

# Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: [www.jacb.journals.ekb.eg](http://www.jacb.journals.ekb.eg)

## Molecular and Genotoxic Effects of Sildenafil Citrate and Tramadol on Testis and Sperm Quality in Rat Males: Role of Apoptosis

Eltohamy, E. A.; A. M. Hassan; Hoda A. S. Elgarhy and A. M. Serag\*



Cross Mark

Department of genetics and genetic engineering faculty of agriculture Benha university

### ABSTRACT

Men are using oral medications like tramadol and sildenafil citrate more frequently these days to treat erection issues and achieve the right level of potency for sexual performance. This study evaluated the effect of persistently administering tramadol and sildenafil citrate on the testis and sperm quality of scoundrel males. 40 rats were assigned into 4 groups: control, group treated with tramadol, sildenafil citrate was given to one group, and tramadol and sildenafil mixture was given to the other; each group had ten rats. After the sacrifice of rats at the end of the experiment, biochemical, histopathological, and comet assays were evaluated. The treatments illustrated a significantly lower sperm count and a rise in aberrant forms compared to the control. The testis' histopathology revealed a decrease in the quantity of spermatogenic cells; additionally, the interstitial tissue between tubules in the tramadol-treated group increased, leading to the loss of the majority of spermatogenic cells, worsening alterations, disrupted seminiferous tubule architecture, and oedema of interstitial matter, more than in the sildenafil citrate group. The combination of the two drugs caused the most significant impairment in all measured parameters. Therefore, limiting the concomitant use of both drugs is recommended to diminish the potentiated toxic effect on the testis and sperm quality, especially tramadol.

**Keywords:** Sildenafil, Tramadol, Histopathology, Comet assay.



### INTRODUCTION

The synthetic opioid analgesic tramadol has a central action and is widely used. Although its exact mechanism of action is unknown, Raffa *et al.*, (1992) found two potential complementary mechanisms: binding to mu opioid receptors (MOR) and preventing noradrenaline and serotonin from being reabsorbed. Numerous instances of tramadol toxicity and abuse have been documented in the literature. Central nervous system depression, biliousness, nausea, tachycardia, and seizures are the major signs of poisoning (Spiller *et al.*, 1997).

Tramadol overdoses have been linked to fatal incidents. In those cases, hypoglycemia, hepatic failure, cardiac arrest, and other causes of death have all been implicated (Daubin *et al.*, 2007).

Sildenafil citrate is a drug used to treat erectile dysfunction. PDE5, an enzyme that degrades cyclic guanosine monophosphate (c-GMP) in the cavernosum, is inhibited by the drug (Boolell *et al.*, 1996). For a penile erection to occur, the corpus cavernosum must emit nitric oxide (NO). By blocking PDE5, which oversees breaking down cGMP in the corpus cavernosum, sildenafil enhances the effects of NO, which in turn causes horizontal muscle moderation and blood inflow (Moncada and Higgs, 1993).

Treatment with Sildenafil Citrate is efficient for erectile dysfunction caused by organic factors (Morales *et al.*, 1998); though, side impacts have been noted, including clearing, worry, obstruction, and heartburn. Even though healthy men who used sildenafil did not experience any clinically harmful homodynamic effects, the drug's systematic vasodilator effect is what causes these side effects (Marmor and Kessler, 1999). During the post-sildenafil exercise test, both systolic and

diastolic universal arterial pressure dropped by 6% from baseline (Conti *et al.*, 1999) This means that the heart's ability to pump blood was reduced by 11%.

The current study aimed to estimate and balance how tramadol and sildenafil citrate affect male rats' sperm characteristics (such as sperm count and sperm malformation) and testes architecture. Moreover, apoptosis is sought as a mechanism of toxicity.

### MATERIALS AND METHODS

#### 1. Animals and ethical protocol

Male albino rats that were 8 weeks old and weighed 200–250 g were utilized. They were given unlimited access to food and water after obtaining it from the Egyptian Atomic Energy Authority (EAEA). They were housed in continuous environments with 12/12 h light/dark cycles and given a week to acclimatize before the trial began. The animals were handled and cared for in accordance with the recommendations of the Benha University Animal Care and Use Committee and the National Institutes of Health's (Maryland, USA) handbook for the care and use of laboratory animals. Every attempt was made to minimize the quantity of animals utilized and their experiences. The research was exploratory in nature and was carried out at the Faculty of Veterinary Medicine, Benha University.

#### 2. Drugs and Chemicals

Sildenafil citrate (Pfizer, Egypt) in 100 mg tablets and tramadol hydrochloride (Mina Pharma, Egypt) in 50 mg capsule preparations were used. Normal saline, 0.9%, and other used chemicals were commercially obtained.

#### 3. Animal grouping and handling protocol

The following groups were formed with 10 animals per group:

\* Corresponding author.

E-mail address: [Ahmed.serageldin@fagr.bu.edu.eg](mailto:Ahmed.serageldin@fagr.bu.edu.eg)

DOI:10.21608/jacb.2023.228192.1063

1. The control group was administered normal saline orally for 8 weeks.
2. The initial treatment group (n = 10) received oral sildenafil citrate (10 mg/kg body weight) for 8 weeks (Nna *et al.*, 2017).
3. The subsequent considered group (n = 10) received oral tramadol at a dose of 20 mg/kg body weight for 8 weeks (Nna *et al.*, 2017).
4. The third treated group (n = 10) received oral sildenafil citrate and tramadol doses of 10 and 20 mg/kg body weight each for a period of eight weeks.

