APB *Advanced Pharmaceutical Bulletin,* **2012**, 2(2), 197-200
doi: 10.5681/apb.2012.030
http://apb.tbzmed.ac.ir/

Protective Effect of Ginger on Gentamicin-Induced Apoptosis in Testis of Rats

Afshin Zahedi [1], Fatemeh Fathiazad [2*], Arash Khaki [3], Behnam Ahmadnejad [4]

[1] *Faculty of Veterinary Medicine, Islamic Azad University, Rasht Branch, Rasht, Iran.*

[2] *Department of Pharmacognosy, faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

[3] *Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

[4] *Faculty of Veterinary Medicine, Islamic Azad University, sciences & Research campus Branch, Tehran, Iran.*

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 15 July 2012
Accepted: 30 July 2012
ePublished: 15 Aug 2012

Keywords:
Gentamicin
Ginger
Apoptosis
Antioxidant
Zingiber officinale
Testis

ABSTRACT

Purpose: Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used spices for various foods and as an herbal medicine in Asian countries. It has been shown that ginger has antioxidant power. Gentamicin is an aminoglycoside antibiotic with a very broad spectrum against microbial pathogens, especially the gram-negative. Many studies revealed that gentamicin induces an oxidative stress-status in the testis by increasing free radical formation and lipid peroxidation. The present study was designed to investigate on the effects of Ginger as a natural anioxidant on testis apoptosis after treatment with gentamicin in rats. ***Methods:*** In order to study the recovery effects of ginger on testis apoptosis after treatment with gentamicin 40 adult Wistar male rats were selected and randomly divided into four groups. Normal salin control (group I) (n=10), gentamicin control (group II), ginger control (group III) and gentamicin + ginger (group IV) each 10 rats. There was observation of negative effect of Gentamicin on testis histology in rats. ***Results:*** The results revealed that there was a significant increase in apoptosis in group III when compared with other groups ($P<0.05$).However, ginger could decrease apoptosis in group IV that received 100mg/kg/rat of Ginger. ***Conclusion:*** Regarding the results, it is recommended that administration of ginger with gentamicin might be beneficial in men who receive gentamicin to treat infections.

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Introduction

Ginger root (actually the rhizome, family: zingiberaceae), is a common kitchen spice widely used worldwide as powder or as the whole fresh root. The surface of the rhizome is grayish-white with pale brown rings. Ginger has a long history of medicinal use in Chinese traditional medicine and Ayurvedic medicine for conditions such as headaches,toothache, nausea, rheumatism, colds and to improve circulation to the limbs. The main constituents of ginger are the essential oil (1-3%) and pungent compounds, including zingibere, sesquiphellandrene and beta-bisabololene, gingerols and shogaols.

Early animal studies demonstrated the antiinflamatory activity of ginger. Ginger and its extract inhibit cyclooxygenase and lipooxygenase enzymes in biosynthesis of prostaglandin and leukotriene. It helps in lowering blood cholesterol, blood clotting and pressure.The effectiveness of ginger as an antiemetic agent was also proved in several studies for motion sickness, postoperative nausea and vomiting during pregnancy. It was found ginger is an effective treatment for rheumatic disorders.[1] Research suggests the antioxidant activity of ginger, ginger extract and its pungent components.[2] Since, lipooxygenase inhibitors are strong antioxidant, such activity might be expected. Androgenic activity of ginger was also reported in animal models.[3]

The spermatozoa are susceptible to oxidative stress induced damage because of the large polyunsaturated fat content in their membranes.[4] In recent years, many studies revealed that gentamicin induces an oxidative stress-status in the testis by increasing free radical formation and lipid peroxidation.These biochemical changes manifest as structural and cytotoxic changes in the testis. Further, gemtamicin and Ofloxacin affects the spermatozoa by affecting their number, motility and morphology.[5,6] The sperm count and sperm motility were decreased and abnormality was increased. Gentamicin induced structural changes such as sloughing of somniferous epithelium, vacuoles and

***Corresponding author:** Fatemeh Fathiazad (PhD), Department of Pharmacognosy, faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran. Tel: +98 (411) 3341315, fax: +98 (411) 3344798, E-mail: fathiazad@tbzmed.ac.ir

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gaps in the epithelium, nuclear pyknosis and atrophic changes in a few tubules.[5,7] Antibiotics such as gentamicin, neomycin, streptomycin and ofloxacin are routinely used by urologists, andrologist and to treat infections prior to in vitro fertilization treatment or when high concentration of leukocytes is present in the semen of these patients.[8-10] Therefore, the present study was designed to investigate the protective effects ginger rhizome on toxicity of gentamicin on testis of rats.

