Int. J. Morphol.,
31(1):82-86, 2013.

The Effect of *Cannabis sativa* Hydroalcoholic Extract on Sperm Parameters and Testis Histology in Rats

Efecto del Extracto Hidroalcohólico de *Cannabis sativa* sobre los Parámetros Espermáticos e Histología Testicular en Ratas

Nahid Lotfi[*]; Mozafar Khazaei[*]; Sayyed Mohammad Ali Shariatzadeh[**]; Malek Soleimani Mehranjani[**] & Ali Ghanbari[*]

LOTFI, N.; KHAZAEI, M.; SHARIATZADEH, S. M. A.; SOLEIMANI MEHRANJANI, M. & GHANBARI, A. The effect of *Cannabis sativa* hydroalcoholic extract on sperm parameters and testis histology in rats. ***Int. J. Morphol., 31(1)***:82-86, 2013.

SUMMARY: *Cannabis Sativa* is a multiuse herb in traditional medicine, its hydroalcoholic extract (10, 50, and 100 mg/kg) administered interaperitoneally for 14 consequent days to Wistar male rats resulted in significant decrease in progressive motility of sperm. Sperm count and seminiferous tubules diameter decreased significantly in comparison with control group. Also decrease in animal body weight in doses of 50 and 100 mg/kg was observed. Changes in testes weight and serum testosterone were not significant. *Cannabis sativa* extract has negative effect on sperm parameters such as motility, sperm count, and seminiferous tubules diameter.

KEY WORDS: *Cannabis sativa*; Rat; Seminiferous tubules; Sperm parameters.

INTRODUCTION

Cannabis sativa (*C. Sativa*) belongs to the family of Cannabaceae, Rosales order (The Angiosperm Phylogeny Group, 2003). This plant has commercial, pharmaceutical and medical usages (Veldhuis *et al.*, 2003). The first written evidence of the medicinal use of this plant comes from China around 5000 years ago. It was recommended for constipation, malaria, rheumatic pains, distraction, and female diseases (Kraft & Kress, 2009). *C. sativa* compounds were unknown until 1964, when the chemical structure of the main psychotropic compound delta-9-tetrahydrocannabinol (THC) was identified (Gaoni & Mechoulam, 1964). Up to now, more than 60 oxygen-containing aromatic hydro- carbon compounds have been isolated from *C. sativa*, known collectively as cannabinoids. It also contains a number of other compounds, including at least 120 different terpenes and 21 flavonoids (ElSohly, 2002).

The seed of this plant contains 20-25% protein, 20-30% carbohydrate, 10-15% insoluble fiber, and 25-35 percent oil (Theimer &, Mileken, 1995; Small & Cronquist, 1976).

Scientific research in the field of cannabinoids has led to the discovery of a new lipid signaling system in humans and animals, endogenous cannabinoid system with specific receptors, and physiologic ligands (Kraft & Kress). Active compounds of *cannabis sativa* (cannabinoids) bind to and activate specific cannabinoid receptors on the cell surface. There are two types of these receptors which have been cloned and characterized from mammalian tissues: CB1 and CB2 (Matsuda *et al*., 1990; Munro *et al*., 1993). CB1 receptors are extensively expressed in CNS and peripheral neurons and are present in tissues like liver, small intestine, fatty tissue, ovary, endometrium, testis, vas deferens, and bladder (Gaoni & Mechoulam; McPartland, 1999; McPartland & Pruitt, 1999). CB2 receptors are less frequent and found predominately in immune system cells (Munro *et al*.).

The endocannabinoid system, now appears not only as a proper modulator of physiological activities in the central nervous system, but also exerts its role in the endocrine system, immune system, gastrointestinal tract, and reproductive system (Gaoni & Mechoulam).

In many developed countries, medicinal Plants and folk medicine are used experimentally in the treatment of a lot of diseases such as ulcers, blood pressure, diabetes, and

[*] Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Iran.
[**]Department of Biology, Faculty of Sciences, University of Arak, Iran.

function of reproductive system (Ageel *et al*., 1994). In recent years, more attention has been paid to the study of the effect of various plants on the reproduction of laboratory mammals by which precious information has been acquired (Choudhary, 1966; Handelsman, 2000). The aim of the present work was to examine the effect of *Cannabis sativa* extract on sperm parameters, including sperm motility, count, and morphology, testosterone level, testis tissue and weight in male rats.

