Protective effect of pumpkin seed extract against testicular toxicity induced by tramadol in adolescent and adult male albino rats: a light and electron microscopic study

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Received 8 September 2016
Accepted 15 December 2016

Egyptian Pharmaceutical Journal
2017, 16:43–52

Background
Tramadol overdose is one of the most common causes of drug poisoning in Egypt, especially in young men with a history of substance abuse and mental disorders.
Objective
The aim of this study was to investigate the effect of tramadol on the testis and the possible protective role of pumpkin seed extract (PSE) in adolescent and adult albino rats.
Materials and methods
Twenty-four healthy growing male Wistar rats aged 3 weeks (adolescent) and another 24 adult rats aged 6 weeks were included in this study. Each group was divided into four equal groups and treated for 3 weeks; these four subgroups were the control group, the PSE alone-treated group (at 40 mg/kg body weight), the tramadol-treated group (at 20 mg/kg body weight/day for the first week and at 40 and 80 mg/kg for the second and third weeks, respectively) and the combination group treated with PSE (at 40 mg/kg body weight) and tramadol (at 20 mg/kg body weight/day in the first week and 40 and 80 mg/kg in the second and third weeks, respectively). At the end of the experiment, testicular tissue samples were collected and processed for histological and ultrastructural examination.
Results and conclusion
The histopathological examination of the tramadol-treated groups (adults and adolescents) indicated severe structural changes in germ cells in the form of degenerated apoptic cells, exfoliation of germ cells in the lumen of seminiferous tubules, hemorrhage, vacuolization, disorganization, and marked loss of cells. But PSE succeeded in protecting the testicular tissue and diminished the atrophic changes. It could be concluded that PSE has a prophylactic effect against tramadol-induced testicular damage and this action was more pronounced in adults than in adolescents.

Keywords:
adolescent, pumpkin seeds extract, testis, tramadol, transmission electron microscope

Egypt Pharmaceut J 16:43–52

1687-4315

Introduction

Tramadol, synthetic codeine analog of the amino-cyclohexanol group, is a weak μ-receptor agonist and centrally acting analgesic. It was first introduced in 1977 in Germany and received high acceptance in human medicine [1]. It can be administered through several routes – orally, subcutaneously, intravenously, intramuscularly, or rectally [2] – and has a complex analgesic action. Most reports showed that clinical impacts of tramadol are due to both opioid activity and nonopioid serotonergic and noradrenergic pathways [3]. Tramadol is recommended for the management of acute and chronic pain of moderate to severe intensity connected with a variety of diseases including osteo-arthritis, fibromyalgia, diabetic neuropathy, neuropathic pain, low back pain, migraine, and even perioperative pain in human patients [4]. Additionally, it could be powerful for alleviating symptoms of depression, anxiety, and phobias [5] and has a particular role in the treatment of premature ejaculation as well [6]. Unfortunately, multiple cases of toxicity and abuse of tramadol have been reported. The most frequent adverse effects of tramadol include constipation, nausea, dizziness, headache, central nervous system depression, somnolence, and vomiting [7]. However, the most serious adverse reactions include confusion, hallucinations, convulsions, serotonin syndrome, and hypersensitivity reactions. Several reports suggested withdrawal symptoms with long-term usage [8], such as tachycardia and seizures [9,10]. Fatal cases

 DOI: 10.4103/1687-4315.205828

have been reported as a result of tramadol overdose. In those instances, death has been attributed to cardiopulmonary arrest and hepatic failure [11–13] as well as hypoglycemia [14]. Moreover, prolonged use of opioids may induce addiction, resulting in physical and psychological dependence. It is believed that natural products if utilized in the correct form and dosage are less harmful than synthetic products [15]. Pumpkin (*Cucurbita pepo*), belonging to the family Cucurbitaceae, is cultivated throughout the world for use as a vegetable as well as in medicine [16]. It has been used traditionally in medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil, and the USA [17–19]. In Egypt, pumpkin seeds are eaten as a nut as they are valued for their high protein [20] and essential fatty acid, such as linoleic acid, content [21]. Pumpkin seeds also contain relatively large amounts of various essential microelements such as K, Cr, Na, Mg, Zn, Cu, Mo, Se, etc. [22]. Moreover, the seeds have protective effects due to their high content of antioxidants, such as vitamins C, E, and A [23], free radical scavenging ability [24], and stimulatory effect of ATP production [25]. Therefore, this study was designed to investigate the histopathological and ultrastructural features of rat testicular tissue following tramadol administration and the possible protective role of pumpkin seed extract (PSE) coadministration.

Materials and methods

Drug and chemicals

Tramadol hydrochloride (Tramadol HCl; Tramal), 225 mg capsules, was obtained from Minapharm Pharmaceuticals (Cairo, Egypt). Its chemical name is (+) *cis*-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride.

Plant materials

Pumpkin seeds (*Cucurbita Pepo L.*) were purchased from a local Egyptian market.

Preparation of pumpkin seeds extract

White pumpkin seeds (500 g) were obtained from a herbal public market. The outer core of the pumpkin seed was removed manually. They were dried in air and then in an oven at 50°C until completely dried. The dried seeds were crushed to powder form and soaked in 70% ethanol in a specific separating funnel for 24 h with frequent stirring. Ethanolic extract was obtained by repeating the extraction procedure three times. The resulting ethanol extracts were filtered and then concentrated using a rotary evaporator (Heidolph. VV2000, Germany) under reduced pressure at a temperature of 55°C. The residue was lyophilized using a vacuum freeze dryer (Tilburg, Holland; 145Fm-RB) and the final extract was weighed and kept refrigerated until further use, following the modified method of Abd El-Ghany *et al.* [26].

Experimental animals

Forty-eight healthy male Wistar rats (*Rattus norvegicus*) [24 rats aged 3 weeks, weighing 80±20 g (adolescents), and 24 rats aged 6 weeks, weighing 100±19 g (adults)], were obtained from the Animal House Colony, National Research Centre, Cairo, Egypt, and were allowed free access to food and water during the experimental period. The animals were housed in a quite nonstressful environment for 1 week before the study. All animals received humane care in compliance with the guidelines of the National Academy of Sciences, published by the National Institutes of Health (NIH publication 86-23 revised 1985), and the protocol was approved by the Animal Care and Use Committee of the National Research Centre, Cairo, Egypt.

