http://www.cjmb.org

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 1, No. 3, Summer 2014, 103-109

eISSN: 2148-9696

Comparison of the Effects of Methotrexate and Ginger Extract on Reproductive Parameters in Rats

Amir Mahdi Imani[1], Nava Ainehchi[2*]

Abstract

Objective: Methotrexate is an anticancer drug used in chemotherapy. The purpose of this study was to evaluate the effect of ginger extract on sex hormones of male rats treated with methotrexate.

Materials and Methods: In this experimental study, 56 male Wistar rats (10-12 weeks old) weighing 200-220 g were randomly divided into control and experiment groups. Experiment group 1 was administered 5 mg methotrexate intraperitoneally daily, experiment groups 2 and 3 were administered 20 mg and 40 mg of ginger extract orally daily, and experiment groups 4 and 5 received methotrexate and ginger extract. Sex hormones were measured after 8 weeks. One-way analysis of variance (ANOVA) was used data analysis.

Results: The results showed that serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone decreased significantly in the group receiving methotrexate compared with the control group. The concentration of these hormones in experimental groups 2 and 3, which received ginger extract, increased compared with the control group. The serum levels of these hormones in groups 4 and 5, which received methotrexate and ginger, increased compared with the group receiving methotrexate.

Conclusion: Ginger extract reduced the adverse effects of methotrexate on sex hormone-producing cells. This effect is probably due to the antioxidant property of ginger.

Keywords: Hormone, Ginger, Methotrexate, Wistar Rats

Introduction

Methotrexate is an anticancer drug used in chemotherapy. This drug is well absorbed from the gastrointestinal tract and is widely distributed in tissues and body fluids, and also crosses the blood-brain barrier. This drug is converted in the liver to active metabolites, and is eventually excreted through the kidneys (1). Methotrexate is typically used as an anticancer drug and an immune suppressant, has alkylation property, and is able to establish covalent bonds in nucleophilic position of DNA strands and proteins and transverse ties. This ultimately leads to DNA strand break and inactivation, stopping DNA synthesis, inhibition of cell proliferation, formation of small nuclei, and ultimately cell death (2). This medication was synthesized in 1985 and was used for tumor treatment and is now widely used in human drug therapy (3). Despite broad clinical applications, methotrexate can cause many side effects including reproductive toxicity in organisms that are exposed to the drug (4). Methotrexate is metabolized in the liver. This combination, affected by microsomal enzymes, breaks down in the liver and is converted to its active metabolites, meaning phosphoramide mustard and acrolein (5). Studies have shown that methotrexate reduces the luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and spermatogenesis (6). Ginger (Terrestris Tribulus) is a native shrub herb which self-propelled in tropical desert areas and in Iran it is found in the central desert and Dasht-e Lut. The soft four part fruit with

Received: 16 Feb 2014, **Revised:** 12 Apr 2014, **Accepted:** 25 May 2014, **Available online:** 15 Jul 2014

[1] Department of Histopathology, Sari Branch, Islamic Azad University, Sari, Iran
[2] Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
*Corresponding Author: Nava Ainehchi, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Tel: +98 9143110316, Email: ainehchi.nava@gmail.com

its sharp spines is the most affective part used for medication (7-9). Studies have shown that ginger contains steroids, saponins, flavonoids, alkaloids, polyunsaturated fatty acids, vitamins, tannins, resin potassium nitrate, aspartic acid, and glutamic acid (10). This plant, due to containing protodioscin and saponins which increase testosterone and LH hormone levels, has long been used in traditional Chinese and Indian medicine to treat sexual dysfunction and increase libido (11). Tribestan is a patented extract of ginger that increases libido and has a counteractive effect on cold nature, infertility, and menopausal disorders (12). Researchers have shown that the dioxin existing in ginger, through increasing free testosterone levels, and estrogen and pregnenolone regulation, increase sexual potency in men (13). Few studies have been conducted and showed that ginger extract improved folliculogenesis in female mice after treatment with methotrexate (14). Since the therapeutic and prophylactic effects of ginger on sex hormones after taking methotrexate have not been investigated, this study was conducted to examine the effects of ginger on sex hormones.

