Ameliorative Effect of *Taraxacum officinale* Leaves on Hormonal Status of Hyperglycemic Rats

Reham, A. Mostafa[1], Doaa R. Negm[1], Eslam A. Header[2], Wafaa F. Abusudah[1], Wedad F. Azhar[1]

[1]Assistant Professor in Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al Qura University, Makkah, Kingdom of Saudi Arabia, [2]Professor in Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al Qura University, Makkah, Kingdom of Saudi Arabia & Department of Nutrition and Food Science, Faculty of Home Economics, Menofia University, Egypt

Abstract

Acute or chronic side effects of hyperglycemia may affect all systems and organs, such as sexual glands. Approximately 90 percent of diabetic patients suffer from sexual dysfunction, including libido and fertility. This study is designed to investigate the effect of diabetes on the sex hormones levels ad to evaluate the ameliorative effect of dandelion leaves powder (DLP) on diabetes-induced changes of gonadal hormones and glucose serum levels in male rats. Experimental diabetes mellitus was induced by subcutaneous injection with 65mg streptozotocin STZ/kg body weight. DLP was given in pelleted food at ratio of 5% for 28 days. DLP was effective in lowering Follicle-stimulating hormone (FSH) serum level in dandelion-treated rats compared to controls in diabetic group. DLP was also able to reverse the progesterone lowering effect of diabetes. In all of the classes, the estrogen level did not show any noticeable difference. It is suggested that DLP can have a regulatory effect in male rats on diabetes-induced changes in gonadal hormone levels, especially progesterone. Our research has shown that long-term administration of DLP in the diabetes experimental model could be used as a good candidate for reproductive hormone deficiency therapy.

Keywords: *Sex hormone - Dandelion (Taraxacum official) - Diabetes-Glucose - Rats.*

Introduction

Diabetes mellitus (DM) is metabolic disorder currently affecting over 350 million peoples worldwide. It costs about 1,200 billion USD to diagnose and treatments. The left untreated, DM can lead to a variety of diseases and long-term complications that lead to death afterwards.[1] As well as, heart disease, stroke, renal failure, foot ulcers and eye damage may be severe long-term complications caused by DM.[2] DM is either because the pancreas does not produce enough insulin, or the body's cells do not respond correctly to the insulin released.[3] DM has become the third greatest "killer" after cancer and cardio-/cerebro-vascular diseases.[4] It is estimated that 5% of the world's deaths are due to diabetes, a figure that will grow by 50 percent in the next 10 years.[5] Increasing evidence shows that the excess generation of reactive oxygen species (ROS) in diabetes causing oxidative stress may contribute entirely or partially to the development of complications in a variety of tissues.[6,7]

Hyperglycemia caused by diabetes mellitus induces acute or chronic side effects that can affect all processes and organs, such as sexual glands.[8] Around 90% of diabetic patients suffer from deficiency in sexual function including libido and fertility.[9] In experimental model, DM induced reproductive dysfunction, but did not compromise the capacity of sperm fertilisation in the cauda epididymis.[10] In addition, DM is accompanied by hyperglycemia and hyperlipidemia. In hyperglycemia and hypercholesterolemia, one of the problem mechanisms for DM is oxidative stress that has consequences that contribute to destruction and dysfunction.[11]

As DM regulation without side effects is a challenge, plant-based drugs may play an important role in DM treatment.[12] For the prevention and treatment of different diseases/pathologies, including cancers, heart disease, diabetes mellitus and high blood pressure, natural products isolated from medicinal plant sources have been used.[13,14] More than 800 species have

been investigated and their hypoglycemic effects were reported.[15]

Experimental studies express evidences about the effect of Dandelion *(Taraxacum officinale)* leaves powder on the gonadal hormones and sexual behaviors. Studies show that some increases in hypophysial gonadotropins are caused by DLP, followed by an increase in sperm in male rats and foliclagenesis in immature female rats. [16] Therefore, the present study aimed to evaluate the effect of DLM on hormonal status for hyperglycemic rats.

Materials and Method

Material

Dandelion Leaves: dandelion leaves was provided from local marketand was identified by Institute of food technology, Giza, Egypt. All chemicals used in this experiment were of analytical grade. Biodiagnostic Co., Dokki, Giza, Egypt, has purchased kits used to quantitatively evaluate the various parameters.