The drugs were orally administered, three times a week for 8 weeks and were dissolved in 0.2 ml of saline.

The animals in this study group were given an intraperitoneal injection of 30 mg per kg of nembutoal to induce anaesthesia at the end of the course of therapy. A thoracotomy was used to expose the heart. The testicles of the animals were removed after the blood transfusion set's needle was inserted into the left ventricle (apex), a pinch in the right atrium, and these procedures.

For routine histological analysis, organs were saved in 10% PSB formalin (pH 7.4). Blood samples were kept at -20 °C until further use.

#### **4. Biochemical assay**

Using commercial ELISA kits, a hormonal profile was performed, including testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin. FSH kits (catalog no. KA0213) from Abnova Company, Taoyuan City, Taiwan, LH kits from Novus biological company, Colorado, USA (catalog No. NBP2-61257).

Cholesterol ELISA kits (catalog No. K4436-100) from Bio Vision company, Massachusetts, USA were used. LDL was measured spectrophotometrically using commercial kits from Crystal Chem Company, Elk Grove Village, USA (catalog No. 79960).

#### **5. Semen samples evaluation**

##### **Semen quality test**

Semen quality was measured colour-metrically using commercial kits purchased from Biodiagnostic Co., Cairo, Egypt (catalog No. SQ2311). The test depended on resazurin reduction and samples were measured at 580 nm and 615 nm. The resazurin reduction test (RRT) ratio was expressed as absorption at 580 nm/ absorption at 615 nm via the manufacturer instructions. Azospermic = 0.70 – 1.16; Oligoathenozoospermic = 1.10 – 1.35; Oligospermic = 1.50 – 2.00; Normozoospermic = 2.25 – 6.00.

##### **Fructose in semen**

Fructose was measured in semen using the colorimetric kits purchased from Biodiagnostic Co., Cairo, Egypt (catalog No. FR2310).

##### **Sperms microscopic examination**

Semen evaluation included the following points:

##### **Individual motility**

According to the strength of the sperms' motility, a drop of newly obtained semen was deposited on a slide held at a temperature close to body temperature (37°C to 38°C) and evaluated under low magnification (X120).

1. Seminal samples that moved extremely quickly were given a rating of 5.
2. Seminal samples with lively mobility received a rating of 2.
3. Seminal samples with modest mobility received a rating of 3.
4. Seminal samples that moved slowly were given a rating of 2.

5. Seminal samples with extremely slow motion received a rating of 1.

##### **Progressive motility of spermatozoa:**

Fresh semen (a tiny drop) was situated on a spotless, warm glass slide at 37–38° C, diluted with a few warm NaCl (0.9%) drops, and covered with a cover slip to conduct microscopic inspection immediately after each collection. According to Soad *et al.*, (1996), examination is conducted at a high magnification (x400).

##### **Sperm cell count in a millilitre (x106/ml):**

Two hundred times with NaCl (0.9%) and one drop of eosin were diluted into the semen, and the hemocytometric estimation of the sperm-cell content in millimeter was performed. Examining was done using an X675 high-power magnifier.

##### **Percentages of live spermatozoa (LS%):**

Eosin-nigrosine staining was used to determine if living and dead spermatozoa could be distinguished from one another. Using the oil sader (X1350), duplicate smears were prepared, and 200 spermatozoa were counted on each slide. Live spermatozoa were unstained, while dead spermatozoa were stained (Dott and Foster, 1972).

##### **Percentage of healthy spermatozoa:**

One hundred sperm were randomly selected and 100 were analyzed after each ejaculate had one smear stained with the eosin nigrosine stain. The estimated proportion of healthy sperm is as follows, based on Khalifa (1977):

$$\text{Normal sperm (\%)} = 100 - \text{Abnormal sperm.}$$

#### **6. Evaluation of DNA fragmentation in rat testis using single-cell gel electrophoresis (comet assay)**

Single-cell gel electrophoresis test known as Comet: Spermatozoa were tested using the alkaline comet assay, as described by Hughes *et al.*, 1996. 100 µl of 0.5% normal melting point agarose (Sigma) were applied on fully opaque glass slides before a coverslip was put on top and the agarose was allowed to set. The coverslips were taken off, and the second layer was created by combining 1x10<sup>5</sup> sperm cells with 50 µl of 1.2% low melting point agarose in 50 µl of PBS (7.2 pH). The slides were then put in lysis buffer (2.5 M NaCl, 100 mM Na EDTA, 10 mM Tris, and 1% Triton X at a pH of 10) with the coverslips removed for 1 hour. Next, 100 l/ml of proteinase K in lysis solution was applied to the slides for an overnight duration at 37 °C. After the proteinase K solution was drained from the slides, they were placed in a horizontal electrophoresis apparatus filled with freshly produced alkaline electrophoresis solution containing 300 mM NaOH and 1 mM EDTA for 20 min. to allow the DNA to denature the buffer level was adjusted to produce the electrophoresis conditions of 25 V (0.714 V/cm), 300 mA, and room temperature for 10 minutes. After that, detergents and alkalis were removed from the slides by washing them in a neutralizing solution of 0.4 M Tris at pH 7. Each slide was stained with 50 µl of 20 g/ml ethidium bromide after neutralization, and then it was mounted on a coverslip. 200 sperm cells in total were seen under a fluorescent microscope (400X). Using an image analysis system (the Comet-Score program), DNA damage is assessed by measuring the proportion of DNA in the tail, the length of the tail, and the tail moment. It is assumed that the DNA concentration is related to the stain's intensity in the comet tail region. While spermatozoa with undamaged (non-fragmented) DNA do not create a "comet," they do show accelerated DNA migration from the nucleus to the anode (Fraser, 2004).