Materials and Methods

This was an experimental study. In this study, all moral principles on handling laboratory animals have been considered. The animals used in this study were 56 adult male Wistar rats with an average weight of 200-220 g and 10-12 weeks of age. During the whole period of 56 days of the study, the animals were exposed to 12 hours of darkness and 12 hours of light. The drinking water for the animals throughout the experiment was municipal tap water and they were fed special rat food. The temperature during testing was 22 ± 24 °C.

The rats were randomly divided into 7 groups of 7; control groups 1 and 2, and experiment groups 1, 2, 3, 4, and 5.

In the control group, standard water and food was used.

Control groups 1 and 2 received 1 ml of distilled water daily and 1 ml of distilled water + alcohol intraperitoneally.

Experiment group 1 received 5 mg per kilogram of body weight of methotrexate daily intraperitoneally.

Experiment group 2 and 3 received 20 and 40 mg per kilogram of body weight of ginger extract orally.

Experiment group 4 and 5, 1 hour after receiving methotrexate 5 mg per kilogram of body weight intraperitoneally, received 20 mg and 40 mg per kilogram of body weight of ginger extract orally.

After 56 days, the mice were anesthetized and blood samples were collected directly from the heart and blood serum was separated. By using ELISA method, the LH, FSH, and testosterone concentrations were measured and the data were entered into a computer. Data were analyzed using SPSS for Windows (version 17, SPSS Inc., Chicago, IL, USA), and one-way ANOVA statistical test and Duncan's post-hoc test. All P values of less than 0.05 were considered significant.

Results

The results of this study are presented in table 1 and figures 1, 2, and 3. According to table 1, serum concentrations of LH, FSH, and testosterone in the experiment group 1 (methotrexate) were significantly decreased compared with the control group ($P < 0.05$).