Experimental design

After a 1-week acclimatization period, each of the two main animal groups (adolescents and adults) was divided to four subgroups (six animals per subgroup).

(1) Subgroup I: The control group received orally 1 ml of 0.9% saline.
(2) Subgroup II: The rats in this group received a daily oral dose of 20, 40, or 80 mg/kg body weight tramadol in 1 ml of distilled water during the first, second, and third week, respectively.
(3) Subgroup III: The rats in this group received a daily oral dose of PSE (40 mg/kg body weight in 1 ml of distilled water).
(4) Subgroup IV: The rats in this group received a daily oral dose of tramadol (20, 40, and 80 mg/kg body weight during the first, second, and third week) plus PSE (40 mg/kg body weight).

At the end of the experimental period (21 days), all animals were killed by sudden decapitation. Their testes were quickly removed and fixed immediately in 10% formalin saline for 48 h for histological examination. The fixed tissues were then thoroughly washed in running water, processed routinely for paraffin embedding, and stained with Harris hematoxylin and eosin [27]. They were then examined under a light microscope (Olympus Cx41) and photographed using a camera (Olympus DP12, Tokyo, Japan) at the Pathology Department, National Research Centre.

Transmission electron microscopy

Other samples of testes from each rat were fixed in 2.5% glutaraldehyde in 0.1 mol/l PBS at 4°C for 3 h and postfixed in 1% osmium tetraoxide for electron microscopic examination. After dehydration in graduated series of ethanol, the tissues were embedded in Epon 812. The blocks were cut into 1-µm-thick semithin sections with an LKB ultrotome V using a glass knife and stained with 1% toluidine blue, as done by Meek [28]. Under light microscopic examination ultrathin sections (80–90 nm) were cut out with an LKB Ultrotome V using a diamond knife, picked up on naked copper grids (Cu 3 mm), stained [29], and then examined with a transmission electron microscope (Joel TEM CS 100) at the Electron Microscopic Unit, National Research centre.

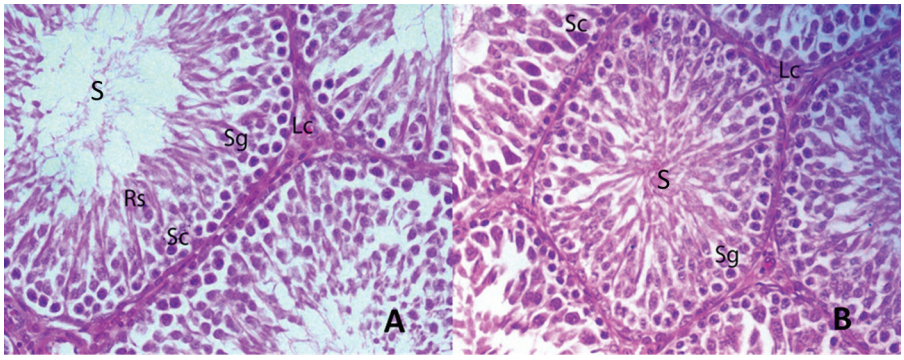
Results

Light microscopic examination

Adult group

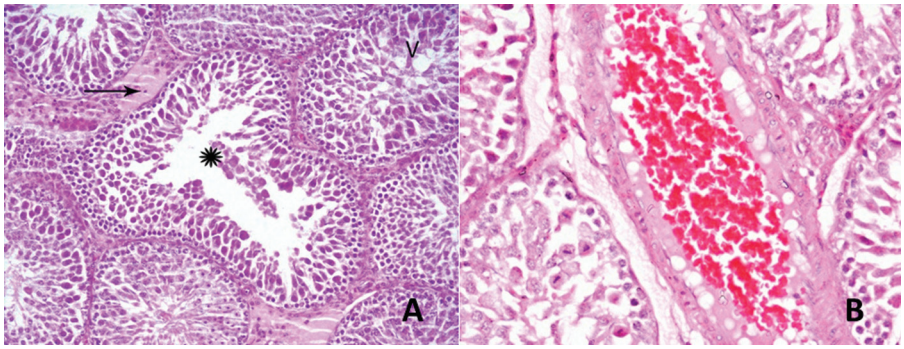
The epithelium of the seminiferous tubules of control rats showed normal cellular architecture for the testis, comprising spermatogonia, Sertoli cells, Leydig cells, primary spermatocytes, round spermatids, late spermatids, and spermatozoa. Leydig cells and blood vessels were on the interstitium between the seminiferous tubules (Fig. 1a). The testis of the rats that received PSE showed seminiferous tubules with normal appearance of the spermatogenic cells (Fig. 1b). The tramadol-treated adult group showed disorganization of seminiferous tubules, with atrophy, exfoliation, and disorganization of the damaged spermatocytes and spermatids within the tubular lumen of many seminiferous tubules. Loss of spermatogenic cells was also seen. Necrotic changes and apoptosis of germ cells also appeared. There were intercellular spaces between germ cells and interstitial spaces and some showed congested and enlarged blood vessels. Some seminiferous tubules had normal architecture, and mature sperms were present in their lumens (Fig. 2a and b). The histological testicular tissue sections of tramadol+PSE-coadministered rats revealed a clear absence of testicular architecture. Many tubules showed normal spermatogenesis (Fig. 3).

Figure 1



Photomicrographs of histological sections of the testis from the adult group: (a) control; (b) pumpkin seed extract-treated testis showing normal seminiferous tubules with normal arrangement of spermatogenic cells [Sertoli cell (Sc), spermatogonia (Sg), spermatocytes, round (Rs) and spermatozoa (S)]. The interstitial tissue appears rich with Leydig cells (Lc) and blood capillaries (hematoxylin and eosin, ×400).

