Zingiber officinale Protective Effects on Gentamicin's Toxicity on Sperm in Rats

Zahedi A (D.V.M; Ph.D)[1], Khaki A (D.V.M; Ph.D.)[2]*,Ahmadi-Ashtiani HR (Ph.D. Student)[3, 5], Rastegar H (Ph.D.)[4] , Rezazadeh Sh (Ph.D.)[5]

1- Faculty of Veterinary Medicine, Islamic Azad University, Rasht Branch, Rasht, Iran
2- Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
3- Biochemistry and Nutrition Department of Zanjan University of Medical Sciences, Zanjan, Iran & Department of Clinical Biochemistry, School of Medical Science, Tarbiat-e-Modarres University, Tehran, Iran
4- Ministry of Health and Medical Education, Food & Drug Laboratory Research Center, Tehran, Iran
5- Institute of Medicinal Plants, ACECR, Tehran, Iran
* Corresponding author: Faculty of Veterinary Medicine, Tabriz Branch of Islamic Azad University, Tabriz, Iran, No:1 etekali La, South Shareati Street, 5138815941
Tel: +98- 914-3138399
Email: arashkhaki@yahoo.com

Receive: 22 Jun 2010 **Acceptance:** 8 Sep. 2010

Abstract

Background: Ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae) is used medicinally and as a culinary spice and has anti-oxidant and cell-protective effects in animals and humans body.

Objective: The aim of this study was to investigate the influence of Ginger rhizome toxicity of Gentamicin on sperm parameters in male rats.

Methods: Forty Wistar male rat (n=40) were allocated into four groups, control (n=10) and test groups (n=30), that subdivided into groups of 3 that received ginger rhizome powder (100 mg/kg/day), gentamicin group that received, 5 mg/kg/day and ginger group that received, 5 mg/kg/day gentamicin additionally, for 30 consequence day. Animals were kept in standard conditions. In thirty day the testes tissue of Rats in whole groups were collected.

Results: Ginger administration caused a marked increase in the testosterone concentrations of the rats even in spite of receiving 5 mg/kg/day gentamicin in compared with the control and gentamicin treated groups.

Conclusion: Ginger rhizome is able to overcome reproductive toxicity of gentamicin and induces spermatogenesis probably mainly through the elevation of testosterone levels.

Keywords: Ginger rhizome, Male fertility, Testosterone, Toxicity

[DOR: 20.1001.1.2717204.2010.9.35.10.0]

Introduction

Ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae), is used worldwide as a spice. Both antioxidative [1] and androgenic activity [2] of *Z. officinale* were reported in animal models. All major active ingredients of *Z. officinale*, such as Zingerone, Gingerdiol, Zingibrene, gingerols and shogaols, have antioxidant activity [3]. Besides, other researches showed that ginger oil has dominative protective effect on DNA damage induced by H_2O_2 and might act as a scavenger of oxygen radical and might be used as an antioxidant [4]. 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 aminoglycosides (gentamicin, neomycin, streptomycin) and fluoroquinolones (ofloxacin) are routinely used by urologists, andrologist and fertility specialists to treat such bacterial infections occurring prior to in vitro fertilization treatment, or when high concentration of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection[5, 6, 7, 8].

Therefore, the present study was designed to investigate about protective effects Ginger rhizome on toxicity of gentamicin on cauda epididymal sperm reserves in rats.

Materials and Methods

Experimental animals

Forty adult male Wistar rats weighing 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 ad libitum 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 07.00-19.00 h light and 19.00 - 07.00 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. At the end of 4 weeks of treatment, testis, cauda epididymides and sperm ducts were dissected from each rat under anesthesia exactly 24h after the last administration. Before anesthesia, 5 ml blood sample was collected from each rat in heparinzed tubes, allowed to settle for 5 minutes at room temperature. The sample was centrifuged at 750 g for 10 minutes and the plasma was separated and kept at -70°C for hormonal assays.

Assessment of sperm count and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin [9]. After 5 min incubation at 37°C (with 5% CO_2), the cauda epididymis sperm reserves were determined using the standard hemocytometric methodand sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO method.

Serum total Testosterone hormone

Serum concentration of total testosterone was measured by use a double antibody RIA from immunotech Beckman Coulter Company. The sensitivities of hormone detected per assay tube were 0.025ng/ml [10].

Statistical analysis

Assessment of the results was performed using one-way ANOVA followed by Tukey HSD as Post-ANOVA test. The 0.05 level of probability was used as the criterion for

[DOR: 20.1001.1.2717204.2010.9.35.10.0]

significance. All data are presented as mean ± SD.

