

Full Length Research Paper

Recovery effect of *Zingiber officinale* on testis tissue after treatment with gentamicin in rats

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Ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae) is used medicinally and as a culinary spice. Gentamicin is synthetic antibacterial agent antibiotic with a very broad spectrum against microbial pathogens, especially the gram-negative. The aim of this study was to investigate the recovery effect of ginger on testis after treatment with gentamicin in rats. The forty male Wistar rats were selected and randomly divided into control (n = 10) and experimental (n = 30) groups. The experimental groups were split into three groups. First group, second experimental group which received 50 mg/kg (IP) gentamicin and one group treated with 100 mg/kg/rat/day of ginger rhizome via gavages daily for 30 days, respectively. However, the control group just received normal saline (IP). On the thirteenth day, after taking biopsy from testis of each group, tissue preparation was performed and analyzed for apoptosis. There was a significant increase in apoptosis in gentamicin groups when compared with other groups (P < 0.05). Gentamicin antibiotic have negative effect on sperm parameters and testis histology in rats. However, these side effects are less seen in the gentamicin group that received 100 mg/kg/rat of ginger. Therefore, it is recommended that usage of ginger with gentamicin has fewer side effects on male fertility.

Key words: Apoptosis, gentamicin, *Zingiber officinale*, testis tissue.

INTRODUCTION

Ginger rhizome (*zingiber officinale* R., family: zingiberaceae) is used worldwide as a spice. Both antioxidative (Khaki et al., 2009) and androgenic activity (Kirtikar et al., 1991) of *Z. officinale* were reported in animal models. All major active ingredients of *Z. officinale* such as zingerone, gingerdiol, zingibrene, gingerol and shogaols have antioxidant activity (Nassiri et al., 2009). Besides, other researchers showed that ginger oil has dominative protective effect on DNA damage induced by H₂O₂ and might act as a scavenger of oxygen radical and might be used as an antioxidant (Khaki et al., 2009).

Antibiotics are commonly prescribed for a multitude of everyday condition. Not surprisingly, a proportion of male patients attending fertility clinics may have been prescribed antibiotics by their general practitioner to treat these unrelated infections. In addition, some patients requiring assisted conception occasionally show evidence of infection of the male reproductive tract. The antibiotic aminoglycoside (gentamicin, neomycin, streptomycin and ofloxacin) are routinely used by urologists and fertility specialists to treat such bacterial infections occurring prior to *in vitro* fertilization treatment or when

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Table 1. Apoptotic cells percent of male rats exposed to ginger rhizome.

Group	Control	Gentamicin+Ginger	Ginger	Gentamicin	P-level
Apoptotic cell (%) (spermatogonia and spermatocytes)	6±2.11	15.05±1.11	0.15±0.14	22.11±1.11	p<0.05

high concentration of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection (Khaki et al., 2009, 2008; Mosher and Pratt, 1991).

Therefore, the present study was designed to investigate the protective effects of ginger rhizome on toxicity of gentamicin on testis of rats.

MATERIALS AND METHODS

Forty adult male Wistar rats weighting 200 ± 10 g (Tabriz university of medical sciences, Iran) were used in this study. They were fed with standard diet pellets and allowed food and water for an acclimation period of two weeks. The animals were maintained in a strictly controlled temperature ($18 \pm 1^\circ\text{C}$). Humidity was kept at 50% and the lighting cycle was 7.00 to 19.00 h light and 19.00 to 7.00 h dark, with adequate ventilation. Animals were handled with human care in accordance with the National Institutes of Health guidelines. The rats were randomly divided into 4 groups each consisting of ten animals. The experimental groups were split into three groups. The first group, second experimental group which received 50 mg/kg (IP) gentamicin and the other group treated with 100 mg/kg/rat/day of ginger rhizome via gavages daily for 30 days, respectively. However, the control group just received normal saline (IP). At the end of 4 weeks of treatment, testis was dissected from each rat under anesthesia exactly 24 h after the last administration then tissue preparation was performed to investigate apoptosis by TUNEL.