Materials and Methods

Plant material

Ginger rhizome was purchased from local market in Tabriz and grounded before commencing experiments.

Animals

Forty adult male wistar rats weighting 200±10 g 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± 1°c). Humidity was kept at 50% and the lighting cycle was 7.00-19.00 h light and 19.00-7.00 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. Group I received normal salin, group II received 50 mg/kg (i.p.) gentamycin, group III received 100mg/kgBW/day of ginger rhizome via gavages for 30 days at an interval of 24 hr between subsequent treatments and group IV received gentamicin (50 mg/kg, i.p.) and ginger (100mg/kgBW/day). Animals were sacrificed on day 30, testes were removed and then tissue preparation was performed to investigate apoptosis by TUNEL.

TUNEL analysis of apoptosis

The in-situ DNA fragmentation was visualized by TUNEL method.[11] 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 % H2O2 for 10 min to block the endogenous peroxidase activity. The sections 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-H2O2 (DAB, Roche, Germany) chromogenic reaction was added on sections and 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 cells were quantified by counting the number of TUNEL stained nuclei per testis tissues cross sections. Cross sections of 100 testis tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross- section was calculated.

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant.

Results

Compared to the control group, apoptotic cells percent decreased following administration of 100mg/kgBW/day of ginger rhizome. Administration of 50 mg/kg/day gentamicin caused a significant increase in the apoptotic cells percent. When gentamicin at dose of 50 mg/kg (i.p.) was administrated with 100 mg/kg/rat/day of ginger rhizome, apoptotic cells percent was significantly ($P<0.05$) decreased from 22.11± 1.11 to 15.05± 1.11 indicating the protective effect of ginger rhizome against gentamicin- induced apoptosis (Table 1).

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

groups	Gentamicin + Ginger	Ginger	Gentamicin	Control
Apoptotic cell (%)	15.05±1.11	0.15±0.14	22.11±1.11	6±2.11

Discussion

Infertility is one of the major problems in match's life. About 25 and 35 percent of infertility is regarded to man and woman respectivity.[12] 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 importance to improve diagnosis and treatment. Smart choices for better foods might prevent body from many diseases.[13,14] 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.[15] The sperm count is considered as an important parameter to assess the effects of chemicals on spermatogenesis.[16] It has also been reported that there is a direct correlation between the epididymal sperm count and motility with fertility in animals.[17-19] The oxidative damage, elevated lipid peroxidation and the alteration of memberane properties. This subsequently can lead to germ cell death at different stages of development and the sperm count decrease.[20] 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

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been shown by researchers 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.[15] 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.[18]

The most reported effects of ginger are immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic activity. Ginger is a powerful antioxidant and may either mitigate or prevent generation of free radicals. It is considered as a safe herbal medicine with only few and insignificant adverse side effects.[17] 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.[6] Gentamicin can reduce the sperm count.[8] The role of gentamicin in the induction of apoptosis and oxidative damage has been reported. Recently, researchers also reported that ciprofloxacin, gentamicin, neomycin, streptomycin and ofloxacin induce apoptosis in testis.[21] Accordingly, the administration of carrot seed extract with gentamicin showing the effectiveness of this extract in the prevention of cell necrosis and apoptosis.[22] This could be indicative of free radical scavengering properties of carrot seeds which has been reported previously.[19] The results of other study showed the ability of ginger in the enhancement of caudaepididymal sperm reserves of rats resulting from increased testicular resulting from decreasing of apoptosis in testis. In fact, administration of ginger with gentamicin was able to counterbalance the negative effect of gentamicin on sperm count.[5] This study demonstrated that the administration of ginger can overcome toxicity of gentamicin on testis tissue and can protect testicular tissue from the detrimental effects of gentamicin.

Acknowledgment

We would like to thank of Islamic Azad University, Rasht branch, Rasht, Iran, for their financial support.

Conflict of interest

The authors report no conflicts of interest in this work.

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