**7. Testicular histopathology**

The testis was removed, thinly sliced, and left to fix for 24 hours in a 10% formaldehyde buffer. Before being embedded, the tissues were kept in 70% alcohol after being cleaned to remove the 10% formaldehyde. The tissues were dehydrated in a series of alcohols before being paraffin embedded. On glass slides, tissue sections with a thickness of 5 mm were produced. The sections were mounted in mounting media after being stained with hematoxylin and eosin. The light microscope was used to inspect the slides. The presence of interstitial oedema, somniferous tubule deterioration, and blocking were investigated in multiple cross sections made up of 20–50 tubule sections from each testis.

**8. Bcl-2 Associated X-protein (BAX) histopathological examination**

To determine the degree of apoptosis, additional sections were prepared and treated with the BAX monoclonal primary antibody, Dako, code M 0887 (Clone 124) (Dakocytomation, Copenhagen, Denmark), diluted at a 1:50 ratio.

**9. Statistical analysis**

During the data analysis process, the statistical software SPSS (version 17.0) was utilised. The data were presented utilising Microsoft Excel 8.0, with the mean ± standard

deviation (SD). The statistical approach employed in this study was the use of one-way analysis of variance (ANOVA), followed by Tukey's test for post hoc analysis. The data were considered statistically significant at a P value of 0.05.

**RESULTS AND DISCUSSION**

**Results**

**1. Biochemical analysis**

Regarding the hormonal profile parameters, the sildenafil and tramadol groups showed decreased LH, FSH, and testosterone levels in comparison to the control group. Furthermore, prolactin was meaningfully increased. The combined group showed less affected parameters as LH, FSH, and testosterone showed less decreased values that were still statistically different from the control, sildenafil, and tramadol groups. Prolactin increased in the combined group more than in the sildenafil and tramadol groups. All these changes were of statistical significance as shown in Table 1.

Lipid profile parameters showed a mild increase in total cholesterol and low-density lipoprotein (LDL) cholesterol in the sildenafil and tramadol groups. Moreover, these parameters were more expanded in the combined group than in the sildenafil and tramadol groups (Table 1).

**Table 1. Hormonal and lipid profile parameters in studied groups (measured in milligrams per deciliter of blood - mg/dL)**

Treatments	LH	FSH	Prolactin	T. Cholesterol	LDL Cholesterol	Testosterone
Control	6.8±0.6 <sup>c</sup>	8.0±0.5 <sup>d</sup>	64.9±0.2 <sup>a</sup>	3.60±0.1 <sup>a</sup>	26.05±2.3 <sup>a</sup>	8.6±0.9 <sup>d</sup>
S	3.96±1.2 <sup>a</sup>	2.3±0.9 <sup>a</sup>	76.7±3.1 <sup>b</sup>	4.80±0.2 <sup>b</sup>	36.66±1.7 <sup>c</sup>	3.1±0.8 <sup>b</sup>
T	3.50±0.4 <sup>a</sup>	2.9±0.8 <sup>b</sup>	77.2±1.5 <sup>b</sup>	4.95±0.4 <sup>b</sup>	31.45±2.1 <sup>b</sup>	2.9±0.9 <sup>a</sup>
S+T	4.83±1.1 <sup>b</sup>	4.0±1.1 <sup>c</sup>	84.7±1.8 <sup>c</sup>	5.93±0.6 <sup>c</sup>	39.20±3.1 <sup>d</sup>	4.0±0.7 <sup>c</sup>

S: Sildenafil; T: Tramadol; S+T: Sildenafil+ Tramadol

Each number is the mean ±SE of the species under various treatments. Significant differences between treatments examined using the Turkey post-hoc test is represented by small letters. At p <0.05, all tests were deemed significant.

**2. Semen evaluation results**

**Semen quality test**

Data in Table 2 revealed that the calculated RRT ratio didn't differ significantly in the tramadol, sildenafil,

and mixed groups from the saline-treated group. The percentage of live sperm, dead sperm, individual motility, and progressive motility either didn't differ significantly or showed minimal variation among the groups.

