Journal of Medicinal Plants Research Vol. 6(10), pp. 2004-2010, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1732
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effect of *Fumaria parviflora* alcoholic extract on male rat's reproductive system

Mitra Heydari Nasrabadi*, Helia Aboutalebi and Maryam Naseri

Department of Biology, Faculty of Basic Science, Islamic Azad University- Parand Branch, Parand, Iran.

Accepted 12 January, 2012

This study was undertaken to study the effect of *Fumaria parviflora* alcoholic extract on male rat spermatogenesis. *F. parviflora* was administered orally at doses of 750 and 1050 mg/kg b.w. for 3 days and 250 mg/kg b.w. for 5 days through oral gavage. Experimental and control rats were sacrificed on day fifteenth after the first gavage. The testes were removed and analyzed. The weight and volume of the testes was increased in experimental groups but these increases were not significant. Moreover histopathological analysis showed that *F. parviflora* significantly increased the number of spermatogonium, spermatocytes, spermatozoids and Leydig cells (P<0.001). Also in these groups, formation of blood vessels was obvious. Results of this study indicate positive effect of *F. parviflora* alcoholic extract on male reproductive system because the number of sexual cells has increased significantly.

Key words: *Fumaria parviflora*, alcoholic extract, spermatogenesis, fertility.

INTRODUCTION

In drug discovery, medicinal herbs have regularly been considered the leading source of pharmaceuticals, employed in the treatment of various human diseases due to their high chemical diversity and broad biological functionality (Jaroszewski, 2005). The genus *Fumaria* L. (Papaveraceae) comprises of 60 species, which most of them grow around the Mediterranean region (Zargari, 1990). Until now, 8 *Fumaria* species have been reported from Iran. Wendelbo (1974) reported 7 species from Iran and recently Lidén (2000) reported *Fumaria officinalis* L. as a new species for the flora of the country (Ebrahimzadeh et al., 2011). *Fumaria parviflora* (Fumariaceae) is an herbaceous plant that grows in wide variety parts of Iran and has been used in Iranian folk medicine in dermatological diseases, for stimulation of liver function and gall bladder, also as antiscabies, antiscorbite, antibronchite, diuretic, expectorant, antipyretic, diaphoretic, appetizer and antineoplastic (Zargari, 1989; Barimani, 1982; Amin, 1991). Phytochemical analyses of some plants of genus Fumaria, including *F. parviflora* has demonstrated the presence of isoquinoline alkaloids (Deng et al., 2001; Hentschel et al., 1995) namely protropine, cryptopine, sinactine, stylopine, bicu-culine, adlumine, parfumine, fumariline, fumaro-phycine, fumaritine, dihydro-fumariline, per-fumidine and dihydrosanguirine in these plants (Suau et al., 2002; Suan, 2003).

Oral antipyretic activity has been shown by hexane-chloroform- and water-soluble extracts of *F. parviflora* in rabbits (Khattak et al., 1985). In addition, aqueous-methanolic extract of this plant (500 mg/kg, orally twice daily for 2 days) prevented the paracetamol-induced hepatotoxicity, but had no effect against hepatotoxicity which was induced by CCl_4 (Gilani et al., 1996). In other studies hepatoprotective effects of several other species of Fumaria have been demonstrated (Rao and Mishra, 1998; Aktay et al., 2000). There are some contradictory reports about toxic effects of *F. parviflora* extracts, while no obvious toxic effects for hexane-, chloroform and water-soluble extracts of *F. parviflora* up to dose of 1.6 g/kg have been reported (Khattak et al., 1985). In one study dose of 500 mg/kg (p.o.) of aqueous-methanolic extract caused significant prolongation in pentobarbital-induced sleep as well as increased strychnine-induced lethality in mice (Gilani et al., 1996; Heidari et al., 2004). Since this species has been used in folk medicine as sexuality multiplier, the objective of this study is to

*Corresponding author. E-mail: heydari_nasr@yahoo.com. Tel: +989123138954. Fax: +982294723254.

