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Reproductive Parameters in Diabetic Male Rat after Exposure to Cannabis Sativa Hydroalcoholic Extract

Nahid Lotfi[a], Mozafar Khazaei[b*], Sayyed Mohammad Ali Shariatzadeh[c], Malek Solmani Mehranjani[c], Faramarz Piri[d], Atefe Ansarian[e]

[a]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b]Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[c]Department of Biology, Faculty of Sciences, University of Arak, Arak, Iran
[d]Kermanshah branch, Islamic Azad University, Kermanshah, Iran
[e]Department of Cardiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

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A B S T R A C T

Diabetes is a metabolic disorder inducing undesirable effects on male reproductive system. Cannabis sativa (C. sativa) is a plant that affects diabetes and decreases blood glucose. The present study was aimed to investigate the effect of Cannabis sativa hydroalcoholic extract on sperm parameters in diabetic rats. Thirty five male Wistar rats (210-240 gr) were divided into five groups: control (distilled water) and four diabetic (60 mg/kg STZ) groups. Diabetic groups were assigned to one diabetic and three experimental groups (receiving 10, 50, and 100 mg/kg extract). Extract was administered interaperitonealy (IP) for 14 days. The animals were weighed and sacrificed at the end of the study. Blood sample was taken and serum testosterone was measured via ELISA method. Also, testes were weighed and sperms were collected from cauda epididymis and analyzed in terms of count, motility and morphology. Histological slides were prepared from testis and seminiferous tubules diameter was determined using Motic camera and software. Data were analyzed using One-way ANOVA method and $P<0.05$ was considered statistically significant. The mean of blood glucose in experimental groups indicated a significant decrease compared to diabetic group (P=0.00). The mean of sperms count and motility, percentage of sperms with normal morphology, testosterone hormone level, seminiferous tubules diameter, and final body weight in diabetic and experimental groups decreased significantly compared to control group (P=0.00). Cannabis sativa hydroalcoholic extract can induce a moderating effect on hyperglasemia resulting from diabetes, but has no ameliorating effect on diabetic reproductive dysfunctions.

***Corresponding Author: Mozafar Khazaei**, **E-mail:** mkhazaei1345@yahoo.com

Introduction

Diabetes mellitus (DM) is an essential and chronic endocrine disorder which is induced as a result of defects in insulin production [1]. Lack of insulin leads to chronic hyperglasemia and metabolic dysfunctions of carbohydrates, lipids and proteins [2]. Diabetes affects virtually all body systems [3]. It has various functional and structural effects on male reproductive system. Decrease in testosterone production and sexual functions [4], and decrease of seminiferous tubules diameter have been reported in diabetic patients [5].

Diabetes also influences spermatogenesis. Low quality of semen, including decreasing sperm count [6, 7] and motility, and increasing abnormal sperms [8, 9] has been reported in diabetic patients. Also, body weight loss and epididymis shrinkage that result in decrease of head, body, and cauda epididymis weight have been reported in diabetic rats [10]. Further, significant increase in blood glucose, decrease in serum levels of LH, FSH and testosterone, number of spermatogonia cells, and seminiferous tubules diameter in the infants of diabetic women have been reported [11].

In most of the developed countries, medicinal plants and traditional medicine are used experimentally for the treatment of many diseases such as ulcers, hypertension, diabetes, and reproductive system dysfunctions. World Health Organization (WHO) has also emphasized the development of plant antidiabetic drugs.

Cannabis sativa is a plant with commercial, pharmaceutical, and medicinal uses [12]. This plant belongs to the family of Cannabaceae, Rosales order [13]. *Cannabis* has been used to reduce chemotherapy related nausea and vomiting, and to treat pain and muscle spasticity [14].

The active components of *Cannabis*, Δ-9 tetra-hydrocannabinol and cannabidiol, and other derivatives named cannabinoids have been identified and specified, and it is evident that cannabinoids possess remarkable therapeutic capability [15, 16]. Cannabinoids can serve as appetite stimulants, antiemetics, antispasmodics, and have some analgesic effects [17], Laboratory studies have showed that cannabis and cannabinoids have anticancer effects, although there has been little research in human cancer treatment [18].

Some studies indicate that cannabinoids may moderate diabetes progress and consequently ameliorate the disease in diabetic patient [19, 20]. We studied Cannabis effect on some reproductive parameters in normal male rat previously [21]. Since the effect of cannabis sativa on reproductive dysfunctions resulting from diabetes has not been investigated so far, the present paper was aimed to investigate the effect of Cannabis hydro-alcoholic extract on serum testosterone, sperm parameters (count, motility, and morphology), seminiferous tubules diameter, testis weight, and body weight.

