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The Effects of Methanolic Extracts of Ginger (*Zingiber officinale*) on Human Sperm Parameters; An *in vitro* Study

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Abstract: This study was conducted on *Zingiber officinale* or ginger on motility, grading and morphological aspects of human sperm. Thirty human semen samples were obtained from the local hospital. The samples were swim up by Ham's F₁₀. The samples (0.9 mL) were treated by 0.1 mL of ginger methanolic extracts (0.1, 0.2, 0.4 and 0.6% concentration). Sperm motility, grading and morphology parameters were assessed using light microscope at 0, 2, 4, 8 and 24 h after treatment. Dose and time-dependent decreases in motility accompanied by concomitant decrease in grading 3 and 4 were noticed. Morphologic profiles of the sperms were changed under different doses of ginger on the basis of time of assess. These data indicate that some exhibits a lower percentage of motility and grading when methanolic ginger is added to semen fluid. According to the results it would be concluded that ginger can induce the toxic effects on sperm parameters.

Key words: Ginger, human sperm, motility, grading, morphology

INTRODUCTION

Our understanding of male reproductive function and the importance of male factors in infertility has advanced significantly over the last decade. Infertility is defined as the inability to achieve a pregnancy after one year of unprotected intercourse (Jain *et al.*, 2004). A multi faceted therapeutic approach to improving male fertility involves identifying harmful environmental and occupational risk factors, while correcting underlying nutritional imbalances to encourage optimal sperm production and function (Sheweita *et al.*, 2005). A number of nutritional therapies have been shown to improve sperm counts and motility, including carnitine (Sigman *et al.*, 2006), folic acid and zinc (Wong *et al.*, 2002), selenium (Hawkes and Turek, 2001), vitamin B₁₂ (Boxmeer *et al.*, 2007). Numerous antioxidants have also proven beneficial in treating male infertility such as vitamin C (Sonmez *et al.*, 2005), vitamin E (Keskes-Ammar *et al.*, 2003), glutathione (Meseguer *et al.*, 2004) and coenzyme Q₁₀ (Angelitti *et al.*, 1995). One type of alternative medicine, herbal therapy for medical ailments, recently has become popular despite the lack of scientific experimentation to assess its effectiveness and safety (Ondrizek *et al.*, 1999). Specific botanical medicines have been documented in several studies as having a positive

effect on sperm parameters. Chen *et al.* (1999) found extracts of panax notoginseng were capable of significantly enhancing *in vitro* sperm motility. When 18 water extracts of Chinese medicinal herbs were evaluated for their effect on sperm motility *in vitro*, Astragalus membranaceus was the only herb with a significant stimulatory effect (Hong *et al.*, 1992). On the other hand, high concentration extract of some medicinal plants such as saw-palmetto, Echinacea Purpura, ginkgo biloba, st. John's wort induced inhibitory effects on sperm motility (Ondrizek *et al.*, 1999).

Ginger (*Zingiber officinale*), a well-known spice, sweet, pungent, heating appetizer has been used in traditional oriental medicines for long time. Its extract and major pungent principles have been shown to exhibit a variety of biological activities (Ghayur and Gilami, 2005; Wei *et al.*, 2005). It has been used to treat a number of medical conditions, including headache, colds and arthritis (Grant and Lutz, 2000). Ginger reduces symptoms in patients with nausea of pregnancy, motion sickness and postoperative nausea and vomiting (Grontved *et al.*, 1988; Phillips *et al.*, 1993). Limited *in vitro* studies on ginger have shown that water and organic solvent extracts of ginger possess antioxidant active components (Masuda *et al.*, 2004; Reddy and

Lokesh, 1992; Krishnakantha and Lokesh, 1993). Ginger significantly decreases lipid preoxidation by maintaining the activities of the antioxidant enzymes (Liu *et al.*, 2003).

The main antioxidant active materials in ginger are the gingerols and shogaols and some related phenolic ketone derivatives (Cao *et al.*, 1993). Oxidative stress can induce injuries on the function of cells. Research indicates that oxidative stress can be harmful to sperm survival and fertility and defective sperm function is the most common cause of infertility (Aitken and Baker, 2004). On the basis of the antioxidative effect of ginger, this study was performed to investigate the effect of methanolic extract of ginger on human sperm parameters *in vitro*.

MATERIALS AND METHODS

Semen sampling: Semen samples were obtained from 30 healthy men who referred to IVF center of Babolclinic hospital (located in Babol town, the North of Iran) during 2006. The men who had varicocele, azoospermia or any male factors and age less than 20 years and more than 40 years were excluded from the study.

Sperm preparation and parameters: Semen samples were collected by natural coitus in sterile containers. Semen Fluid Analysis (SFA) was done according to the WHO standards criteria (WHO, 1992). In this procedure fresh sperm specimens were washed using by Ham's F₁₀ medium and placed in testing tubes. After swim up several smears were prepared from each specimen to record possibly abnormal morphologic pattern. A total of 200 sperms were evaluated per smear in all tests. The sperm count was obtained as million per milliliter. Motility was evaluated by direct examination and morphology by Papanichola staining technique (WHO, 1992). The sperm morphologic assessment at 0 and 8 h of incubation was performed following the method by Kruger *et al.* (1988). In brief, 5 μ L semen were used to make the smears using two morphology slides cleaned thoroughly with 70% ethanol before use. The slides were air-dried at room temperature and fixed for 15 sec.

