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Original Article

Ginger (zingiber officinale) might improve female fertility: A rat model

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Abstract

Background: Ginger (*Zingiber officinale*) is a well known and extensively used antioxidant in traditional remedies. In this study, we aimed to investigate the effects of ginger powder on ovarian folliculogenesis and implantation in rats.
Methods: There were two study groups. In the 5-day treatment group (one estrous cycle), 100 mg ginger powder, 200 mg ginger powder or distilled water was given for 5 days to the three subgroups each containing seven rats. In the 10-day treatment group, same doses were given for 10 days (two estrous cycle) to the three subgroups each containing seven rats. At the end of the 5th and 10th days, ovarian volumes, ovarian weights, primordial follicles, antral follicles, atretic follicles, and corpus luteum counts were assessed. To evaluate the angiogenic effects of ginger, vascular endothelial growth factor (VEGF) and for the antioxidant effects of ginger endothelial nitric oxide synthase (eNOS) were examined in the ovaries and in the endometrium immunohistochemically.
Results: In the 5-day treatment group, antral follicle count and ovarian stromal VEGF were significantly high in the 100 mg ginger subgroup in comparison to the control group ($p < 0.05$). In the 10-day treatment group, endometrial VEGF and ovarian stromal eNOS were significantly high in the 100 mg ginger subgroup in comparison to the control group ($p < 0.05$). There was no statistically significant difference at 200 mg ginger dose both in 5-day and 10-day treatment groups.
Conclusion: The increases in the antral follicle count and ovarian stromal VEGF in the 100 mg/5-day treatment subgroup indicate that ginger have positive effects on folliculogenesis in short term with low dose. Additionally, ginger may enhance implantation in rats in long term with low dose.

Keywords: eNOS; Folliculogenesis; Ginger; Implantation; Oxidative stress; VEGF

1. Introduction

Herbal medicine is very popular and gains much attention nowadays. It has been believed that it is much more safer than synthetic drugs. Ginger (*Zingiber officinale*) has a long historical medicine use dating back 2500 years in China and India.[1] Its pharmacological properties are varied including antioxidant, anti-inflammatory, anticancer and antimicrobial activities.[2−5]

More than 60 active constituents are known to be present in ginger, which have been broadly divided into volatile and nonvolatile compounds. Hydrocarbons mostly monoterpenoid hydrocarbons and sesquiterpene include the volatile component of ginger and impart distinct aroma and taste to ginger. On the other hand, nonvolatile compounds include gingerols, shogaols, paradols, and also zingerone. The active ingredients

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like gingerols, shogaols, zingerone, and so forth present in ginger exhibit antioxidant activity. It inhibits an enzyme xanthine oxidase, which is mainly involved in the generation of reactive oxygen species.[6]

Antioxidant applications are important for protecting the human body from various sources of oxidative damage and are used extensively for prevention of a variety of diseases. In order to protect the human body from various forms of oxidative damage, recently there has been a noticeable increase in the search and identification of natural and safe antioxidants.

Oxidative stress can significantly negatively impact cellular survival and longevity and lead to programmed cell death.[7] The generations of reactive oxygen species (ROS) that result in oxidative stress include nitrogen based free radical species such as nitric oxide and peroxynitrite as well as superoxide free radicals, hydrogen peroxide, and singlet oxygen.[8] Physiological levels of ROS are required for proper functioning of different biological pathways and in maintaining homeostasis within the human body. Low levels of free radicals act as modulators in female reproductive pathways such as oocyte maturation, physiological follicular atresia, ovulation, fertilization, luteal regression, and corpus luteum formation during pregnancy.[9] ROS is also believed to play a role in the different phases of the endometrial cycle. Disruption in physiological levels of ROS leads to female reproductive dysfunction.[10]

Nitric oxide (NO) is known to mediate physiological functions, such as vasodilation, regulation of angiogenesis, and blood flow in many tissues, including the ovary.[11] Endothelial NO synthase (eNOS) was detected in ovarian follicles and in the corpus luteum during the estrous cycle in several species. It has been demonstrated that NO plays a role in the regulation of angiogenesis, steroidogenesis, apoptosis, and luteolysis.[12]

