Original Article

Tramadol Abuse Induced Testicular Toxicity in Adult Male Albino Rats: An Experimental Histopathological Ultrastructural Study

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ABSTRACT

Introduction: The synthetic opioid tramadol works on the brain and spinal cord to lessen pain in adults. It is applied therapeutically in order to relieve moderate to severe pain that other painkillers are unable to relieve. Even so, it has the potential to become addictive and habit-forming.

Aim of the Work: Our present investigation aimed to explore the effects of three different therapeutic and narcotic drug-addicts doses of tramadol, through three different durations, using two different modes of administration on testicular structure.

Materials and Methods: For 10, 20, and 30 days, 120 male adult rats (Rattus norvegicus) were divided into control and treatment groups and administered varied dosages of tramadol (20 mg, 40 mg, and 80 mg/kg/day, i.p. and oral). For the ultrastructural study, tissue from the testicles was collected.

Results: Tramadol doses of 40 and 80 mg/kg were administered by either i.p. injection or oral treatment for 20 and 30 days caused significant histopathological degenerative changes on rat's testes. The most common findings were alterations on the cellular membranes combined with apoptotic nuclei, vacuolated mitochondria and cytoplasmic vacuoles with degeneration of cells matrices. It also led to ruptures of nuclear and cell membranes with exudation of nuclear matrix, degeneration of the rough endoplasmic reticulum, Smooth endoplasmic reticulum proliferation, cellular necrosis and degeneration of mitochondrial cristae. There were degenerated Leyding cells with large vacuoles and enlarged Sertoli cells.

Conclusion: These findings might provide a reasonable justification for the delayed or even complete infertility linked to tramadol abuse, which may highlight the need for developing a national awareness campaign to highlight the risks of tramadol abuse on sexual functions in order to enact more stringent regulations and laws against tramadol abuse on both therapeutic and narcotic-addict levels.

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Key Words: Dose; rats; testis; tramadol; ultrastructure.

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INTRODUCTION

Governments throughout the world are worried about the problem of drug misuse as one of the most dangerous issues, and they are now working to eradicate it[1]. This problem is no longer limited to one type of drug, one country, or one class of society due to the severe harm it causes to health, social, and economic aspects. Instead, it was emerged that directly affect male fertility rates and testicular cancer[2]. Numerous medication kinds may be harmful to men, such as opioids and analgesics, which cause oxidative stress and male infertility[3].

Tramadol hydrochloride, however, is a centralized opioid that is administered parenterally (by injection) and orally to treat pain. as of right now[4]. With fewer concerns of respiratory depression, tolerance, and dependency, tramadol is a useful analgesic for treating moderate to severe postoperative acute pain in adults, especially in those with impaired cardiorespiratory, hepatic, or renal functions[5]. Tramadol aids in pain relief by preventing the absorption of serotonin and norepinephrine and acting as an agonist for the opioid receptor that increase the inhibitory effects on pain transmission in the spinal cord[6].

At 2014, according to the World Health Organization (WHO), tramadol can be used as a therapy of pain in several international medical guidelines. In the WHO recommendations for therapy of pains of cancer, this drug is described as a step-two analgesic. Additionally, tramadol is included on numerous national essential medication lists[7]. Several reports indicated that many youth can be died because of overdose of tramadol[8].

In addition, there are many indicators that tramadol drug is taken in different African and West Asian nations, particularly in view of the expanded utilization of these medications in North and West Africa. Gaza, Egypt, Lebanon, Jordan, Mauritius, Libya, Togo, and Saudi Arabia all reject tramadol abuse. Egypt has raised the tramadol table for 2009 given to the rising rate of abuse of it. Given to several websites' extensive user testimonials on tramadol's non-medical uses, tramadol is easily offered online without a prescription[9].

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According to the results of a national survey, 9.6% of Egyptians used illicit drugs (drugs that are illegal to have), at least once in their lifetime[10]. Since 2008, tramadol use has increased dramatically in Egypt, prompting many people to consent to entering addiction treatment[11]. For instance, it was found that tramadol was used by 21% of permanent cleaners and 40% of temporary cleaners employed at government hospitals in Zagazig, Egypt[12].

Blood-cerebrospinal fluid and blood-brain barrier penetration of tramadol were both rapid, and the presence of its metabolites in cerebrospinal fluid (CSF) was less than that in plasma[13].

The histological changes in testes of the adult male rats shed insight on the potential hazards of increased liver, renal, neurological, and sexual dysfunction brought on by long-term, repetitive tramadol administration[14]. Generalized tonic clonic seizures are the most frequent manifestation of tramadol neurotoxicity, which often occurs within 24 hours of tramadol administration. These seizures were more prevalent in participants who were also taking alcohol, illegal drugs, antipsychotic medications, or antidepressants[15].

The mechanism behind tramadol's analgesic effects is too complex since the liver is now in charge of tramadol's metabolism and excretion to lessen the danger of major liver damage[16]. N-desmethylation to N-desmethyltramadol (M2) by CYP2B6 (Cytochrome P450 Family 2 Subfamily B Member 6) and CYP3A4 and O-demethylation to O-desmethyltramadol (M1) by CYP2D6 are the two main mechanisms by which tramadol is metabolised in the liver (drug-metabolizing enzyme, Cytochrome P450 Family 2 Subfamily D Member 6).

Among the tramadol metabolites, only M1 (the sole tesofensine metabolite found in human plasma) is pharmacologically active. Compared to the parent medication, it is more selective for receptors and has a greater affinity for opioid receptors[17]. It is converted in the liver to O-desmethyl-tramadol, a substance that is 2-4 times more effective and influential than[18].

Numerous investigations have shown that the therapeutic effects of tramadol, including its analgesic effectiveness, may result from both opioid and non-opioid routes. Tramadol, a drug, binds to the -opioid receptor even though it is far less potent than morphine. Similar to antidepressant medications like amitriptyline and desimipramine, it inhibits norepinephrine and serotonin neuronal reuptake[19].

Genital examinations showed that taking tramadol at a dose of 80 mg per kilogramme of body weight for about a month had an effect on the activity of sex hormones in male rats at 20 and 30 days of therapy compared to the control group, while tramadol at a dose of 40 mg per kilogram of body weight had a less significant effect compared to both the 80 mg tramadol treated and the control groups[20].