Tunel analysis of apoptosis

The *in situ* DNA fragmentation was visualized by TUNEL method. Briefly, dewaxed tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3% H_2O_2 for 10 min to block the endogenous peroxidase activity. The section were incubated with the TUNEL reaction mixture, fluorescein-dUTP (*in situ* cell death detection, POD kit, Roche, Germany), for 60 min at 37°C . The slides were then rinsed three times with PBS and incubated with secondary antifluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine- H_2O^2 (DAB, Roche, Germany) chromogenic reaction was added on sections and was counterstained with hematoxylin.

As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections and nucleotide mixture in reaction buffer was used instead. Apoptotic germ cells were quantified by counting the number of TUNEL stained nuclei per seminiferous tubular cross section. Cross section of 100 tubules per specimen were assessed and the mean number of TUNEL positive germ cells per tubule cross section was calculated. In this method, the apoptotic cells can be identified by their darkly stained nuclei (Khaki et al., 2008).

RESULTS

Compared to the control group, number of apoptotic germ cells (spermatogonia and spermatocytes) per tubule cross section decreased following administration of 100 mg/kg/rat/day of ginger rhizome. Administration of 50 mg/kg/day gentamicin caused a significant increase in the apoptotic germ cells percent. When this dose of gentamicin was administrated together with 100 mg/kg/rat/day of ginger rhizome, apoptotic cells percent was significantly decreased from 22.11 ± 1.11 to 15.05 ± 1.11 indicating the protective effect of ginger rhizome against gentamicin-induced apoptosis (Table 1).

DISCUSSION

Infertility is one of the major problems in match's life, about 25 and 35% of infertility is regard to man and woman receptivity (Carlsen et al., 1992; Cummings and Bingham, 1998). The importance of many of these factors is not yet clearly understood. A better understanding of underlying mechanisms in fertility and better study results clarifying the effectiveness of nutritional and biochemical factors are important to improve diagnosis and treatment. 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 seminiferous 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; 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. The ability of antioxidants such as ascorbic acid in semen to protect spermatozoa from oxidative damage has been shown by some authors (Timmermans, 1989).



Figure 1. (A) Photomicrograph of testis in Ginger group, there are no apoptotic germ cells(tunel method) $\times 320$. (B) Photomicrograph of testis in Gentamicin group that received Ginger, there are apoptotic germ cells, arrow (tunel method) $\times 320$. (C) Photomicrograph of testis in Gentamicin group, there are apoptotic germ cells, arrow (tunel method) $\times 320$.

The main pharmacological actions of ginger and compounds isolated there include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic action. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Dawson et al., 1992). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radical, is an essential attribute of aerobic life (Acharya et al., 2008). A disturbance in the pro-oxidant/antioxidant system has been defined oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, administration of this extract with gentamicin was also able to counterbalance the negative effect of gentamicin on sperm count.

Gentamicin can reduce the sperm count as it was demonstrated in this study and others (Khaki et al., 2009). Gentamicin is able to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical and frequently used to produce oxidative and necrotic damages (Khaki et al., 2008). The role of gentamicin in the induction of apoptosis and oxidative damage has also been reported. Ciprofloxacin, gentamicin, neomycin, streptomycin and ofloxacin induce apoptosis in testis (Hong et al., 2006). Accordingly, the administration of carrot seed extract with gentamicin showing the effectiveness of this extract in the prevention of cell necrosis and apoptosis. This could be indicative of free radical scavenging properties of carrot seeds which has been reported previously (Polat et al., 2006).

The results of other study showed the ability of ginger in the enhancement of caudal epididymal sperm reserves of rats resulting from increased testicular resulting

decrease of apoptosis in testis. Our finding showed that ginger can cause a decrease in rat male germ cell apoptosis rate which is in agreement with other studies (Morakmyo et al., 2008; Khaki et al., 2008). Morakmyo et al. (2008) showed that ginger caused a significant increase ($P < 0.05$) in the weight of the testis, epididymis and serum testosterone level. Khaki et al. (2008) showed that ginger caused increase of spermatogenesis. This study demonstrated that the administration of ginger can overcome reproductive toxicity of gentamicin. This natural extract was also able to reduce apoptosis in testis (Figure 1).

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