Figure 2

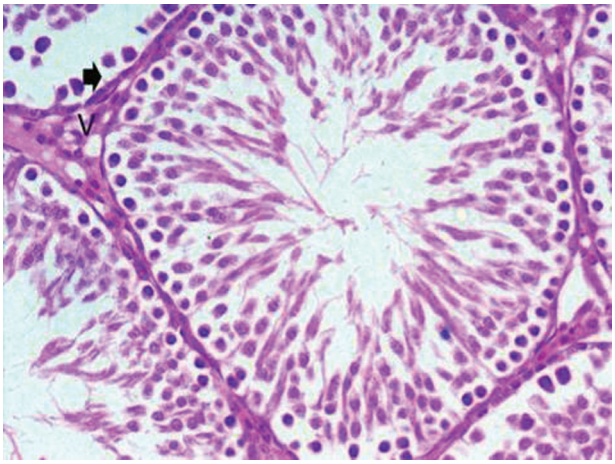


Photomicrographs of histological sections of adult tramadol-treated testis showing (a) irregular shape of seminiferous tubules (arrow), exfoliation of germ cells in the lumen of seminiferous tubules (star), huge vacuolation of germ cell cytoplasm, marked loss of cells and increase in the number of Leydig cells and dilatation of interstitial tissue (arrow); (b) congestion of blood vessels and hemorrhage and vacuolization of germ cells (hematoxylin and eosin, ×400).

Adolescent group

As found in the adult group, histological examination of the testicular section of control rats (Fig. 4a) and PSE-treated groups (Fig. 4b) revealed a normal structure for this tissue, with normal arrangement of germ cells. Tramadol-treated animals showed marked tissue damage in the rat testis in the form of marked depletion of the spermatogenic cell populations from the germinal epithelium, which was also irregular. Disturbance of spermatogenic layers and exfoliation of germ cells within many seminiferous tubules, as well as reduction of mature spermatozoa, were noticeable. There was apoptosis of some spermatogenic cells, dilated and congested blood vessels in interstitial tissue, and large vacuoles on Leydig cells and intercellular spaces (Fig. 5a). The group treated with tramadol and PSE showed more improvement compared with the tramadol group (Fig. 5b).

Figure 3



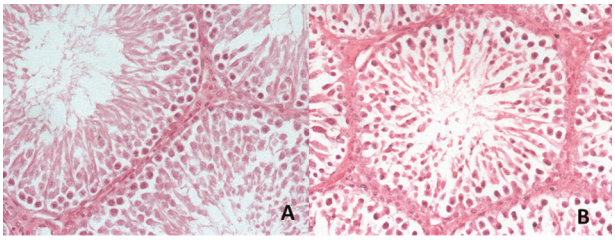
Photomicrographs of histological sections of the testis of the tramadol +pumpkin seed extract-treated adult group showing the presence of normal testicular architecture and regular seminiferous tubules with some degenerative changes like vacuoles (v) and intercellular spaces (arrow) (hematoxylin and eosin, ×400).

Electron microscopic examination

Adult group

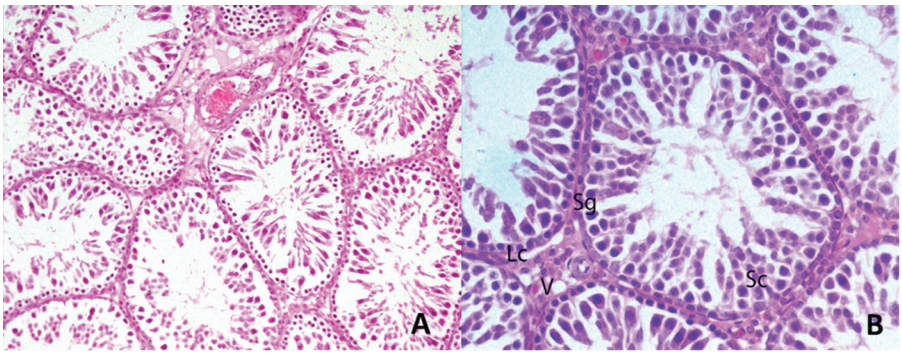
The control group showed seminiferous tubules with regular basement membrane, which included myoid cells. Sertoli cells showed euchromatic nuclei with its characteristic longitudinal fold and conspicuous nucleoli, mitochondria, smooth endoplasmic reticulum (SER), a few strands of rough endoplasmic reticulum, and lysosomes dispersed on the cytoplasm. Spermatogonia showed rounded nuclei with clumps of heterochromatin. The cytoplasm showed mitochondria and free ribosomes. The primary spermatocytes had large rounded nuclei and peripherally arranged mitochondria in the cytoplasm (Fig. 6a and b). Well-developed elongated spermatids showed completely formed acrosome, pyriform nuclei with condensed chromatin, nuclear rings on both lateral sides, and manchettes attached to it posteriorly on the cytoplasm for formation of the tail. Centrioles occupied the implantation fossa on the posterior region (Fig. 7a). The interstitial tissue revealed Leydig cells

Figure 4



Photomicrographs of testicular histological sections of the adolescent group: (a) control group; (b) pumpkin seed extract-treated rats showing the presence of normal testicular architecture and regular seminiferous tubules (hematoxylin and eosin, ×400).

Figure 5



Photomicrographs of testicular sections of adolescent rats: (a) tramadol-treated rats showing irregular shape of seminiferous tubules, congestion of blood vessels and hemorrhage, vacuolated cytoplasm of germ cells and Leydig cells (Lc), widening of intercellular spaces, sloughing of germ cells. (b) Tramadol+pumpkin seed extract cotreatment showing lesser atrophic and degenerative changes like vacules (v) (hematoxylin and eosin, ×200). Sc, Sertoli cell; Sg, spermatogonia.

having large euchromatic nucleus with a prominent nucleolus and a rim of peripheral dense chromatin (Fig. 7b).

Deteriorating effects were remarkably observed on the ultrastructure of the testis of tramadol-treated rats. Inter-Sertoli junctional specialization was interrupted by empty spaces (Fig. 8a). There was absence of acrosomal cap on most of the round spermatids and vacuolated mitochondria on the margins of the cell cytoplasm with interrupting cristae. Malformation and degeneration of the axonemal structure of spermatozoa and mitochondrial sheath on the middle piece cross section (Fig. 8b).