MATERIAL AND METHOD

Preparation of plant extract. *C. Sativa* was purchased from a traditional medicine center and identified and authenticated by a botanist. Extracting method was described previously (Khazaei & Salehi, 2006). In this method, *C. sativa* seeds (200 g) were powdered and added to 400 cc of 70% ethanol and were left to macerate at room temperature for 4 hours. Then, the soaked seeds were extracted by percolation method and the obtained extract was concentrated in the vacuum and was dried in the flat surface. The weight of the obtained extract was 8.5 g. The extract was dissolved in distilled water and was immediately administered interaperitoneally (IP) to rats, expressed as mg of extract per kg of body weight.

Animals. Twenty-eight Wistar male rats with weight of 210-240 g were used. Animals were kept in the temperature of 22±2ºC, under controlled environmental conditions, 12/12 h light/dark cycle and free access to water and food *ad libitum*. The rats were randomly assigned to four groups (n=7). The control group received distilled water and the experimental groups 1, 2, and 3 received *cannabis sativa* extract with doses 10, 50, and 100 mg/kg, respectively, for 14 consequent days.

The animals were weighed and anesthetized 24 hours after the last injection. Blood was taken from heart and preserved in the temperature of 37ºC for 30 minutes and was centrifuged (1000 *g*) for 15 minutes. Its serum was collected and preserved in -20ºC until measuring the testosterone hormone. Testosterone hormone measurement was performed by ELISA method. The testes were separated and weighed separately and their means were used. Then, testes were preserved in 10% neutral buffered formalin.

Cauda epididymis was separated and segmented in DMEM/F12 containing 5% FBS which had been balanced in the incubator previously; it was then put in incubator with the temperature of 37ºC and 5% CO_2. The prepared suspension was used for the analysis of sperm parameters including: motility, count and morphology. Sperm motility was divided into four levels (Mehrabi nasab *et al.*, 2010): (0): without motility, (I): minor *in situ* motility. (II): circumferential motility and (III): progressive motility.

To count the sperms, after putting the sperm suspension on Neubauer's chamber, the sperms on the four corners of the central square were counted. To examine sperm morphology, smear was prepared from the samples and was stained and investigated by Papanicohaou method.

After fixation of testes, tissue processing, including dehydration, clearing, and embedding were performed. Microscopic sections (5μm) were prepared and stained using H&E method. Twenty full linear sections were prepared from each tissue block and sections numbers 5, 10, 15, and 20 were selected and photographed separately from three random scopes. Diameter of seminiferous tubules was measured by Motic camera and software (Moticam 2000, Spain). The mean seminiferous tubule diameter in micrometers was determined for each testis.

The obtained data were analyzed by One Way ANOVA and $P<0.05$ was considered significant.

RESULTS

The effect of extract on body and testis weight- By increasing the dose of the extract, the final body weight decreased significantly ($P<0.05$) compared to control group. During the experiment, the rats receiving higher doses of extract indicated symptoms such as lack of appetite and motility and gradual weight loss (Table I and Fig. 1A). However, relative increase in body weight was observed in 10 mg/kg dose. Also, the means of testes weight in control and experimental groups revealed no significant differences (Table I).

Changes of serum testosterone hormone-Testosterone hormone did not show significant difference in control and experimental groups ($P>0.05$) (Table I).

Sperm count, motility, and morphology-Sperm count decreased significantly ($P<0.05$) in experimental groups in comparison with control group (Table I and Fig. 1B). Further, sperm progressive motility had a significant decrease in experimental groups 1, 2, and 3 compared to control group (Table I and Fig. 1C). Sperm morphology in control and experimental groups was similar and no noticeable differences were observed between groups.

Seminiferous tubules diameter decreased significantly ($P<0.05$) in experimental groups 1, 2, and 3 (Fig. 2) in comparison with control group (Table I, and Fig. 1D).

Table I. The results of body and testis weight, testosterone hormone, sperm count and motility, and seminiferous tubules diameter in control and experimental groups (results are indicated as mean ± SE).