Experimental Animals: A total of thirty two adult male Albino rats of Sprague Dawley strain weighing (200±10g) obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The animals were kept under observation for 1 week before experiment and fed on standard diet according to **Reeves *et al.*,(1993)**[17], and water *ad libitum.*

Method

Preparation of dandelion Powder: Dandelion were washed with running tap water and dried. The dried plant was ground into fine powder.

Chemical composition of dandelion: Dandelion was chemically analyzed to determine its protein, fat, carbohydrate, fiber and ash content according to **A.O.A.C. (2005).**[18]

Diabetes Mellitus Induction (DM): Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal (i.p.) Streptocytocin (STZ) injection (65 mg/kg b.w.).[17] The rats were given 5% glucose solution in feeding bottles for the next 24 h to prevent hypoglycemia after STZ injection. After 72 hours of the injection, Using a retro-orbital approach to estimate fasting serum glucose, fasting blood samples were collected. Rats were considered diabetic with fasting serum glucose of more than 180 mg/dL and were used for the experiment.**[19]**

Experimental Design: After 1 week, The rats were divided into four equal groups in each group (8 rats) as follows: the first group was fed with a basal diet and kept negative as a control, The 2nd group was left as a control positive and fed on basal diet + DLP (the non-diabetic dandelion-treated group), the 3rd group (diabetic rats) was fed on basal diet only, the 4th group was (diabetic group treated with DLP) fed on basal diet + DLP for 4 weeks.

Hormonal Assay: To assess the sexual hormone levels, blood samples from all rats were collected from their hearts at the end of the experimental period. The serum levels of estrogen, progesterone, FSH, LH and testosterone were determined by radioimmuno-method according to the procedures of **AOAC (2003)** provided in the kits. In addition, the serum levels of glucose was also measured in all groups according to the procedures provided in kits.[20]

Histopathological Examination: In 10 percent neutral buffered formal in, testis specimens were mounted. The set tissues were then trimmed, washed with ice saline and dehydrated in ascending grades of isopropyl alcohol and cleared in xylene. Using the same grade wax, the wax impregnated tissues were embedded in paraffin blocks and the paraffin blocks were cut with a 3-5µ thickness of rotary microtome. The pieces were floating at 40 ° C on a tissue float bath and taken on glass slides. The sections were then melted in an incubator at 60°C and after 5 min. they were allowed to cool and stained with Hematoxylen and Eosin according to **Bancroft and Cook, (1998),**[21] and examined microscopically.

Statistical Analysis: Statistical analysis of the findings was carried out using statistical package for social science (SPSS), version 20. The results were expressed as mean ± standard deviation (SD).One way analysis of variances (ANOVA) were used to compare the parameters between controls positive group and diabetic rats groups. A *P*-value less than 0.05 was considered statistically significant.

Results

Chemical analysis of dandelion/100 g: Data in table (1) showed the chemical analysis of dry dandelion powder (g/100g), protein (18.19%), fat (1.0%), ash (20.46%), fiber (10.86%), carbohydrates (48.00%), and total phenols (2.49%).

Table 1. Chemical composition of dry powder Dandelion (g/100g).

Components	Protein	Fat	Ash	Fiber	Total Carbohydrate	Total Phenols
Value %	18.19	1.0	20.46	10.86	48.00	2.49

Effect of sex hormones: A comparison of sexual hormone levels between control and dandelion -treated group in animal samples indicated that there was no significant difference in the estrogen level between treated and non-treated groups (table 2). In the non-diabetic dandelion -treated group, serum levels of progesterone, testosterone and LH have significantly increased compared to the control group (as it is shown in table 2). On the other hand, in the dandelion - treated group, serum level of FSH has been significantly decreased. Statistical analysis of sexual hormone levels in diabetic animals indicated that there was no significant difference in the estrogen level between treated and non-treated diabetic groups (table 2). Furthermore, in dandelion -treated diabetic group, compared to diabetic group, serum levels of progesterone, testosterone and LH have significantly increased. On the other hand, in dandelion -treated group compared to diabetic group, serum level of FSH has been significantly decreased.