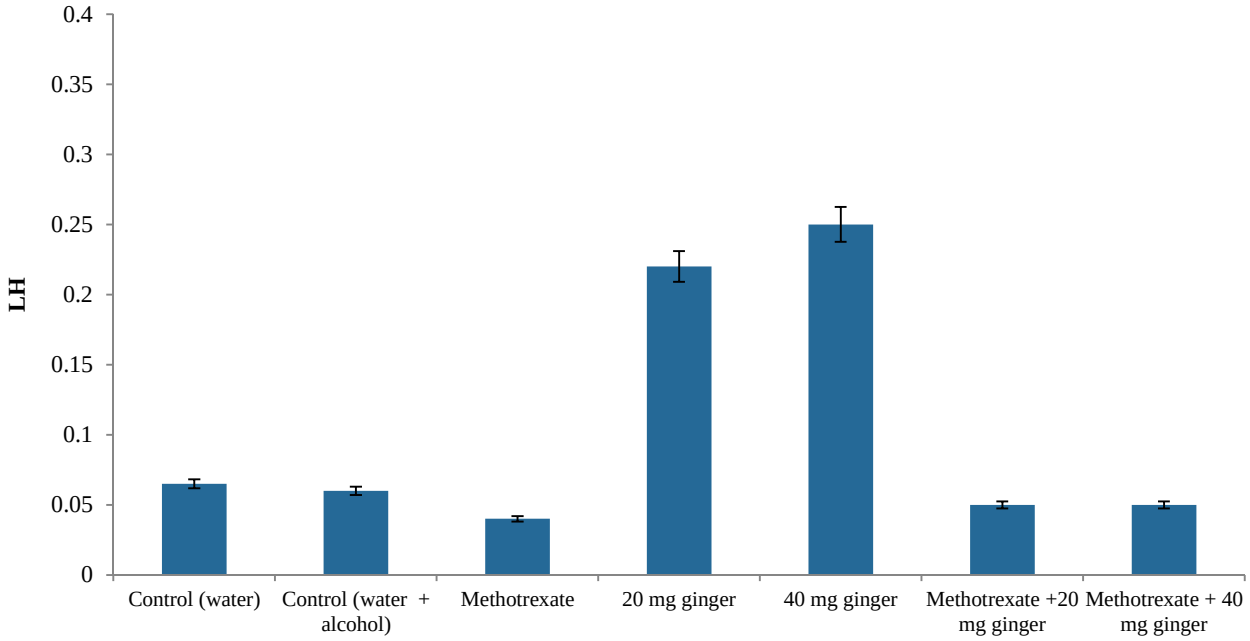

Figure 1. The mean plasma concentration of luteinizing hormone (LH) in terms of IU/l in different experiment groups compared with the control group

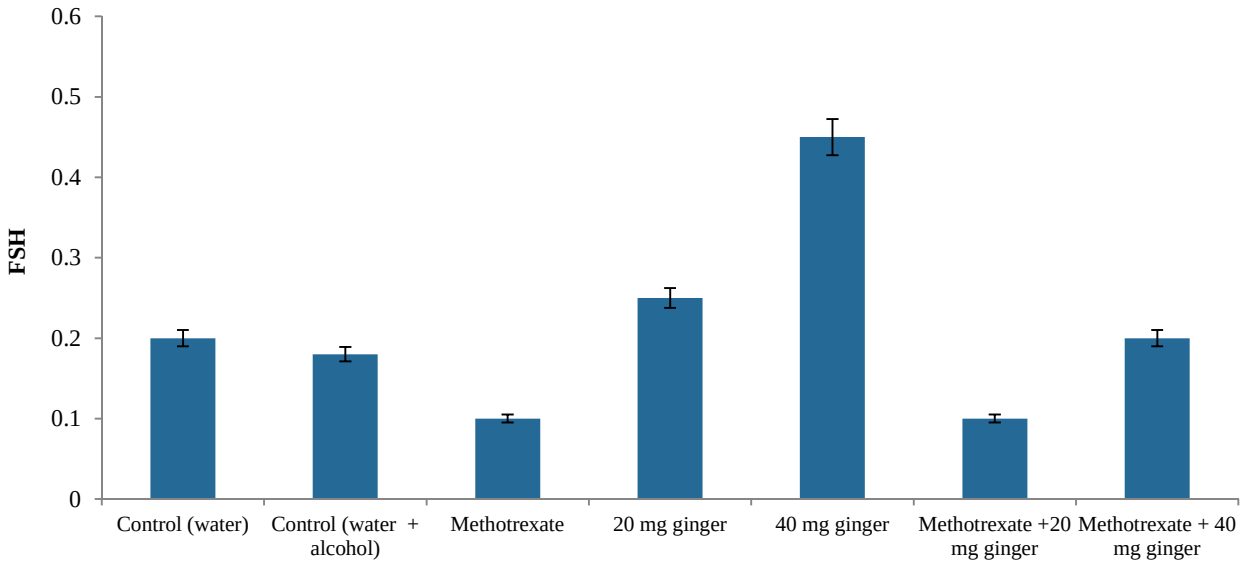

Figure 2. The mean plasma concentration of follicle stimulating hormone (FSH) in terms of IU/l in different experiment groups compared with the control group