Table 1. Testis diameters in control and experimental I, II, III groups.

Group	Long diameter	Short diameter
Control	1.802 ± 0.121	1.063 ± 0.051
Experimental I	1.728 ± 0.097	1.050 ± 0.045
Experimental I I	1.824 ± 0.081	1.094 ± 0.039
Experimental I I I	1.834 ± 0.088	1.138 ± 0.063

Figure 1. Formation of blood vessels in experimental I and II groups Compared to the control group. Experimental I and II groups received 750 and 1050 mg/kg b.w. doses of *F. parviflora* extract for 3 days. Control group animals only received distilled water.

investigate the role of *F. parviflora* alcoholic extract in spermatogenesis process.

MATERIALS AND METHODS

Plant and extraction

F. parviflora was collected from Heiran region of Ardabil province (Iran). The whole parts of the plant were air-dried in shadow followed by grinding. Extraction was performed by adding ethanol to 100 g of dried Plant, heated in bain-marie (45ºC for 24 h), then leaved at room temperature (1 h). The obtained extracts were concentrated by rotary evaporator apparatus *in vacuo* (60 rev/min, 64ºC) (Naseri et al., 2011).

Animals

The use of animals in this study was approved by the committee on the Use of Live Animals in Teaching and Research of Islamic Azad University-Parand Branch. Male Wistar rats (230±5 g) were reared on a standard laboratory diet and given tap water. They were kept in a room where humidity (65 to 70%), temperature (22±2°C) and day/night cycle (12:12 light/dark) were controlled. Rats were divided into 4 groups; control, experimental I, experimental II and experimental III (n=7).

The control group received distilled water, the experimental I and II groups received 750 and 1050 mg/kg b.w. *F. parviflora* alcoholic extract for 3 days and the experimental III group received 250 mg/kg b.w. *F. parviflora* alcoholic extract for 5 days through oral gavage.

Sample preparation

Rats were anesthetized and sacrificed fifteenth day after the first gavage and the testes were removed. Samples were washed with normal saline, the volume and weight of the testes were measured and the tissues were fixed. After fixation of tissues by formaldehyde 10% solution, they were dehydrated in a graded series of ethanol and embedded in paraffin. Thin sections (4 to 5 µm) were cut using a microtome and stained with H and E method. Germinal cells was counted in the surface unit by light microscope (Naseri et al., 2011).

Statistical analysis

The data were expressed as Mean±SEM Statistical analysis was performed with ANOVA followed by Post-Hoc Tukey multiple range tests using the Statistical Package for the Social Sciences (SPSS) for Windows. P <0.05 were considered statistically significant.

RESULTS

The effect of *F. parviflora* on testis volume is shown in Table 1. The morphometric studies show that testes volume increased in experimental groups compares to the control group but these increases is not significant. Also increase in formation of blood vessels was obvious (Figure 1). The histophatological analysis showed that alcoholic extracts administration of *F. parviflora* at doses of 750, 1050 and 250 mg/kg b.w. have increased germinal