**Table 2. Semen samples evaluation in the studied groups.**

Treatments	RRT	Live %	Dead %	Fructose	Fructose Con mg/dl	SCC*1 <sup>6</sup>	IM	PM
Control	3.74±1.1 <sup>b</sup>	74.6±6.3 <sup>b</sup>	25.6±3.4 <sup>a</sup>	0.22±0.09 <sup>a</sup>	311.7±7.4 <sup>a</sup>	113.7±6.2 <sup>a</sup>	77.2±2.8 <sup>b</sup>	3.7±0.7 <sup>a</sup>
T	3.64±0.2 <sup>a</sup>	74.3±3.2 <sup>b</sup>	26.1±1.9 <sup>b</sup>	0.22±0.06 <sup>a</sup>	317.2±2.8 <sup>c</sup>	113.2±2.9 <sup>a</sup>	76.3±3.5 <sup>a</sup>	3.7±0.6 <sup>a</sup>
S	3.68±0.8 <sup>a</sup>	73.8±1.8 <sup>a</sup>	26.5±1.5 <sup>b</sup>	0.22±0.07 <sup>a</sup>	315.7±7.1 <sup>b</sup>	114.3±3.5 <sup>b</sup>	76.7±7.1 <sup>a</sup>	3.7±0.7 <sup>a</sup>
S+T	3.67±0.9 <sup>a</sup>	74.4±1.4 <sup>b</sup>	26.1±2.2 <sup>b</sup>	0.22±0.09 <sup>a</sup>	318.1±2.8 <sup>c</sup>	113.4±4.7 <sup>a</sup>	76.7±7.7 <sup>a</sup>	3.7±0.7 <sup>a</sup>

S: Sildenafil; T: Tramadol; S+T: Sildenafil+ Tramadol

IM: individual motility, PM: progressive motility

The mean ±SE is shown by each value. Significant differences between treatments examined using the Turkey post-hoc test is represented by small letters. At p <0.05, all tests were deemed significant.

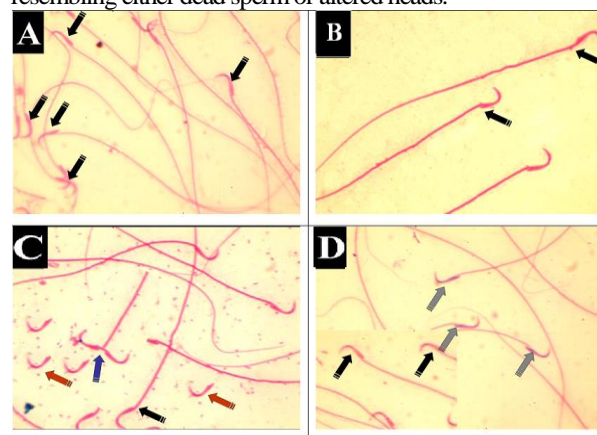
**Fructose in semen**

According to table 2, fructose in semen didn't show obvious variations in the studied groups. Either minimal or statistically insignificant variations were observed.

**Sperms microscopic examination**

Figure 1 (A, B, C, and D) illustrates the observation that the sperm in the control group (A), which were subjected to eosin nigrosine staining, had normal morphology and intact tails, with no instances of detached heads (shown by black arrows). Group B, which received sildenafil citrate treatment, exhibited a comparable sperm count, while maintaining a normal morphology and indicating in the group (C), which received tramadol HCL treatment, several sperm malformations were observed, including detached heads (indicated by brown arrows) and double heads (indicated by blue arrows). Conversely, in group (D), which received a combination treatment of sildenafil citrate and tramadol, the sperm exhibited a normal shape but displayed a significant presence of dark,

nearly blue-colored heads (indicated by grey arrows), resembling either dead sperm or altered heads.