	Control	10 mg/kg of extract	50 mg/kg of extract	100 mg/kg of extract
Final body weight (g)	237.14 ± 7.1	244.71 ± 7.2	218.14 ± 5.8*	215.71 ± 7.6*
Testis weight (g)	1.36 ± 0.05	1.29 ± 0.06	1.42 ± 0.03	1.38 ± 0.02
Testosterone (ng/ml)	2.10 ± 0.26	1.54 ± 0.07	1.81 ± 0.24	1.75 ± 0.20
High motility (%)	29.57 ± 4.5	12.43 ± 2.4*	9.14 ± 1.6*	6.14 ± 1.2*
Sperm count ($x10^{6}$)	68.43 ± 2.2	54.00 ± 4.4*	53.59 ± 2.3*	56.66 ± 1.6*
Diameter of ST (μm)	82.11 ± 0.43	79.42 ± 0. 34*	78.46 ± 0.28*	70.94 ± 0.38*

*=P-value < 0.05 was considered significant (One-Way ANOVA). ST= Seminiferous Tubules.

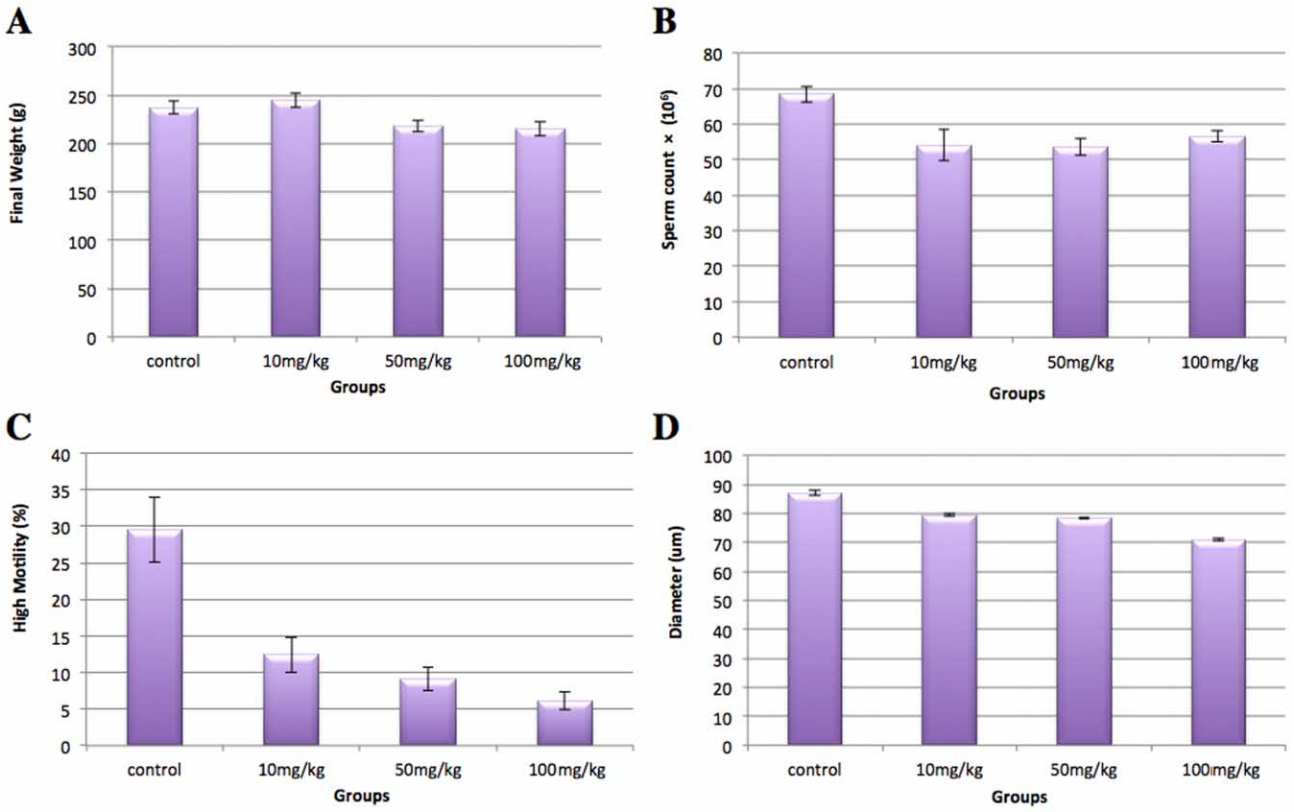

Fig. 1. Effect of *C. sativa* extract on: A) body weight in control and experimental groups. B) sperm count in control and experimental groups. C) sperm motility in control and experimental groups. D) seminiferous tubules diameter in control and experimental groups.