Data also showed that the serum levels of gonadal hormones in control and diabetic control groups were totally different. In this regard, progesterone (table 2) and FSH levels in diabetic groups compared to control group were significantly decreased ($P<0.05$). However, testosterone level in diabetic group showed significant increment. In addition, the level of LH in the diabetic group was markedly higher than that of the control group. There was no significant difference in the estrogen level between control and diabetic groups.

Table 2. Serum FSH, LH, testosterone, estrogen and progesterone levels of control, diabetic, and DLP–treated rats.

Groups	FSH (mlu/ml)	LH (mlu/ml)	Testosterone (ng/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)
Control	0.32 ± 0.001^{a}	0.20 ± 0.001^{d}	1.21 ± 0.01^{d}	68.12 ± 4.01^{a}	7.02 ± 1.25^{b}
Control + DLP 5%	0.25 ± 0.003^{bc}	0.57 ± 0.003^{a}	1.75 ± 0.04^{c}	67.54 ± 3.17^{a}	8.16 ± 2.65^{a}
Diabetes	0.29 ± 0.013^{b}	0.41 ± 0.002^{c}	2.83 ± 0.07^{b}	66.24 ± 2.49^{c}	3.04 ± 0.98^{d}
Diabetes + DLP 5%	0.17 ± 0.001^{d}	0.54 ± 0.006^{ab}	3.74 ± 0.09^{a}	66.71 ± 4.65^{cd}	4.17 ± 1.04^{c}

Values are expressed as mean ± SD.A P-value <0.05 was reflected significant. Values which don't share the same letter in each column are significantly different. FSH: Follicle-stimulating hormone. LH: Luteinizing hormone. DLP: DLP.

Effect on serum glucose level: Untreated and dandelion -treated diabetic rats had elevated serum glucose level over those of control rats. However, in both control and diabetic groups, dandelion did not induce any significant change in serum glucose levels (Table 4).

Table 3. Serum Glucose (mg/dl) levels of control, diabetic, and DLP – treated rats.

Groups	Control	Control + DLP 5%	Diabetes	Diabetes + DLP 5%
Glucose	128.6 ± 25.6^{c}	123.4 ± 12.1^{dc}	381.6 ± 39.2^{a}	344.8 ± 63.4^{ab}

Values are expressed as mean ± SD. A P-value <0.05 was reflected significant. Values which don't share the same letter in each column are significantly different.

Histopathological examination of testes: The normal histological structure of the seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis was revealed microscopically by rat control experiments. (Fig. 1). However, testes of rats from group control & dandelion treated revealed no histopathological changes (Fig. 2).

In contrary, testes of rats from diabetes group revealed degeneration and necrosis of spermatogoneal cells lining seminiferous tubules with interstitial edema (Figs. 3 & 4).

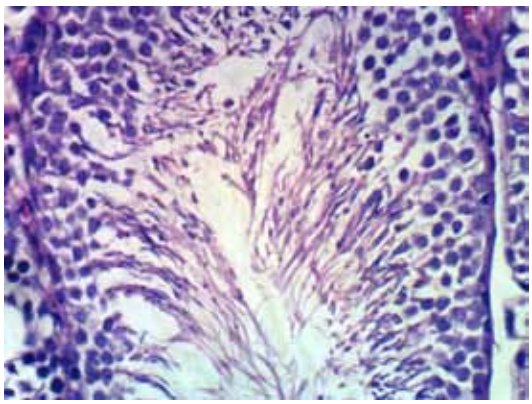

Fig. (1): Testis section from control (-) rats showing the normal histological structure of seminiferous tubule with normal spermatogoneal cells and complete spermatogenesis (H & E X 400).

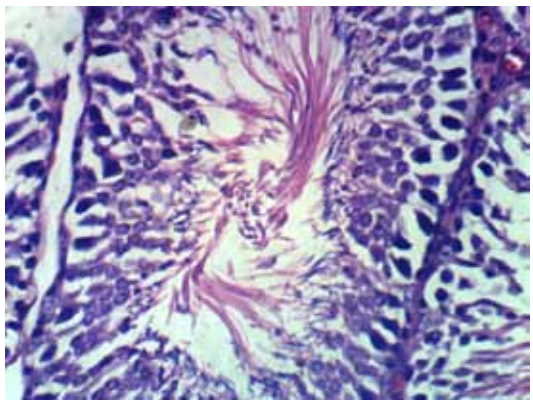

Fig. (2): Testis section from control & dandelion treat-rats showing no histopathological changes (H & E X 400).