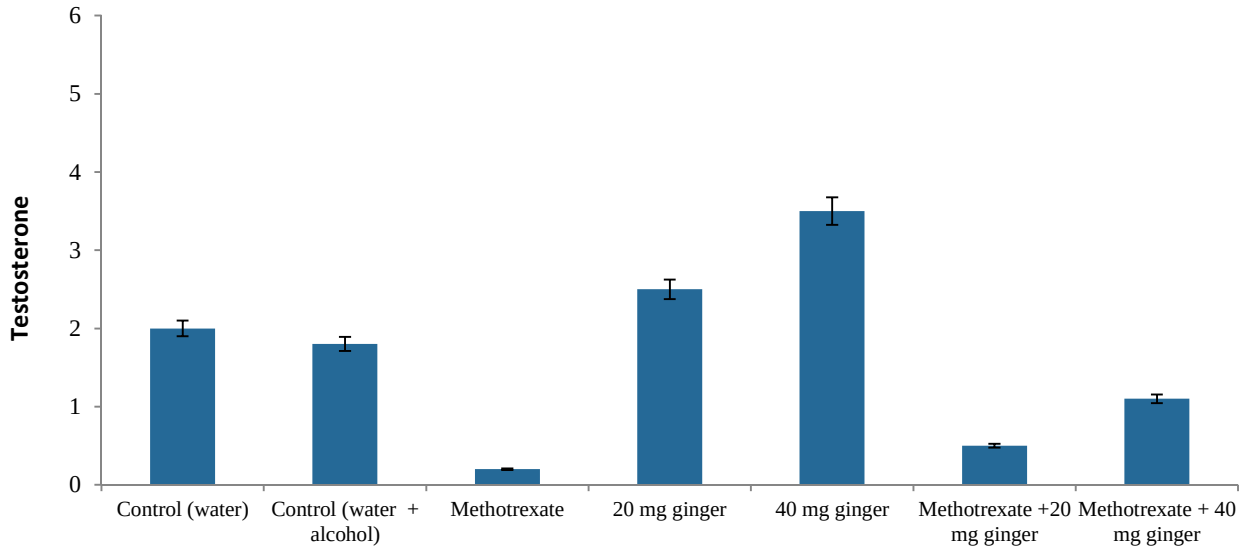

Figure 3. The mean plasma concentrations of testosterone in IU/l in different experiment groups compared with the control group

Table 1. The mean plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels in different groups of rats

Groups	Parameters		
	LH	FSH	Testosterone
Control 1 (Water)	0.065	0.20	2.00
Control 2 (Water + Alcohol)	0.060	0.18	1.80
Methotrexate (5 mg)	0.040	0.10	0.18
Ginger (20 mg)	0.220	0.25	2.50
Ginger (40 mg)	0.250	0.45	3.54
Methotrexate (5 mg) + Ginger (20 mg)	0.050	0.10	0.47
Methotrexate (5 mg) + Ginger (40 mg)	0.050	0.20	1.10

The means that have at least one letter in common are not significantly different at $P < 0.05$; LH: Luteinizing hormone; FSH: Follicle stimulating hormone

The concentration of LH in experiment groups 2 and 3 (20 and 40 mg ginger) was significantly increased compared with the control group ($P < 0.05$). The serum concentrations of this hormone in groups that received methotrexate + 20 mg ginger and methotrexate + 40 mg ginger, compared with the control group, had decreased, but this decrease was not significant. In addition, the

concentration of LH in groups that received methotrexate + 20 mg ginger and methotrexate + 40 mg ginger, compared with the methotrexate group, had increased, but this increase was not significant. Serum concentrations of FSH and testosterone in experiment groups 2 and 3, compared with the control group, had increased. This increase was significant in experiment group 3 ($P < 0.05$). Serum concentrations of FSH hormone in the groups that received methotrexate + 20 mg ginger and methotrexate + 40 mg ginger had, respectively, decreased and increased neither of which were significant. The serum concentration of testosterone in the methotrexate + 20 mg ginger group was significantly reduced compared with the control group ($P < 0.05$). Moreover, in the methotrexate + 40 mg ginger group, its concentration increased compared with the control group; however, this increase was not significant. Serum concentrations of FSH and testosterone in the methotrexate + 20 mg ginger and methotrexate + 40 mg ginger groups increased compared to the methotrexate group. This increase was significant in the methotrexate + 40 mg ginger group.