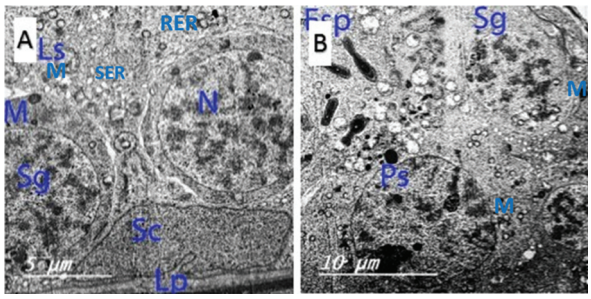
The PSE+tramadol-treated group showed normal ultrastructure for most seminiferous tubules, although some revealed alterations. There was a huge increase in the number of Sertoli cells dispersed on the stroma, with indented nuclei. Basal lamina of the seminiferous tubules appeared irregular and differently thickened. Degenerated mitochondria with vacuoles and disturbed cristae in the cytoplasm of primary spermatocytes (Fig. 9a), complete degeneration, disorientation of 9+2 microfibers and outer dense fibers, and rupture of the plasma membrane sheath in a cross-section of the middle piece of the spermatozoa were also observed (Fig. 9b).

Adolescent group

Ultrastructural examination of the adolescent control group revealed normal architectural features (Fig. 10a and b).

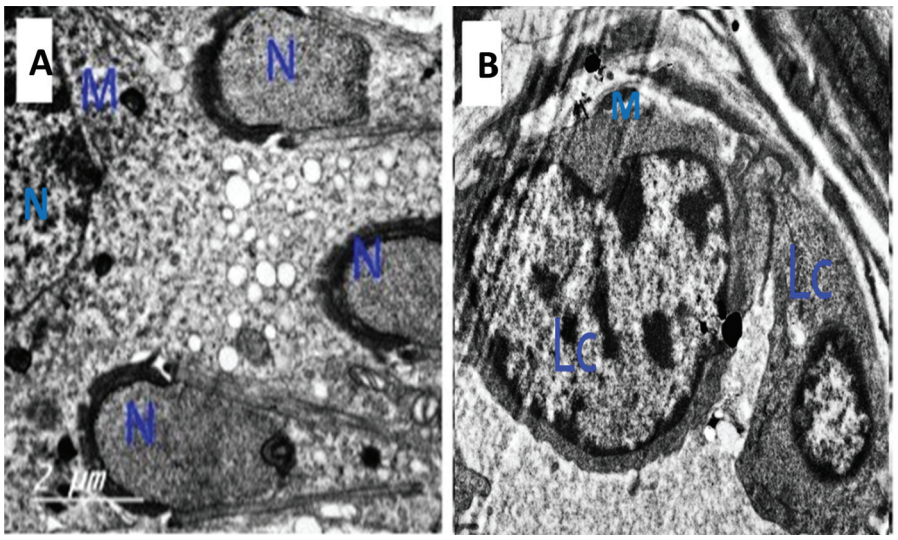
The adolescent tramadol-treated group revealed ultrastructural alterations similar to those of the adult group, but to a higher extent. There were many shrunken and malformed spermatogonia with elongated nuclei and clumped marginated heterochromatin, ill-defined nuclear envelope, and rarified cytoplasm, (Fig. 11a). Elongated spermatids showed partially perserved ectoplasmic specialization, complete disruption of axonemal structure and mitochondrial membrane of

Figure 6



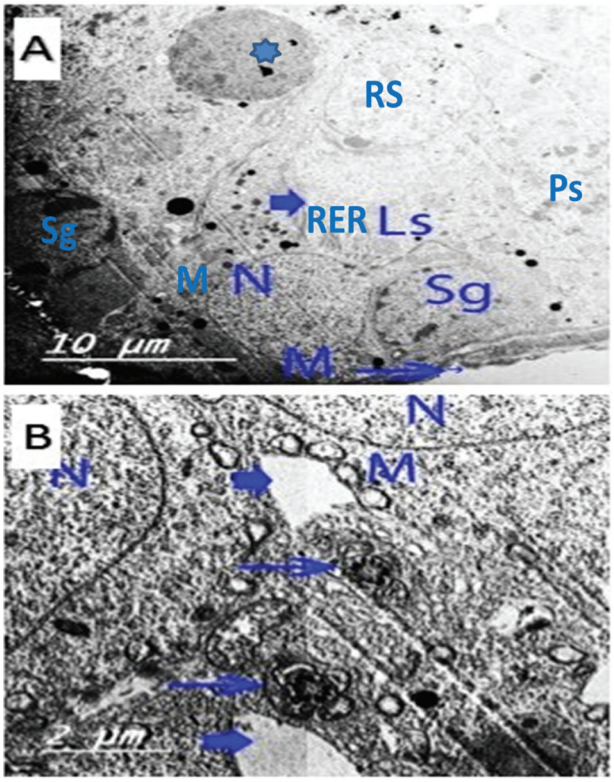
Transmission electron micrographs of rat testis from adult control group; a) Spermatogonia (Sg) rested on basement membrane (Lp); round nucleus (N) with clumps of heterochromatin, mitochondria (M) in cytoplasm, Sertoli cell (Sc) triangular euchromatic nucleus (N) appear on between and processes upward into the lumen of seminiferous tubule and SER, RER and ribosomes and mitochondria in the cytoplasm b) Different stages of germ cells appear, spermatogonia rested on the basement membrane (Sg), primary spermatocyte (Ps) showing round nucleus with clumps of heterochromatin and, mitochondria, RER on cytoplasm, elongated spermatids (Esp) also appear.