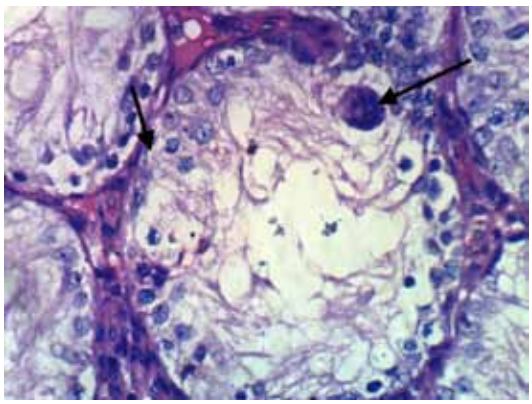

Fig. (3): Testis section from diabetic rats showing degeneration of spermatogoneal cells lining seminiferous tubules with formation of spermatid giant cells (H & E X 400).

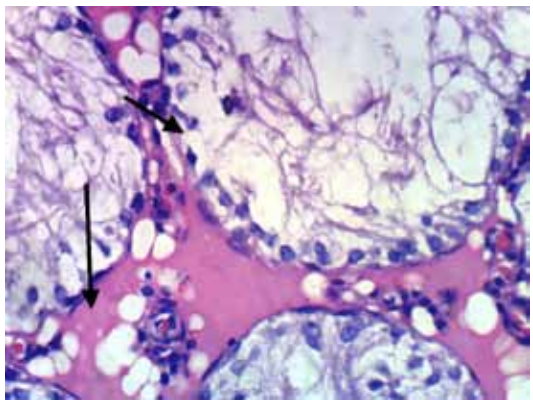

Fig. (4): Testis section from diabetic rats showing necrosis of spermatogoneal cells lining seminiferous tubules with interstitial oedema (H & E X 400).

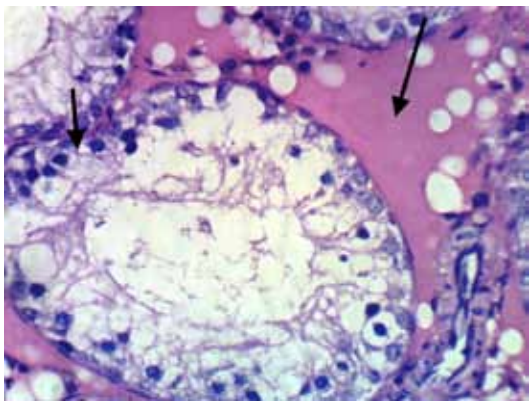

Fig. (5): Testis section from diabetic & dandelion–treated rats showing degeneration and necrosis of spermatogoneal cells lining seminiferous tubules with interstitial oedema (H & E X 400).

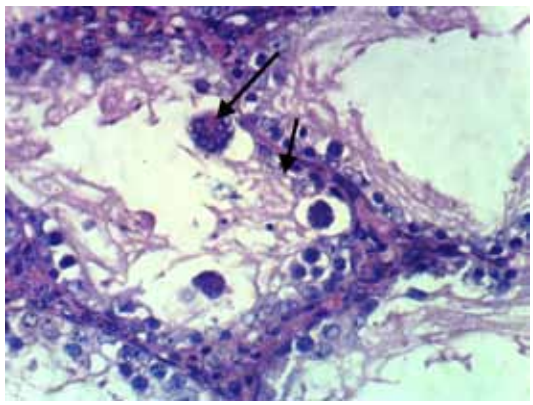

Fig. (6): Testis section from diabetic & dandelion–treated rats showing degeneration and necrosis of spermatogoneal cells lining seminiferous tubules with formation of spermatid giant cells (H & E X 400).