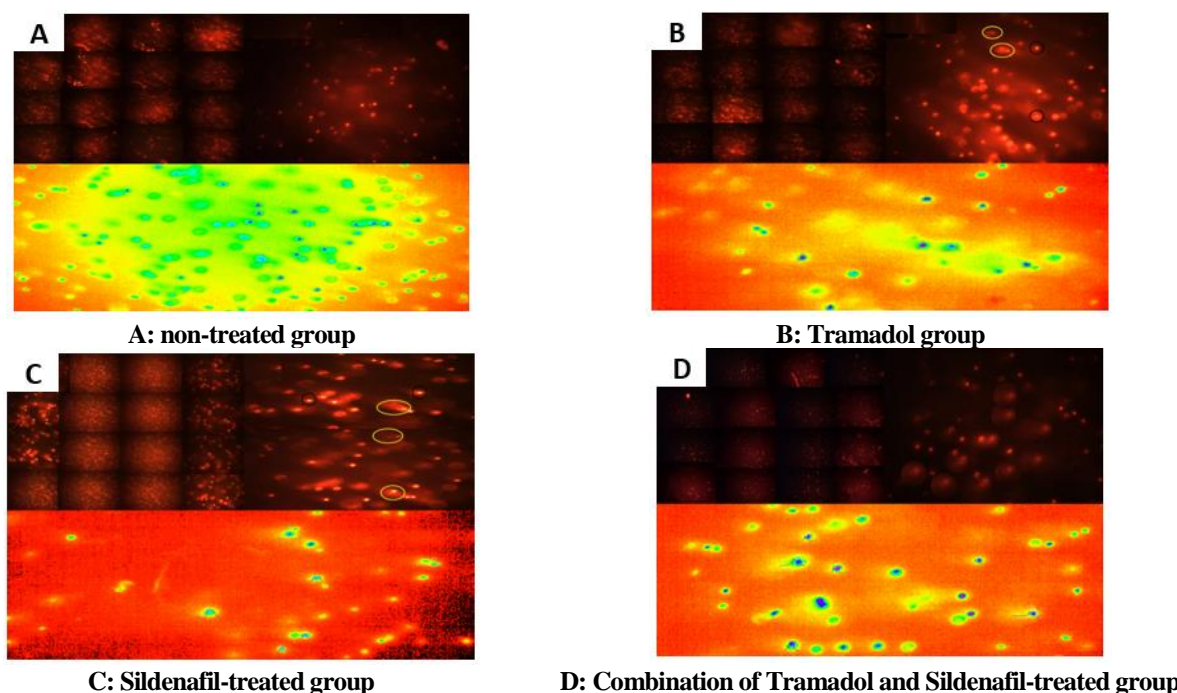


**Figure 1. The spermatic stained with Eosin nigrosine for 4 groups.**

**Using a comet assay to measure DNA fragmentation.**

The findings presented in Figure 2 and Table 3 indicate that the control group had a robust cellular structure and intact cells that conformed to the established criteria of the comet experiment. In the control group, the measurements of comet length, height, area, head diameter, tail length, tail area, percentage of DNA in the tail, tail moment, and olive moment, if applicable, exhibit a deceleration. In contrast to the control group, the groups treated with Sildenafil, Tramadol, and their combination had pronounced comet expression. This was indicated by a

statistically significant increase in comet length, height, area, and percentage of DNA in the tail, along with a substantial reduction in head diameter. The group administered with Tramadol had relatively low effects in comparison to the combination group, however these effects remained statistically significant when compared to the control group. On the other hand, the combination group primarily demonstrated substantial effects related to comet disintegration and leaking of single-strand DNA, as visually depicted in figure 2.



**Figure 2. Evaluated Comet Assay Parameters.**

**Table 3. Detection of DNA fragmentation by the comet assay, assessed as tail moment and tail length in testis rats treated with sildenafil citrate and tramadol (chronic treatments).**

Parameters (px)	Treatments			
	Control	Sildenafil	Tramadol	Combined
Comet Length	13.05 ± 0.38d	20.96 ± 0.69c	27.12 ± 0.78b	57.27 ± 2.23a
Comet Height	19.7 ± 0.61d	33.6 ± 0.85c	43.9 ± 0.8b	55.17 ± 1.93a
Comet Area	341.4 ± 13.98d	557.05 ± 20.02c	709.09 ± 24.96b	3053.79 ± 114.93a
Head Diameter	19.47 ± 0.61d	33.32 ± 0.59c	42.73 ± 0.76b	54.67 ± 1.69a
Tail Length	0.84 ± 0.02d	1.42 ± 0.04c	1.82 ± 0.06b	4.11 ± 0.16a
Tail Area	0.96 ± 0.03c	1.61 ± 0.06b	2.09 ± 0.1a	1.02 ± 0.04c
%DNA in Tail	11.81 ± 0.32c	19.14 ± 0.51b	21.59 ± 0.84b	26.43 ± 0.58a
Tail Moment	1.05 ± 0.04c	1.71 ± 0.1b	2.29 ± 0.14a	0.75 ± 0.03d
Olive Moment	0.34 ± 0.01d	0.58 ± 0.03c	0.75 ± 0.04b	3.02 ± 0.1a

The mean ±SE is shown by each value. Significant differences between treatments examined using the Turkey post-hoc test is represented by small letters. At p <0.05, all tests were deemed significant.

**3. Histopathology of testis**

The microscopic examination of testis of control rats exhibited normal histological findings of multiple rounded to oval seminiferous tubules and little interstitial spaces. The seminiferous tubules were lined with stratified germinal epithelium at different stages of differentiation accompanied with supporting Sertoli cells and spermatozoa in their lumen, as well as bounded by a basal lamina. Spermatogenic cells and Sertoli cells were the two types of cells that made up the seminiferous epithelium. Spermatogenic cells, which were organized from the basal compartment toward the lumen, included spermatogonia, spermatocytes, spermatids, and spermatozoa. Spermatogonia, which are characterized by