Discussion

This study examined the effect of ginger on sex hormones in male rats after treatment with methotrexate. Considering the results of measuring the testosterone, LH, and FSH hormone concentrations in this study, the concentration of these hormones in the methotrexate group, compared with the control group, decreased. This finding was consistent with the results of other researchers (6,15,16). The study by Cao et al. showed that enzymatic and non-enzymatic antioxidant levels in interstitial cells decreased. Thus, they decreased the synthesis and secretion of testosterone and were effective risk factors for impaired spermatogenesis, and consequently, in the significant reduction of epididymal sperm number (17). The toxic effects of chemotherapy drugs, such as methotrexate, can directly and indirectly damage the seminiferous epithelium on interstitial cells (18). Testosterone steroid hormone, which plays an important role in the evolution and differentiation of sperm cells, is secreted by interstitial cells. Therefore, with the loss of sexual cells and Sertoli cell damage, atrophy of interstitial cells and the synthesis and secretion of testosterone decreases (19). Oxidative damage, after administration of methotrexate, decreases cellular processes and steroidogenesis by interstitial cells (20). Studies have shown that low levels of testosterone, in addition to disrupting spermatogenesis, could have a negative effect on the function of the epididymis, and impair sperm maturation and quality (21).

Decreased serum concentrations of FSH and LH after administration of methotrexate can result from the effect of active metabolites, such as acrolein, resulting from the metabolism of methotrexate in the body. This drug can have many side effects by affecting the DNA molecule and breaking it, and influencing RNA molecules and protein synthesis. This subject is justifiable based on the pharmacology book by Katzung-Bertram and Iranpharma by Shahraz and Ghaziyani as well as studies conducted in the past (22-24). The FSH and LH levels are reduced according to their chemical structure which is polysaccharide.

The results in table 1 and figure 3 showed that during the study, the testosterone concentrations of animals that received 40 mg/kg of ginger extract had increased. Several other studies also indicated this increase following the use of ginger (25,26). Gauthaman and Ganesan demonstrated that ginger in castrated mice can also increase testosterone (27). This finding was in agreement with that of the present study. Ginger plant increased testosterone level due to containing glycosides estradiol the most important of which is protodioscin. Natural estradiol, in this compound, may act as an intermediary, and facilitate the direction of androgen production of estradiol (28). Therefore, it will increase testosterone hormone level, and this hormone, in turn, will increase spermatogenesis. Ginger also contains unsaturated fatty acids (29). Polyunsaturated fatty acids increase 17-beta-hydroxy-steroid dehydrogenase enzyme activity and since this enzyme is involved in the production of testosterone, therefore, testosterone also increases (30). In another study, it was reported that these acidic compounds inhibit the activity of the aromatase enzyme. Considering the fact that this enzyme leads to the conversion of androgens to estrogens, inhibition of its activity results in increased levels of androgens (testosterone) in the blood (31). Studies have shown that the alcohol extract of plant parts of the same ginger family plant at a dose of 50 mg/kg, causes a significant increase in serum levels of free testosterone in the body. This extract is an aphrodisiac which is likely to result in an increase in androgens (32).

Results of the present study showed that 20 and 40 mg/kg doses of ginger increased serum concentrations of LH and 20 mg/kg doses increased the serum concentration of FSH in rats receiving the hydroalcoholic extract of this plant. Gauthaman and Ganesan, in a study on primates, rabbits, and rats, demonstrated that this extract has the ability to increase some sex hormones; this was due to its protodioscin content (27). Ginger plant, due to its saponin content, increases lutein hormone from the pituitary gland. Lutein hormone is also a stimulant of testosterone production, and hence, is able to improve sexual function, including increased sperm production, and improved erectile function and sexual desire (33). Furostanol is one of the saponins found in ginger that have a stimulating effect on spermatogenesis through increasing the production of gonadotropins by the pituitary gland, which can also stimulate testosterone hormone production. This substance significantly improves the quality and quantity of sperm (34). The increase in concentration of gonadotropin was probably due to the active ingredient in the extract.