Figure 7



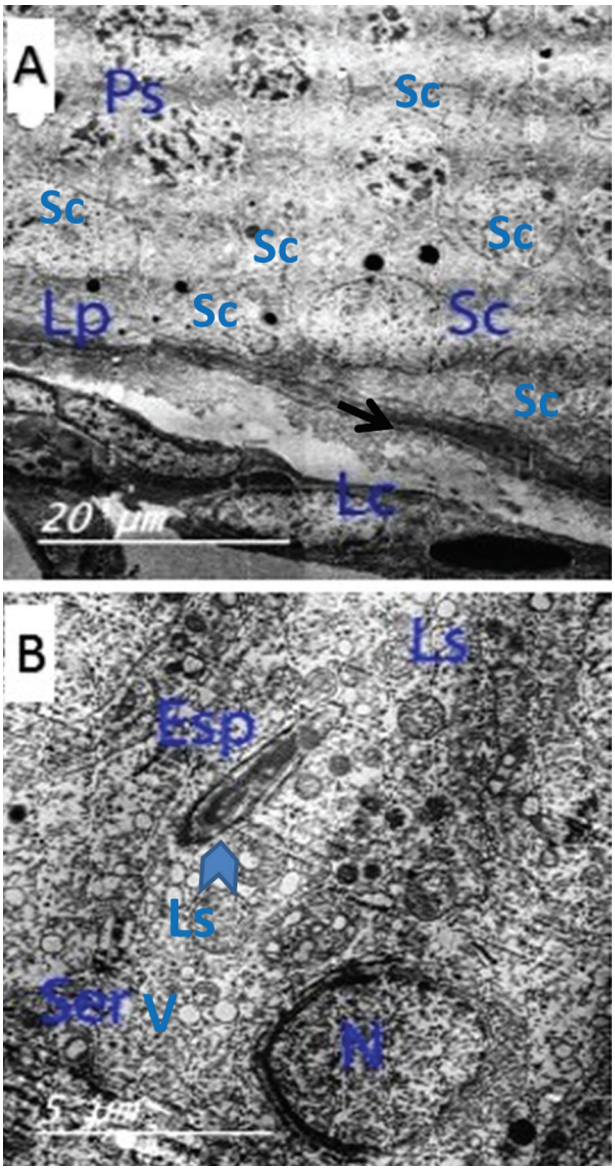
Transmission electron micrographs of rat testis from adult control group a) Well developed elongated spermatid showing pyriform nucleus (N) with condensed chromatin and completely formed acrosome (arrow) covered with head cap all covered with nuclear membrane then plasma membrane, nuclear rings (crossed arrow) on both lateral sides and manchettes (arrow head), Centriole (C) occupy the implantation fossa on the posterior region. b) Leydig cells (Lc) in the interstitial tissue between the convoluted seminiferous tubules showing eccentric nucleus with marginated heterochromatin, Mitochondria (M) in fine granular cytoplasm.

Figure 8



Transmission electron micrographs of rat testis from tramadol treated adult group; A) different thickened basement membrane (arrow), Spermatogonia (Sg) with clumped hetero chromatin oval nucleus, vacuolated mitochondria (M) in rarified cytoplasm, Sertoli cell showing round indented euchromatic nucleus (N), mitochondria (M), RER, lysosomes (Ls), lipid droplets, disorganization of germ cells; round spermatid (RS) appear between primary spermatocytes (Ps) showing preacrosomal vesicle, Pyknotic germ cell (star), another degenerated (Sg) on left side with shrunken nucleus. B) Cross section of middle piece of spermatozoa showing complete degeneration and disorientation of axonemal structure microfilaments with rapture of plasma membrane sheath (arrow), Others showing normal appearance, intercellular spaces between spermatids (thick arrow) also vacuolated marginated mitochondria.

Figure 9



Transmission electron micrographs of rat testis from PSE + tramadol co-treated adult group; A) Leydig cells (Lc) appeared on the interstitial spaces showing elongated nucleus with marginated heterochromatin and mitochondria on cytoplasm (M), Basal lamina (LP) of the seminiferous tubule appear irregular and different thickened (arrow), huge increase of sertoli cell (Sc) number notified dispersed on the stroma. B) Mature elongated spermatid (Esp) showing pyriform nucleus with condensed chromatin and acrosomal cap, partially preserved ectoplasm specialization (arrow head), round spermatid (N) with ruptured nuclear envelope and rarified reduced cytoplasm, loss of mitochondria, vacuolated mitochondria (V) of another round spermatid and swollen lysosomes (Ls).

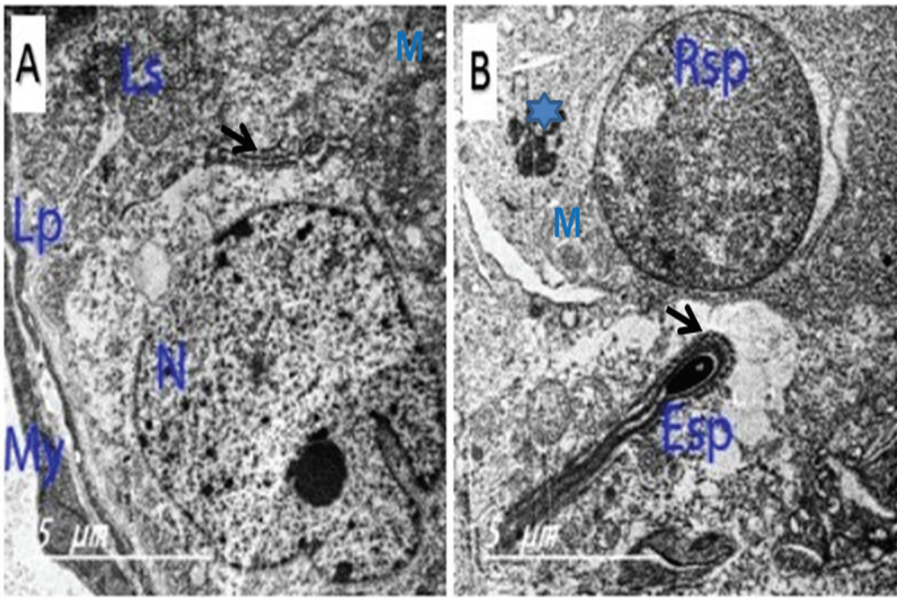
the mid piece. There was fusion of the outer dense fibers with 9+2 microfilaments, as well as disorientation. Vacuolated mitochondria with disrupted cristae on the margins of round spermatids and dilatation of SER were also observed (Fig. 11b).