Experimental groups: The prepared sperm samples were randomly divided into six groups. Different concentrations of methanolic extract of ginger (0.1, 0.2, 0.4 and 0.6%) in sunflower oil as vehicle were prepared. Then 0.9 mL of final sperm in medium was separately mixed with 0.1 mL of vehicle oil and the ginger extracts. Four groups of sperm samples were mixed with different concentrations of ginger extracts. One group of sample was mixed with vehicle and the remaining had no received any treatment. The sperm parameters including motility and

grading in 5 steps at 0, 2, 4, 8 and 24 h were evaluated in all study groups. Then smear was provided at 0 and 8 h phases and sperm morphology evaluated by papamichola staining.

Statistical analysis: Sperm motility and grading values are presented as mean \pm SD. To analysis of within group differences in baseline data repeated measures analysis of variance and for between groups analysis, one way ANOVA with post-hoc Tukey test were done. The difference at 0 and 8 h in sperm morphology was analyzed by Wilcoxon U-test. The different between data with p-value under 0.05 was considered statistical significant.

RESULTS

There was not a significant change in the sperm counts in both of no-treatment and treatment medium with vehicle oil. But the different concentrations of ginger could reduce the count of sperms. The count was significantly reduced with higher concentration of ginger ($p < 0.05$).

The parameter of motility was investigated. No effects were seen with vehicle and no-treatment arms of study. On the other hand, ginger can dose-dependently exert a decreasing effect on sperm motility. On the basis of grading of motility, there were not detected spermatozoa with grade 4 (Table 1).

According to Table 1, as the dose of ginger become increased the level of motility will be decreased. This reduction profile of the sperm motility was significantly time dependent ($p < 0.001$).

In this investigation all sperms which were exposed to the different concentration of ginger were classified under grading levels of motility. With except of grade 4, other grades of motility were presented in Table 2-4. All sperms were exposed to the ginger concentrations showed a reduction in the motility grading. Long duration of exposure of the sperms to different concentrations of ginger can resulted in formation low grade motility of sperms. The grade 1 of motility was seen after exposure to ginger concentration. But this reduction in grade 1 of motility with treatment of 0.6% concentration of ginger was seen higher than other concentrations of ginger. On the other hand, the trend of reduction of motility from grade 3 and 2 into grade 1 with 0.6% concentration was quicker than other concentration. After time consuming of exposure to high concentration of ginger all sperms became immotile.

The mean percentage of sperm frequency in Grade 1 motility increased after exposure to different concentrations of ginger. This increase at hour 2, 4 and

Table 1: The mean±SD of percent sperm motility in various concentrations of methanolic extract of ginger by times

Concentration	Time (h)				
	0	2	4	8	24
No treatment	92.3±6.7	91.2±3.2	88.9±5.0	84.4±6.2	75.0±11.0
Vehicle (oil)	91.4±3.4	90.2±4.0	88.5±4.7	85.3±6.2	74.4±10.7
0.1	87.7±5.3	79.7±10.4	65.8±23	47.8±27.5	6.1±14.4
0.2	84.4±8.4	71.8±13.4	44.3±18.4	14.1±18.2	1.2±2.7
0.4	89.5±5.7	69.8±18.2	40.5±27.2	18.2±23.3	0.9±2.4
0.6	84.7±5.3	48.8±21.1	17.3±17	2.8±9.1	0.1±0.7

Table 2: The mean±SD percentage of sperm frequency in grade 1 motility in different concentrations of methanolic extract of ginger in various times

Concentration	Time (h)				
	0	2	4	8	24
No treatment	5.8±2.3	7.5±2.5	9.2±2.7	13.2±5.7	23.7±8.8
Vehicle (oil)	7.0±3.6	8.2±2.5	9.5±3.7	12.1±4.3	24.9±9.3
0.1	12.6±7.3	19.5±10.4	31.2±22	42.4±29.7	46.5±48.9
0.2	15.3±6.0	34.5±18.2	64.5±21.6	59.3±43.4	17.8±36.8
0.4	9.8±4.9	32.0±18.4	66.1±28.5	64.2±43.0	16.5±35.5
0.6	19.2±16	50.3±20.1	85.1±30.6	28.3±44.8	3.3±18.2

Table 3: The mean±SD percentage of sperm frequency in grade 2 motility in different concentrations of methanolic extract of ginger in various times

Concentration	Time (h)				
	0	2	4	8	24
No treatment	39.1±7.9	41.9±7.7	43.2±7.3	42.5±6.1	46.2±7.7
Vehicle (oil)	41.3±9.8	41.5±7.5	42.4±6.9	43.4±7.5	49.4±7.5
0.1	46.9±10.2	52.2±11.5	50.6±15.7	33.6±21.8	4.8±13.9
0.2	52.5±10.9	57.1±12	33.9±19.8	10.5±19.0	2.1±7.6
0.4	47.5±7.9	56.7±13.7	28.4±23.4	8.9±17.7	3.3±12.6
0.6	53.2±9.2	47.7±18.9	4.8±10.3	1.6±9.1	0

