

Investigating the prenatal exposure of hydro-alcoholic extract of ginger on the function of Pituitary – Gonad axis in male mature offspring rats

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Abstract

Background and Objective: Ginger has been used for a long time as a spice in food as well as pharmaceuticals in a wide variety of diseases. Ginger has been shown to prevent the nausea in pregnant women. The aim of the present study was to examine the effect of hydro-alcoholic extract of ginger (HEG) on function of pituitary – gonad axis in mature male rats exposed to HEG in the prenatal period.

Method: In this experimental study, 40 pregnant female rats were divided into four groups. Groups contained control, placebo (daily received 0.5 ml normal saline), and treatment groups receiving 50 mg/kg and 100 mg/kg of HEG in pregnancy period. At the end of the infancy period, males of groups were separated and 10 rats out of each group were randomly chosen in maturity.

Upon the time of maturity of chosen male rats, blood samples were drawn for measurement of the levels of sex hormones in male rats. Also the number of Leydig, Sertoli, spermatogonia, spermatocytes and spermatide cells were counted.

Results: Administration of HEG in groups received 50mg/kg and 100 mg/kg which resulted in a significant increase in concentration of testosterone, LH and FSH hormones in male progenies. HEG administration also significantly increased the number of Leydig and Sertoli cells, spermatogonia, spermatocytes and spermatid.

Conclusion: With regard to the results of this research, consumption of HEG in the perinatal period results in increase in function of Pituitary – Gonad axis in born male adult rats.

Key words: Ginger; testosterone; Luteinizing Hormone; Follicle Stimulator Hormone; Spermatocyte,

Introduction

Despite many achievements in modern medicine, the main problem is still the usage of synthetic chemical drugs for the treatment of diseases causing serious side effects in patients receiving them (1). Nowadays traditional medicine has been revisited as the use of herbal medicine is markedly increasing due to it possessing lower side effects (2). So the current approach is to apply herbal medicine to obtain a high standard of therapy for the treatment of myriad diseases with minimum side/adverse effects.

Ginger (*Zingiber officinale*) belongs to the *Zingiberaceae* family. As spice, ginger has been used in the diet and pharmaceutical components. Historically medicine has shown that ginger has been widely used in China, Japan and India for the treatment of nausea and vomiting in women during pregnancy (3).

The ingredients of ginger contain water, protein, fat, minerals (including iron, calcium and phosphorus), vitamins (such as thiamine, riboflavin, niacin and vitamin C), fiber and carbohydrate. These compounds may be different as a result of variation in agricultural, drying and storage conditions. In the rhizome of fresh ginger, gingerols are the main components. Special aroma and the taste of ginger come from the mixture of gingerols, shogaol and zingerone which are found in ginger (4 and 5).

Until now, more than 50 types of antioxidants have been isolated from the rhizome of ginger. Gingerols are the most important components of ginger that have significant antioxidant properties. In addition, they have a high antioxidant activity due to containing vitamins such as A, B, C and E as well as flavonoids and glutathione (6). Ginger has a wide variety of pharmaceutical properties to treat many health problems over the years, including stomach ache and intestinal problems. Because of possessing cholinergic and anti-histamine properties, ginger has desirable effects on reducing the nausea and vomiting in women during pregnancy (7). Ginger is able to stimulate blood circulation, increase cell activity and metabolism. It also can have anti-cancer property because of antioxidant activity and ability in inactivating the effective factors on carcinogenesis (8). Regular intake of ginger in the dietary regimen can improve the activity of heart and circulation system (9). Ginger causes a significant decrease in the physical signs of initial dysmenorrhoea (10). Studies indicate that the extract of ginger stimulates menstruation, eliminates the irregularities in the menstruation cycle, increases spermatogenesis and enhances sperm fertility factors (11, 12). Furthermore, ginger is used to treat fever, rheumatism, neuronal diseases, gingivitis, tooth pain, asthma and coughing (13).

There are many concerns about using chemical drugs in women during pregnancy for the nausea and vomiting owing to possible deformities in fetus. Hence, using herbal components paves the way for alternative treatment in pregnant women. Based on the above evidence, having less side effects for ginger usage has opened a new

horizon to treat such complications in women during pregnancy (14).