Materials and Methods

Animals

Thirty five male Wistar rats (210-240 gr) were purchased from Pasture Institute and were kept in the temperature of 22 ± 2ºC, under controlled environmental conditions, 12/12 hr light-dark cycle with free access to water and food ad libitum for the period of one week before the start of the experiment so that the animals could be adapted to the environment. The ethical committee of the university approved animal experiments in this study.

Preparation of extract

The seeds of *Cannabis sativa* were prepared from a traditional medicine center and were identified and approved by a botanist. Extracting was performed by percolation method. *C. sativa* seeds (200 gr) were powdered and soaked in 400 cc ethanol 70% for four hours. It was then extracted, concentrated in the vacuum and left to dry on the flat surface [22].

Induction of diabetes

To induce type I diabetes, streptozotocin (STZ) (60 mg/kg) (USA Sigma co.) dissolved in buffer citrate (0.1 M, pH=4.5) was administered (IP) in single dose. One week after STZ administration, blood glucose was determined by glucometer and glucose level over 200 mg/dL was considered

diabetic [21]. Then, diabetic and control rats were weighed.

Experimental design

The rats were divided into five groups: one control and four diabetic. Diabetic rats were randomly assigned to one diabetic and three experimental groups (n=7) as it follows: Control (A) and diabetic groups (B) received distilled water and experimental groups received 10 (C1), 50 (C2) and 100 (C3) mg /kg extract. The extract was dissolved in distilled water and administered (IP) to rats daily with specified doses for two weeks. The rats were weighed (secondary weight) and anesthetized 24 hours after the last injection. Blood was taken from the heart and its serum was separated by centrifuge. The serum samples were kept in -20°c. Testosterone hormone level was determined by ELISA method (Monobind kite, Italy).

Semen analysis

Cauda Epididymis was separated and cut in DMEM/F12 containing 5% FBS and was put in incubator (37ºc and 5% CO_2) for thirty minutes. The prepared suspension was used for the analysis of sperm parameters, including motility, count, and morphology. Sperm motility was divided into four levels [21, 24]: 0: immotile, I: limited in situ motility, II: slow motility and III: progressive (fast) motility. Neubauer slide was used for counting sperms. Smear was prepared from the samples to examine sperm morphology and was stained and analyzed by Papanicolaou method.

Tissue analysis

Testis were extracted and weighed, and after tissue preparation (fixation, dehydration, clearing, and embedding), histological slides were prepared and H & E staining was performed. According to our previous work, ten slides were selected from each sample and five images were taken from each slide by Motic camera, and seminiferous tubules diameter was determined by special software (Motic, Spain) [21].

Statistical analysis

The results were presented as Mean± SE and data were analyzed by One-way ANOVA and Tukey test using SPSS software (version 16). $P<0.05$ was considered statistically significant.

Results and Discussion

Body weight

The mean weight of diabetic rats one week after STZ administration indicated a significant decrease in comparison with control group (**P=0.00). Also, the mean of final weight in experimental groups (10, 50, and 100 mg/kg extract) revealed a significant decrease compared to control group (**P=0.00) (Table 1). The final weight of rats receiving extract decreased in comparison with diabetic group, but it was not statistically significant.

Table 1. Mean of body weight (gr) for control, diabetic and diabetic treated groups

Groups	Primary weight (gr)	Weight after STZ injection (gr)	Final weight (gr)
Control	220.86±5.6	-	237.14±7.1
Diabetic	229.57±3.7	206.57±6.9	183.86±6.8
Diabetic + extract (10 mg/kg)	234.14±2.2	213.71±4.2	180.71±9.4
Diabetic + extract (50 mg/kg)	223.14±6.7	188.14±3.0	160.43±9.0
Diabetic + extract (100mg/kg)	230.71±5.3	177.71±6.5	176.71±5.3

Significant weight decrease were seen after STZ administration in diabetic and diabetic treated groups (**P=0.00). Results are presented as mean± SE. The final weight of rats receiving extract decreased none significantly.

Blood glucose

The mean of blood glucose in diabetic group increased significantly compared to control and extract groups at the end of the study (P=0.00). The mean of blood glucose in the rats receiving

extract (10, 50, and 100 mg/kg) decreased significantly compared to diabetic group (P=0.00) (Table 2). It seems that 50 mg/kg is the most effective dose in decreasing blood glucose in STZ-induced diabetic rats.