Experimental studies[21] examining the testicles of tramadol-treated rats revealed that the seminiferous tubules had irregularly shaped, widely separated separations from one another, and were devoid of spermatozoa. Multi-nucleated big cells, apoptotic cells, and spermatogenic cells all had an abundance of vacuoles. There were less Leydig (interstitial) cells overall, and the intertubular tissue showed degeneration and an abundance of inflammatory cells. The majority of the spermatogenetic cells were found out to be disorganized and injured, as shown by the wounded germ cells' propensity to exfoliate in the seminiferous tubules' core cavity and thicken the basal lamina. Despite the fact that certain spermatids in this collection of rat testes seemed to have decreased nuclei, the basal lamina and spermatogonic cells of the seminiferous tubules were found to be unaltered. In the majority of the seminiferous tubules, they also counted the quantity of sperm tufts and extra layers of spermatogenic cells. The seminiferous tubules were spaced closer together.

After receiving an oral tramadol dose of 40 mg/kg, a second experimental study found that the testicles exhibited acute widespread testicular degeneration, spermatid giant cell formation, and an abundance of spermatocytes. There was no spermatogenesis, however. spermatocytes often had a necrotic appearance[22].

Later, Abdellatief and his colleagues arrived to the conclusion that tramadol affects the normal histology of rat testicles and alters the amounts of sex hormones. Issues with male infertility might result from this result[23].

However, Ahmed & Kurkar mentioned that tramadol significantly increased testicular lipid peroxidation and nitric oxide levels while decreasing the activity of anti-oxidant enzymes when compared to the control group[24]. Their immunohistochemical investigations revealed an increase in endothelial nitric oxide synthase expression in the testicular tissues. They came to the conclusion that the function of adult male rats' testicles is impacted by tramadol use, and that this impact may be caused by the drug's activation of oxidative stress and excessive nitric oxide generation. There have been cases of sexual dysfunction caused by the administration of different medications during pharmacological therapy.

Minisy and colleagues' recent research[25] found that despite tramadol toxicity being higher in adolescence to be totally protected, pomegranate seed extract has a protective effect against tramadol-induced testicular damage that occurs in both adult and teenage ages.. Pomegranate seeds' high concentrations of antioxidant compounds may have this protective effect.

Most recently, Adelakun and colleagues[26] found that Tramadol significantly lowers sperm quality. Tramadol-treated mice also exhibited problems with spermatogenesis and testicular degeneration.

AIM OF THE WORK

Our investigation examined the effects of acute tramadol injection and oral usage on rat testes and their

reproductive rates in response to the recent rise in tramadol addiction. Furthermore, we attempted to employ reliable ultrastructural techniques to assess all possible traumatic histopathological tramadol toxic effects on adult male rats' testes. This was done to contribute onto the public's growing awareness of tramadol's risky side effects and the potential for drug abuse by addicts.

MATERIALS AND METHODS

Drugs

a. Solutions of tramadol (100 mg/2ml ampoules, tramadol hydrochloride) was purchased from ADWIA Co. S.A.E. in 10th of Ramadan City, Egypt.

b. Tablets containing 225 mg of tramadol hydrochloride from Sigma Pharmaceutical Industries (S.P.I) SAE, Egypt, was utilised.

- All other compounds were of the analytical kind and were obtained from regular industrial vendors.
- Animal Grouping & Experimental Protocol:

120 adult male Rattus norvegicus rats weighing 180 to 200g were used in the current study (Provided from Animal house of the Faculty of Agriculture Suppliers, Minia University, Minia, Egypt). All animal experiments were performed upon approval by the Animal Experiment Committee of National Research Center, Egyp. Rats were randomly assigned to one of three main groups (I, II, or III) and four subgroups (a, b, c, or d). There were a total of "9" experimental groups (Ia, Ib & Ic; IIa, IIb & IIc and IIIa, IIIb & IIIc) and "3" control groups (Id, IId & IIId) with ten rats in each (n=10). Five rats of each group received the calculated dose of tramadol through intraperitoneal injection, and the other five were given the calculated dose of tramadol orally by gastric gavages; as 20, 40, 80 mg (1 ml T HCL/day for sub-groups a, b, & c respectively, while the control sub-groups d were given by 1 ml/day of the saline)

Rats were sacrificed after they were anesthetized by ether inhalation (2 ml) for about 2 min in a transparent acrylic jar[27] and their testes were collected from the main groups (I, II & III) after both intra-peritoneal injection and oral gastric gavages administration of the calculated dose for 10, 20 & 30 days respectively (Table I).

Table I: Tramadol Administration Grouping, Dosage and Duration

Ser.	Group	Intra-peritoneal Injection			Oral Administration via Gastric Gavage		
		No. of Rats	Dose Tramadol HCL 100 mg/2 ml Ampoules in Saline Solution	Duration	No. of Rats	Dose Tramadol HCL 100 mg/ Tablet Suspended in Saline Solution	Duration
1	Group I a	5	20 mg (T HCL 1 ml/day)	10 days	5	20 mg (T HCL 1 ml/day)	10 days
2	Group I b	5	40 mg (T HCL 1 ml/day)	10 days	5	40 mg (T HCL 1 ml/day)	10 days
3	Group I c	5	80 mg (T HCL 1 ml/day)	10 days	5	80 mg (T HCL 1 ml/day)	10 days
4	Group I d	5	Control (1 ml/day Saline Solution)	10 days	5	Control (1 ml/day Saline Solution)	10 days
5	Group II a	5	20 mg (T HCL 1 ml/day)	20 days	5	20 mg (T HCL 1 ml/day)	20 days
6	Group II b	5	40 mg (T HCL 1 ml/day)	20 days	5	40 mg (T HCL 1 ml/day)	20 days
7	Group II c	5	80 mg (T HCL 1 ml/day)	20 days	5	80 mg (T HCL 1 ml/day)	20 days
8	Group II d	5	Control (1 ml/day Saline Solution)	20 days	5	Control (1 ml/day Saline Solution)	20 days
9	Group III a	5	20 mg (T HCL 1 ml/day)	30 days	5	20 mg (T HCL 1 ml/day)	30 days
10	Group III b	5	40 mg (T HCL 1 ml/day)	30 days	5	40 mg (T HCL 1 ml/day)	30 days
11	Group III c	5	80 mg (T HCL 1 ml/day)	30 days	5	80 mg (T HCL 1 ml/day)	30 days
12	Group III d	5	Control (1 ml/day Saline Solution)	30 days	5	Control (1 ml/day Saline Solution)	30 days