The ultrastructural changes of the PSE+tramadol-treated group were comparable to those of the tramadol group: shrunken spermatogonia with clumped heterochromatin and rounded nuclei, numerous Sertoli cells with rough granulated chromatin on nuclei, swollen vacuolated lysosomes and mitochondria on the cytoplasm, apoptic pyknotic germ cells with shrunken vacuolated nuclei and numerous swollen mitochondria with disrupted cristae, swollen vacuolated SER (Fig. 12a), normal elongated spermatids with normal appearance showing pyriform nuclei with condensed chromatin and acrosomal cap, postacrosomal bodies and manchettes, round spermatids with ill-defined nuclear envelope and absence of acrosomal cap, and mitochondria on the cytoplasm (Fig. 12b).

Discussion

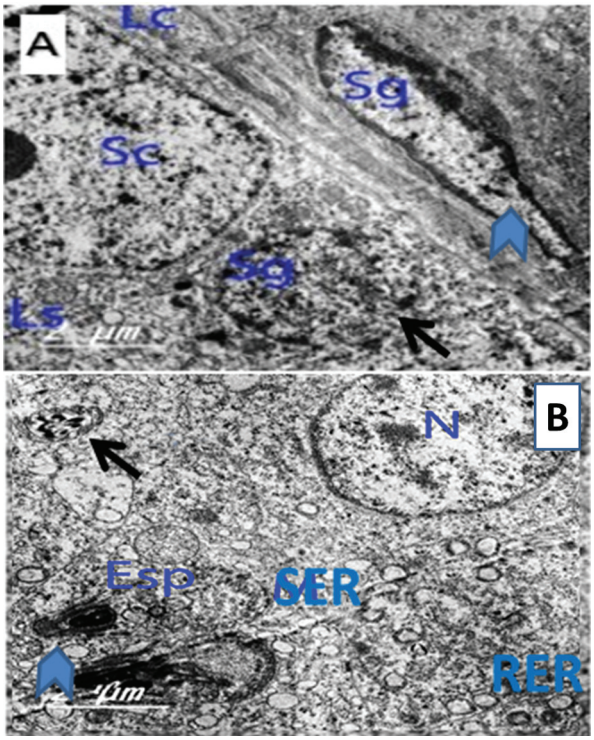
Nowadays narcotic drugs are widely misused by different age groups, especially young men, and

Figure 10



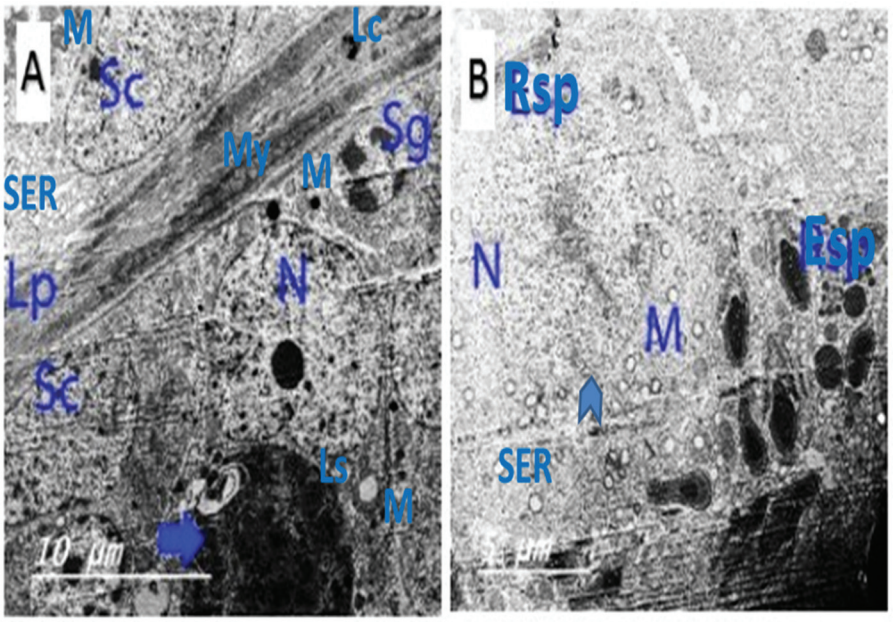
Transmission electron micrographs of rat testis from control adolescent group; a) Basal lamina (Lp) of seminiferous tubule and myoid cell (My), sertoli cell showing triangular euchromatic nucleus (N), mitochondria (M), RER and lysosomes (Ls) in cytoplasm, Part of tight junction is also seen (arrow), b) Mature spermatid (Esp) showing pyriform nucleus with condensed chromatin, acrosomal cap (arrow) and manchettes or tail region, round spermatid (Rsp) also appear with round nucleus, dense bodies (star) on cytoplasm, Parts of tight junctions appear.

Figure 11



Transmission electron micrographs of rat testis from tramadol treated adolescent group; a) showing 2 seminiferous tubules and interstitial cells (LC) in-between containing blood vessels, sertoli cell (Sc) resting on cell membrane showing nucleus (N) with granulated chromatin and prominent nucleolus and spermatogonia (Sg) showing ill-defined nuclear envelope (arrow), malformed spermatogoia resting on cell membrane showing elongated nucleus with clumped heterochromatin marginated on the periphery and rarified cytoplasm (arrow head), b) Round spermatid (Rsp) showing granulated nucleus with complete loss of acrosomal cap, mitochondria (M) and dilatation of SER on the cytoplasm, cross section of spermatozoa showing complete disruption, mitochondrial membrane got degenerated, fusion of outer dense fibers with 9+2 microfilaments and disorientation, rapture of plasma membrane (arrow) and partially preserved ectoplasm specialization (arrow head).

Figure 12



Transmission electron micrographs of rat testis from PSE + tramadol co-treated adolescent group; a) Basal lamina (Lp) with myoid cell (My) and leydig cell (Lc) on interstitial showing marginated clumped heterochromatin on nucleus, blood vessel also appear. Shrunken spermatogonia (Sg) with clumped heterochromatin rounded nucleus, pyknotic germ cell (arrow), numerous sertoli cells (Sc) with fine chromatin on nucleus and prominent nucleolus, swollen vacuolated lysosomes and mitochondria on cytoplasm. b) Elongated spermatid (Esp) with normal appearance showing pyriform nucleus with condensed chromatin and acrosomal cap, post acrosomal bodies and manchettes, Round spermatid with round nucleus (Rsp), ill-defined nuclear envelope (arrow head) and lack acrosomal cap, mitochondria on cytoplasm.

many recent studies have shown that it affects fertility in different ways. Data from the present study showed that tramadol negatively affects the testicular function in both adolescent and adult male rats. It caused many alterations, such as irregular shape of seminiferous tubules with marked depletion of the spermatogenic cell populations. Marked loss of germ cells and degeneration of many cells, apoptosis, pyknotic nucleus, vacuolated cytoplasm, wide intercellular spaces, congested blood vessels, and hemorrhage were noticed. Exfoliation of germ cells and spermatids in tubular lumen were found also in many seminiferous tubules. These results match those of previous studies [30,31].