Table 2. Blood glucose level in Control, Diabetic and Extracts groups

Groups	primary blood glucose (ng/dl)	secondary blood glucose (ng/dl)
Control	104.00±2.8	117.86±5.6
Diabetic	382.57±41.9	517.71±78.4
Diabetic + extract (10 mg/kg)	296.57±31.9	277.43±61.5
Diabetic + extract (50 mg/kg)	309.17±28.5	118.33±18.1
Diabetic + extract (100 mg/kg)	340.17±30.9	178.67±28.3

Results are presented as mean± SE. Blood glucose increased significantly one week after STZ administration and decreased significantly in treated groups (P=0.00).

Testis weight

The mean of testes weight in diabetic and experimental groups decreased in comparison with control group, but it was not statistically significant (P=0.25).

Seminiferous tubules diameter

Seminiferous tubules diameter in diabetic and experimental groups indicated a significant decrease compared to control group (P=0.00) (Table 3).

Serum testosterone level

The mean of testosterone level in diabetic and experimental groups decreased significantly in comparison with control group (P=0.00) (Table 3).

Table 3. Seminiferous Tubules Diameter and Testosterone Hormone in different groups.

Groups	Testis weight (gr)	Seminiferous tubule diameter (µm)	Testosterone hormone level (ng/ml)
Control	1.36±0.05	87.12±0.89	2.1±0.26
Diabetic	1.22±0.05	58.96±0.69	1.18±0.11
Diabetic + extract (10 mg/kg)	1.23±0.07	60.87±0.64	1.24±0.10
Diabetic + extract (50 mg/kg)	1.15±0.04	65.31±0.54	1.28±0.18
Diabetic + extract (100 mg/kg)	1.18±0.05	61.35±0.61	1.27±0.16

Results are presented as mean±SE. The testes weight decreased in diabetic and experimental groups significantly (P=0.25). Diameter of seminiferous tubules and testosterone level decreased significantly in diabetic and experimental groups in comparison with control group (P=0.00).

Sperm parameters

The mean of sperm count in diabetic and experimental groups decreased significantly in comparison with control group (P=0.00) (Table 4). Analysis of sperm motility indicated that percentage of progressive motile sperms (fast) in diabetic and experimental groups decreased significantly compared to control group (P=0.00). The percentage of sperms with normal morphology in diabetic and experimental groups revealed a significant decrease in comparison with control group (P=0.05) (Table 4). Sperm abnormal morphology included twisted cauda sperm, round cauda sperm, and headless sperm.

The findings of the present study reveal that *Cannabis* hydroalcoholic extract can decrease blood glucose resulting from diabetes. Analysis of the effect of extract on the groups receiving the extract indicated a decrease in testis weight, seminiferous tubules diameter, serum testosterone level, sperm count, sperm progressive motility, and percentage of normal sperms compared to control group. These, in turn, show that although *C. sativa* extract could

Table 4. Sperm parameters (Count and Motility).

Groups	Sperm count ($X10^6$)	Immotile sperms (%)	Sperm progressive motility (%)	Sperm abnormal morphology (%)
Control	68.71±2.2	19.28±3.2	29.57±4.5	19.38±3.4
Diabetic	27.71±1.5	38.00±2.1	5.71±1.9	47.86±7.2
Diabetic + extract (10 mg/kg)	35.95±6.3	55.86±8.7	4.43±1.7	38.17±2.7
Diabetic + extract (50 mg/kg)	36.06±8.7	61.00±8.3	2.33±2.0	42.93±4.8
Diabetic + extract (100 mg/kg)	30.09±8.9	57.67±6.7	1.33±0.57	57.58±4.9

Count, percentage of progressive motion (fast) and normal morphology of sperms decreased significantly in diabetic and experimental groups (P=0.00).

decrease blood glucose in diabetic rats, it has had no ameliorating effect on sperm parameters. Some finding of present study is same as our previous work on normal (non diabetic) rat which *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 [21].

STZ can quickly destroy pancreas beta cells which results in prevention of insulin production and its consequent dysfunctions [25]. Partial or total lack of insulin in diabetic people and experimental animals leads to hyperglasemia inducing malfunctions in all organs, including reproductive organs [26, 27]. *C. sativa* extract significantly decreased blood glucose in diabetic groups receiving extract in comparison with diabetic group which is indicative of the therapeutic effect of this extract on diabetic hyperglasemia. Administration of 5 mg cannabidiol (CBD), one of the most important non-psychoactive compounds of *C. sativa*, per day significantly decreases diabetes in mice [28]. Also in the study by Weiss et al., it was indicated that CBD can be effective in curing and preventing diabetes type I [29].