O Treated but without ultrastructural alterations
O Treated & revealed ultrastructural alterations

For transmission electron microscopy (TEM), 2-3 mm pieces of testicles were bathed for two hours at room temperature in 2.5% glutaraldehyde buffered with 0.1M cacodylate (pH 7.2). The samples were post-treated in phosphate-buffered 1% osmium tetroxide for two hours at room temperature after being submerged in buffer solution for three 15-minute washes, followed by a final 12-minute rinse. Fixed tissues are dried in ethanol at increasing concentrations, up to 100%, embedded in epoxy resin, trimmed, and then finally cut into 50-70 nm slices using an ultratome[25]. Then, ultrathin slices were gathered on copper grids with a mesh size of 300, coloured with 2% aqueous uranyl acetate, and examined with an 80 kV Joel electron microscope (Japan).

Statistical analysis

The data was displayed as mean standard deviation. One-way analysis of variance (ANOVA), followed by the LSD post hoc test, was used to analyse the data and information in order to determine the significance of the mean between the groups (SPSS 16.0). *P* 0.05 was used to determine statistical significance.

RESULTS

Control group

The control group (Figure 1), essentially displayed the normal seminiferous tubules, which were found to be encased by myoid cells and bordered by basement membranes. Spermatogenic Sertoli cells lined them. The Sertoli cells had large, indented nuclei that were covered in euchromatin. The main spermatocyte had large, euchromatin-containing nuclei that was rounded to oval. In the cytoplasm, one can observe numerous rounded to oval mitochondria with expanded cristae, well-defined golgi apparatus, and rough endoplasmic reticula.

The initial characteristics of spermatogonia were large, spherical nuclei with peripheral heterochromatin in their borders and a normal basement membrane The initial characteristics of spermatogonia were large, spherical nuclei with peripheral heterochromatin on their borders and a normal basement membrane. Additionally, normal Gologi complexes, mitochondria with normal cristae, smooth and rough endoplasmic reticula, were seen also normal (Figure 2). Additionally, normal Golgi complexes, mitochondria with normal cristae, smooth and rough endoplasmic reticula with normal size were observed as well.

Secondary spermatocytes were distinguished by having acrosomal caps on one side of the cells, large, spherical nuclei, and mitochondria that seemed small and peripheral (Figure 3).

When spermatids initially began to form, their cells were long and elongated, with an acrosomal cap and large, oval euchromatic nuclei. Their cytoplasm seemed granular and contained vesicular mitochondria near the periphery (Figure 4).

Ultrastructural Findings in Treated Groups

Our findings for all experimental subgroups "Ia", "Ib", and "Ic" (rats were given the tramadol calculated dose, by either intraperitoneal injection or orally by gastric gavage, as 20, 40, or 80 mg/kg of tramadol for 10 days, respectively), showed almost normal ultrastructural appearances as the subgroup "Id" with negligible ultrastructural variations.

Additionally, almost identical results were found in groups "IIa" (rats received either an intraperitoneal injection or oral treatment with 20 mg/kg of tramadol for 20 days), and group "IIIa" (rats received either an intraperitoneal injection or oral treatment with 20 mg/kg of tramadol for 30 days), at which rats testes ultra thin sections appeared normal rather than modified.

However, the testes of rats in other treated groups clearly displayed ultrastructural abnormalities in the testicular tissue architecture when similar comparisons are made with the testes of the control group. According to the dosage given, the method of administration, and the length of the therapy, these structural changes were detailed in this inquiry.

Testes from rats in the group II b that had received i.p. injections of 40 mg/kg of tramadol for 20 days were examined in detail. Sections showed some ultrastructural alterations on the spermatogonial cells membrane along with apoptotic nuclei, vacuolated mitochondria, an increase in the cytoplasmic vacuoles, and a degeneration of cells matrices (Figure 5).

The primary spermatocytes in group II c (rats were injected intraperitoneally with 80 mg/kg tramadol for 20 days) displayed apoptotic nuclei, ruptured nuclear and cell membranes, nuclear matrix exudation, degeneration of the rough endoplasmic reticula, proliferation of the smooth endoplasmic reticula, cellular necrosis, and degeneration of mitochondrial cristae (Figure 6).

Testicular tissue sections from group IIb rats that received 40 mg/kg of tramadol orally for 20 days showed generally rarified cytoplasm with irregularly defined shrunken nuclei. Additionally, cell matrices were degrading and some spermatogonia seemed to have vacuolated cytoplasm. Nuclear membranes of other spermatogonia may also have an accumulation of peripheral chromatin (Figure 7).

In (Figure 8), interstitial hyperplasia was seen in Leydig cells, and the nuclei of these cells were shown to be somewhat elongated and compressed, (Rats were administered oral tramadol at a dosage of 80 mg/kg for 20 days). Leydig cell degeneration, interstitial hyperplasia, and smooth endoplasmic reticulum hyperplasia were all seen in spermatogonia encircled by connective tissue.

However, after receiving an intraperitoneal injection of 40 mg/kg tramadol for 30 days, the testes of the rats in group IIIb exhibited vacuolated mitochondria, increased cytoplasmic vacuolation, and apoptotic nuclei. Cell membranes were ruptured, releasing cellular contents, and macrophages could be seen. Additionally, smooth endoplasmic reticulum growth and rough endoplasmic reticulum degradation were observed (Figure 9).

The mitochondrial cristae seemed damaged and vacuolated, and the initial spermatocytes featured irregularly shaped membranes, a degenerating nuclear membrane, and the ejection of nuclear material into the cytoplasm. Additionally, group III c, spermatocytes were likewise surrounded by necrotic cells and collagenous fibres after receiving an intraperitoneal injection of 80 mg/kg tramadol for 30 days (Figure 10).

Secondary spermatocytes showed degradation of the acrosomal caps in addition to the findings previously described by the same group, although in a different field, III c"2" (rats were i.p., treated with 80 mg/kg of tramadol for 30 days) (Figure 11).

Moreover, some group III c "Three secondary spermatocytes were found in a third area. These spermatocytes exhibited scattered remnant bodies in their cytoplasm and degraded nuclei (rats received intraperitoneal injections of tramadol for 30 days).