Defects in ovarian angiogenesis may contribute to a variety of disorders including anovulation and infertility, pregnancy loss, ovarian hyperstimulation syndrome, and ovarian neoplasms.[13] Vascular endothelial growth factor (VEGF), during gonadotropin surge, controls the crucial follicles transition from preovulatory to periovulatory stage that precedes ovulation.[13] Besides, VEGF is known to play an essential role in the regulation of angiogenesis in the endometrium. Its expression increases during the proliferative phase and has a second expression peak later during the mid-secretory phase, being responsible for maturation of spiral arteries during the "implantation window".[14]

The effects of ginger on male infertility and sperm parameters were investigated in a few studies.[15−19] The results showed favorable outcomes on sperm indices.[15−18] However, the effects of ginger on ovarian functions have not been studied so far. In this study, we aimed to investigate the effects of ginger powder on ovarian folliculogenesis and implantation in rats. We evaluate the effects of ginger in the ovaries and in the endometrium by VEGF and eNOS levels. This is the first study in the literature that investigates this topic.

2. Methods

The experiments were approved by the Experimental Animal Ethics Committee of Ankara Training and Research Hospital (protocol no: 0019/23.10.2014). There were 42 female albino rats in estrous cycle, each weighing approximately 200 gr (28 in the study groups and 14 in the control groups). The animals were housed in standard propylene cages in the same animal facility under conventional conditions (12:12-h light:dark; room temperature: 22 ± 2 °C). Specific pelleted food and filtered bottled tap water were supplied *ad libitum*. The animals were allowed to acclimatize for 2 weeks. Three days before the beginning of the experiments, the female rats were exposed to soiled bedding of a mature male rat to synchronize their estrous cycles.[20] Estrous phase was confirmed by vaginal smear examinations. Organic ginger roots were rinsed with distilled water. After drying, the roots were grated into small pieces and dried again using a dehydrator. Then, a mixer was used to grind the small ginger pieces until a powder was obtained. There were two study groups, each with a different length of treatment.

Group 1 (5-day treatment group): 100 mg ginger powder, 200 mg ginger powder or 2 cc distilled water (control group) was given to three subgroups, each containing seven rats, daily for 5 days (one estrous cycle). Ginger powder was mixed with 2 cc distilled water and administered by gavage. The control group also received 2 cc distilled water by gavage.

Group 2 (10-day treatment group): 100 mg ginger powder, 200 mg ginger powder or 2 cc distilled water (control group) were given to three subgroups, each containing seven rats, daily for 10 days (two estrous cycle). Like the 5-day treatment group, the ginger powder was mixed with 2 cc distilled water and administered by gavage. The control group also received 2 cc distilled water by gavage.

At the end of the 5th and 10th days, the rats were sacrificed and the inner genital organs were removed. All surgeries were performed under sodium pentobarbital anesthesia and all efforts were made to minimize suffering. The ovarian volumes and ovarian weights were measured. The primordial, antral, and atretic follicles and the corpus luteums were counted using histopathological examination stained with hematoxylin eosin in the entire cross-sectional area of both ovaries for each rat. To evaluate the angiogenic effects of ginger, immunohistochemical assessment of ovarian cortical, ovarian stromal, and endometrial VEGF were done by anti-VEGF receptor 2-antibody kit (catalog number ab15292, Abcam, Cambridge, UK). For both groups, eNOS was immunohistochemically examined in the ovaries (cortical and stromal) and in the endometrium by eNOS antibody kit (catalog number ab66127, Abcam, Cambridge, UK). For each rat, the entire cross-sectional area of the ovaries and endometrium were scanned consecutively and the stained cells were counted at ×200 magnification. All pathological and immunohistochemical examinations were done by the same pathologist who was blinded to the codes given to the rats.

Statistical analyses were performed using the Statistical Package for the Social Sciences version 15.0 (SPSS, Chicago, IL, USA). The variables are expressed as mean ± standard

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