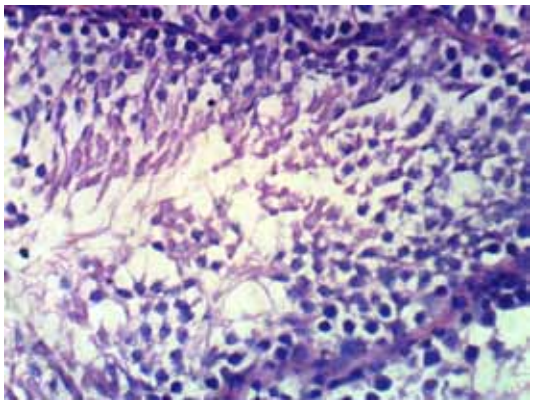

Fig. (7): Testis section diabetic & dandelion – treated rats showing no histopathological changes (H & E X 400).

Most of the examined segments of the diabetic & dandelion treatment community demonstrated degeneration and necrosis of spermatogoneal cells lining seminiferous tubules with spermatid giant cell formation. (Fig. 5). Moreover, some examined cases from diabetic & dandelion treated with showed degeneration and necrosis of spermatogoneal cells lining seminiferous tubules with interstitial oedema (Fig. 6), whereas, other sections revealed no histopathological changes (Fig. 7).

Discussion

Our study has indicated that serum levels of progesterone, testosterone and LH have significantly increased for dandelion-treated diabetic group, compared to diabetic group, on the other hand, in dandelion -treated group compared to diabetic group, serum level of FSH has been significantly decreased. Our result revealed that agree with **Wang *et al.,* (2018)** they demonstrated that progesteon, estogediol and progesterone levels increased with the increasing dandelion extract concentration. [22]

Tahtamouni *et al.,* (2016) who reported that a substantial decrease in testis and seminal vesicle weight, a decrease in serum testosterone concentration, damaged sperm parameters, and a decrease in pregnancy parameters have been caused by oral administration of dandelion (whole plant and leaf aqueous extract). Structural changes such as germ cell hypoplasia, reduced germ epithelium thickness, spermatogenesis arrest at the spermatid stage (late maturation arrest) and decreased Leydig cell count have been shown in the testicular histology of treated rats.[23]

The findings of the current study concerning the effect of dandelion on reducing FSH and raising the level of LH plasma are in line with previous work by **Abdel-Magied*et al.,* (2001),**[10].Dandelion may have inhibitory and exhibitory effects on FSH and LH gonadotrophs, respectively, due to the effect of dandelion on FSH and LH levels. Regarding the fact that ovulation and gestation are regulated by the LH/progesterone system, at least in the early stages in women,**[24]** one may conclude that dandelion has a positive effect on reproduction by increasing LH and progesterone.

The effect of dandelion on testosterone level in this research is not in accordance with previous study by **Abdel-Magied *et al.,* (2001)**in which a reduction has been shown in the testosterone level of dandelion -treated rats. This discrepancy might be related to the type of dandelion administration (in food in our experiment and in water and stomach tube in their experiment).[10]On the other hand, we have treated the rats for four weeks, whereas in the previous study rats have been treated for six days. It is notable that dandelion contains a steroidal lactone (withaferin A)**.**[25]From this study and the presence of steroidal compound in dandelion, it appears that Ashwagandh is mimicking the steroidal hormones.

Untreated and dandelion -treated diabetic rats had elevated serum glucose level over those of control rats, these results agree with **Davaatseren *et al.,* (2013)** they indicate that dandelion leaf extract significantly suppressed lipid accumulation in the liver and reduced insulin resistance.[26]

Conclusion

Our study has indicated that long time administration of DLP in experimental model of diabetes, could be used as a good candidate in the treatment of reproductive hormones deficiency.

Ethical Clearance: All procedures performed on animals were in accordance with ethical standards of the institution at which the study was conducted.

Source of Funding: Self-funding.

Conflict of Interest: Nil.

References

1. Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. Chronic Complications of Diabetes Mellitus: A Mini Review. Curr Diabetes Rev. 2017;13 (1):3-10.
2. WHO (2014): World health organization, (Diabetes

Fact sheet. 312).