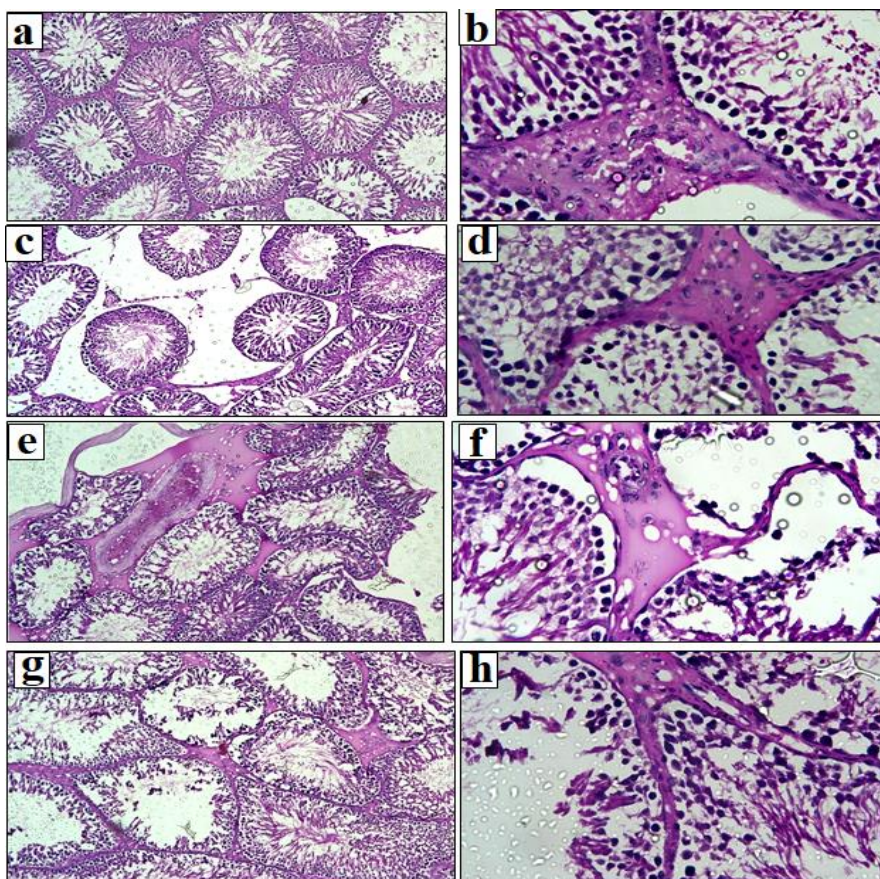
tiny, oval to round, highly stained nuclei, and faintly stained cytoplasm, were seen resting on the basal portion of the tubules. The spermatogonia, which were enormous in size and had large, spherical nuclei in the center, were found near to the main spermatocytes. The little, spherical spermatids with rounded ends were then discovered. Following them were elongated spermatids, which could be distinguished by their lengthy, darkly pigmented nuclei. Additionally, Sertoli cells with triangular nuclei were discovered between spermatogonia sitting on the basement membrane. Leydig cells, either singular or in clusters, encircled the blood vessels in the interstitial tissue. They had big nuclei, granular cytoplasm, and looked polygonal or rounded (Figure 3 a, b).

Testicular tissue samples from rats given Tramadol exhibited irregularities in the configuration of the seminiferous tubules along with the disarray and deterioration of spermatogenic cells under a microscope. In certain tubules, there was exfoliation of germ cells in the tubular lumen and dissociation of the germinal epithelium from the underlying basement membrane. Most of the tubules that were studied appeared to have fewer germ cells. Basal cell spermatogonia's strongly pigmented nuclei were seen in a few tubules. Spermatogenic cells in seminiferous tubules were seen to vacuolate. Some tubules were free from sperms. Thick corrugated contours of seminiferous tubules were seen. Also, there were prominent Leydig cells with multiple vacuoles around the interstitial tissue blood vessels. Marked congestion in interstitial blood vessels accompanied with vasculitis. The interstitial tissue was exhibited widening and contained vacuolated homogenous acidophilic material. In addition to that, a thick capsule of testis was observed (Figure 3 c, d).

The histopathological effect of sildenafil on testis appeared as disorganization of spermatogenic layers with thick and irregular basement membrane of seminiferous tubules. There was separation of the germinal epithelium from the underlying basement membrane with germ cells and cell debris desquamation in the tubular lumen in some tubules. There was a clear decrease in the quantity of spermatogonial cells, necrosis, and vacuolar degeneration of

the spermatogenic cells. Except for sporadic spermatogonia cells along the basement membrane in a few tubules, all interior layers were absent. Also visible was a localized tear of the basement membrane. Moreover, widening of the interstitium with the appearance of vacuoles and inflammatory infiltration was observed. The interstitial tissue was exhibited vacuolated homogenous acidophilic material. Interstitial blood vessel showed marked congestion, vasculitis and had thick wall. Thickening of tunica albuginea were seen (Figure 3 e, f).

The microscopic examination of the testis of the rats in the tramadol and Sildenafil-treated group indicated irregularity and degradation of spermatogenic cells of the seminiferous tubules. Numerous tubules showed spermatogenic cell separation. The exfoliation of the injured spermatogenic cells with cellular debris in the lumens revealed the lack of sperm. Between the deteriorated spermatogenic cells, vacuoles developed. Some tubules' lumen exhibited the presence of enormous cells. With the swelling of the tubules' basement membrane, some seminiferous tubules broke. The interstitial tissue between the seminiferous tubules showed markedly congested blood vessels associated with vasculitis and vacuolated homogenous acidophilic material as well as Leydig cells with dark stained nuclei was also detected. Mild leucocytic infiltrations were detected in the testicular stroma. Moreover, thickening of capsule of testis was obvious (Figure 3 g, h).



