-hoc tests and presented as mean ± SEM. $P < 0.05$ was considered to denote significant differences between groups.

## RESULTS AND DISCUSSION

The results of the ginger on sex hormones and blood biochemical levels in rat are presented in tables 1-5. As seen in table 1, a dose dependent increase observed on spermatozoa forward movement (P=0.0001). Also, spermatozoa motility without movement significantly increased by administration of the ginger (100, 200 and 300 mg) compared to the control group (P=0.0001). Also, spermatozoa without movement significantly decreased in rats treated with ginger (100, 200 and 300 mg) compared to the control group (P=0.0024). A dose dependent increase observed on spermatozoa viability (P=0.0001) and decreased dead sperm (P=0.0001).

Effect of the ginger on blood biochemical levels in rat is presented in table 2. According to the results, orally

Table 2. Effect of the ginger on blood biochemical levels in rat

| Groups | Total protein (g/dl) | Albumin (g/dl) | Glucose (mg/dl) | Urea (mg/dl) |
|---|---|---|---|---|
| Control | 7.68[c] | 5.2 | 105.85[a] | 29.53 |
| Ginger (100 mg) | 8.24[b] | 5.3 | 113.62[a] | 41.2 |
| Ginger (200 mg) | 8.82[a] | 5.46 | 110.62[a] | 36.95 |
| Ginger (300 mg) | 8.22[b] | 5.46 | 72.98[b] | 36.95 |
| P value | 0.0008 | 0.65 | 0.014 | 0.4 |
| SEM | 0.17 | 0.16 | 9.53 | 2.05 |

SEM standard error of mean. Different letters (a, b and c) indicate significant differences between treatments at each time ($p< 0.05$).

Table 3. Effect of the ginger on blood lipid profile in rat

| Groups | Cholesterol (mg/dl) | Triglyceride (g/dl) | HDL (mg/dl) | LDL (mg/dl) |
|---|---|---|---|---|
| Control | 253.7 | 51.65$^{b}$ | 46.55$^{b}$ | 23.61 |
| Ginger (100 mg) | 215.5 | 71.73$^{a}$ | 55.65$^{a}$ | 27.65 |
| Ginger (200 mg) | 214.80 | 55.66$^{b}$ | 45.78$^{b}$ | 26.74 |
| Ginger (300 mg) | 218.50 | 62.51$^{ab}$ | 48.78$^{b}$ | 25.98 |
| P value | 0.15 | 0.01 | 0.0006 | 0.60 |
| SEM | 13.7 | 4.22 | 1.66 | 2.18 |

SEM standard error of mean. Different letters (a and b) indicate significant differences between treatments at each time ($p< 0.05$). HDL: high density lipoprotein; LDL: low density lipoprotein.

administration of the 100, 200 and 300 mg of the ginger significantly increased serum total protein levels compared to the control group (P=0.0008). Serum glucose significantly decreased in rat received ginger (100, 200 and 300 mg) compared to the control group (P=0.014). No significant difference observed on serum albumin and urea levels in rat treated with ginger in comparison to the control group (P>0.05).

Administration of the ginger in a dose dependent manner increased serum triglyceride (P=0.01) and HDL (P=0.0006) levels in rat compared to the control group. No significant difference observed on serum cholesterol and LDL in animals received ginger than control group (P>0.05) (table 3).

As seen in table 4, orally gavage of the ginger significantly increased semen testosterone levels in comparison to the control group (P=0.009) while had no significant effect on LH and FSH levels (P>0.05).

According to the table 5, ginger (100, 200 and 300 mg) had no significant effect on serum LH, FSH and testosterone levels compared to the control group (P>0.05).

Infertility is one of the major problems which the both male and female related factors are not yet clearly understood (Nassiri et al. 2009). Better understanding of underlying mechanisms in fertility is important to improve diagnosis and treatment (Nassiri et al. 2009). Male factor is involved in 40-50% of infertility and numerous conditions include in spermatogenesis and sperm quality (Mazaheri et al. 2014). Despite many achievements in modern medicine, side effects of synthetic chemical drugs are still the main problem (Lim, 2016). Hence, there are growing interests to use of herbal medicine due to it possessing lower side effects (Lim, 2016). Ginger is a famous medical plant by anti-oxidant, anti-serotonergic and anti-inflammatory properties (Lim, 2016).