Acrosomal cap degeneration and an increase in the smooth endoplasmic reticulum with a decrease in the rough endoplasmic reticulum were further results. There formed a second secondary spermatocyte with a ruptured nuclear membrane and collagen fibers (Figure 12).

Rats in group III b, however, were given a 40 mg/kg dosage of oral tramadol for 30 days. Along with cell multiplication and a rise in cytoplasmic vacuoles, degradation of the cell matrix and dense bodies were seen. Primary spermatocytes (PSs) of the testicular tissue showed nuclear matrices that were leaking, apoptotic nuclei, and damaged nuclear membranes. Degeneration of the rough endoplasmic reticulum was also seen. Lysosomal growth was detected along with smooth endoplasmic reticulum (sER) degradation. (Figure 13).

Testes from rats in group IIIc with a 30-day oral tramadol dosage of 80 mg/kg had deteriorated Leydig cells with expanded vacuoles. The smooth endoplasmic reticulum and cytoplasmic vacuoles of Sertoli cells, however, seemed to multiply even as the cell matrices and nuclear deterioration (Figure 14).

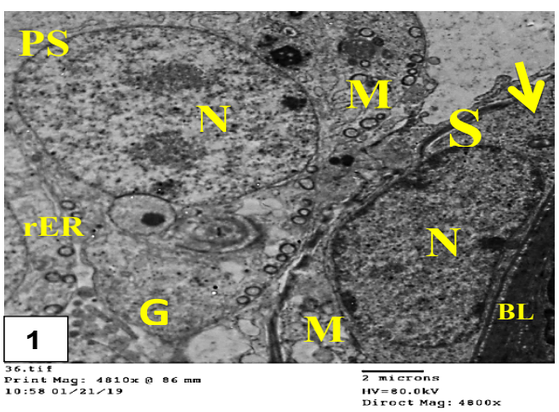

Fig. 1: An electron micrograph of a control group rat's testis reveals: Sertoli cell (S) with many rounded to oval mitochondria (M) with extended cristae, smooth endoplasmic reticulum (arrows), basal lamina (BL), and primary spermatocyte (PS) with large rounded to oval nucleus (N) with euchromatin (EU), Golgi apparatus(G) and rough endoplasmic rstuculm (rER).

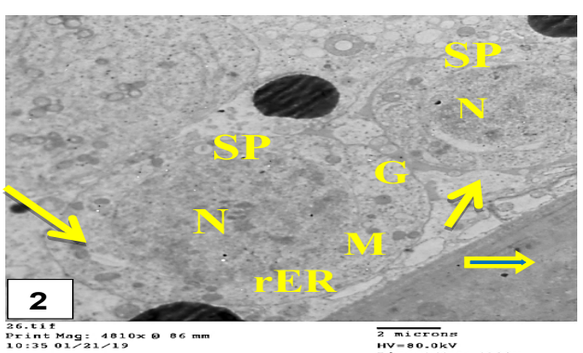

Fig. 2: An electron micrograph of the testis of a control group rat reveals spermatogonia (SP) with a round nucleus (N), mitocndaria (M), nuculous (Nu), smooth endoplasmic reticulum (thin arrows), rough endoplasmic reticulm (rER), normal basement membrane (thick arrows), and Gologi complexes (G).

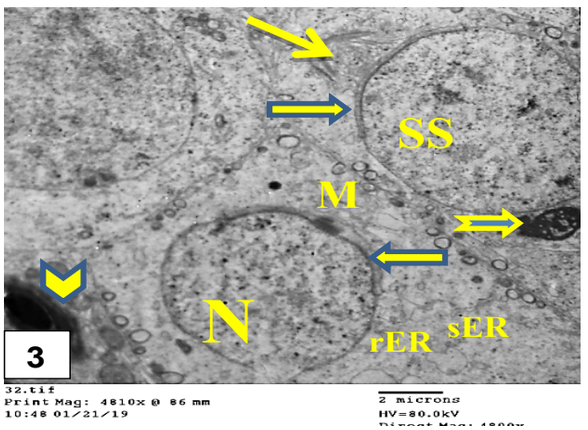

Fig. 3: An electron image of a control rat's testis shows secondary spermatocytes (SS) with an acrosomal cap (thick arrows) and a round nucleus (N), mitocndaria (M), smooth endoplasmic reticulm (sER), rough endoplasmic reticulm (rER), and Gologi complexes (arrow tails). An acrosomal cap protects the pyramidal, black nucleus mature sperm head (heads of arrows)

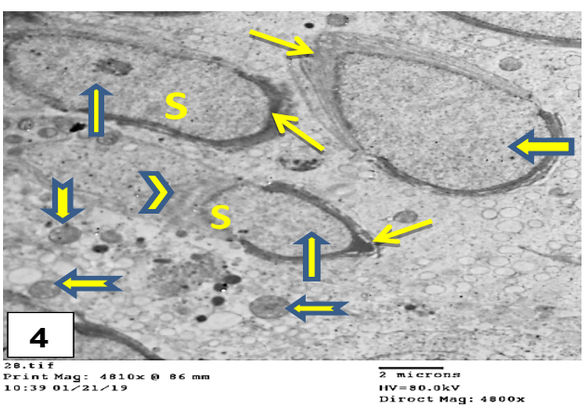

Fig. 4: An electron micrograph of the testis of a control rat reveals spermatoides (S) with an acrosomal cap and an oval nucleus, mitocndaria (arrow tails), and Gologi complexes (arrow heads).

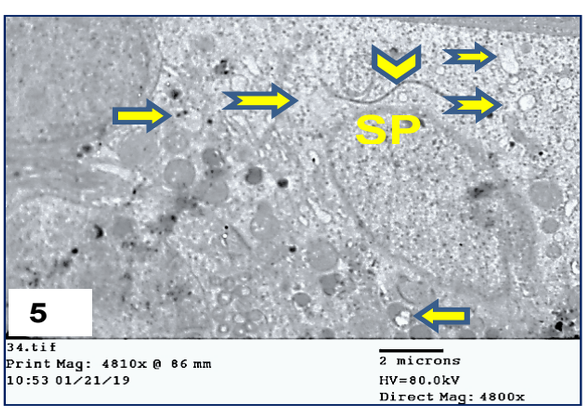

Fig. 5: An electron micrograph of rat testis section (group II b, rats were treated with 40 mg/kg of tramadol injected for 20 days) showing: A rat testis ultrathin section (group II b; rats got tramadol injections at a dosage of 40 mg/kg daily for 20 days) showing matrix degradation as well as spermatogonial membrane degeneration (arrow heads), apoptotic nuclei (N), vacuolated mitochondria (thick arrows), and enlarged cytoplasmic vacuoles (arrow tails).

