

Combined Effect of Ginger and Pumpkin Seed Extracts on Rat Testis and Serum Biochemical Parameters after Cyclophosphamide Treatment

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Article info:

Received: 14 Aug 2013

Accepted: 24 Dec 2013

Key Words:

Testis,
Ginger,
Pumpkin,
Cyclophosphamide,
Biochemical parameters.

ABSTRACT

Introduction: Cyclophosphamide is a chemotherapy drug with several side effects on various organs such as male reproductive system that can cause infertility. In this study, we assessed combined effect of ginger and pumpkin extracts on rat testis after CP injection.

Methods: Forty adult male rats were randomly divided into 4 groups: The control group received intraperitoneal injection of isotonic saline solution. The cyclophosphamide (CP) group received a single dose of cyclophosphamide (100 mg kg⁻¹BW) intraperitoneally. Combined extracts (ginger+ pumpkin) group received orally 300 mg combined extracts and combined extracts(ginger+ pumpkin)+CP groups received orally 300 mg combined extracts for a period of 6 weeks after CP injection.

Results: Our results showed that although ginger extract could not change testis weight, testosterone, malondialdehyde (MDA) and ROS, antioxidant level in serum was increased significantly. Epithelium thickness and tube diameter were decreased in combined groups with or without CP in comparison to control group. The combined extract could improve histological changes in both combined extract and combined extract+ CP compared to CP group, which could be attributed to the higher serum level of antioxidants.

Conclusion: The administration of combined extracts can increase the serum antioxidant level and decrease the side effects of CP on testis.

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1. Introduction

An inability to conceive after 12 months of sexual practice without using any contraception is defined as infertility[1]. World Health Organization reported that 10–15% of young couples are faced with infertility and each gender shows 50% of the related causing factors[2]. It has been reported that environmental factors such as pesticides, exogenous oestrogens, heavy metals and chemotherapy are the reasons for declining male sperm count which may have a negative impact on male fertility[3]. cyclophosphamide (N, N-bis (2-chloroethyl) tetrahydro-2H-1, 3, 2-oxazaphosphorin-2-amine-2-oxide), a cytotoxic alkylating agent is a nitrogenous mustard belonging to the group of cytotoxic or cytostatic drugs[4]. Studies have shown that generation of free radicals and reactive oxygen species is associated with CP treatment as well. It is known that CP disrupts the redox balance of tissues resulting in oxidative stress. It has been reported that oxidative DNA damage is caused by hydroperoxide derivatives of CP through generation of H₂O₂. Also, acrolein, another component of CP, has been found to interfere with the tissue antioxidant defense system and produces highly reactive oxygen free radicals, which are mutagenic to mammalian cells[5]. Lipid peroxidation has been suggested to be closely related to CP-induced testicular damage, and malondialdehyde (MDA) is a good indicator of lipid peroxidation that could induce sperm abnormality[4]. CP treatment is associated with oligozoospermia and azoospermia, as well as biochemical and histological alterations in the testis and epididymis of human and rats [6, 7]. Furthermore, disturbance in gonadotropin secretion, testicular damage, and decreased plasma testosterone levels are found in patients enduring treatment with CP[8]. Medicinal herbs have been popular among people from ancient times, and in recent years, a new interest has emerged to use medicines with natural and especially herbal origin like pumpkin and ginger [9]. Medicinal plants contain phytochemicals and numerous chemical compounds, which can be implemented in pharmacology by isolating the active compounds to generate new medicines and provide alternative healing methods[10]. In most cases, herbal medicine offers less invasive and less costly physical and emotional treatment compared with other procedures.