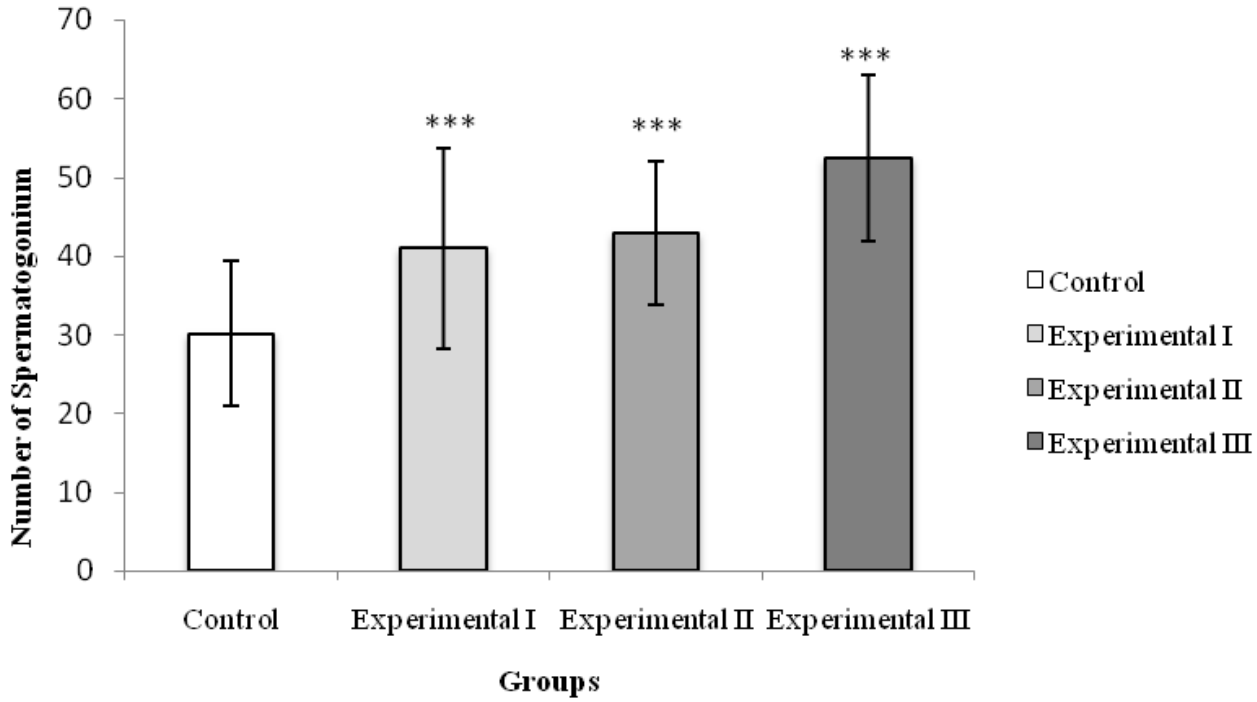

Figure 2. Number of spermatogonium in control and experimental (I, II and III) groups. Results are presented as Mean±SEM *P<0.05, ** P<0.01, ***P<0.001 Experimental (I, II and III) groups compared to the Control group, one-way ANOVA.

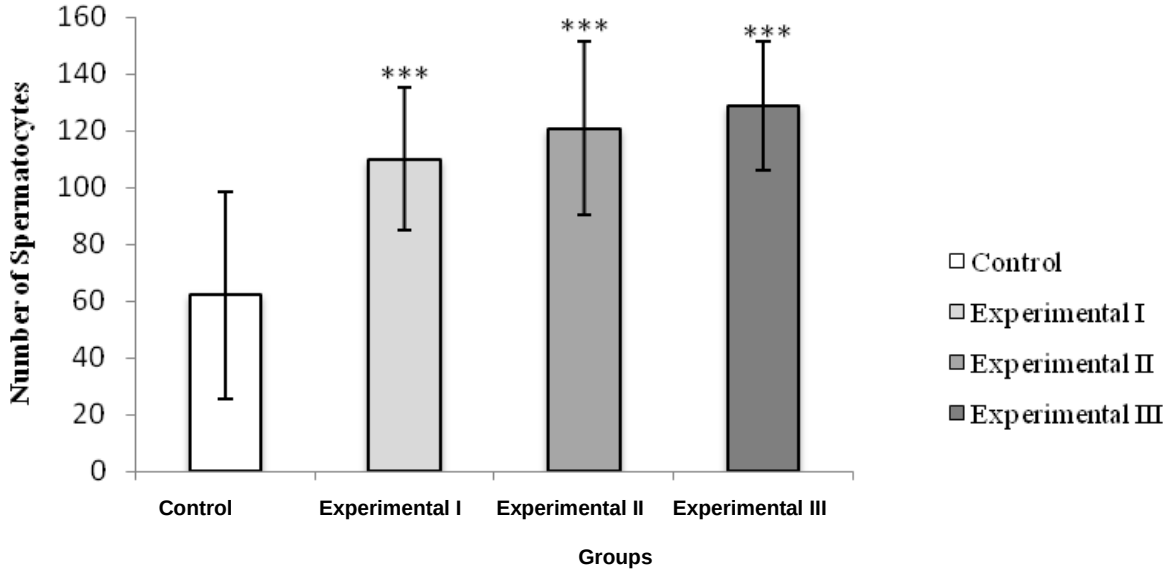

Figure 3. Number of Spermatocytes in control and experimental (I, II and III) groups. Results are presented as Mean±SEM *P<0.05, ** P<0.01, ***P<0.001 Experimental (I, II and III) groups compared to the control group, one-way ANOVA.