Research has shown that ginger increases spermatogenesis and effectiveness of sperm fertility parameters. Regarding high prevalence of men's infertility in the world which is resulting from the production of damaged sperm and malfunction of the cells responsible for spermatogenesis, there is a need for an alternative therapy for the treatment of infertility due to the high cost of therapeutic agents and severe side effects. Several lines of evidence suggest ginger has a beneficial role in spermatogenesis and sperm parameters because of the antioxidant properties of ginger. In light of the effectiveness of ginger on spermatogenesis, the present study was established to examine the effect of prenatal exposure to HEG on pituitary – gonad axis hormones and spermatozoa of young adult male rats.

Materials and Methods

In this study, 40 Wistar adult female rats weighing between 200-220 gr were grouped into 4 groups (10 per group) including control, placebo and two treatment groups. In addition, 8 Wistar adult male rats were used for crossbreeding.

Rats were kept in Animal House of Azad Islamic University of Falavarjan in a 12 hour light/12 hour dark cycle in 25 ° c and relative humidity ranging from 40-60% in order to be acclimatized in their new place. Also, water and food were provided *ad libitum*.

To prepare the HEG, the rhizome of ginger was obtained from the Agricultural Research Center of Isfahan. For this purpose, fresh and intact plants were used. Then herbarium no.128/3/001/001 was approved by the plant specialists of Islamic Azad University of Falavarjan. After that, ginger rhizome was dried and then ground and finally the resulting powder was extracted by maceration.

Before crossbreeding the female rats with males, in order to synchronize the menstrual cycles of female rats, 100 mg estradiol valerate was dissolved into 0.2 ml olive oil and then was injected into the rat muscles using insulin syringes. After 42 hours of the first injection 50 mg of progesterone was injected into the muscles of rats. After 6 hours of the second injection, a vaginal smear was obtained from the rats. Marcondes' method was used for recognizing the steps of estrous cycle. In this method, each step of the cycle is recognized based on the proportion among three cell populations including epithelial, horny and leukocytes observed in the vaginal smear (15).

Microscopic observations showed that synchronizing the cycle of rats had occurred in the estrous s step. Then, for crossbreeding, 8 adult male rats were co-caged with female rats (16). Upon observing sperm in vaginal smears the day 0 of pregnancy was defined. During pregnancy for two treatment groups 50 mg/kg and 100 mg /kg HEG were intraperitoneally injected every day. The control group received no treatment. The placebo group daily

received 0.5ml normal saline. At the end of the infancy period, male and female offspring were separated and kept without any treatment for up to 2 months. Then, 10 male rats of each four groups were randomly selected for analysis of hormones. To do that, blood was obtained from their hearts after being anesthetized. Blood samples were centrifuged at 300 rpm for 5 minutes and kept in -20° C until usage.

The levels of FSH, and LH hormones were measured by electro-chemiluminescence luminescence method (ECL) and the levels of testosterone hormones were measured by enzyme linked immunosorbent assay (ELISA). Kits used for measuring FSH and LH were purchased from (Cusabio, USA) and one for testosterone with mark IBL, GmbH made in Germany, respectively.

In order to count the number of sexual dynastic cells, at first, right testicles of male rats were removed and placed in a 10% solution of formalin. Soon after, dehydration by ethanol, clearing by xylene alcohol and tissue embedding were done. Next, using Rotary Microtome (LEIYZ Australian model 1512) 5 micron thickness tissue sections were provided. Then, the obtained sections were stained by hematoxylin and eosin. The number of Leydig, Sertoli, spermatogonia, spermatocytes and spermatide cells were counted using light microscopy. Finally, data were analyzed by SPSS software by one-way analysis of variance (ANOVA) and Tukey HSD test. Significance level was considered if p value was less than 0.05.

Results

Results obtained from data analysis showed that there wasn't any significant difference between serum mean of LH, FSH and testosterone hormones and sex lineage cells, while HEG in both used doses resulted in significant increase in serum mean of LH and testosterone hormones and also in serum mean of FSH hormones in $P \leq 0.01$ in the groups receiving a high dose of HEG (Table 1).

In addition, results obtained from counting lineage cells showed that administration of HEG in both doses resulted in significant increase in the number of Leydig, spermatogonia, Sertoli, spermatocytes and spermatide cells in these groups in comparison to controls in $P \leq 0.01$ level (Table 2 and Figure 1).