Ginger rhizome (*Zingiber officinale* R., family: Zingiberaceae) is used worldwide as a spice. Both antioxidative and androgenic activity of ginger have

been reported in animal models[11]. It contains several compounds including acids, resins, vitamin C compounds, folic acid, inositol, choline, pantothenic acid, gingerol, sesquiterpene, vitamin B3 and B6 volatile oils and bio-trace elements like Ca, Mg, P and K[12]. This plant has been considered a safe herbal medicine with few side effects[9]. Ginger has been previously shown to stimulate spermatogenesis[12]. Morakinyo et al. (2008) suggested that ginger extract (500 and 1000 mg kg⁻¹ B.W doses) has a beneficial effect on male reproductive functions in rats, which is confirmed by other studies showing an increase in sperm count, motility, testosterone, antioxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH) and a decrease in malondialdehyde lipid peroxidation levels [13, 14]. We recently showed that ginger extract at doses of 300 and 600 mg/kg BW has a positive effect on recovery of spermatogenesis in adult rats after cyclophosphamide (CP) treatment. Also, we found that co-administration of this extract with CP can counterbalance the negative effect of CP on testis parameters demonstrated in our study[1].

Pumpkin (*Cucurbitapepo* var. *styriaca*) family cucurbitacea is an important leaf and seed vegetable tropical vine grown and highly reputed in traditional medicine and largely consumed in many countries such as Iran [15]. The seeds are a rich natural source of proteins, phytosterols, polyunsaturated fatty acids, phytochemicals, sterols, antioxidant vitamins such as carotenoids and tocopherol and trace elements such as zinc and selenium[4]. It has been demonstrated that pumpkin seeds and daily rich diet of zinc can decrease the undesirable side effect of lead contaminants and improve the sexual health status[16]. Pumpkin seeds improve sexual stimulation and intromission and ejaculatory latency[17]. Pumpkin causes a significant reduction in sperm count with primary and secondary abnormalities by producing further zinc and protein. Therefore, pumpkin is proposed for both the prevention and treatment of infertility in male animals[18]. The findings of our recent study indicated that pumpkin seed extract could recover the side effects of CP, epididymis histology and sperm parameters through preventing oxidative stress[4].

Although it is now broadly accepted that ginger and pumpkin seeds have a positive effect on fertility, this study is the first study that evaluates the effects of the combined extracts of ginger and pumpkin seeds on CP-treated rat testis. Therefore, the present study was designed to investigate any possible protective effects

Table 1. Administration effect of the combined extracts of ginger and pumpkin seeds on histological parameters in rat seminiferous tubes.

Parameters	Groups			
	Control	CP	Combined Extract 300	CP+300 Combined Extract
Tube diameter(μm)	286.20 \pm 13.74	270.80 \pm 19.81	253.18 \pm 35.6*	245.57 \pm 18.72*
Epithelium thickness (μm)	152.79 \pm 7.21	131.16 \pm 6.60†	114.60 \pm 6.50†	111.42 \pm 2.8†
Lumen diameter(μm)	133.41 \pm 7.93	139.65 \pm 18.21	139.99 \pm 31.02	134.14 \pm 18.20

*P < 0.05 compared with control group.

†P < 0.001 compared with control group.

of the combined extracts of ginger and pumpkin on biochemical parameters and testicular histology of CP-treated male rats.

2. Materials and Methods

Cyclophosphamide was purchased from Baxter Oncology GmbH, Frankfurt, Germany. Pumpkin seeds (*Cucurbita Pepo* var. *Styriaca*) were purchased from local Iranian markets. Ginger was obtained from Natural Remedies Company in India that has been standardized as 'total Gingerol 5%'. MDA, ROS, antioxidant and testosterone kits were purchased from Glory Science Co. Ltd, China.

Animal experiment : In this study, forty healthy adult male Wistar rats (8–10 weeks old, 300–350 g) were obtained from Kashan University of Medical Sciences. Rats were in wire-wooden cages under controlled light schedule (12 h light and 12 h darkness). The animals were allowed to acclimatize for a period of 7 days before starting the experiment. During the treatment period (Six weeks) they were fed with the supplied food pellets and had free access to water. All experiments were implemented in accordance with the guidelines and were approved by the Local Committee on Animal Research in Kashan University of Medical Sciences.

Study design and treatment: The rats were randomly divided into four groups of ten. Control group received a single intraperitoneally injection of isotonic saline solution (1 ml). CP Group received a single dose of cyclophosphamide (100 mg kg⁻¹ body weight) intraperitoneally [19]. Group ginger +pumpkin 300 received 300 mg/kg BW of ginger extract plus pumpkin seed extract. Group CP+ginger +pumpkin 300 received CP

plus 300 mg/kg BW ginger and pumpkin seed extract orally for a period of 6 weeks after CP injection.