3. Gardner DG and Shoback D. Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical, 2011;17: ISBN 0-07-162243-8.
4. Ketan H. and Annapurna A. The effect of quercetin on blood glucose levels of normal and streptozotocin induced diabetic (type i & type ii) rats. IJPCBS. 2014; 4(3):613-619.
5. Piya MK, Tahrani AA and Barnett AH. Emerging treatment options for type 2 diabetes. J. Clin. Diabetes Br. Pharmacol. 2010; 70(5):631-644.
6. Rochette L, Zeller M, Cottin Y and Vergely C. Diabetes, oxidative stress and therapeutic strategies. Biochim. Biophys. Acta., 2014;1840 (9):2709-2729.
7. Sandireddy R, Yerra VG, Areti A, Komirishetty P, Kumar A. Neuroinflammation and oxidative stress in diabetic neuropathy: Futuristic strategies based on these targets. Int J Endocrinol. 2014;2014:674987.
8. Mallick C, Mandal S, Barik B, Bhattacharya A, Ghosh D. Protection of testicular dysfunctions by MTEC, a formulated herbal drug, in streptozotocin induced diabetic rat. Biol Pharm Bull. 2007; 30: 84-90.
9. Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. Int J Androl 2006; 29: 482-488.
10. Abdel-Magied EM, Abdel-Rahman HA, Harraz FM. The effect of aqueous extracts of Cynomorium coccineum and Withaniasomnifera on testicular development in immature Wistar rats. J Ethnopharmacol. 2001; 75(1):1-4.
11. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant effect of Withaniasomniferaglycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol. 2001;74(1):1-6.
12. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. Phytother. Res. 2006; 20(9):717-724.
13. Arise RO, Malomo SO, Adebayo JO, Igunnu A. Effects of aqueous extract of Eucalyptus globules on lipid peroxidation and selected enzymes of rat liver. J. Med. Plant Res. 2009;3(2):77-81.
14. Ogbourne SM and Parsons PG. The value of nature's natural product library for the discovery of New Chemical Entities: the discovery of ingenolmebutate. Fitoterapia. 2014;98:36-44.
15. El-Abhar HS and Schaalan MF. Phytotherapy in diabetes: review on potential mechanistic perspectives. World J. Diabetes.2014;5(2):176–197.
16. Hemalatha S, Wahi AK, Singh PN, Chansouria JP. Hypoglycemic activity of WithaniacoagulansDunal in streptozotocin-induced diabetic rats. Journal of Ethnopharmacol. 2004; 93: 261-264.
17. Reeves PG, Rossow KL and LindlaufJ . Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J. Nutr. 1993; 123:1923-1931.
18. A.O.A.C. Official method of analysis of the association of official analytical chemistry. (19th ed.). Virginia: AOAC International Suit; 2005.
19. Manickam D and Periyasamy L. Antidiabetic effect of methanolic extract of Decalepishamiltonii root (Wight and Arn) in normal and STZ-induced diabetic rats. J. Pharm. Res. 2013; 6 (1): 166-172.
20. A.O.A.C. Official method of analysis of the association of official analytical chemistry. (15th ed ed.). Washington: Washington, DC; 2003.
21. Bancroft JD, Cook HC. Manual of histotechnological techniques. New York: Churchi Livingstone;1998.
22. Wang T, Xue B, Shao H, Wang SY, Bai L, Yin CH, et al. Effect of Dandelion Extracts on the Proliferation of Ovarian Granulosa Cells and Expression of Hormone Receptors. Chin Med J (Engl). 2018; 20;131(14):1694-1701. doi: 10.4103/0366-6999.235864.
23. Tahtamouni LH, Al-Khateeb RA, Abdellatif RN, Al-Mazaydeh ZA, Yasin SR, Al-Gharabli S, et al. Anti-spermatogenic activities of Taraxacum officinale whole plant and leaves aqueous extracts. Vet Res Forum. 2016; 7(2):89-97.
24. Devi PU, Akagi K, Ostapenko V, Tanaka Y, Sugahara T. Withaferin A: a new radiosensitizer from the Indian medicinal plant Withaniasomnifera. Int J Radiat Biol. 1996; 69:193-197

25. Ballester J, Muñoz MC, Domínguez J, Palomo MJ, Rivera M, Rigau T. Tungstate administration improves the sexual and reproductive function in female rats with streptozotocin-induced diabetes. Hum Reprod. 2007; 22 (8): 2128-2135.

26. Davaatseren M, Hur HJ, Yang HJ, Hwang JT, Park JH, Kim HJ, et al. Taraxacum official (dandelion) leaf extract alleviates high-fat diet-induced nonalcoholic fatty liver. Food Chem Toxicol. 2013;58:30-6.