cells significantly (P<0.001) (Figures 2 to 6). The experimental group III (250 mg/kg b.w. extract for 5 days) showed the most increase in the number of spermatogonium, spermatocytes, spermatozoids and Leydig cells in comparison to the other groups (P<0.001). These results show that less, but continuous dosage of

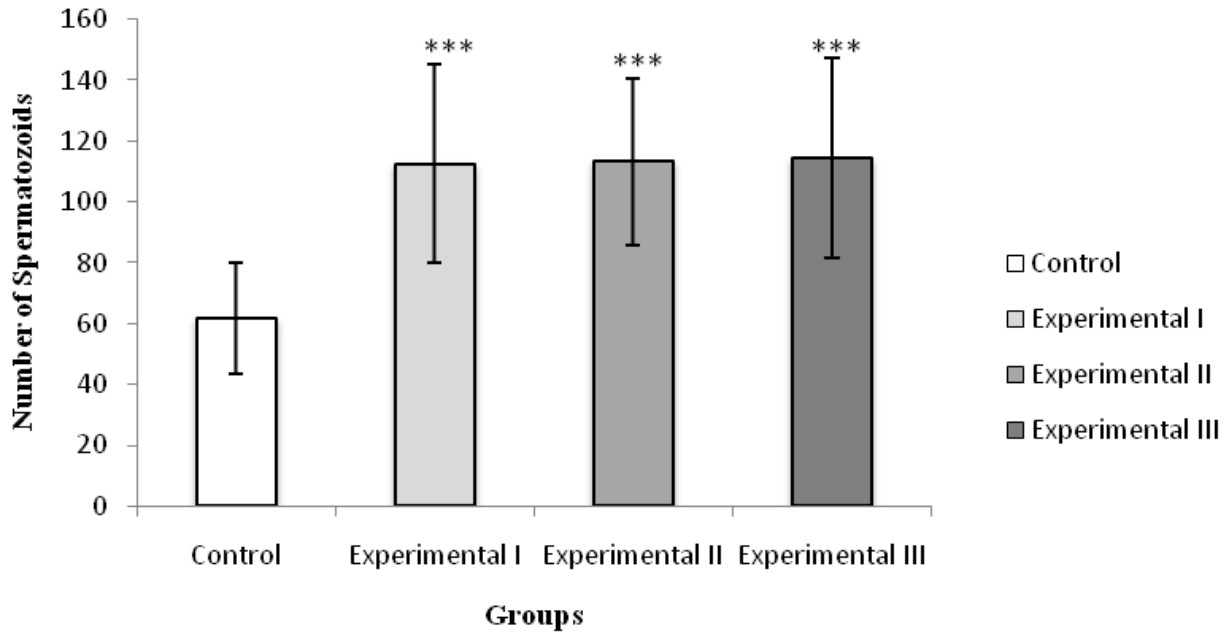

Figure 4. Number of spermatozoids in control and experimental (I, II and III) groups. Results are presented as Mean±SEM *P<0.05, ** P<0.01, ***P<0.001 Experimental (I, II and III) groups compared to the Control group, one-way ANOVA.