Food regimens: The synthetic diet was purchased from Behparvar Company in Iran. Pumpkin seed without oil (cold pressed oil) was obtained from Barij Essence Company in Kashan, Iran. The pumpkin seed without oil was mixed with 70% alcohol in a blender and incubated for 72 hours at room temperature. Then ethanol liquid was separated with a filter paper. The extract was standardized to contain amino acid, total flavonoid and total phenolic compound concentration [20, 21]. One kg of the synthetic diet was mixed with 512cc of pumpkin seed extract (2.6%) for 300 mg/kg body weight dosage. Also, ginger extract was dissolved in 50% ethanol and with pumpkin seed extract, was added to powdered food pellets of rats. The mixture was dried in oven under 50°C and stored at 4°C. The animals were fed based on a daily consumption of 15-17 gr of dried diet/rat. Before starting the experiment, we measured the food intake in control and CP-treated rats. In case of CP-injected rats, the food intake was decreased to about 8 grams in the first 5 days after injection. So, the ginger+ pumpkin content of the food was adjusted to give the right dose during the experiment. Fresh diet was prepared weekly.

Sample collection: Since spermatogenic period in rats is 48 days [22], in our study, rats were weighed and killed under anaesthesia 42 days after CP injection. Blood samples were collected from the left ventricle, and plasma was immediately separated for biochemical examinations. After weighing, testis was fixed with Bouin's solution for histology.

Biochemical studies: The level of rat testosterone, total antioxidant capacity (T-AOC), MDA and reactive

Table 2. Administration effect of the combined extracts of ginger and pumpkin seeds on cell count of germ and Sertoli cell parameters in rat seminiferous tubes.

Parameters	Groups			
	Control	CP	Combined Extract 300	CP+300 Combined Extract
Spermatogonia(n)	63.15±8.15	51.55±3.51†	64.17±4.8††	61.50±5.04††
Spermatocytes(n)	74.20±11.16	57.24±2.15*	71.03±6.5††	71.41±4.5††
Spermatid(n)	230.0±38.27	196±4.5	211.9±15.7	201.74±13.9
Sperm(n)	180.07±5.5	161.11±4.6††	190.2±9.5*††	175±4.57††
Sertoli(n)	17.75±3.89	15.22±2.99	18.82±2.81	18.56±1.47

*P < 0.05 compared with control group.

†P < 0.001 compared with control group.

††P < 0.001 compared with CP group.

oxygen species were determined using an enzyme-linked immunosorbent assay (ELISA) method. Blood was collected from left ventricle of anaesthetized rats in a tube and centrifuged immediately at 3000 rpm for 10 min. The separated serum was deposited in -20 °C freezers for the biochemical tests. The biochemical tests were carried out according to the kit instruction. Briefly, a series of standard solutions were prepared. First, the primary antibody was added to the sample wells of a 96-well plate (not to control or standard wells). 40 ul of samples or 50 ul of standard solutions were added to the designated wells. After adding the secondary antibody (labelled with Strep-tavidin-HRP) to the standard and sample wells, the plate was incubated at 37 °C for 60 min. Plate was washed; chromogenic solutions (A, B) were added and incubated at 37 °C in the dark for 10 min. To the control wells, only chromogenic and stop solutions were added. After adding the stop solutions to the wells, the absorbance of wells at 450 nm wavelengths was read by an Elisa Reader (Model StatFax 2100). A standard curve was extrapolated based on the readings of the standard wells, and linear regression equation was calculated. The concentration levels of samples were calculated based on OD readings of the samples and the standard curve.

Histology and light microscopy: The left testis was carefully dissected, trimmed of all fats and blotted dry to remove any blood. The testis was divided into three parts, and the middle section was fixed in Bouin's fluid for 48 h. The fixed samples were dehydrated in graded levels of ethanol, cleared in xylene and embedded in

paraffin wax for sectioning. Five µm thick sections were prepared and stained with Hematoxylin and Eosin (H&E) and observed under a light microscope.