DISCUSSION

In the present study, *C. sativa* extract decreased body weight, sperm count and progressive motility, and seminiferous tubules diameter significantly, but it had no significant impact on testis weight and serum testosterone. Nowadays, plant extracts have been largely taken into consideration and their positive and negative effects on various organs and tissues of the body have been identified. One of the target tissues of plant extracts is the tissue of organs of reproduction system such as testis and sperm parameters. Sperm motility is introduced as an important factor in the success of natural and experimental fertilization. In fertile individuals, sperm motility levels especially progressive sperms is directly related to the ability of fertilization (Aitken *et al.*, 1989; Aitken 1995). *C. sativa* contains various active compounds named Cannabinoids and tetrahydrocannabinol (THC) has been identified as its main active molecule (Di Marzo *et al.*, 2005). Cannabinoid receptors (CBR) have been identified in male reproductive tract. Cannabinoid receptors (CBR) have been observed in testis, prostate, and Vas deferens, tract (Pertwee *et al.*, 2002; Gye *et al.*, 2005; Ricci *et al.*, 2007). Although there are few studies, which have investigated the

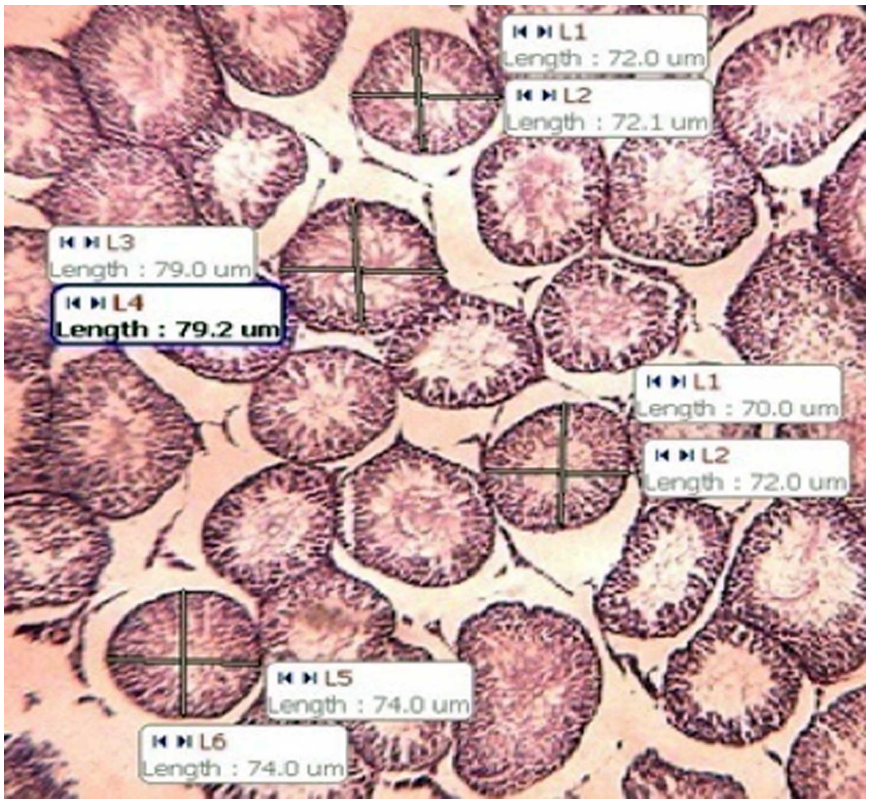

Fig. 2. Photomicrograph of testis tissue (10 mg/kg *C. sativa* extract).

effects of cannabinoids on sperm motility, it seems that activation of CB1 receptors inhibits sperm motility in vertebrates and invertebrates at least experimentally (Whan *et al.*, 2006). The available evidence regarding animal studies has shown that CB1 receptors in germ cells are expressed from spermatogony to mature sperm. Studies have shown the presence of this receptor in spermatogonia, primary spermatocytes, and sperm in rat's testes (Gye *et al.*; Cobellis *et al*., 2006).

Sustentocytes (Sertoli cells) are one of the major components of seminiferous tubules and their number is greatly linked to total sperm production. Sustentocytes encompass and take care of germ cells during their maturity period. Modulating the function of all hormone stimuli which regulate spermatogenesis process, Sustentocytes provide a completely regulated environment that result in the maturation of germ cells from spermatogony to mature sperm (Brinster 2007). Further, CB2 receptors are expressed in Sustentocytes in which they regulate apoptosis (Maccarrone *et al.*, 2003).