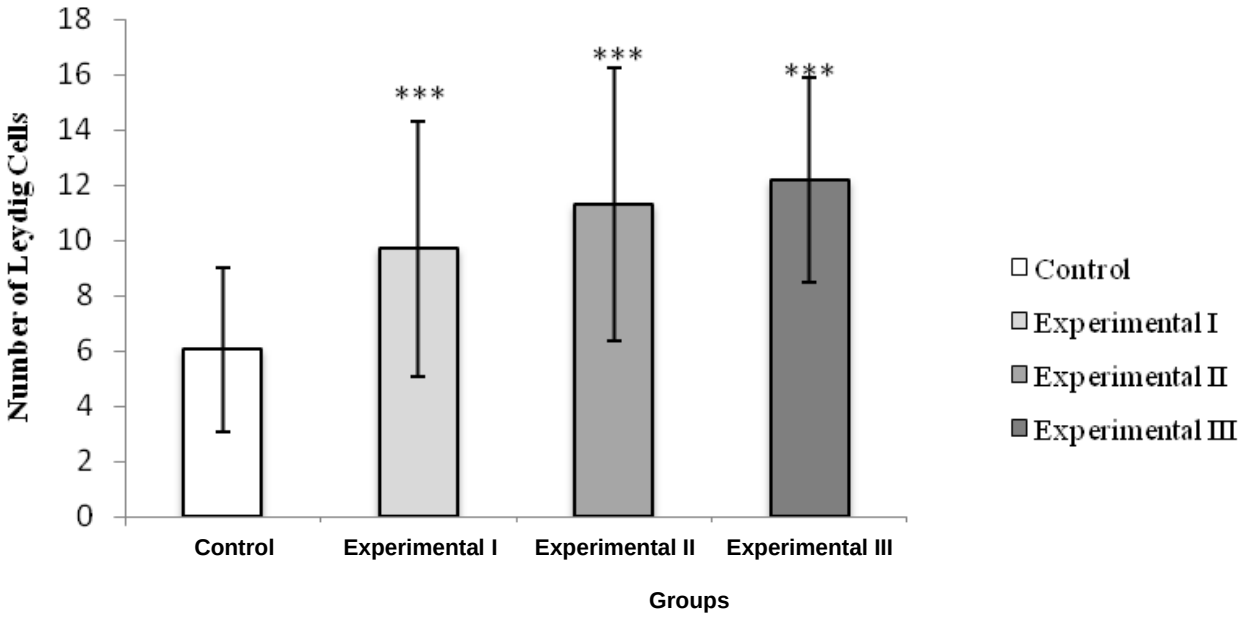

Figure 5. Number of Leydig cells in control and experimental (I, II and III) groups. Results are presented as Mean±SEM *P<0.05, ** P<0.01, ***P<0.001 Experimental (I, II and III) groups compared to the Control group, one-way ANOVA.

this extract has been more effective. Experimental animals received doses of 750, 1050, 250 mg/kg b.w. of *F. parviflora* alcoholic extract for 3 and 5 days intragastrically. Control animals received only distilled water. Volumes of testes were expressed as long and short diameters. Data are expressed as Mean±SEM.

DISCUSSION

F. parviflora is an annual herb creeper mainly found in the skirt of Zagros especially in Eghlid plain. This herb is used in folk medicine and has a remarkable place of new medicine in the last two decades. One of the most