Morphometric study: An optical microscope (Zeiss/German) with an objective lens ×40 was used for cell counting. Spermatogonia, primary spermatocyte, spermatid, spermatozoa and Sertoli cells were counted in 10 seminiferous tubules in stages VII or VIII in each animal. The diameter of the round or nearly round seminiferous tubules was estimated as the average of two perpendicular longer and shorter diameters. Epithelium thickness was obtained by the same method. The size of the lumen was calculated by subtracting epithelium thickness from the diameter. A mean value of each of these parameters was calculated for each group and was compared with the other groups.

Statistical analyses: The data are presented as mean ± SEM (standard error of mean). Statistical analyses were carried out using ANOVA test and a P value of P < 0.05 was considered as statistically significant.

3. Results

Morphometry: Testicular weight did not change significantly between the control and other groups. The general effects of CP were some hair loss and a decreased appetite in the first 5 days after CP injection only seen in CP group. CP treatment decreased the number of germ cells as well as epithelium thickness of seminiferous tubules (Tables 1 and 2). Other features

Table 3. Administration effect of the combined extracts of ginger and pumpkin seeds on serum levels of testosterone, anti-oxidant, Malondialdehyde (MDA) and ROS in rat seminiferous tubes.

Parameters	Groups			
	Control	CP	Combined Extract 300	CP+300 Combined Extract
MDA(nmol/ml)	2.42±0.46	2.71±0.42	2.05±0.56	2.50±0.40
ROS	3.40±0.85	2.60±1.03	4.17±0.67	4.24±0.56
Antioxidant(μl)	5.51±3.52	5.20±2.43	11.97±3.6*††	11.92±3.22*††
Testosterone(ng/dl)	7.38±2.09	8.39±1.43	9.45±1.93	2.06±7.93

*P < 0.05 compared with control group.

††P < 0.001 compared with CP group.

of the CP-treated seminiferous tubules were exfoliation of germ cells and vacuolated appearance of the epithelium. The administration of combined extract to CP-treated or normal rats could significantly increase germ cells count in seminiferous tubules (spermatogonia, spermatocytes, sperm) compared to CP group. However, epithelium thickness and tube diameter were decreased in combined groups with or without CP in comparison to control group.

Biochemical Parameters

Antioxidant levels: Cyclophosphamide treatment did not change the antioxidant level significantly in comparison with the control group. However, administration of the combined extracts 300 mg kg⁻¹ BW⁻¹ strongly increased antioxidant levels compared with that of control or CP group (Table 3).

Testosterone, ROS and MDA: Testosterone, Ros and MDA levels did not change significantly in the different groups (Table 3).

Histology: Cyclophosphamide treatment caused a reduction in the size, epithelium thickness and the number of different types of cells in the seminiferous tubules. Degeneration, vacuolation and exfoliation of germ cells into the lumen of seminiferous epithelium were other features of the CP group samples. However, administration of the combined extracts 300 mg kg⁻¹ BW⁻¹ to CP group rats and rats without CP caused an improvement in the germ cells count of the seminiferous tubules compared with the CP group but epithelium thickness and tube diameter decreased in comparison to control group in these groups.

4. Discussion

Using chemotherapy drugs like CP for cancer treatment is limited by their side effects. The side effects of chemotherapy include reproductive toxicity that has been documented in different studies[19]. This study was completed to investigate the effects of the combined extracts of ginger plus pumpkin seeds on CP-injected and normal rat testis. To our knowledge, this is the first study investigating the effects of this combined extract against testicular damage caused by CP in rats. Our results showed that in combined extract-treated rats (300 mg kg⁻¹ BW⁻¹), the number of germ cells in seminiferous tubules was increased significantly in comparison to CP group alone. This result reconfirms our last report indicating that administration of ginger or pumpkin increases the number of germ cells in seminiferous tubes and has a positive effect on recovery of spermatogenesis in adult rats after cyclophosphamide (CP) treatment[1, 23].