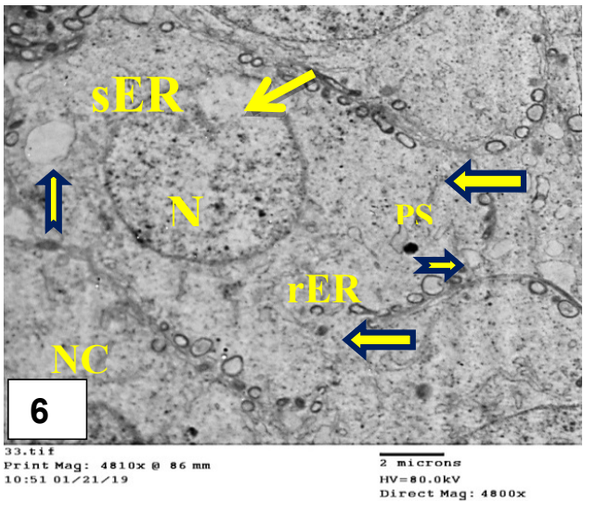

Fig. 6: An electron micrograph of a rat testis segment from group IIc animals that received tramadol (80 mg/kg intravenously over a 20-days) exhibits primary spermatocytes (PS) with apoptotic nuclei, nuclear membrane rupture, and nuclear matrix exudation (thin arrow). Rough endoplasmic reticulum (rER) degeneration, smooth endoplasmic reticulum (sER) incresase and rupture of cell membrane (thick arrows), necrotic cells (NC) and degeneration of mitochondrial cristae (arrow tails).

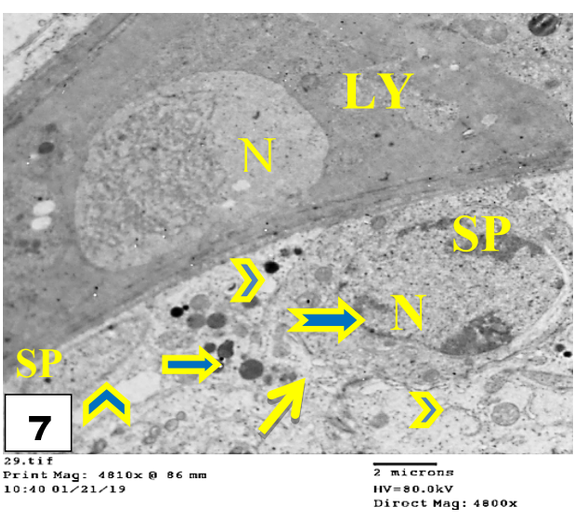

Fig. 7: In the testis of group IIb, rats administered 40 mg/kg of tramadol orally for 20 days, spermatogonia (SP) were smaller, and there were more dense bodies (thin arrows), lipid droplets (thick arrows), and hyperplasia interstitial Leydig cells, as well as cytoplasmic vacuoles with an elongated, indented nucleus (N). On the margin of the nuclear membrane, extra spermatogonia (SP) nuclei were seen. They have chromatin accumulation (arrow tails).

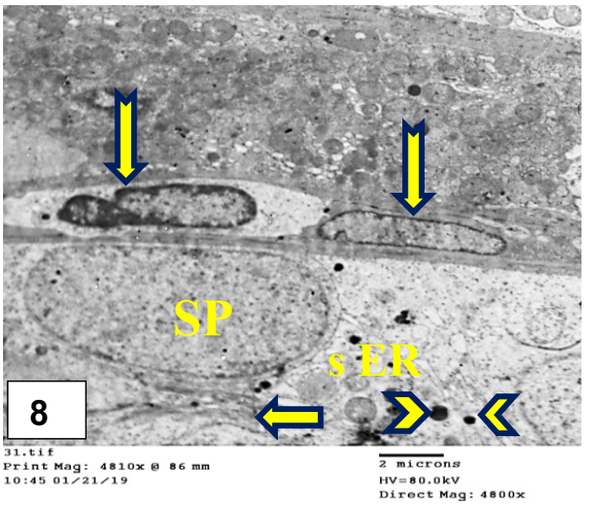

Fig. 8: Lipid droplets (arrow heads), hyperplasial interstitial degeneration of the Leydig cell's nucleus (arrow tails), and smooth endoplasmic reticulum (sER) can be seen in an electron micrograph of a rat testis segment (group II c, treated for 20 days with oral tramadol 80 mg/kg).

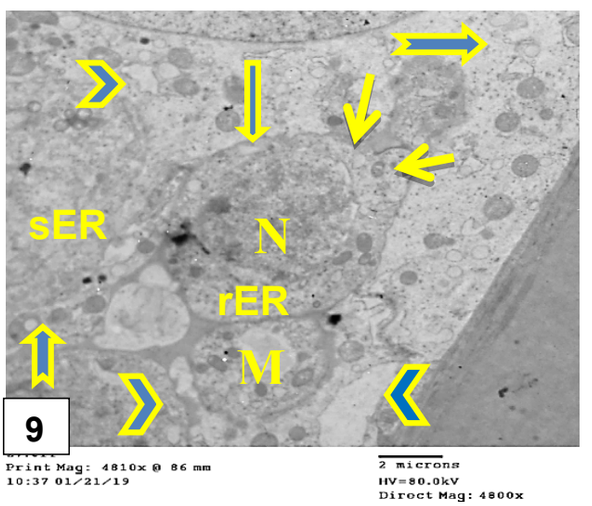

Fig. 9: An EM image for group III b, (rats were treated with 40 mg/kg of tramadol injected for 30 days): spermatogonia (SP) with matrix degeneration, dense bodies (thin arrows), ruptured cell membranes (thick arrows), and cell content exudation, as well as vacuolated macrophages (M), smooth endoplasmic reticulum (sER) proliferation, degeneration of rough endoplasmic reticulum (rER), apoptotic nuclei (N), vacuolated mitochondria (arrow tails), and increased cytoplasmic vacuoles (arrow heads).