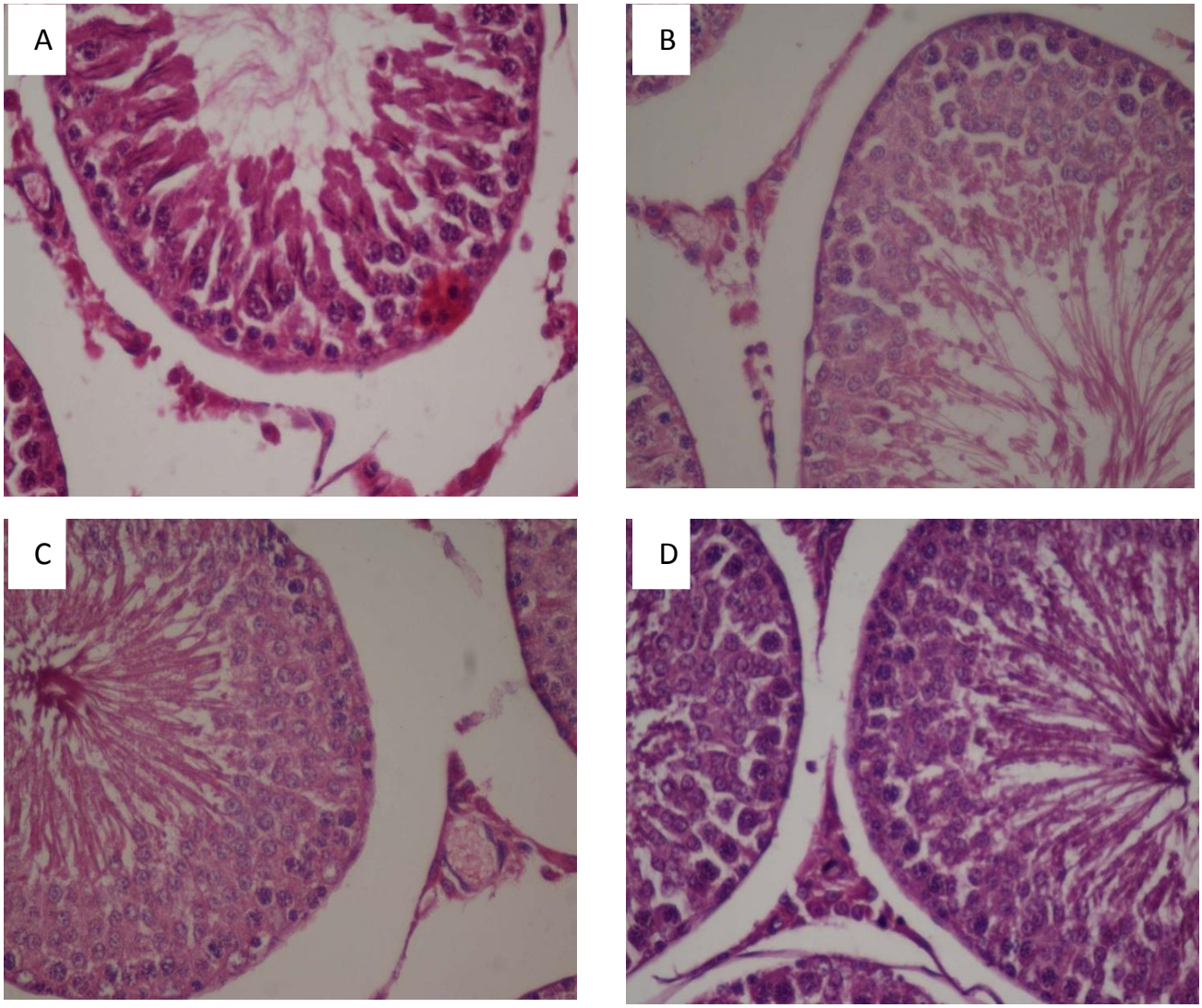

Figure 6. Somniferous tubules in A: Control group, B: experimental I group, C: experimental II group, D: experimental III group.

important implicit pharmaceutical effects of *F. parviflora* is increasing sexuality (Amin, 1991). In the present study it was shown that in rats treated with *F. parviflora* for 3 and 5 days, there were significant increase in number of spermatogonium, spermatocytes, spermatozoids and Leydig cells. The most significant increases were observed in experimental III group animals which have received 250 mg/kg b.w. alcoholic extract of *F. parviflora* for 5 days. These changes were in association with increased testes weight and volume, however they were not significant. The results of the present study also supported our previous finding on the protective role of *F. parviflora* against chlorambucil side effect (Souri et al., 2009).