Table 1. Comparison of the mean serum level of LH, FSH hormones and testosterone in the groups treated with HEG

Hormones	N	LH (IU/dl)Mean \pm SD	FSH (IU/dl)Mean \pm SD	testosterone(ng/ml)Mean \pm SD
Control	10	0.841 \pm 0.04	0.950 \pm 0.09	0.292 \pm 0.03
Experimental	10	0.866 \pm 0.04	1.06 \pm 0.32	0.270 \pm 0.04
Ginger extract 50 mg/kg	10	0.958 \pm 0.07**	1.10 \pm 0.13	0.600 \pm 0.08**
Ginger extract 100 mg/kg	10	1.140 \pm 0.09**	1.27 \pm 0.07**	0.780 \pm 0.07**

* Shows the significant difference in level ($P < 0.05$) in comparison to control

** Shows the significant difference in level ($P < 0.01$) in comparison to control

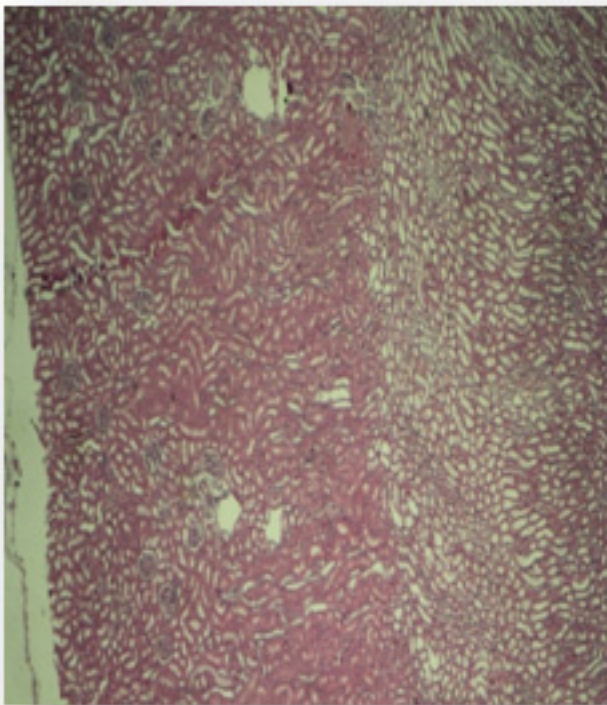
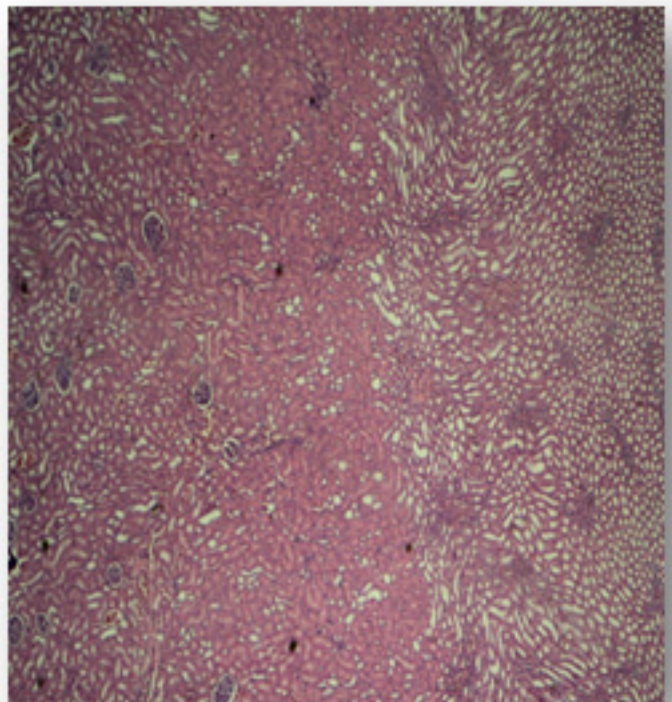
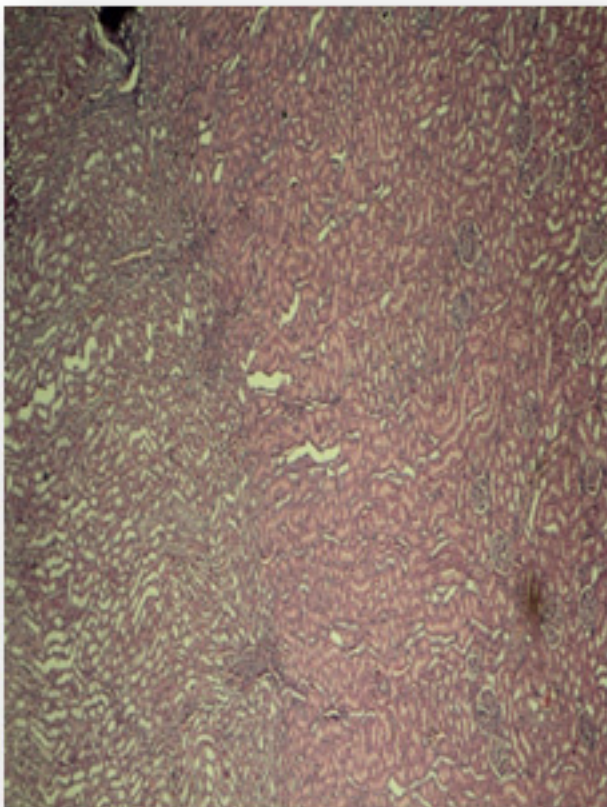
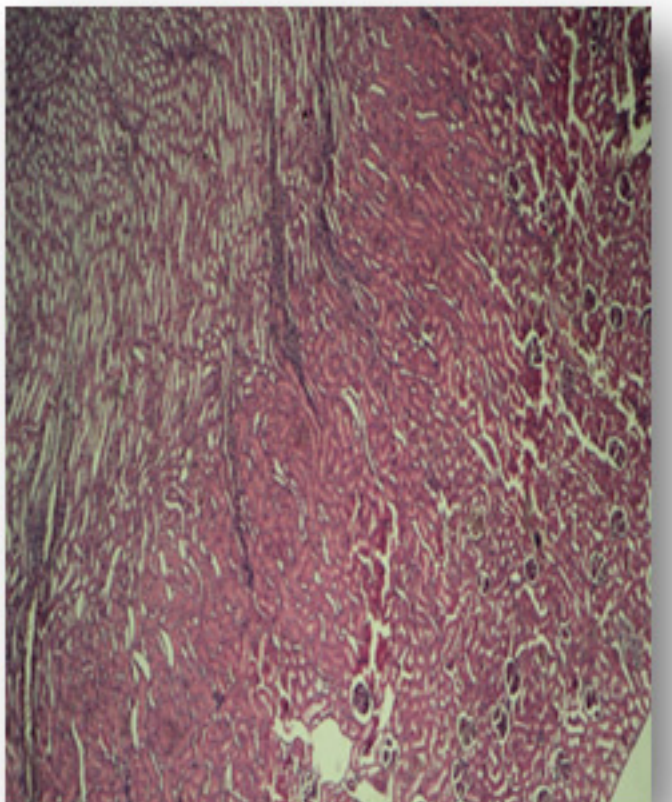
Table 2: the number of lineage sex cells in the groups treated with HEG in comparison to control