Epithelium thickness and tube diameter decreased in comparison to control group in these groups. Our results support those of [24] Saalu reported decrease in tubular diameter and epithelium thickness after administration of fluted pumpkin extract 400 mg/kg/day/oral and our result in another study where we found that administration of ginger extract 300 mg/kg/day/oral decreased epithelium thickness in comparison to control group [1].

In our study, the weight of the testis did not change significantly between the different groups. A decreased testis weight has been reported in CP-treated rats [25]. In that study a dose of 15 mg kg⁻¹ BW of CP was

given to rats by oral gavage once a week for 10 weeks (in total 150 mg kg⁻¹ BW⁻¹), while in our study, 100 mg kg⁻¹ BW⁻¹ of CP was injected in a single dose. The chronic low-dose administration of CP to male rats could be the reason for decreased reproductive organ weights[26]. In another study, two different doses of CP (100 and 200 mg kg⁻¹) were injected to male rats. A decrease in the weight of testis was detectable 1 week after the injection for both doses, but after 5 weeks, the reduced weight of testis was only detectable in 200 mg kg⁻¹ injected rats[27]. In our study, the samples were collected and studied 6 weeks after the injection of 100 mg kg⁻¹ of CP; therefore, we could not see any difference in testis weight. Of course, the reason that samples were studied after 6 weeks was that spermatogenesis (development of mature spermatozoa from diploid spermatogonial cells) in rats takes 48 days[22].

In CP treated rats the toxic effect was indicated by significant reduced spermatogonia, spermatocyte and sperm count. Our results support those studies reporting irregular and diminished seminiferous tubules containing only a few germ cells in the CP group[19]. Our results provided no evidence for those studies [19] which reported that MDA and ROS levels were increased significantly in CP-treated rats. Since we examined the rats 42 days after chemotherapy, probably the levels of these parameters have been recovered in the first or second weeks of the experiment. This recovery most probably has been done because of the effective help of the mixed extract. In our study, testosterone, ROS and MDA levels neither changed significantly in CP group nor in combined extract-treated groups (with and without CP).

Our results showed that the combined extract with dose (300 mg kg⁻¹ BW⁻¹) could increase antioxidant to a higher level. This result confirms the antioxidant effects of *Telfairia occidentalis* (fluted pumpkin) extracts reported by Nwanna and Oboh[28] and zingiber reported by Morakinyo[12]. Antioxidant therapy improves fertility parameters through a protective mechanism against oxidative stress[11]. Phenolic compounds such as polyphenols flavonoids as well as vitamins and zinc in medicinal plants such as ginger and pumpkin are attributed factors for antioxidant activity [4, 12]. In studies by Tsai et al. (2006) and Gossell-Williams et al. (2006), it was reported that Pumpkin seed oil is rich in many powerful antioxidants and useful nutritional supplements such as essential fatty acids and polyunsaturated fatty acids including linoleic acid, oleic acid,

palmitic acid, omega 3, 6 and 9, carotenes, lutein, gamma and P-tocopherols, phytosterols, chlorophyll, selenium and zinc[29, 30]. Also, the presence of oleic acid, monounsaturated fatty acid in pumpkin reduces the susceptibility of the testis and epididymis to lipid Peroxidation[31]. Zinc in pumpkin seeds is an essential trace mineral that acts as an antioxidant by neutralizing free radical generation. Also, Zinc could play a direct antioxidant action by engrossing the iron or copper binding sites of lipids, proteins, and DNA[14]. On the other hand, all major active ingredients of *Z. officinale* such as Zingerone, Ginger-diol, Zingibrene, gingerols and shogaols, have antioxidant properties[9]. Besides, other studies have shown that ginger oil has a protective effect on DNA damage against Hydrogen Peroxide (H₂O₂) and might decrease oxygen radical and could be used as an antioxidant[32, 33]. In previous studies, we showed that the higher dose of ginger extract (600 no 300mg kg⁻¹ BW⁻¹) alone could increase testosterone level. Because only the higher dose of ginger could induce the higher level of testosterone. Ginger extract might act in a dose-dependent manner[1]. In our previous work, only the lower dose (300 mg/kg) of pumpkin seed showed antioxidant activity but not the higher dose (600 mg/kg), which even increased serum free radical level. Based on these results, the combined extract was prepared from the lower dose of each plant. Since in Iranian family gatherings normally both pumpkin seeds and ginger flavored tea are served, we decided to study the effects of the combined extract on testis after chemotherapy.