Weight loss may be another reason for decreasing blood glucose in diabetic rats receiving extract. The results of the comparison of final body weight in diabetic group receiving extract and control group indicates that extract has decreased the weight of the aforementioned groups. During the experiments, symptoms such as lack of appetite and immobility were observed in diabetic and diabetic receiving the extract groups. Appetite decline can be due to weight loss. This manifests the role of main compounds of cannabis sativa, namely cannabinoids, on appetite and weight regulation [30].

Sperm count and progressive motility in diabetic group dimensioned compared to control group that is similar to the results of other researchers [31, 32]. These findings are compatible with previous results about the effect of diabetes on decreasing testis weight, seminiferous tubules diameter, and serum testosterone in diabetic rats in comparison with control group [33]. Also, our findings are in line with the findings of studies that indicate high doses STZ-induced diabetes in the testis of male rat results in testosterone decrease which shows functional decrease in leydig and sertoli cells probably created due to insulin secretion drop.

Changes of STZ-induced diabetes in count and function of leydig cells can partly be explained by LH controlling leydig cells. Accordingly, researchers have concluded that in insulin-dependent diabetes: 1. leydig cells function and testosterone production decrease owing to lack of insulin stimulation impact on these cells, 2. insulin-dependent decrease is present in FSH which, in turn, decreases LH, 3. sperm production level and reproduction decreases due to FSH decline that is the result of insulin decline[27]. Diabetes induces changes in testis tissue through apoptosis, seminiferous tubules atrophy, tubule

diameter decrease, and spermatogenic cells decrease [34]. (Fig 1)