Follicles Groups	The total number of spermatogenic cells Mean \pm SD	The total number of spermatocyte Mean \pm SD	The total number of spermatid cells Mean \pm SD	The total number of Sertolic cells Mean \pm SD	The total number of Leydig, cells Mean \pm SD
Control	67.74 \pm 3.10	80.28 \pm 2.55	109.19 \pm 1.73	16.33 \pm 0.34	26.97 \pm 0.47
Experimental groups	64.99 \pm 3.94	83.67 \pm 2.58	110.39 \pm 1.83	17.37 \pm 0.35	28.02 \pm 0.415
Ginger extract 50 mg/kg	78.00 \pm 3.23**	93.06 \pm 2.73**	124.05 \pm 3.51**	18.53 \pm 0.35**	29.01 \pm 0.70**
Ginger extract 100 mg/kg	84.77 \pm 3.33**	97.98 \pm 2.82**	133.66 \pm 3.24**	19.99 \pm 0.76**	30.48 \pm 1.22**

* Shows significant difference in level (P <0.05) in comparison to control

** Shows significant difference in level (P <0.01) in comparison to control

Figure 1: Light photomicrograph of thyroid tissue in group 1(control), Placebo group (2) (3) group received the extract of HEG with dose 50 mg/kg (4) group received HEG with dose 100 mg/kg H & E was used for staining the tissues.

**1****2****3****4**

Discussion

Results of this study showed that administration of HEG in both doses resulted in significant increase in the levels of testosterone, LH and FSH hormones. HEG also increased the number of germ cell lineage. In view of increase in number of Leydig, spermatogonia, spermatocytes and spermatid cells along with increase in testosterone hormone, it seems that testosterone hormone plays an important role in division of Gametes, nourishing the dividing gametes. HEG could have a beneficial role in sperm through direct effect on Sertoli cells and excretion of tube fluid and various proteins including growth factor and transferrin (17). Subsequently, given the important role of testosterone in spermatogenesis, it is obvious that increasing the levels of this hormone could increase the number of sperm. Our findings show a significant increase in the count of sperm after treatment with ginger.