Researchers have found that initiating and maintaining spermatogenesis requires normal FSH and LH in the time before and after sexual maturation (35). The distinction of spermatocytes and spermatid stage 7 is under the direct influence of testosterone (36), or the absence of FSH, LH can indirectly affect them (35). Therefore, as was mentioned, ginger extract contains furostanol that stimulates spermatogenesis through increased production of gonadotropins by the pituitary gland (34). Thus, the increase in FSH concentration was probably because of the effects on the pituitary-gonadal axis, and this effect is due to the increased secretion of gonadotropins. Findings suggested that testosterone levels in the methotrexate + 20 mg ginger group decreased compared to the control group. Moreover, according to previous studies, which suggested methotrexate caused biochemical changes and testicular and tissue destruction (37), one other reason for the reduction of testosterone was testicular tissue destruction by methotrexate. In the methotrexate + 40 mg ginger group, compared to methotrexate group, there was a significant increase in testosterone concentrations. This, certainly, suggested the improvement of testicular tissue due to testosterone secretion from testicular interstitial cells (38). Furthermore, in this study, changes in FSH levels indicated that the methotrexate + 40 mg ginger group had a significant increase compared to the methotrexate group. This reflected the positive effects of antioxidants of ginger extract. Investigations found that methotrexate reduced gonadal and libido function (21). In researches on the effect of methotrexate on rat ovarian follicles, it was stated that methotrexate can cause an increase in apoptotic cells in the ovary and lead to dysfunction of the ovaries. The most important oxidative system is in the glutathione peroxidase in the sexual organs, and this medication can reduce the activity of this system (39,40). Based on the results of the study by Sabik and Abd El-Rahman, catalase and superoxide dismutase activity reduced due to methotrexate use, and levels of malondialdehyde increased, indicating the production of free radicals, oxidative stress, and lipid peroxidation. They evaluated the protective effects of vitamin E and ginger as antioxidants and introduced ginger as an herbal remedy more effective than vitamin E in reducing oxidative stress induced by methotrexate (41). Thus, prescription of antioxidants during chemotherapy to reduce oxidative stress resulting from the administration of methotrexate and removing tissue toxins seems necessary (42).

Conclusion

It can be conclude that chemotherapeutic agents, such as methotrexate, used to inhibit cancer cells, in addition to their therapeutic effects also have some side effects. In this study, the use of methotrexate for 56 days decreased FSH, LH, and testosterone levels. Ginger extract increased these three hormones. Combined use of these two resulted in the ginger compound reducing metabolites of methotrexate, and thereby, increasing testosterone and FSH.

Ethical issues

We have no ethical issues to declare

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

Our appreciation goes to the Reproductive Health and Women's Health Center of Tabriz University of Medical Sciences, Iran.