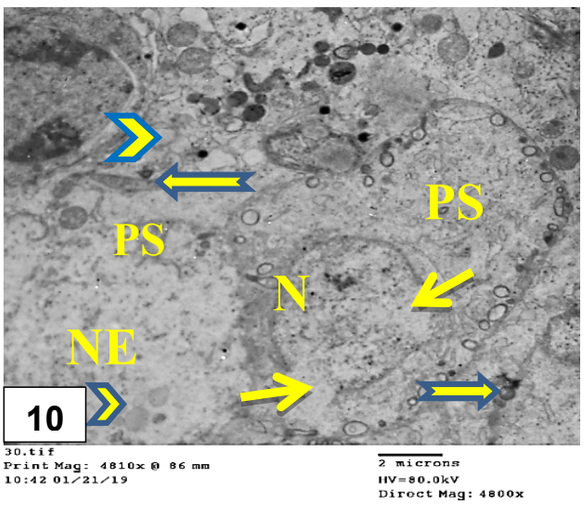

Fig. 10: An electron microscope image of a rat testis primary spermatocytes (PS) howing apoptotic nuclei, nuclear membrane rupture, nuclear matrix exudation (arrows), necrosis (NE), collagen fibres (arrow tails), and vacuolated mitochondria (arrow heads), (group III c"1" animals were given an injection of 80 mg/kg tramadol for 30 days).

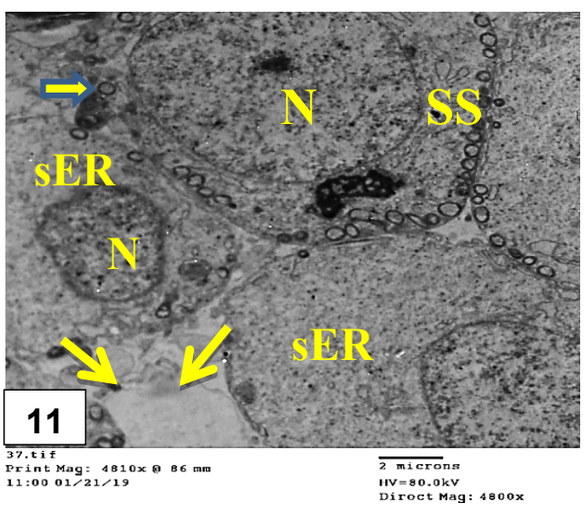

Fig. 11: A testis ultrathin section from a group III c"2 (rat that received tramadol injections at an 80 mg/kg dose for 30 days) showing secondary spermatocytes (SS) with degenerated acrosomes (thick arrows), oval nuclei (N).However, primery spermatocytes exhibited apoptotic nuclei (N), and proliferationof smooth endoplasmic reticulm (sER) with large nuclei (thin arrows).

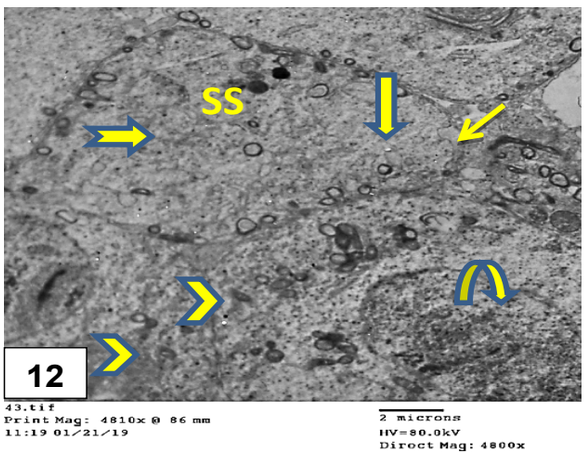

Fig. 12: In a testis slice from a rat treated with 80 mg/kg tramadol for 30 days (III c 3), secondary spermatocytes (SS) have degenerated acrosomes (thin arrows), and degenerated nuclei (N), smooth endoplasmic reticulum proliferation (thick arrows) and rough endoplasmic reticulum degeneration (arrow tails). There were more secondary spermatocytes with collagen fibres and apoptotic nuclei (curved arrows) and collagen fibers (arrow heads).

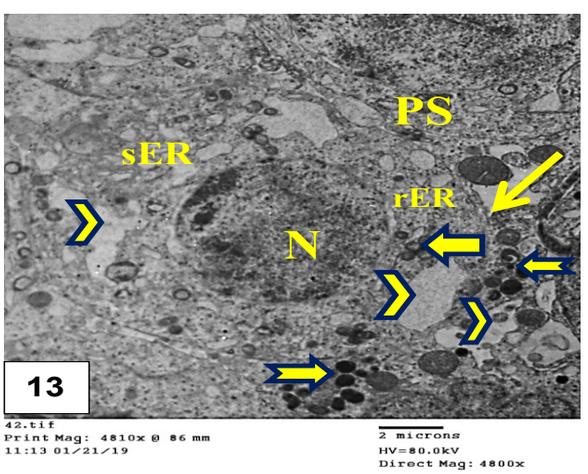

Fig. 13: An electron microscope image of a rat testis section from group III b shows primary spermatocytes (PS) with apoptotic nuclei, nuclear membrane rupture, and nuclear matrix exudation (thin arrows), rough endoplasmic reticulum (rER) degeneration, increased cytoplasmic vacuoles (arrow heads) together with cells matrices degeneration, dense bodies (arrows tails), lysosomes (thick arrows) and degenerated smooth endoplasmic reticulum (sER) .

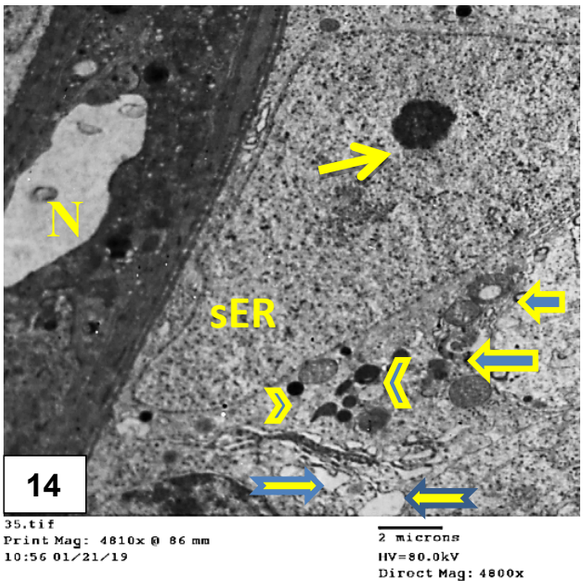

Fig. 14: A rat testis section from group IIIc(rats that given oral tramadol at a dose of 80 mg/kg for 30 days) shows interstitial Leydig cell hyperplasia with degenerated nuclei (N), Sertoli cell hyperplasia with smooth endoplasmic reticulum (sER) proliferation and degenerated nucleus (thin arrows), vacuolated mitochondria (thick arrows), and lipid accumulation (arrow heads), increased cytoplasm vacuoles (arrow tails) and degenerated cellular matrices.