In similar studies, the concentration of testosterone and LH in groups receiving ginger extract was significantly increased in comparison to controls. Since the testosterone is an androgenic hormone produced by Leydig cells which belong to testis stimulated by the excretion LH from pituitary gland, it would be plausible that the mechanism underlying this phenomenon might be due to the direct effect of HEG on luteotropic cells of anterior pituitary increased levels of LH (17).

Ginger has many types of antioxidants. Among these compounds, gingerols are of note. Gingerols possess anti-oxidant, anti-serotonergic and anti-inflammatory properties. (18) In line with the results of this study, Khaki et al in an experimental study have shown that ginger can increase the number of sperm and their motility which may be attributed to gingerols and shogaol. They stimulate the androgens especially the testosterone (19).

In addition to the above properties, ginger has a high anti-oxidant activity because of the vitamins including vitamin A, B, C and E also flavonoids and glutathione (18). Vitamin E is considered as a strong non-enzyme antioxidant which can inhibit the peroxidation lipids of the cell membrane through scavenging the free radicals (20). In the male genital system, the antioxidant activity of vitamins has been reported and is thought to be a result of inhibition the destructive effects of free radicals in the testis (21) and sperm (22 and 23). Moreover, vitamin E can strengthen the antioxidant defense system of testis and sperm cells (24).

Studies have shown that ROS are produced by two different sources in sperm fluid containing damaged spermatozoa cells and active white blood cells whereby a high amount of them results in male infertility through breakage in the DNA structure, decrease in live sperm percentage and dissociation of sperm from ovule surface (25 and 26). These compounds increase the amount of malone-di-aldehyde. Malone-di-aldehyde leads to distortion in distribution of lipid in cell membrane through penetration of the structure of cell membrane. Furthermore, ROS can

cause chromosome disintegration by intercalating into the DNA. Vitamin E can make a breakage between two peroxide lipid bonds thereby the inhibition producing free radical (27). Another crucial vitamin in ginger is vitamin C that acts as a neutralizing antioxidant scavenging the free radicals produced by ROS. It has been demonstrated that it would facilitate the entrance of other antioxidants such as vitamin E and uric acid to the cycle (28).

Glutathione peroxidase (GPx) enzyme plays an important role in protecting the sperm and epididymis in which the reduction of GPx results in infertility. Extract of ginger increases the activity and expression of GPx enzyme cells of liver, kidney, breast, testis, etc. Studies show that antioxidant enzymes including GPx and superoxide dismutase protect the cell through hindrance of the formation of the peroxide and oxidative reactions. (29) Therefore, GPx prevents the deleterious effects of DNA breakage consequences in the sperm and sperm-producing cells. It also protects the sperm nuclei and epididyme fluid against free radicals, resulting in correct maturation of sperm.

Consistent with these results, Mohammadi et al studied the effect of ginger on testicles of rats by treating the cells with Cyclophosphamide. They found that ginger had protective effect on the testis of rats because of high antioxidant content (30).

In a study conducted by Hafez in Greece on the effect of a ginger and cinnamon combination on infertile diabetic rats, a significant increase was observed in sperm parameters and reproductive behavior in terms of sperm parameters including count, motility and viability (31). Another study was conducted by Abo-Ghanema et al using the combination of ginger and L-carnitine to treat infertile rats. The authors showed that this combination increased the weight of testicles and seminal vesicles, improved the quality and quantity of semen (32).

Selenium is another antioxidant found in ginger. Selenium is a quasi-metal micro-nutrient which is requisite for the dietary regimen of mammals found in plant and animal products. Various reports of research implies that the positive effect of selenium on male health is by reducing the signs and symptoms of diseases such as infertility, viral infections, cancer and cardiovascular diseases. Selenium is an essential antioxidant for spermatogenesis and male fertility (33, 34). Investigation carried out in this regard showed that selenium stimulates the motility of sperm (35).

Conclusion

Tantalizing evidence suggests a key role for oxidative stress in the development of infertility in men implying that further studies are warranted to unravel the role of oxidative stress in the progression of male infertility (36). Regarding the protective role of ginger on sperm through antioxidant properties and deleterious effects of oxidative agents on them, it is suggested that antioxidants found in

ginger could be used as a potential therapeutic agent for the treatment and alleviation of infertility in men.

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