Chlorambucil is a medication used in the treatment of certain types of cancers. This drug can damage spermatozoids and cause infertility. In previous study we demonstrated that administration of Chlorambucil and *F. parviflora* extract at dose of 150 mg/kg simultaneously can protect testes from damage and prevent decreasing in the number of spermatogonium, spermatocytes, spermatozoids and sertoli cells (Souri et al., 2009). The results of studies show that spermatogenesis process and transition from germinal cells to the mature cells depend on protection from pathologic and cytotoxicity damages that threat this process (Nikravesh et al., 2010). Free radicals cause damage to the other molecules including biological membrane fatty acids and oxidize them due to high tendency of getting electron (Modaresi et al., 2010). As a consequence motility, structure and function of the membrane incure damage (Halliwell and Gutterridge, 1989). Antioxidant compositions can protect cell membranes from these damages (Rice Ewans and Eurdon, 1994). Previous studies demonstrate the existence of antioxidant component in *Fumaria* species (Haq and Hussain, 1993). Therefore there is a possibility

that *F. parviflora* reinforcement antioxidant defense system and reduce oxidative stress which result in spermatozoid increasing numbers.

The results of this study showed significant increase in the number of leydig cells in experimental groups. Since *F. parviflora* extract increased leydig cells beside germinal cells which secrete male sexual hormone - testosterone- that cause spermatogenesis stimulation (Hosseini et al., 2011), it can be conclude that the possible mechanism of *F. parviflora* extract effect on the testis tissue is increase in leydig cell numbers and there upon increase in sexual male hormone and more blood flow to the testis via angiogenesis increasing. Both mechanisms affect sertoli cells which control spermatogenesis, and therefore increase germinal cells. Reactive oxygen species (ROS) have been associated with impaired sperm function, including decreased motility, abnormal morphology and decreased sperm egg penetration (Aitken and Clarkson, 1987; Aitken et al., 1989; Iwasaki and Gagnan, 1992; Mazzilli et al., 1994; Sukcharoen et al., 1996). Studies have indicated that excessive concentrations of ROS are present in the semen of 15% of normal patients, 40% of oligospermic patients and 96% of spinal injury patients. Infertile men are more probable than fertile men, to have depressed total antioxidant capacity and lower levels of individual antioxidants (Smith et al., 1996; Lewis et al., 1995; Lewis et al., 1997). Therefore, improved fertility observed in the present study might be due to the antioxidant effect of *F. parviflora*. Note that as our data showed less but continuous dosage of *F. parviflora* extract, is more effective because of more increase in the number of germinal cells.

Conclusion

Since oligospermia and azoospermia are the causes of men infertility, use of this plant will be beneficial as a treatment, however further preclinical studies and clinical trials in humans are suggested.

ACKNOWLEDGEMENT

We are grateful to Young Researchers Club of Islamic Azad University (Iran) for supporting this research by a scholarship.

REFERENCES

Aitken JR, Clarkson JS (1987). Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J. Reprod. Fertil., 81(2): 459-469.

Aitken RJ, Clarkson JS, Hargreave TB (1989). Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia. J. Androl., 10(3): 214-220.

Aktay G, Deliorman D, Ergun F, Yesilada E, Cerik C (2000). Hepatoprotective effects of Turkish folk remedies on experimental liver injury. J. Ethnopharmacol., 73: 121-129.

Amin GH (1991). Popular medical plants of Iran. Tehran: Farhang Publ., 1: 51-52.

Barimani L (1982). Traditional medicine_traditional drugs. Tehran: Gutenberg Publ., 3: 335-336.

Deng M, Song XY, Tiang B (2001). Effects of protopine on proliferation of cultured rabbit aortic vascular smooth muscle cells. Chin. Pharmacol. Bull., 17: 306-309.

Ebrahimzadeh AF, Keshavarzi M, Sheidaii M, Ghadam P (2011). Fruit and seed morphology of the *Fumaria* L. species (Papaveraceae) of Iran. Turk. J. Bot., 35(2011): 167-173.

Gilani AH, Janbas KH, Akhtar MS (1996). Selective effect of an extract from *Fumaria parviflora* on paracetamol-induced hepatotoxicity. Gen. Pharmacol., 27: 979-983.

Halliwell B, Gutterridge JMC (1989). Role of free radical and catalytic metal ions in human disease. Overview Method Enzymol., 186: 1-85.