**Figure 3. Histopathological examination of rat testis by H&E (Hematoxylin and Eosin stain).**

As following; a. In the lumen of the seminiferous tubules, which are firmly packed and bordered by stratified germinal epithelium, are visible sperm in the testes of the positive control rats (H&E, X100). b. Testis from control

rats displaying portions of nearby seminiferous tubules bordered by Sertoli cells and germinal epithelium. Round spermatids, primary spermatocytes, spermatogonia, Leydig cells inside interstitial tissue, and spermatozoa in the lumen

make up the germinal epithelium (H&E, X400). c. Testis of Tramadol-treated rats showing widely separated seminiferous tubules with corrugated and thick contours of seminiferous tubules, some tubules were free from sperms as well as numerous vacuolations and prominent congestion of blood vessels in interstitial tissue (H&E, X100). d. Testicles from rats given Tramadol reveal the germinal epithelium separating from the underlying basal lamina, germ cells exfoliating in the tubular lumen, spermatogenic cells' strongly coloured nuclei and vacuolating material in interstitial tissue (H&E, X400). e. Testis of Sildenafil-treated rats showing disorganization of spermatogenic layers with irregular basement membrane, vacuolated homogenous acidophilic material in interstitial tissue, severely congestion interstitial blood vessel, vasculitis and had thick wall as well as thickening of tunica albuginia (H&E, X100). f. Testis of Sildenafil-treated rats showing disorganization of spermatogenic layers with thick and irregular basement membrane, separation of the germinal epithelium from the basement membrane and germ cells exfoliation in the tubular lumen, necrosis, vacuolar degeneration of the spermatogenic cells, rupture of basement membrane, congested and vasculitis of interstitial blood vessel, vacuolated Leydig cells and vacuolated homogenous acidophilic material with inflammatory infiltration in interstitial tissue (H&E, X400). g. Rats given sildenafil and tramadol had testicular abnormalities including seminiferous tubule degeneration, spermatogenic cell separation, damaged spermatogenic cell cellular debris in lumens, absence of sperm, and interstitial tissue with vacuolated homogenous

acidophilic material (H&E, X100). h. Rats treated with tramadol and sildenafil had testes that displayed fragments of disorganized seminiferous tubules, spermatogenic cells that had degenerated and been separated, cellular debris in the lumens, thickening of the tubules' basement membrane, vacuolated homogenous acidophilic material in interstitial tissue, and mild leucocytic infiltrations (H&E, X400).

#### a. BAX histoimmunochemical study

The microscopic analysis of BAX immunohistochemical staining revealed a diminished response in the rats belonging to the control group. The weak brown coloration is predominantly observed within the cytoplasm of Leydig cells, along with a limited presence in spermatocytes and elongated spermatids. The testicular immunoreactivity of BAX was shown to be diminished in rats treated with saline. The mild brown coloration is observed in the cytoplasm of Leydig cells, spermatocytes, and elongated spermatids (Figure 4a). Tramadol-treated group exhibited moderate immunolabeling positive reactions in the cytoplasm of the spermatogonial cells (spermatogonia, spermatocytes and elongated spermatids), Leydig cells and Sertoli cells as dark brown colour (Figure 4 b).

Sildenafil-treated groups showed moderate immune-positive reactions of the cytoplasm of the spermatogonia, spermatocytes and elongated spermatids as well as Sertoli cells and Leydig cells (Figure 4 c). On the other side, the group that treated with Tramadol and Sildenafil together showed strong positive BAX immunolabeling germ cells, Sertoli cells and Leydig cells (Figure 4 d).

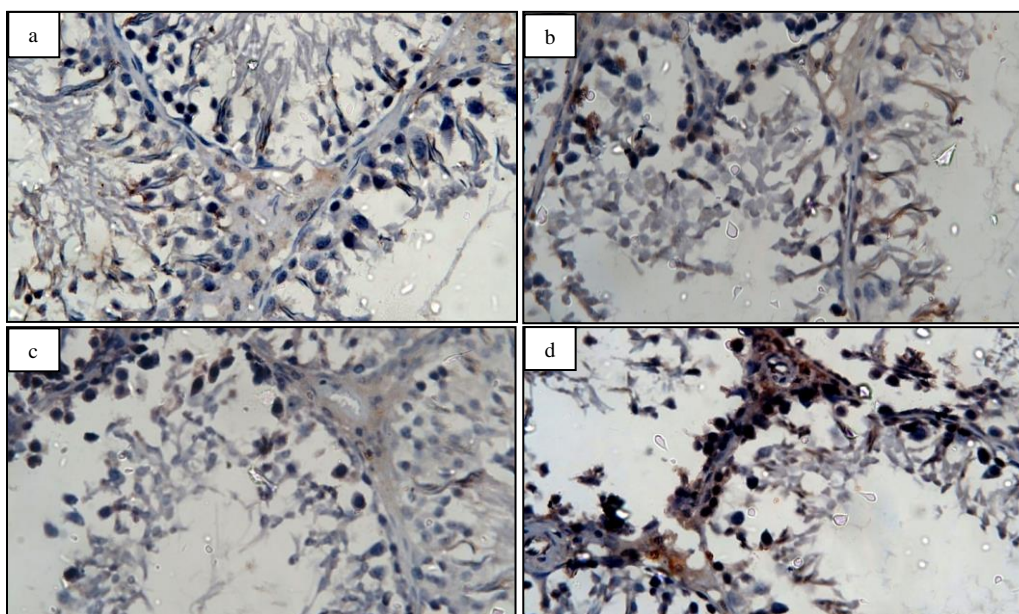


Figure 4. Immunohistochemical study of BAX immunoreactivity in rat testis (BAX immunostaining, x400)