According to the results, dose dependent increase observed on spermatozoa forward movement. Dose dependent increase was observed on sperm viability. In this regard, Khaki et al. (2009) reported administration of the ginger rhizome powder (50 and 100mg/kg) for 20 consequence day increased sperm viability and motility in rat. Orally administration of the *Zingiber officinale* (1000mg/kg for 28 days) increased epididymal sperm count and motility (Morakinyo et al. 2008).which our results were similar to their findings. Orally administration of the 100, 200 and 300 mg of the ginger significantly increased serum total protein and decreased glucose levels. In this regard, it is reported ginger has anti hyper glycaemic effect by decrease glucose levels in rats (Al-Amin et al. 2006). Despite direct mechanism of action ginger on blood glucose level is not fully elicited, Khan et al. (2003) reported ginger increase

Table 4. Effect of the ginger on semen sex hormone in rat

| Groups | LH (Iu/l) | FSH (Iu/l) | Testosterone (ng/ml) |
|---|---|---|---|
| Control | 2.54 | 0.24 | 0.75$^{c}$ |
| Ginger (100 mg) | 1.84 | 0.08 | 1.03$^{bc}$ |
| Ginger (200 mg) | 2.13 | 0.09 | 1.2$^{ab}$ |
| Ginger (300 mg) | 1.34 | 0.11 | 1.48$^{b}$ |
| P value | 0.30 | 0.47 | 0.009 |
| SEM | 0.44 | 0.04 | 0.14 |

SEM standard error of mean. Different letters (a, b and c) indicate significant differences between treatments at each time ($p< 0.05$). LH: luteinizing hormone; FSH: follicle stimulating hormone.

Table 5. Effect of the ginger on serum sex hormone in rat

| Groups | LH (Iu/l) | FSH (Iu/l) | Testosterone (ng/ml) |
|---|---|---|---|
| Control | 4.9 | 0.59 | 1.02 |
| Ginger (100 mg) | 2.52 | 0.34 | 1.44 |
| Ginger (200 mg) | 2.96 | 0.34 | 1.59 |
| Ginger (300 mg) | 2.025 | 0.15 | 2.02 |
| P value | 0.31 | 0.45 | 0.007 |
| SEM | 0.69 | 0.10 | 0.19 |

SEM standard error of mean. Different letters (a and b) indicate significant differences between treatments at each time ($p< 0.05$). LH: luteinizing hormone; FSH: follicle stimulating hormone.

glucose uptake and glycogen synthesis and phosphorylation of the insulin receptor.

Based on the findings of the current study, administration of the ginger in a dose dependent manner increased serum triglyceride and HDL levels in rat. Orally gavage of the ginger significantly increased semen testosterone levels while had no significant effect on LH and FSH levels. In contrast it is reported administration of the 50 and 100mg/kg ginger rhizome powder increased testosterones without effects on LH and FSH hormones (Khaki et al. 2009). According to our findings, no effect observed on serum LH, FSH and testosterone levels in ginger-treated rat.

However, Riaz et al. (2017) reported orall administration of the ginger (1.5gm/kg) significantly decreased plasma testosterone and LH levels in male rats after lead induced toxicity. Imani and Ainehchi, (2014), reported ginger (20 and 40 mg/kg) increased serum LH while only 20 mg/kg increased serum FSH levels in rats. In the current study we used 100, 200 and 300 mg of the ginger powder in water. So, perhaps only low levels of the ginger increase the FSH levels which needs further investigations to determine direct cellular and molecular of actions. Ginger extract has androgenic activity which elevates semen testosterone and accumulation of sperm in the seminiferous tubules in rat (Amr and Hamza et al. 2006; Rekha et al. 2010).

As observed in our study, ginger had no effect on serum and semen LH and FSH levels while increased sperm viability and motility. Ginger extract has antioxidant effect by antioxidant enzymes including super oxide dismutase, glutathione peroxides and catalase in rats (Khaki et al. 2009). It seems, ginger increase sperm motility via protective effect (Amr and Hamza et al. 2006). However, because of the limitations of the current study, we were not able to measure antioxidant enzyme levels in serum and testis tissue of the ginger-treated rat. Ginger roots induce antidiabetic activity and enhance male fertility in diabetic rats (Hafez 2010). As observed in this study, ginger decreased serum glucose level and increased sperm viability and movement. Observed effects of the ginger are attributed to its major ingredients including Zingerone, gingerdiol, Zingiberene, gingerols and shogoals (Morakinyo et al. 2008). In conclusion, these results suggested ginger improves spermatozoa characteristics and semen hormone level.

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