Conclusion

The combined extract of ginger and pumpkin (300 mg kg⁻¹ BW⁻¹) has an antioxidant activity and thus can reduce the adverse effects of CP in testis. Also, these results suggest further studies to evaluate the use of the combined extracts of ginger and pumpkin as a supplement drug to counterbalance the negative effect of CP in human as well.

Acknowledgment

This research is financially supported by the Deputy for Research of Kashan University of Medical Sciences (Kaums) and Kashan Anatomical Sciences Research Center (Grant No 9110).

References

- Mohammadi F, Nikzad H, Taghizadeh M, Azami-Tameh A, Hosseini M. Protective effect of Zingiber officinale extract on rat testis after cyclophosphamide treatment. *Andrologia*. 2014. 46: 680-686.
- Kashani HH, Malekzadeh Shiravani S, Hoshmand F. The effect of aqueous extract of Salep prepared from root-tubers of *Dactylorhiza maculata* (Orchidaceae) on the testes and sexual hormones of immature male mice. *Journal of Medicinal Plants Research*. 2012. 6(24): 4102-4106.
- Lamfon HA. Effect of fenugreek seed extract on carbendazim-inhibited spermatogenesis in albino rats. *Journal of Applied Pharmaceutical Science*. 2012. 2(04): 09-13.
- Aghaei S, Nikzad H, Taghizadeh M, Azami Tameh A, Taherian A. Protective effect of Pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in adult male rats treated with Cyclophosphamide. *Andrologia*. 2014. 46: 927-935.
- Rezvanfar M, Sadrkhanlou RA. Protection of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stress. *Hum Exp Toxicol*. 2008. 27(12): 901-910.
- Masala A, Faedda R, Alagna S, Satta A, Chiarelli G, Rovasio PP. Use of testosterone to prevent cyclophosphamide-induced azoospermia. *Ann Intern Med*. 1997. 126: 292-295.
- Kirkland R, Bongiovanni AM, Cornfeld D, McCormick JB, Parks JS. Gonadotropin responses to luteinizing releasing factor in boys treated with cyclophosphamide for nephrotic syndrome. *J Pediatr*. 1976. 89: 941-944.
- Hoorweg-Nijman JJ. Cyclophosphamide-induced disturbance of gonadotropin secretion manifesting testicular damage. *Acta endocrinologica*. 1992. 126(2): 143-148.
- Mohammadi F, Nikzad H, Taherian A, Amini Mahabadi J, Salehi M. Effects of Herbal Medicine on Male Infertility. *Anatomical Sciences*. 2013. 10(4): 3-16.
- Erasmus N, Solomon M, Fortuin K, Henkel R. Effect of *Eurycoma longifolia* Jack (Tongkat Ali) extract on human spermatozoa in vitro. *Andrologia*. 2012. 44: 308-314.
- Khaki A, Fathiazad F, Nouri M, Afshin A, Hamadeh DVM. The effects of Ginger on spermatogenesis and sperm parameters of rat. *Iranian Journal of Reproductive Medicine*. 2009. 7(1): 7-12.
- Morakinyo OA, Achema PU, Adegoke OA. Effect of Zingiber Officinale (Ginger) on Sodium Arsenite Induced Reproductive Toxicity in Male Rats. *African Journal of Biomedical Research*. 2010. 13(1): 39-45.
- Morakinyo A, Adeniyi OS, Arikawe AP. Effects of Zingiber officinale on reproductive functions in the male rat. *African Journal of Biomedical Research*. 2008. 11(3): 329-334.
- Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T. Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J Reprod Dev*. 2008. 54(2): 129-134.
- Nkang A, Omokaro D, Egbe A, Amanke G. Variations in fatty acid proportions during desiccation of *Telfairia occidentalis* seeds harvested at physiological and agronomic maturity. *African Journal of Biotechnology*. 2004. 2(2): 33-39.