DISCUSSION

Since tramadol is still widely abused in many nations and is not currently under international control as a medication for mild to severe pain in both acute and chronic modes[48], controls have been implemented at the national level in many nations, including Egypt, since 2002, with the strictest controls coming into effect in 2009[49]. Due to the fact that many people become addicted to Tramadol after repeated use, this problem is typically a source of concern.

In order to feel the euphoric and pain killing effects of tramadol, users must take greater dosages as their bodies grow accustomed to it[50]. Based on all of the previously discussed arguments, the discussion of this study was established as a trial to express the severity of tramadol's adverse effects at acute therapeutic doses as well as the drug's abuse effects among drug addicts.

Considerable studies may support the conclusion that the majority of the moderate to severe degenerative changes reported in this study for adult rat testicular tissue modifications following tramadol therapy occurred under nearly comparable conditions in terms of dosage, method of administration, and duration.

Aprioku and his colleagues have found a significant decrease in sperm motility that is obviously dose-dependent. After 30 days of oral tramadol dosing, viability and a reduction in sperm count were also observed. In addition, they found that tramadol markedly increased morphological flaws in sperms, such as deteriorated cell architecture, atrophied and corroded seminiferous tubules, and a decrease in the measure of spermatogenic cells[28]. The long-term use of tramadol at clinical dose levels, which may have a deleterious impact on testosterone levels, sperm parameters, and testicular histology, was the cause of these results, according to Aprioku and his colleagues.

In a relatively recent study, tramadol caused a noticeable decrease in sperm count, motility, and morphology while significantly increasing oxidative stress markers. Additionally, due to the loss of mitochondrial membrane potential and the expansion of mitochondria, tramadol caused mitochondrial dysfunction in the testicles[29].

Ultrastructural study of the testicular tissue cells of rats injected i.p. with 40 mg/kg b.w. for 30 days confirmed our results that tramadol significantly produced degenerative changes in adult testicular tissue cell organelles, including many mitochondria with fewer cristae. The study conducted by Ghoneim and his associates[21] backed up these traits. They administered 50 mg/kg b.w. of tramadol intravenously for 28 days, and they hypothesized that the marked mitochondrial changes could be attributed to the primary appearance of apoptosis and an adaptive response to some unfavourable conditions, such as excessive exposure of the cell to released radicals at the level of the intracellular organelles.

In addition, subcutaneous injections of 40 mg/kg b.w. of tramadol three times per week for eight weeks revealed that this treatment modality has an effect on the testicular function of adult male rats with a decreased sperm count, decreased movement, decreased amounts of early spermatocytes, recycled spermatids, and Leydig cells. They ascribed these effects to the tramadol-induced overproduction of oxidative stress and nitric oxide. The severity of our identical results may be interpreted based on the light microscopic level of investigation they used, as well as the manner and duration of tramadol injection[24].

The correlation between the increase in cellular apoptotic index and pyknotic nuclei seen in the current research and previous studies was also observed. Kroemer and co-researchers[30] subordinated nuclear pyknosis to apoptotic features. In their argument, Kumar and colleagues[31] stated that pyknosis is a process of nuclear changes connected to cell necrosis and characterised by nuclear shrinkage with increased basophilia as its DNA condenses into a solid shrunken mass. In addition, several concentric membrane lamellae were seen in the cytoplasm of primitive spermatozoa. Such structures were reported by Castejón[32] who indicated that myelin figures, which are concentric membrane lamellar formalizations, have been associated to drug administration, anoxic-ischemic circumstances, and a broad spectrum of clinical disorders.

This problem got clearer following Ibrahim and Salah-Eldin[33], they ascribed these results to various enzyme pathways. According to their results, tramadol may trigger apoptotic changes in testicular tissues. They stated that the activity of the antioxidant enzymes decreased and the

MDA (malondialdehyde) level increased, both of which were signs of oxidative stress. They found that tramadol use results in anomalies in testicular tissues connected to oxidative stress, which may support the notion that increased oxidative stress on testicular cells is caused by tramadol addiction.

Other findings of this study regarding spermatogonial cells with wide intercellular spaces, vacuolated cytoplasm, and darkly stained nuclei; and Sertoli cells with cytoplasmic vacuoles are supported by Abou Elnaga and his team[34], who administered 40 mg/kg b.w. orally for 4 and 6 weeks using the same pattern of administration. Regarding the vacuolated cytoplasm of spermatogenic cells, this might be attributable to tramadol-induced lipid peroxidation followed by membrane degradation and subsequent increase in membrane permeability[35].

However, the enlargement of the intertubular gaps seen in this research might be attributable to the precipitation of homogenous acidophilic elements in the majority of the interstitial spaces[36]. This hyaline material may be largely attributable to the overflowing lymphatic secretions penetrated by deteriorated lymphatic arteries, as well as to the free radicals and reactive oxygen species' (ROS) increase in vascular permeability[37].

Additionally, the widening of the intercellular spaces seen in the current study may be related to the disruption of blood-testis tight junctions brought on by exposure to ROS, which allows extra water and toxic agents to enter the sperm cells and ultimately causes the widening of the intercellular spaces. Furthermore, Sertoli cells, which come into touch with germ cell spares, may be responsible for the death of cell processes upon sperm cell exfoliation into the lumen of the seminiferous tubules[38].

On the nuclei of several spermatogonia and primordial spermatocytes, dilated perinuclear cisternae have been identified. Lipid peroxidation of nuclear membranes may be the cause of these modifications[31]. These observations also showed that certain spermatogonial nuclei contain vacuoles. These nuclear vacuoles may be viewed as a genotoxicity indication[39].