References

1. Aguilar-Mahecha A, Hales BF, Robaire B. Chronic cyclophosphamide treatment alters the expression of stress response genes in rat male germ cells. Biol Reprod 2002; 66: 1024-32.
2. Hengstler JG, Fuchs J, Tanner B, Oesch-Bartlomowicz B, Holz C, Oesch F. Analysis of DNA single-strand breaks in human venous blood: a technique which does not require isolation of white blood cells. Environ Mol Mutagen 1997; 29: 58-62.
3. Brock N. Oxazaphosphorine cytostatics: past-present-future. Seventh Cain Memorial Award lecture. Cancer Res 1989; 49: 1-7.
4. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am 1998; 27: 927-43.
5. Ludeman SM. The chemistry of the metabolites of cyclophosphamide. Curr Pharm Des 1999; 5: 627-43.
6. Johari H, Mahmoudinejad F, Amjad G. An Evaluation of the effect of the hydroalcoholic extract of ginger on the hypothalamus-pituitary-gonadal axis in adult female rats (Rat) treated with cyclophosphamide. Pejoda 2011; 6: 62-70.
7. Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A. Protective effect of aqueous saffron extract (Crocus sativus L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. J Pharm Pharm Sci 2005; 8: 387-93.
8. Conrad J, Dinchev D, Klaiber I, Mika S, Kostova I, Kraus W. A novel furostanol saponin from Tribulus terrestris of Bulgarian origin. Fitoterapia 2004; 75: 117-22.
9. Zargari A. Medicinal Plants. Tehran, Iran: Tehran University Press; 1996. p. 1345-52.
10. Yan W, Ohtani K, Kasai R, Yamasaki K. Steroidal saponins from fruits of Tribulus terrestris. Phytochemistry 1996; 42: 1417-22.
11. Koumanov F, Bozadjieva E, Andreeva M, Platonova E, Ankova V. Clinical trial of Tribestan. Exp Med 1982; 4: 211-5.
12. Tomova M. Tribestan. Pharmacy 1987; 37: 40-2.
13. Basic Chemicals P & CEPCISPARC. Selected Medicinal Plants of India: A Monograph of