Table 4: The mean±SD percentage of sperm frequency in grade 3 motility in different concentrations of methanolic extract of ginger in various times

Concentration	Time (h)				
	0	2	4	8	24
No treatment	55.6±8	50.6±8.5	47.2±7.9	44.3±8.7	29.6±10.0
Vehicle (oil)	51.7±10.8	49.9±8.8	48.0±7.4	44.8±7.4	25.9±14.2
0.1	38.4±11.1	27.8±13	18.4±13	9.3±12.1	0.6±3.0
0.2	32.2±11.8	9.0±8.6	1.5±4.7	0.1±0.9	0
0.4	42.6±8.1	11.2±11.3	1.8±3.8	0.2±1.0	0
0.6	29.7±11.6	1.9±4.5	0	0	0

8 but no 24 were seen in concentrations higher than 0.1 (p<0.0001).

This position was not seen in sperm of grade 2. This sperms slightly shifted into grade 1 and after this step all the sperms will be significantly immotile.

The frequency of grade 2 of sperm motility dose dependently was decreased in the present of various ginger concentrations.

With the except of 0.1% concentration of ginger, the motility of grade 3 of sperms was more rapidly reduced to zero in the exposure to higher concentrations.

AS can be seen in Table 4, a dose and time-dependent reduction were observed in percentage of sperm frequency in grade 3 of motility (p<0.0001).

Table 5: The mean±SD percentage of sperm frequency with normal morphology in different concentrations of methanolic extract of ginger

Concentration	Time (h)		p-value (Wilcoxon)
	0	8	
No treatment	10.3±1.6	8.7±1.4	0.0001
Vehicle (oil)	9.0±1.9	8.5±1.5	0.0001
0.1	8.6±1.1	7.1±1.2	0.0001
0.2	8.0±1.7	7.0±1.3	0.0001
0.4	8.4±1.0	7.1±0.8	0.0001
0.6	8.1±1.1	7.1±1.0	0.0001

A significant decrease in the number of spermatozoa with normal morphology is observed after exposure to all concentrations of ginger extracts (Table 5).

DISCUSSION

In this study, the effect of different doses of methanolic ginger extracts were investigated.

Several *in vitro* studies similar to present study investigated the effects of herbal medicine on sperm motility. Amano *et al.* (1996) found that the motility of sperm in 10 and 100 µg mL⁻¹ of Hochu-ekki-to (Astragalus), a Chinese herbal medicine, for 1-6 h was significantly than that of the control groups.

Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities. Ahmed *et al.* (2000) 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. The results indicate that ginger is comparatively as effective as ascorbic acid as an antioxidant. In the other hand, we know that spermatozoa membranes are rich in poly-unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid preoxidation. The cellular damage in the semen is a result of an improper balance between oxygen free radicals generation and scavenging activities. Therefore numerous antioxidants such as vitamin C, vitamin E, glutathione and coenzyme Q₁₀ have proven beneficial effects in treating male infertility (Sheweita *et al.*, 2005). But a number of certain drugs such as diltiazem (Wood *et al.*, 2003), colchicines (Haimov-Kochman and Ben-Chetrit, 1998), lidocaine (Moudgil *et al.*, 2002) and chemotherapeutic agents (Longhi *et al.*, 2003) inhibited sperm motility *in vitro*.

Also smoking and heavy use of alcohol can decrease semen quality (Martini *et al.*, 2004). To evaluate protective effect of ginger against cisplatin-induced reproductive toxicity, ethanolic extract of *Zingiber officinale* (1 g/kg/day) were given P.O. 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

(Amin and Hamza, 2006), which was in contrary with present results. Also the aqueous extract of *Z. officinale* significantly increased weight of the testis, the serum testosterone level and epididymal α -glucosidase activity *in vivo* (Kamtchouing *et al.*, 2002).

Despite the antioxidants effect of ginger which is mentioned above, all concentrations of ginger in this study had time-dependent damaging effects on sperm motility and morphology. These effects could be due to administration of high doses of ginger in this investigation. A clinical trial study showed that high concentrations of saw-palmetto, echinacae, or ginkgo inhibited motility at 24 and 48 h (Ondrizek *et al.*, 1999).

This result is consistent to present study that ginger extract in this investigation can reduce the sperm motility.

In this study, after 8 h the percentage of grade 1 sperms in all concentrations except of 0.6% were increased. This temporary increase can be arise from simultaneous decline in the number of grade 2 sperms that is some of grade 2 sperms were imported in grade 1 phase.

In conclusion, these data indicate that some exhibits a lower percentage of motility and grading when methanolic ginger is added to semen fluid. According to the results it would be concluded that ginger can induce the decreasing effects on sperm parameters. In fact in our study, ginger extracts had adverse effect on sperm motility and morphology *in vitro* which is dose and time dependent.

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