- Abd El-Ghany M, Dalia A, Hafez Soha M. Biological study on the effect of pumpkin seeds and zinc on reproductive potential of male rats. Faculty of Specific Education Mansoura University, Egypt April. 2010: 14-15.
- Gundidza G, Mmbengwa VM, Magwa ML, Ramalivhana NJ, Mukwevho NT. Aphrodisiac properties of some Zimbabwian medicinal plants formulations. *African Journal of Biotechnology*. 2009. 8(22): 6402-6407.
- Oyeyemi, M., S. Olukole, and O. Esan. Sperm morphological studies of West African Dwarf Bucks treated with pumpkin plant (*Cucurbita pepo*). *Int J Morphol*, 2008. 26: 121-126.
- Ilbey YO, Ozbek E, Simsek A, Otunctemur A, Cekmen M, Somay A. Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. *Fertil Steril*. 2009. 92(3): 1124-1132.
- Tsantili E, Shin Y, Nock J, Watkins C. Antioxidant concentrations during chilling injury development in peaches. *Postharvest Biol Technol*. 2010. 57(1): 27-34.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. 2006. 97(4): 654-660.
- George j. The handbook of experimental animals, The laboratory rat. 2000, San Diego, Calif. London: Academic. 145-152.
- Mohammadi F, Nikzad H, Taghizadeh M, Moravveji SA. Effect of pumpkin extract regimen on testicular structure and serum biochemical parameters in cyclophosphamide-treated adult rats. *KAUMS Journal (FEYZ)*. 2013. 17(5): 438-446.
- Saalu L, Kpela T, Benebo AS, Oyewopo AO, Anifowope EO, Oguntola JA. The Dose-Dependent Testiculoprotective and Testiculotoxic Potentials of *Telfairia occidentalis* Hook f. Leaves Extract in Rat. *International Journal of Applied Research in Natural Products*. 2010. 3(3): 27-38.
- Selvakumar E, Prahalathan C, Sudharsan P, Varalakshmi P. Chemoprotective effect of lipoic acid against cyclophosphamide-induced changes in the rat sperm. *Toxicol*. 2006. 217(1): 71-78.
- Das UB, Mallick M, Debnath J, Ghosh D. Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic and androgenic disorders in male rats. *Asian J Androl*. 2002. 4(3): 201-208.
- Elangovan N, Chiou T, Tzeng W. Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. *Toxicol*. 2006. 222(1): 60-70.
- Nwanna EE, Oboh G. Antioxidant and Hepatoprotective Properties of Polyphenol Extracts from *Telfairia occidentalis* (Fluted Pumpkin) Leaves on Acetaminophen Induced Liver Damage. *Pakistan Journal of Biological Sciences*. 2007. 10(16): 2682-2687.

29. Gossell-Williams M, Davis A, O'Connor N. Inhibition of testosterone-induced hyperplasia of the prostate of sprague-dawley rats by pumpkin seed oil. *Journal of Medicinal Food*. 2006. 9(2): 284-286.
30. Tsai YS, Tong Y, Cheng J, Lee C. Pumpkin seed oil and phytosterol-F can block testosterone/prazosin-induced prostate growth in rats. *Urologia Internationalis*. 2006. 77(3): 269-274.
31. Bourre JM, Dumont O, Durand G. Dose-effect of dietary oleic acid: oleic acid is conditionally essential for some organs. *Reproduction Nutrition Development*. 2004. 44(4): 371-380.
32. Khaki A, Iraj S, Bazi P, Imani SAM. Comparative Study of Aminoglycosides (Gentamicin & Streptomycin) and Fluoroquinolone (Ofloxacin) Antibiotics on Testis Tissue in Rats: Light and Transmission Electron Microscopic Study. *Pak. J. Med. Sci*. 2009. 25(4): 624-629.
33. Grzanna R, Lindmark L, Frondoza CG. Ginger-an herbal medicinal product with broad anti-inflammatory actions. *Journal of medicinal food*. 2005. 8(2): 125-132.