Results

Cauda epididymal sperm reserves and relative weights of testes

The effects of Ginger rhizome administration on cauda epididymal sperm reserves (CESR) and motility of the sperms in rats have been tabulated. Compared to the control group, the CESR increased following administration of either 100 mg/kg Ginger rhizome, but only in the later case, this elevation was statistically significant ($p<0.05$). Administration of 5 mg/kg/day gentamicin caused a significant reduction in the CESR. When this dose of gentamicin was administrated together with 100 mg/kg Ginger rhizome, the CESR level was significantly ($p<0.05$) elevated from 10.5±1.8 to 22.5±1.4 ($\times 10^6$) indicating the protective effect of Ginger rhizome against gentamicin-induced necrosis. There were no significant differences ($p>0.05$) between motility of sperms of all groups apart from the group receiving gentamicin. The motility value of this group was different from all other four groups which indicate the effectiveness of Ginger rhizome in retention of the sperm motility of rats in spite of receiving gentamicin.

Hormones

Radioimmunoassay of serum samples revealed significant increases in the testosterone concentrations of the Ginger rhizome**,** Group animals compared with the control and gentamicin treated groups.

Discussion

Infertility is one of the major problems in match's life, about 25 and 35 percent of infertility is regard to man and woman receptivity [11, 12]. The importance of many of these factors is not yet clearly understood. A better understanding of underlying mechanisms in (sub) 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 [13, 14]. As all spermatogenesis stages occur in seminiferous tubules of the testis, it is possible to evaluate the extent of spermatogenesis by determination of the number of spermatozoa produced per one gram of testicular parenchyma [15, 16]. The sperm count is considered as an important parameter to asses the effects of chemicals on spermatogenesis [17]. It has also been reported that there is a direct correlation between the epididymal sperm count and motility with fertility in animals [18 - 20]. The oxidative damage, elevated lipid peroxidation and the alteration of memberane properties can lead to germ cell

Table 1- Cauda epididimal sperm reserves (CESR) and motility of sperms of male rats exposed to Ginger rhizome

Parameter	Control	Ginger (100 mg/kg)	Gentamicin (5 mg/kg) + Ginger (100 mg/kg)	Gentamicin (5 mg/kg)	p Level*
CESR ($\times 10^6$)	28.2 ± 1.8	32.1 ± 2.2	22.5 ± 1.4	10.5 ± 1.8	$p < 0.05$
Motility (%)	41.8±2.6	45.7±2.8	31.0±2.8	15.2±1.6	$p < 0.05$
Serum testestrune	(1.1±0.9)	(1.9±0.8)	(1±0.9)	(0.7±0.9)	$p < 0.01$

* The number of animals per group was eight rats. Data are presented as means ±SE (95% CI for mean).

[DOR: 20.1001.1.2717204.2010.9.35.10.0]

death at different stages of development and the sperm count decrease [21]. 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 [20]. The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant 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 [18].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 radicals, is an essential attribute of aerobic life [16]. A disturbance in the pro-oxidant/antioxidant system has been defined as 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 (O^{-2}), nitrogen oxide (NO) and hydroxyl radical (HO─), 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 [4] 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 [5]. The role of gentamicin in the induction of apoptosis and oxidative damage has also been reported Recently we reported that ciprofloxacin [22] gentamicin, neomycin, streptomycin and ofloxacin due Apoptosis in testis [23] Accordingly, the administration of compounds with antioxidant properties and reactive oxygen species scavengers can ameliorate the severity of [24] 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 scavengering properties of carrot seeds which has been reported previously [22]. The results of this study also showed the ability of ginger in the enhancement of cauda epididymal sperm reserves of rats resulting from increased testicular spermatogenesis. It was also observed that the administration of ginger can elevate significantly testosterone in plasma [25]. On the other hand, the levels of testosterone reduced significantly following exposure to gentamicin. This is probably an indication that interstitial cell necrosis arises from a reduction in the level of this hormone.

Conclusion

It was demonstrated that the administration of ginger can overcome reproductive toxicity of gentamicin. This natural extract was also able to induce spermatogenesis and cauda epididymal sperm reserves probably mainly through the elevation of testosterone levels.

Acknowledgment

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

[DOR: 20.1001.1.2717204.2010.9.35.10.0]

References

1. Khaki A, Fathiazad F, Nouri M Khaki AA, Chelar C, Ozanci, Ghafari-Novin M, Hamadeh M. The Effects of Ginger on Spermatogenesis and Sperm parameters of Rat. *Iranian J. Reproductive Medicine* 2009; 7 (1): 7 - 12.

2. Kirtikar KR, Basu BD, "Indian Medicinal Plants", 2nd ed., Periodical Export, New Delhi, 1991.

3. Nassiri M, Khaki A, Gharachurlu Sh, Ashteani A, Rastegar H, Rezazadeh Sh. Effects of Ginger on spermatogenesis in Streptozotocin-induced Diabetic Rat. *Iranian J. Medicinal Plants* 2009; 8 (31): 118 - 24.