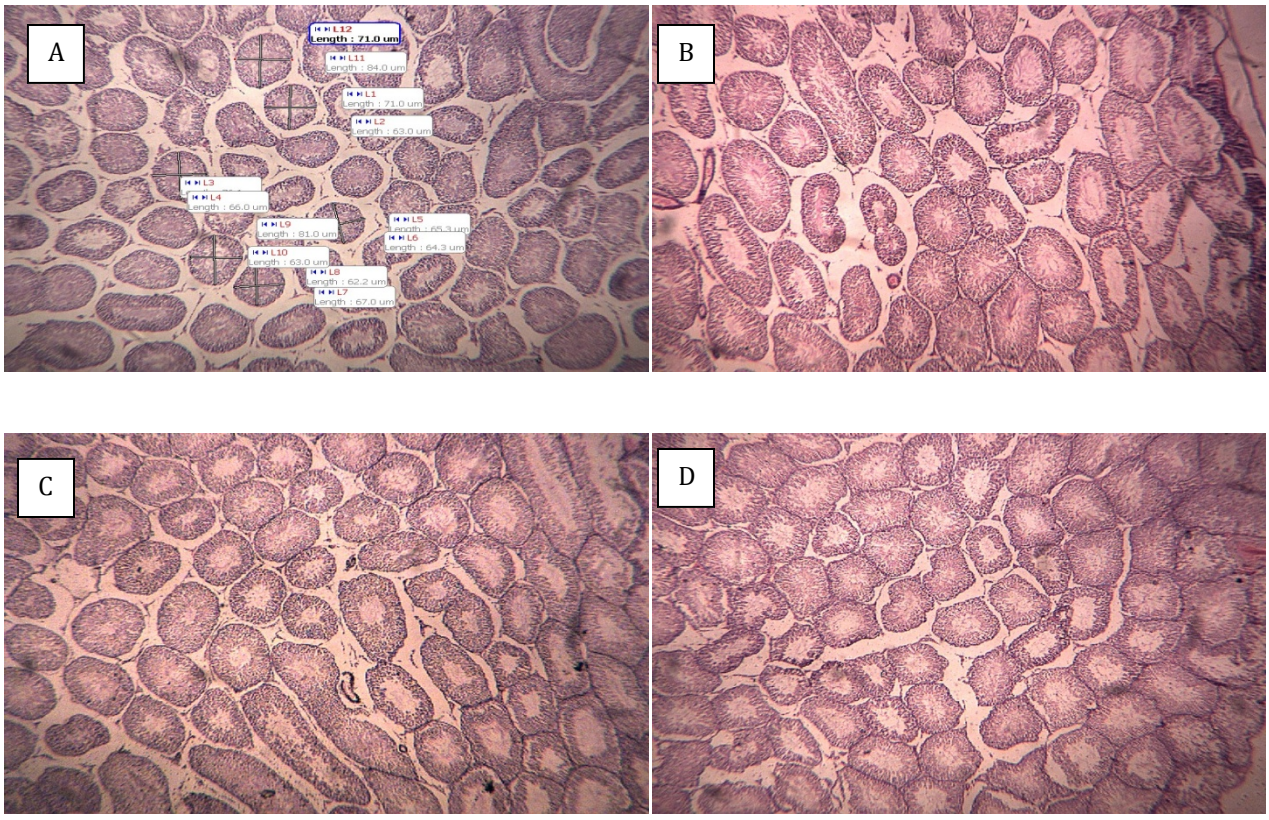

Fig. 1. Seminiferous tubules and calculation of diameter in control (A), 10 (B), 50 (C), 100 (D) mg/kg extract. (X10)

C. sativa contains a number of active compounds called cannabinoids. Cannabinoid receptors (CBR) are identified along male reproductive tract. They have been observed in testis, prostate, and vas deferens [35, 36]. In addition, CB1 receptors are found in the sperm of different vertebrate and invertebrate species. The data reported so far indicates that cannabinoids control male reproductive functions directly and indirectly [37].

It has been reported that cannabinoids decrease plasma level of LH which results in plasma testosterone decrease, although contradictory results have been reported in this regard [38]. Evidence regarding animal studies has revealed that CB1 receptors in germ cells are translated from spermatogonia to mature sperm. For instance, Gye et al. showed the presence of this receptor in spermatogonia, primary spermatocytes, and mouse sperm [35]. Previous research suggests that cannabinoids decrease germ cells proliferation and weight of reproductive organs [39]. Sertoli cells encompass and preserve germ cells during maturation period. CB2 receptors are described in sertoli cells in which they regulate apoptosis [40]. Further, it has been reported that CB1 receptors are translated in leydig cells and decrease testosterone production and consequently prevent spermatogenesis while activated [35].

Conclusion

Cannabis sativa hydroalcoholic extract can induce a moderating effect on hyperglasemia resulting from diabetes, but has no ameliorating effect on diabetic male reproductive dysfunctions.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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References

[1] Tharkar S, Devarajan A, Kumpatla S, Viswanathan V. The socioeconomics of diabetes from a developing country: a population based cost of illness study. Diabetes Res Clin Pract. 2010;89: 334-340.

[2] Davis SN. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. in: Goodman and Gilmans. The pharmacological basis of therapeutics. Brunton, L.L. (Ed.). New York: McGraw-Hill; 2006.

[3] Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Cur Diabetes Rev. 2008;4:46-54.

[4] Soudamani S, Yuvaraj S, Rengarajan S, Sivakumar R, Malini T, Balasubramanian K. Effects of streptozotocin diabetes and insulin replacement on androgen and estrogen receptor concentrations in the epididymis of Wistar rats. JER. 2006;10:59-61.

[5] Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S, Uysal N. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur Surg Res. 2008;40:354-360.

[6] Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG. Sexual behavior, sperm quantity and quality after short-term streptozotocin-induced hyperglycemia in rats. Int J Androl. 2006;29:482-488.

[7] Kim ST, Moley KH. Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. Reproduction. 2008;136:313-322.

[8] Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F et al. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod 2002;17:2673-2677.

[9] Delfino M, Imbrogno N, Elia J, Capogreco F, Mazzilli F. Prevalence of diabetes mellitus in male partners of infertile couples. Minerva Urol Nefrol 2007;59:131-135.

[10] Soudamani S, Malini T, Balasubramanian K. Effects of streptozotocin-diabetes and insulin replacement on the epididymis of prepubertal rats: histological and histomorphometric studies. Endocr Res 2005;31:81-98.

[11] Jelodar G, Khaksar Z, Pourahmadi M. Endocrine profile and testicular histomorphometric in adult rat off spring of diabetic mothers. J Physiol Sci 2009; 59:377-382.

[12] Veldhuis WB, Van der Stelt M, Wadman WM. Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. J Neuroscience 2003;15:4127-4131.

[13] The Angiosperm Phylogeny Group. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGII. Bot J Linn Soc 2003;141:399-436.

[14] Borgelt LM, Franson KL, Nussbaum AM, Wang GS. The pharmacologic and clinical effects of medical cannabis. Pharmacotherapy 2013;33:195–209.