Identity, Safety and Clinical Usage. Bombay, India: Chemexcil; 1992.
14. Rezaie A, Roozbeh M, Goorani Nejad S, Najaf Zadeh Varzi H, Fatemi Tabatabaei SR, Pourmahdi-broojeni M. Effects of Tribulus Terrestris extract and Vitamin C on changes induced by cyclophosphamide in the rat ovary. Physiol Pharmacol 2013; 17: 194-203.
15. Mokhtari M, Johari H, Hematkhah V, Jamali H, Allahverdi Gh. The Effect of Hydro-Alcoholic Garlic Extract on Testis Weight and Spermatogenesis in Mature Male Rats under Chemotherapy with Cyclophosphamide. J Fasa Univ Med Sci 2011; 1: 67-74.
16. Hosseini A, Zare S, Ghaderi Pakdel F, Ahmadi A. Effects of Vitamin E and Ginseng Extract on Fertility Changes Induced by Cyclophosphamide in Rats. J Reprod Fertil 2011; 11: 227-37.
17. Cao L, Leers-Sucheta S, Azhar S. Aging alters the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells. J Steroid Biochem Mol Biol 2004; 88: 61-7.
18. Howell SJ, Radford JA, Ryder WD, Shalet SM. Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. J Clin Oncol 1999; 17: 1493-8.
19. Hosseini A, Ahmadi A, Ghaderi Pakdel F, Zare S. Effects of long-term use of low dose cyclophosphamide on testicular tissue in rats. Physiol Pharmacol 2011; 15: 351-60.
20. Luo L, Chen H, Trush MA, Show MD, Anway MD, Zirkin BR. Aging and the brown Norway rat leydig cell antioxidant defense system. J Androl 2006; 27: 240-7.
21. Hermol RB. Efferent ducts, epididymis, and vas deferens: stracture, functions and their regulation. In: Knobil E, Neill JD, Editors. The Physiology of Reproduction. New York, NY: Raven Press; 1988.
22. Katzung-Bertram J. Basic Medical Pharmacology. Trans. Niyayesh M, ModaresMusavi F, Fathallahi A. Tehran, Iran: Arjomand Publications; 1999. p. 372-8.
23. Shahraz S, Ghaziyani T. Iranpharma: A Comprehensive Textbook of Official Iranian Medications. Tehran, Iran: Tayeb Publications; 2007.
24. Ajith TA, Nivitha V, Usha S. Zingiber officinale Roscoe alone and in combination with alpha-tocopherol protect the kidney against cisplatin-induced acute renal failure. Food Chem Toxicol 2007; 45: 921-7.
25. Morakinyo AO, Adeniyi OS, Arikawe AP. Effects of Zingiber Officinale on Reproductive Functions in the Male Rat. Afr J Biomed Res 2008; 11: 329-34.
26. Kafashelahi R, Mousavi G, Hejazi S, Khayat Nouri MH, Kalantari S. Effects of tribulus tertesris extract on body weight, testis histopathology and size in rats. Veterinary Journal Islamic Azad University of Tabriz 2011; 5: 1043-9. [In Persian].
27. Gauthaman K, Ganesan AP. The hormonal effects of Tribulus terrestris and its role in the management of male erectile dysfunction-an evaluation using primates, rabbit and rat. Phytomedicine 2008; 15: 44-54.
28. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update 2007; 13: 163-74.
29. Adaikan PG, Gauthaman K, Prasad RN, Ng SC. Proerectile pharmacological effects of Tribulus terrestris extract on the rabbit corpus cavernosum. Ann Acad Med Singapore 2000; 29: 22-6.
30. Chung BH, Mitchell SH, Zhang JS, Young CY. Effects of docosahexaenoic acid and eicosapentaenoic acid on androgen-mediated cell growth and gene expression in LNCaP prostate cancer cells. Carcinogenesis 2001; 22: 1201-6.
31. Clinton SK, Mulloy AL, Li SP, Mangian HJ, Visek WJ. Dietary fat and protein intake differ in modulation of prostate tumor growth, prolactin secretion and metabolism, and prostate gland prolactin binding capacity in rats. J Nutr 1997; 127: 225-37.
32. El-Tantawy WH, Temraz A, El-Gindi OD. Free serum testosterone level in male rats treated with Tribulus alatus extracts. Int Braz J Urol 2007; 33: 554-8.
33. Xu YJ, Xie SX, Zhao HF, Han D, Xu TH, Xu DM. Studies on the chemical constituents from Tribulus terrestris. Yao Xue Xue Bao 2001; 36: 750-3.
34. Brown GA, Vukovich MD, Martini ER, Kohut ML, Franke WD, Jackson DA, et al. Endocrine and lipid responses to chronic androstenediol-herbal supplementation in 30 to 58 year old men. J Am Coll Nutr 2001; 20: 520-8.
35. French FS. Hormonal Regulation of Spermatogenesis: Proceedings of the Second Annual Workshop on the Testis Held in Chapel Hill, North Carolina, April 2, 1975. New York, NY: Plenum Press; 1975.
36. Chowdhury AK. Dependence of testicular germ cells on hormones: a quantitative study in hypophysectomized testosterone-treated rats. J Endocrinol 1979; 82: 331-40.
37. Reichman BS, Green KB. Breast cancer in young women: effect of chemotherapy on ovarian function, fertility, and birth defects. J Natl Cancer Inst Monogr 1994; 125-9.
38. Cryptorchidism: a prospective study of 7500 consecutive male births, 1984-8. John Radcliffe Hospital Cryptorchidism Study Group. Arch Dis Child 1992; 67: 892-9.
39. DeLeve LD. Cellular target of cyclophosphamide toxicity in the murine liver: role of glutathione and site of metabolic activation. Hepatology 1996; 24: 830-7.
40. Lopez SG, Luderer U. Effects of

cyclophosphamide and buthionine sulfoximine on ovarian glutathione and apoptosis. Free Radic Biol Med 2004; 36: 1366-77.
41. Sabik LM, Abd El-Rahman SS. Alpha-tocopherol and ginger are protective on Cyclophosphamide-induced gonadal toxicity in adult male albino rats. Basic and Applied Pathology 2009; 2: 21-9.
42. Das UB, Mallick M, Debnath JM, Ghosh D. Protective effect of ascorbic acid on cyclophosphamide- induced testicular gametogenic and androgenic disorders in male rats. Asian J Androl 2002; 4: 201-7.

Citation: Imani AM, Ainehchi N. **Comparison of the Effects of Methotrexate and Ginger Extract on Reproductive Parameters in Rats**. Crescent J Med & Biol Sci 2014; 1(3): 103-9.