The histological findings were confirmed by the ultrastructural examination of the seminiferous tubules. It was noticed that tramadol caused a serious degenerative change in adult testicular tissue like many disrupted mitochondria with fewer cristae, SER dilatation and swollen lysosomes in the Sertoli cells, degeneration of the nuclei of spermatogonia, pyknotic, apoptic, and shrunken nuclei, vacuolation of the cytoplasm, and complete degeneration and disorientation of axonemal structure microfilaments with rupture of plasma membrane sheath in some cross-sections of spermatozoa, as well as head cap loss and degeneration. These features were in agreement with the findings of Ghoneim *et al.* [32], who suggested that the observed mitochondrial changes might be considered as early manifestation of apoptosis and an adaptative process to unfavorable environments such as excess exposure of the cell to free radicals at the level of intracellular organelles. These suggestions might be confirmed by the successful suppression of these changes by free radical scavengers [33]. The present study also showed that PSE administration negated the adverse effect of tramadol on the testes when coadministered. Regarding the wide intercellular spaces, Lanning *et al.* [34] postulated that exfoliation of germ cells may be due to disruption of Sertoli/germ cell junctions leading to loss of adhesion or it may be due to disruption of Sertoli cell cytoskeletal fibers leading to sloughing of apical Sertoli cell cytoplasm and attached germ cells. Lipid peroxidation of the cellular membrane may eventually result in dysfunction and structural damage of the cell [34]. The congested blood vessels and hemorrhage could be due to endothelial and smooth muscle cell apoptosis [35]. Other changes, such as shrunken spermatogonia, separation of spermatogonial cells from each other and from the basal lamina, as well as mitochondrial vacuolization, could also be considered preapoptotic signs [36]. It was reported that the cytoplasmic vacuoles could be a product of a

fusion of several wide cisternae of SER [37]. Regarding the degeneration of the plasma membrane of the head piece, middle piece, and principal piece of sperm cells, peroxidation of sperm lipids destroys the structure of the lipid matrix in the membranes of spermatozoa and it is associated with the rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability, and increased mid-piece morphological defects. It also completely inhibits spermatogenesis in extreme cases [38,39]. All the histopathological changes in the seminiferous tubules in this study could be due to hormonal effect and not a consequence of a direct effect [40]. In addition, authors revealed that tramadol induces a decrease in testosterone and oxidative stress in testicular tissues by increasing nitric oxide and lipid peroxidation levels in the testicular tissue, and decreasing the antioxidant enzyme activities significantly, compared with the control group [31,41].

The oral coadministration of 40 mg/kg of PSE with tramadol was able to protect the testicular tissue from damage caused by tramadol in both adult and adolescent groups. These results were confirmed by the ultrastructural examination. Abd El-Ghany *et al.* [26] also postulated that pumpkin improves the sexual health status. These results were in agreement with those of Xanthopoulou and colleagues [24,25], who reported that the protective and improvability effects of PSE are possibly related to its high amount of antioxidants and free radical scavenging ability and stimulatory effect of ATP production. Flavonoids have been used in the treatment of reproductive endocrine diseases in men and women [42]. Also flavonoids have profound effects on semen quality and the functionality of the accessory sex organs [43].

Conclusion

Tramadol exerts different adverse effects on the testes of both adult and adolescent rats. PSE demonstrated a prophylactic effect on tramadol-induced testicular damage due to antioxidant activity and free radical scavenging ability from the rich content of polyphenols and flavonoids. Apparently PSE showed less improvement in the testes of the adolescent group compared with the adult group.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Osterloh G, Friderichs E, Felgenhauer F, Günzler WA, Henmi Z, Kitano T, *et al.* General pharmacological studies on tramadol, a potent analgetic agent. Arzneimittelforschung 1978; 28:135–151.

2 Leppert W, Łuczak J. The role of tramadol in cancer pain treatment – a review. Support Care Cancer 2005; 13:5–17.

3 El-Gaafarawi II. Biochemical toxicity induced by tramadol administration in male rats. Egypt J Hospital Med 2006; 23:353–362.

4 Lamont L, Mathews K. Opioids, nonsteroidal anti-inflammatories and analgesic adjuvants. In: Tranquilli WJ, Thurmon JC, Grimm KA, editors. Lumb and Jones's veterinary anaesthesia and analgesia. Ames, IA: Blackwell Publishing; 2007:241–271.

5 Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Micó JA. Antidepressant-like effect of tramadol and its enantiomers in reserpinized mice: comparative study with desipramine, fluvoxamine, venlafaxine and opiates. J Psychopharmacol 2004; 18:404–411.

6 Salem EA, Wilson SK, Bissada NK, Delk JR, Hellstrom WJ, Cleves MA. Tramadol HCL has promise in on-demand use to treat premature ejaculation. J Sex Med 2008; 5:188–193.

7 Stitik TP, Altschuler E, Foye PM. Pharmacotherapy of osteoarthritis. Am J Phys Med Rehabil 2006; 85:S15–S28.

8 Adverse Drug Reactions Advisory Committee. Tramadol – four years' experience. Aust Adv Drug React Bull 2003; 22:2.

9 Spiller HA, Gorman SE, Villalobos D, Benson BE, Ruskosky DR, Stancavage MM, Anderson DL. Prospective multicenter evaluation of tramadol exposure. J Toxicol Clin Toxicol 1997; 35:361–364.

10 Shadnia S, Soltaninejad K, Heydari K, Sasanian G, Abdollahi M. Tramadol intoxication: a review of 114 cases. Hum Exp Toxicol 2008; 27:201–205.