Early spherical spermatid cytoplasm included heterogeneous electron-dense structures[40]. This discovery may be caused by the autophagy of damaged cytoplasmic debris[41] Heterogeneous electron-dense entities have been recognised as secondary lysosomes. Certain spermatozoal heads had unusual forms; these irregularities may be attributable to spermatogenesis disruptions, which resulted in the degeneration of spermatozoa's motility and content as well as morphological defects[42].

In addition, it has been established that sperms are particularly vulnerable to oxidative damage due to the high quantity of polyunsaturated fatty acids in their cell membranes and the low levels of sweeping enzymes in their cytoplasm[43]. Some Sertoli cell cytoplasmic sER dilations may be the result of lipid peroxidation of the sER membranes[44]. This water buildup may also be the cause of the dilatations of sER.

In a light microscopic research in which male adult rats were orally fed 40 mg/kg bw for 30 days, seminiferous tubules with markedly diminished spermatogenic cell populations were observed[45]. Many seminiferous tubules lacked sperm, spermatids, and secondary spermatocytes, and the tubular lumina of many seminiferous tubules displayed exfoliation of wounded spermatocytes and spermatids. These findings coincide with those of a parallel group in which male adult rats were orally fed 40 mg/kg bw for 30 days. Our other findings concerning nuclear, endoplasmic reticulum, lysosomal, and cytoplasmic abnormalities may be attributable to our ultrastructural technique for visualising cell organelles and their interactions.

As a general note, our results differed across and among different studies based on dose and mode of administration. This was corroborated by Cannon and colleagues[46], who noted that more volumes of medication can be delivered into the abdominal cavity using the intraperitoneal (i.p.) injection technique, which is faster than taking them orally. In addition, they noted that the administration of 12.5 mg/kg b.w. of tramadol intraperitoneally did not result in any adverse effects when compared to 25 mg/kg b.w. and 50 mg/kg b.w.

Spermatogenesis is significantly regulated by extracellular matrix, particularly spermatogonia and Sertoli cells. Sertoli cells make up the blood-testis barrier (BTB), as they are in direct contact with the basement membrane, which is in turn in intimate contact with the underlying collagen network. Cross-talk between the seminiferous epithelium is facilitated by the basement membrane and the underlying collagen network, promoting Sertoli and germ cell activity[47]. This may interpret the severe connective tissue inflammation that results in the cessation of spermatogenesis in rat testes.

CONCLUSION

Our findings demonstrated that Tramadol treatment of 40 and 80 mg/kg b.w. either i.p. or oral route for 20 and 30 consecutive days displayed various unfavourable modulations on testicular ultrastructure - which may lead to cell death and therefore to reproductive difficulties - in adult male rats. This may suggest the need for a national awareness campaign that highlights the hazards of tramadol misuse on sexual functions, as well as stricter limitations and legislations on both the therapeutic and narcotic-addict levels of tramadol consumption.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة تجريبية بالمجهر الإليكتروني على ذكور الجرذان البيضاء عن أمراض وتسمم انسجة الخصية الناتجة عن سوء إستخدام الترامادول

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خلفية الدراسة: يعتبر الترامادول من المواد الأفيونية الإصطناعية التي تستخدم كمسكن للآلام لدى البالغين عن طريق تاثيره على المخ والحبل الشوكي، وبالرغم من أنه يستخدم طبيا لتخفيف الآلام الحادة والمتوسطة التي لا يمكن تخفيفها بأنواع أخرى من مسكنات الألم، إلا أنه من الممكن أن يسبب الإدمان عند الإفراط في إستخدامه.

الهدف من الدراسة: استهدفت دراستنا الحالية استكشاف الآثار الناتجة عن تعاطي ثلاث جرعات علاجية مختلفة من الترامادول لتسكين الآلام وكذلك تأثير نفس الجرعات - على سمية الخصيتين - لدى مدمني الترامادول الذين يتعاطونه كمخدر، وذلك خلال ثلاث فترات زمنية مختلفة وباستخدام طريقتين مختلفتين لتعاطيه.

التقنيات والمواد المستخدمة: تم تقسيم إجمالي عدد ١٢٠ من ذكور الجرذان البالغة من نوع (Rattus norvegicus) بشكل عشوائي إلى مجموعات للضبط والتحكم، ومجموعات أخرى معالجة تتلقى جرعات مختلفة من الترامادول (٢٠ و ٤٠ و ٨٠ ملغم / كغم / يوميا ، عن طريق الحقن داخل الغشاء البريتوني وكذلك عن طريق الفم) لمدة ١٠ و ٢٠ و ٣٠ يوم متتالية. ثم جمع أنسجة الخصية وإعدادها للفحص بالمجهر الإليكتروني النافذ.

النتائج: أوضحت الدراسة أن حقن ٤٠ و ٨٠ ملغم / كغم من الترامادول داخل الغشاء البريتوني، أو تعاطي نفس الجرعات عن طريق الفم لمدة ٢٠ و ٣٠ يومًا قد سبب حدوث تغييرات نسيجية مرضية سلبية في خصيتي الجرذان البيضاء. وكانت النتائج الأكثر شيوعًا هي التغييرات في الأغشية الخلوية وكذلك أنوية الخلايا التي عانت من الموت المبرمج، كما ظهرت تأثيرات على الميتوكوندريا على شكل فراغات وكذلك فجوات سيتوبلازمية مع خلل في مكونات الخلايا، كما أدى إلى تمزق الأغشية النووية والخلوية مع وتآكل مكونات الأنوية ، وتلاشي الشبكة الإندوبلازمية الخشنة ، وانتشار الشبكة الإندوبلازمية الملساء ، والنخر الخلوي ، وتحلل أعراف الميتوكوندريا. كما كانت هناك فجوات وتدهور في خلايا Leyding مع وجود فجوات كبيرة فيها وتضخم لخلايا Sertoli .

الخلاصة: هذه النتائج قد تعطي تفسيراً محتملاً لتأخر الخصوبة أو حتى العقم المرتبط بتعاطي الترامادول ، الأمر الذي قد يظهر ضرورة إعداد حملة توعية قومية لتسليط الضوء على مخاطر إساءة استخدام الترامادول على الوظائف الجنسية ووضع قيود وتشريعات أكثر صرامة على تعاطي الترامادول على المستوى العلاجي وكذلك على إستخدامه كمخدر لدى مدمني الترامادول.