Cannabinoids have negative effects on sperm function including sperm motility. These inhibitory effects are moderated by the functioning of cannabinoids on sperm through activation of CB1 receptors which are expressed in mature sperm. Using experimental, pharmaceutical methods, the inhibitory functions of cannabinoids on sperm motility in mammals has been attributed to CB1 receptors (Rossato *et al*., 2005).

The findings obtained from examining the body weight of rats indicated a significant difference in the final weight of rats receiving doses 50 and 100 mg/kg *cannabis sativa* extract. During experiment, symptoms such as lack of appetite and motility was observed in experimental groups 2 and 3 that could be a reason for the decrease of the weight of above mentioned groups. This is indicative of the role of the major components of *Cannabis sativa* (i.e. cannabinoids) in appetite and weight regulation (Fu & Longhurst, 2009). Since, in this study, *Cannabis sativa* extract could decrease the quality of sperm parameters including sperm count and motility, the negative effects of *Cannabis sativa* on sperm parameters and fertility should be taken into account. Further studies are recommended in this regard.

ACKNOWLEDGEMENTS. We thank Mr. Faramarz Piri for helpful suggestions on the manuscript. This study was approved and financially supported by the Fertility and Infertility Research Center, Kermanshah University of Medical Sciences. There is no conflict of interest in this study.

LOTFI, N.; KHAZAEI, M.; LOTFI, N.; KHAZAEI, M.; SHARIATZADEH, S. M. A.; SOLEIMANI MEHRANJANI, M. & GHANBARI, A. Efecto del extracto hidroalcohólico de *Cannabis sativa* sobre los parámetros espermáticos e histología testicular en ratas. ***Int. J. Morphol., 31(1)***:82-86, 2013.

RESUMEN: La *Cannabis Sativa* es una hierba de múltiples usos en la medicina tradicional. Su extracto hidroalcohólico (10, 50, y 100 mg/kg) administrado intraperitonealmente durante 14 días consecutivos a ratas Wistar macho produjo una disminución significativa en la motilidad progresiva de los espermatozoides. El recuento de espermatozoides y el diámetro de los túbulos seminíferos se redujo significativamente en comparación con el grupo control. También se observó disminución del peso corporal de los animales en dosis de 50 y 100 mg/kg. Cambios en el peso de los testículos y la testosterona sérica no fueron significativos. El extracto de *Cannabis sativa* tiene un efecto negativo sobre los parámetros seminales tales como la motilidad, conteo espermático, y el diámetro de los túbulos seminíferos.

PALABRAS CLAVE: *Cannabis sativa*; Rata macho; Túbulos seminíferos; Parámetros espermáticos.

REFERENCES

Ageel, M. A.; Islam, M. W.; Ginawi, O. T. & Al-Yahia, M. A. Evaluation of the aphrodisiac activity of *Litsea chinensis* (Lauraceae) and *Orchis maculate* (Orchidaceae) extracts in rats. *Phytother. Res., 8(2)*:103-5, 1994.

Aitken, R. J. Free radicals, lipid peroxidation and sperm function. *Reprod. Fertil. Dev., 7(4)*:659-68, 1995.

Aitken, R. J.; Clarkson, J. S. & Fishel, S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biol. Reprod., 41(1)*:183-97, 1989.

Brinster, R. L. Male germline stem cells: from mice to men. *Science, 316(5823)*:404-5, 2007.

Choudhary, R. R. Plants with possible antifertility effect. ICMR. Spl. Report. Series. New Delhi: *ICMR, Special Report Series, 55*:1-6, 1966.

Cobellis, G.; Cacciola, G.; Scarpa, D.; Meccariello, R.; Chianese, R.; Franzoni, M. F.; *et al*. Endocannabinoid system in frog and rodent testis: type-1 cannabinoid receptor and fatty acid amide hydrolase activity in male germ cells. *Biol. Reprod., 75(1)*:82-9, 2006.

Di Marzo, V.; De Petrocellis, L. & Bisogno, T. The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handb. Exp. Pharmacol., (168)*:147-85, 2005.