11 Loughrey M, Loughrey C, Johnston S, O'Rourke D. Fatal hepatic failure following accidental tramadol overdose. Forensic Sci Int 2003; 134:232–233.

12 Garrett P. Tramadol overdose and serotonin syndrome manifesting as acute right heart dysfunction. Anaesth Intensive Care 2004; 32:575–577.

13 Daubin C, Quentin C, Goullé JP, Guillotin D, Lehoux P, Lepage O, Charbonneau P. Refractory shock and asystole related to tramadol overdose. Clin Toxicol 2007; 45:961–964.

14 Mugunthan N, Davoren P. Danger of hypoglycemia due to acute tramadol poisoning. Endocr Pract 2012; 18:e151–e152.

15 Adisa W, Okhiai O, Bankole J, Iyamu O, Aigbe O. Testicular damage in *Telfairia occidentalis* extract treated Wistar rats. Am J Med Biol Res 2014; 2:37–45.

16 Oboh G. Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (*Telfairia occidentalis*) leaves against garlic-induced oxidative stress. J Med Food 2005; 8:560–563.

17 Popovic M. On growing squash and pumpkin (*Cucurbita sp.*) in Yugoslavia. Contemp Agr 1971; 11:59–71.

18 Jia W, Gao W, Tang L. Antidiabetic herbal drugs officially approved in China. Phytother Res 2003; 17:1127–1134.

19 Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 2005; 99:325–348.

20 Mansour E, Dworschak E, Pollhamer Z, Gergely A, Hovari J. Pumpkin and canola seed proteins and bread quality. Acta Alimentaria 1999; 28:59–70.

21 Glew R, Glew R, Chuang LT, Huang YS, Millson M, Constans D, *et al.* Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita spp*) and *Cyperus esculentus* nuts in the Republic of Nigeria. Plant Foods Hum Nutr 2006; 61:49–54.

22 Yadav M, Jain S, Tomar R, Prasad G, Yadav H. Medicinal and biological potential of pumpkin: an updated review. Nutr Res Rev 2010; 23: 184–190.

23 Christian A. Fluted pumpkin (*Telfairia occidentalis* Hook F.) Seed: a nutritional assessment. Electron J Environ Agric Food Chem 2007; 6:1787–1793.

24 Xanthopoulou MN, Nomikos T, Fragopoulou E, Antonopoulou S. Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Res Int 2009; 42:641–646.

25 Egbunike G, Branscheid W, Pfisterer J, Holtz W. Changes in porcine sperm lactate dehydrogenase isoenzymes during sperm maturation. Andrologia 1986; 18:108–113.

26 Abd El-Ghany M, Dalia AH, Soha M. Biological study on the effect of pumpkin seeds and zinc on reproductive potential of male rats. In: The 5th Arab and 2nd International Annual Scientific Conference on Recent Trends of Developing Institutional and Academic Performance in Higher Specific Education Institutions in Egypt and Arab World; 14–15 April 2010; Mansoura, Egypt: Faculty of Specific Education, Mansoura University; 2010. pp. 2384–2404.

27 Robinson G, Gray T. Electron microscopy 2. Tissue preparation, sectioning and staining. In: Bancroft JD, Stevens A, editors. Theory and practice of histological techniques. London, UK: Churchill Livingstone; 1990:525–562.

28 Meek GA. Practical electron microscopy for biologist. New York, NY: Wiley Interscience; 1970: 406.

29 Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 1963; 17:208–212.

30 El-Ghawet HA. Effects of tramadol on the reproductive function of Wistar albino rats. Eur J Exp Biol 2015; 5:56–64.

31 Ahmed MA, Kurkar A. Effects of opioid (tramadol) treatment on testicular functions in adult male rats: the role of nitric oxide and oxidative stress. Clin Exp Pharmacol Physiol 2014; 41:317–323.

32 Ghoneim FM, Khalaf HA, Elsamanoudy AZ, Helaly AN. Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. Int J Clin Exp Pathol 2014; 7:7323–7341.

33 Wakabayashi T. Megamitochondria formation physiology and pathology. J Cell Mol Med 2002; 6:497–538.

34 Lanning LL, Creasy DM, Chapin RE, Mann, Peter C, Barlow, Norman J *et al.* Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol 2002; 30:507–520.

35 Beltrame FL, Cerri PS, Sasso-Cerri E. Cimetidine-induced Leydig cell apoptosis and reduced EG-VEGF (PK-1) immunoexpression in rats: evidence for the testicular vasculature atrophy. Reprod Toxicol 2015; 57:50–68.

36 Soliman HM, Wagih HM, Attia GM, Algaidi SA. Light and electron microscopic study on the effect of antischizophrenic drugs on the structure of seminiferous tubules of adult male albino rats. Folia Histochem Cytobiol 2014; 52:335–349.

37 Goluža T, Boscanin A, Cvetko J, Kozina V, Kosović M, Bernat MM, *et al.* Macrophages and Leydig cells in testicular biopsies of azoospermic men. BioMed Res Int 2014; 2014:1–14.

38 Lenzi A, Gandini L, Maresca V, Rago R, Sgro P, Dondero F, *et al.* Fatty acid composition of spermatozoa and immature germ cells. Mol Hum Reprod 2000; 6:226–231.

39 Türk G, Sönmez M, Aydin M, Yüce A, Gür S, Yüksel M, *et al.* Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin Nutr 2008; 27:289–296.

40 Movahed E, Nejati V, Sadrkhanlou R, Ahmadi A. Toxic effect of acyclovir on testicular tissue in rats. Iran J Reprod Med 2013; 11:111–118.

41 Abdellatief R, Elgamal D, Mohamed E. Effects of chronic tramadol administration on testicular tissue in rats: an experimental study. Andrologia 2015; 47:674–679.

42 Qin DN, She BR, She YC, Wang JH. Effects of flavonoids from Semen Cuscutae on the reproductive system in male rats. Asian J Androl 2000; 2:99–102.

43 Das S, Parveen S, Kundra CP, Pereira BM. Reproduction in male rats is vulnerable to treatment with the flavonoid rich seed extracts of *Vitex negundo*. Phytother Res 2004; 18:8–13.