Haq LU, Hussain M (1993). Medicinal plants of mansehra. Hamdard Med., 36: 78-79.

Heidari MR, Mandgary A, Enayati M (2004). Antinociceptive effects and toxicity of *Fumaria parviflora lam*. In mice and rats. DARU, 12(4): 136-140.

Hentschel C, Dressler S, Hahn EG (1995). *Fumaria officinalis* (fumitory)- clinical applications. Fortschr. Med., 113(19): 291-292.

Hosseini A, Zarei S, Ghaderi PF, Ahmadi A (2011). Study the effect of anti-oxidant Ginseng extract and vitamin E on rat's fertility through long term treatment with Cyclophosphamide. J.R.I., 11(4): 227-237.

Iwasaki A, Gagnan C (1992). Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil. Steril., 57: 409-416.

Jaroszewski JW (2005). HPLC–SPE–NMR in natural products research. Abstracts of short lectures, SL 016, final program and books of Abstracts, 53rd Annual Meeting of the Society of Medicinal Plant Research (GA) and Joint Congress with the Italian Society of Phytochemistry (SIF), Florence, Italy, August 21st–25th. (electronically accessible at http://www.farmacia.unifi.it/gacongress2005.html).

Khattak SG, Gilani SN, Ikram M (1985). Antipyretic studies on some indigenous Pakistani medicinal plants. J. Ethnopharmacol., 14: 45-51.

Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W (1995). Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil. Steril., 64: 868-870.

Lewis SE, Sterling ES, Young IS, Thompson W (1997). Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil. Steril., 67: 142-147.

Mazzilli F, Rossi T, Marchesini M, Ronconi C, Dondero F (1994). Superoxide anion in human semen related to seminal parameters and clinical aspects. Fertil. Steril., 62: 862-868.

Modaresi M, Messripour M, Rajaei R (2010). Effect of Cinnamon extract on the number of spermatocyte and spermatozoa cells in mice. Iran. J. Med. Aromat. Plants, 26(1): 83-90.

Naseri M, Heydari Nasrabadi M, Khodarahmi P, Ahmadi F, Mojibi P, Aboutalebi H (2011). Study of the effect of Fumaria parviflora alcoholic extract on spermatogenesis in male rats. New Cell. Mol. Biotechnol., 1(2): 61-66.

Nikravesh MR, Jalali M, Mohammadi Sh (2010). Study the effect of raw extract onion on rat's testis. J.R.I., 10(4): 239-244.

Rao KS, Mishra SH (1998). Antinociceptive activity of monomethyl fumarate isolated from *Fumaria indica*. J. Ethnopharmacol., 60: 207-213.

Rice ECA, Eurdon RM (1994). Free radical damage and it's control. Elsevier Amsterdam, 113: 46-44.

Smith R, Vantman D, Ponce J, Escobar J, Lissi E (1996). Total antioxidant capacity of human seminal plasma. Hum. Reprod., 11: 1655-1660.

Souri M, Heydari NM, Shiravi A, Habibi Z (2009). Interaction between *Fumaria parviflora* aqueous extract and cholorambocil on spermatogenesis in rats. J. Animal. Biol. (IAU-Damghan branch), 2(1): 11-18.

Suau R, Cabezudo B, Rico R, Najera F, Lopez-Romero JM (2002). Direct determination of alkaloid contents in *Fumaria* species by GC-

MS. Phytochem. Anal., 13: 363-367.
Suan R (2003). Alkaloids from *Fumaria sepium* and *Fumaria agaria*. Biochem. Syst. Ecol., 30: 263-265.
Sukcharoen N, Keith JH, Irvine DS, Aitken RJ (1996). Prediction of the *in vitro* fertilization (IVF) Potential of human spermatozoa using sperm function tests: the effect of the delay between testing and IVF. Hum. Reprod., 11: 1030-1034.
Zargari A (1990). Medicinal plants. Tehran university press. Sixth Ed., 1: 72-166.
Zargari A (1989). Medicinal Plants. Tehran: Tehran Univ. Publ., 2: 170-171.