ElSohly, M. A. *Chemical constituents of cannabis. In*: Grotenhermen, F. & Russo, E. (Eds.). Cannabis and Cannabinoids. Pharmacology, Toxicology and Therapeutic Potential. New York, Haworth Press, 2002.

Fu, L. W. & Longhurst, J. C. Electroacupuncture modulates vlPAG release of GABA through presynaptic cannabinoid CB1 receptors. *J. Appl. Physiol., 106(6)*:1800-9, 2009.

Gaoni, Y. & Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc., 86(8)*:1646-7, 1964.

Gye, M. C.; Kang, H. H. & Kang, H. J. Expression of cannabinoid receptor 1 in mouse testes. *Arch. Androl., 51(3)*:247-55, 2005.

Handelsman, D. J. Hormonal male contraception. *Int. J. Androl., 23(Suppl 2)*:8-12, 2000.

Khazaei, M. & Salehi, S. Protective effect of Falcaria vulgaris extract on ethanol- induced gastric ulcers in rat. *Iran J. Pharmacol. Ther., 5(1)*:1-4, 2006.

Kraft, B. & Kress, G. Cannabinoids in pain and palliative care medicine – hope or dope? *Memo Mag. Eur. Med. Oncol., 2(3)*:166-72, 2009.

Maccarrone, M.; Cecconi, S.; Rossi, G.; Battista, N.; Pauselli, R. & Finazzi-Agrò, A. Anandamide activity and degradation are regulated by early postnatal aging and follicle-stimulating hormone in mouse Sertoli cells. *Endocrinology, 144(1)*:20-8, 2003.

Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C. & Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature, 346(6284)*:561-4, 1990.

McPartland, J. Marijuana and medicine: the endocrine effects of cannabis. *Alternative Ther. Wom. Health, 1(6)*:41-4, 1999.

McPartland, J. M. & Pruitt, P. L. Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. *Altern. Ther. Health Med., 5(4)*:57-62, 1999.

Mehrabi nasab, E.; Khazaei, M. & Khazaei, S. The effect of pentylenetetrazol kindling induced epilepsy on hypogonad hormones and sperm parameters of rats. *Arak Medical University Journal, 12(4)*:105-12, 2010.

Munro, S.; Thomas, K. L. & Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature, 365(6441)*:61-5, 1993.

Pertwee, R. G.; Ross, R. A.; Craib, S. J. & Thomas, A. (-)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. *Eur. J. Pharmacol., 456(1-3)*:99-106, 2002.

Ricci, G.; Cacciola, G.; Altucci, L.; Meccariello, R.; Pierantoni, R.; Fasano, S. & Cobellis, G. Endocannabinoid control of sperm motility: The role of epididymus. *Gen. Comp. Endocrinol., 153(1-3)*:320-2, 2007.

Rossato, M.; Ion Popa, F.; Ferigo, M.; Clari, G. & Foresta, C. Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function. *J. Clin. Endocrinol. Metab., 90(2)*:984-91, 2005.

Small, E. & Cronquist A. A practical and natural taxonomy for *Cannabis. Taxon, 25(4)*:405-35, 1976.

The Angiosperm Phylogeny Group. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc., 141(4)*:399- 436, 2003.

Theimer, R. R. & *Mileken, H. Analysis of the oil from different hemp cultivars- perspective for economical utilization. In*: Bioresource Hemp. 2nd ed. Rosenstr, Nova-Institute, 1995.

Veldhuis, W. B.; van der Stelt, M.; Wadman, M. W.; van Zadelhoff, G.; Maccarrone, M.; Fezza, F.; *et al.* Neuroprotection by the endogenous cannabinoid anandamide and arvanil against *in vivo* excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. *J. Neurosci., 23(10)*:4127-33, 2003.

Whan, L. B.; West, M. C.; McClure, N. & Lewis, S. E. Effects of delta-9-tetrahydrocannabinol, the primary psychoactive cannabinoid in marijuana, on human sperm function *in vitro*. *Fertil. Steril., 85(3)*:653-60, 2006.

Correspondence to:
Mozafar Khazaei, Ph.D.
Fertility and Infertility Research Center
Medical Faculty
University Avenue
Shahid Shiroodi Boulevard, Postal Code: 6714869914,
Kermanshah
IRAN

Tel: 0098-9183360835 Fax: 0098-8314271563

Email: mkhazaei1345@yahoo.com

Received: 25-03-2012
Accepted: 25-09-2012