4. Khaki A, Khaki AA, Iraj S, Bazi P, Imani SAM, Kachabi H. Comparative Study of Aminoglycosides (Gentamicin & Streptomycin) and Fluoroquinolone (Ofloxacin) Antibiotics on Testis Tissue in Rats: Light and Transmission Electron Microscopic Study. *Pak. J. Med. Sci.* 2009; 25 (4): 624 - 9.

5. Khaki A, Heidari M, Ghaffari Novin M, Khak AAi. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. *Iranian J. Reproductive Medicine* 2008; Vol. 6 (2): 14 – 20.

6. Khaki A, Ghaffari Novin M, Khak AA, Fathiazad F, Khabiri M, Hossinchi J. Ultra Structural Study of Gentamicin and Ofloxacin Effect on Testis Tissue in Rats: light and Transmission Electron Microscopy. *AJPP.* 2009; Vol. 3 (4): 105 - 9.

7. Khaki A, Fathiazad F, Nouri M, Khaki AA, Jabbari khamenhi H, Hammadeh M. Evaluation of Androgenic Activity of Allium cepa on Spermatogenesis in Rat. *Folia Morphologica* 2009; 68 (1): 45 - 51.

8. Mosher WD, Pratt WF. Fecundity and infertility in the United States: incidence and trends. *J. Fertil Steril*. 1991; 56 (2): 192 - 3.

9. Huang HFS, Linsenmeyer TA, Li MT, Giglio W, Anesetti R, von Hagen J, Ottenweller JE, Pogach L. Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study. *J. Androl*. 1995; 16: 148 - 57.

10. Amann RP, Almquist JO. Reproductive Capacity of Dairy Bulls. I. Technique for Direct Measurement of Gonadal and Extra-Gonadal Sperm Reserves. *J. Dairy Sci*. 1961; 44: 1537 - 45.

11. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *B. M. J*. 1992; 12: 305: 609 - 13.

12. Cummings JH, Bingham SA. Fortnightly review - diet and the prevention of cancer, *British Medical J.* 1998; 317: 1636 – 40.

13. Suryavathi V, Sharma S, Sharma S, Saxena P, Pandey S, Grover R, Kumar S, Sharma KP. Acute toxicity of textile dye wastewaters (untreated and treated) of Sanganer on male reproductive systems of albino rats and mice. *Reprod. Toxicol*. 2005; 19: 547 - 56.

14. Reddy PS, Pushpalatha T, Reddy PS. Reduction of spermatogenesis and steroidogenesis in mice after fentin and fenbutatin administration. *Toxicol. Lett*. 2006; 166: 53 - 9.

15. Hew KW, Heath GL, Jiwa AH, Welsh MJ. Cadmium in vivo causes disruption of tight junction-associated microfilaments in rat Sertoli cells. *Biol. Reprod*. 1993; 49: 840 – 9.

[DOR: 20.1001.1.2717204.2010.9.35.10.0]

16. Acharya UR, Mishra M, Patro J, Panda MK. Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. *Reprod. Toxicol*. 2008; 25: 84 – 8.

17. Yousef MI. Protective role of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. *Toxicol*. 2005; 207: 81 – 9.

18. Dawson EB, Harris WA, Teter MC, Powell LC. Effect of ascorbic acid supplementation on the sperm quality of smokers. *Fertil. Steril.* 1992; 58: 1034 – 9.

19. Yu LL, Zhou KK, Parry J. Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chem*. 2005; 91: 723 - 9.

20. Timmermans LM. Modifications in spermatogenesis following antibiotic therapy. *Acta Urol. Belg.* 1989; 57: 35 - 46.

21. Bestas A, Bayar MK, Akpolat N, Okuducu MN. Effect of sevoflurane anesthesia on the severity of renal histopathologic changes in rabbits pretreated with gentamicin: A controlled, investigator-blinded, experimental study. *Curr. Therapeutic Res*. 2006; 67: 386 - 95.

22. Polat A, Parlakpinar H, Tasdemir S, Colak C, Vardi N, Ucar M, Emre MH, Acet A. Protective role of aminoguanidine on gentamicin-induced acute renal failure in rats. *Acta Histochemica.* 2006; 108: 365 - 71.

23. Hong SH, Park SK, Cho Y, Lee H, Kim KR, Kim MG, Chung W. Gentamicin induced nitric oxide-related oxidative damages on vestibular afferents in the guinea pig. *Hearing Res.* 2006; 211: 46 - 53.

24. Ademuyiwa O, Ngaha EO, Ubah FO. Vitamin E and selenium in gentamicin nephrotoxicity. *Toxicol.* 1990; 9: 281 – 8.

25. Anahara R, Toyama Y, Mori C. Review of the histological effects of the anti-androgen, flutamide, on mouse testis. *Reprod. Toxicol*. 2008; 25: 139 - 43.

[DOR: 20.1001.1.2717204.2010.9.35.10.0]