[15] Mechoulam R. The pharmacohistory of Cannabis sativa. In: Mechoulam R, (ed). Cannabinoids as therapeutic agents. Boca Raton (FL): CRC Press. 1986;1-19.

[16] Beaulieu P, Ware M. Reassessment of the role of cannabinoids in the management of pain. Curr Opin Anaesthesiol. 2007;20:473-477.

[17] Ben Amar M. Cannabinoids in medicine: a review of their therapeutic potential. Journal of Ethnopharmacology 2006;105:1–25

[18] Cannabis (marihuana, marijuana) and the cannabinoids". Health Canada. February 2013.

[19] Lu D, Vemuri VK, Duclos RI Jr, Makriyannis A. The cannabinergic system as a target for anti-inflammatory therapies. Current Topics in Medicinal Chemistry 2006;13:1401-1426.

[20] Croxford JL, Yamamura T. Cannabinoids and the immune system: Potential for the treatment of inflammatory diseases. J Neuroimmunol. 2005;166: 3-18.

[21] Lotfi N, Khazaei M, Shariatzadeh SMA, Soleimani Mehranjani M, Ghanbari A. The effect of Cannabis sativa hydroalcoholic extract on sperm parameters and testis histology in rats. Int J Morph 2013;31:82-88.

[22] Khazaei M, Salehi H. Protective effect of Falcaria vulgaris extract on Ethanol- induced gastric ulcers in rat. IJPT. 2006;5:1-4.
[23] Nouri M, Khaki A, Fathi Azar F, Rashidi MR. The protective effects of carrot seed extract on spermatogenesis and cauda epididymal sperm reserves in gentamicin treated rats. Yakhteh Medical Journal 2009;3:327-333.
[24] Ghanbari M, Mortazavi SB, Khavanin A, Khazaei M. The effects of cell phone waves (900 MHz-GSM band) on sperm parameters and total antioxidant capacity in rats. Int J Fertil Steril. 2013;7:21-28.
[25] Szkudelski T. The mechanism of alloxan and stereptozotocin action in B cells of the rat pancreas. Physiology Res. 2001;50:536-546.
[26] Agbaje IM, Rogers DA, McVicar CM, McClure N, Atkinson AB, Mallidis C, Lewis SE. Insulin dependent diabetes mellitus: implications for male reproductive function. Hum Reprod. 2007;22:1871-1877.
[27] Ballester J, Munoz MC, Dominguez J, Rigau T, Guinovart JJ, Rodriguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH and LH-linked mechanisms. J Androl. 2004;25:706-719.
[28] Weiss L, Zeira M, Reich S, Har-Noy M, Mechoulam R, Slavin S, Gallily R. Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. Autoimmunity 2006;39:143-151.
[29] Weiss L, Zeira M, Reich S, Slavin S, Raz I, Mechoulam R, Gallily R. Cannabidiol arrests onset of autoimmune diabetes in NOD mice. Neuropharmacology. 2008;54:244-249.
[30] Fu L, Longhurst JC. Electroacupuncture modulates vlPAG release of GABA through presynaptic cannabinoid CB1 receptors. J Appl physiol 2009;106:1800-1809.
[31] Shrilatha B, Muralidhara. Occurrence of oxidative impairments, response of antioxidant defenses and associated biochemical perturbations in male reproductive milieu in the streptozotocin-diabetic rat. Int J Androl 2007;30:508-518.
[32] Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: A prospective study. Fertil Steril. 2002;78:491-499.
[33] Navarro-Casado L, Juncos-Tobarra MA, Chafer-Rudilla M, Iniguez de Onzono L, Blazquez- Cabrera JA, Miralles- Garcia JM. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. J Androl 2010;31:584-592.
[34] Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S, Uysal N. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur Surg Res 2008;40:354-360.
[35] Gye MC, Kang HH, Kang HJ. Expression of cannabinoid receptor 1 in mouse testes. Arch Androl. 2005;51:247-255.
[36] 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. 2007;153:320-322.
[37] 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. 2005;90:984-991.
[38] Park B, Mc Partland JM, Glass M. Cannabis, cannabinoids and reproduction. Prostaglandins Leukot Essent Fatty Acids. 2004;70:189-197.
[39] Brinster RL. Male germ line stem cells: from mice to men. Science. 2007;316:404-405.
[40] Maccarrone M, Cecconi S, Rossi G, Battista N, Pauselli R, Finazzi-Agro A. Anandamide activity and degradation are regulated by early postnatal aging and follicle-stimulating hormone in mouse sertoli cells. Endocrinol. 2003;144:20-28.