#### Discussion

The present investigation sought to assess the impact of sildenafil citrate and tramadol medication on parameters related to male rat sperm count, sperm deformities, and testicular histological abnormalities. The presence of histological abnormalities, such as hypertrophy cells, necrosis of seminiferous tubules, destruction of the testis, and the presence of inflammatory cells, provided evidence to support the conclusion that sildenafil citrate induced sperm abnormalities, characterised by a decrease in sperm quantity

and an increase in sperm deformities. The administration of orally ingested sildenafil citrate may potentially affect seminal parameters in individuals with erectile dysfunction, as indicated by the limited existing data about the influence of sildenafil citrate on seminal characteristics. The presence of at least two different PDE isoforms (PDE1 and PDE4) in human sperm cells has been established. Rolipram and 8-methoxy-isobutyl methylxanthine, which specifically inhibit PDE1 and PDE4, respectively increase the acrosome response as well as sperm motility (Fisch *et al.*, 1998). When

17 healthy male volunteers aged 19 to 34 were randomized to receive a single dose of 100 mg of sildenafil for two periods and a single dose of placebo for two periods, with each period separated by at least 5-7 days, it was found that sildenafil had no statistically significant effect on sperm motility, count, or density, the percentage of abnormal sperm, or the percentage of living sperm (Burger *et al.*, 2000). Previous research has shown that sildenafil has no impact on sperm motility or count in males of reproductive age. In a study involving 20 healthy male volunteers, there was no difference in the amount of sperm, progressive motility, or morphological abnormalities between semen samples collected 1 hour after consuming a 100 mg dose of sildenafil or a double-blind placebo (Zavos and Zarmakoupis-Zavos, 2000; Aversa *et al.*, 2000). The histological changes caused by sildenafil in the seminiferous tubules' tubular and interstitial tissues, increased Leydig cell cellularity, and tubular degeneration may ultimately result in the full halt of spermatogenesis (Jarrar, 2011).

Tramadol medication was significantly associated with poorer sperm quality measures in this study's analysis when equated to the normal control group and even more significantly when compared to the sildenafil group. The findings of the sperm quality examination revealed a substantial decline in sperm dose, motility, and vitality, which suggests that tramadol use over an extended period may have negative effects on rat epididymal spermatozoa. The majority of the spermatogenic cells were lost in the tramadol-treated group, along with degenerative alterations, altered seminiferous tubule architecture, and interstitial tissue edema.

Drug use considerably lowers blood testosterone levels, which compromises the secondary sex organs' structural and physiological health, according to prior study. These medications prevent luteinizing hormone (LH) secretion, which reduces blood testosterone levels, by acting either directly on the pituitary gland or in the hypothalamus (Bliesener *et al.*, 2005). Reuhl *et al.*, 2001 observed that the tubular diameter and height of the germinal epithelium in drug users were the shortest and had the thickest interstitial tissue. Cell development was also arrested in these individuals. Testicular metabolism can be impacted by changes in basement membrane thickness, which is widely acknowledged, which in turn encourages greater tubular atrophy and germinal cell hypoplasia. The duration of drug use is closely related to the degree of testicular injury (Sorge and Stewart, 2006). Inducing apoptosis in the seminiferous tubules of mouse testis and altering the histology of the testis in experimental animals are both effects of amphetamine compound administration (Yamamoto *et al.*, 2002). In contrast to the normal and sildenafil-treated groups, the tramadol-treated group's blood testosterone levels were significantly lower, according to this study. The histopathology-detected testicular tissue degeneration may be the cause of this. Recently, the effects of prolonged tramadol administration on testicular function were examined in the work by Ahmed and Kurkar, 2014.

Throughout the experiment, the rat was given tramadol subcutaneously for 8 weeks at a dose of 20 mg/kg. In testicular tissues, endothelial nitric oxide synthase was found to be more expressed. In testicular tissues, endothelial

nitric oxide synthase was found to be more expressed in response to tramadol treatment, according to the research findings. In addition, tramadol decreased the amount of sperm and their motility, as well as the quantity of primary spermatocytes, spherical spermatids, and Leydig cells. They found that chronic tramadol usage changes the way adult male rats' testicle's function, and they theorized that these alterations may be brought about by the drug's development of oxidative stress and excessive NO production.

## CONCLUSION

Both tramadol and sildenafil have been found to negatively affect testis function in male rats. Tramadol specifically leads to a considerable decrease in sperm counts, as well as the production of aberrant morphologies. Additionally, tramadol has a more pronounced impact on the histological architecture of the testis. In comparison to the cohort administered with sildenafil, who exhibited testosterone levels within the normal range, the group receiving tramadol saw a notable decrease in serum testosterone levels.

## REFERENCES

- Ahmed MA and Kurkar A (2014). Effects of opioid (tramadol) treatment on testicular functions in adult male rats: Role of oxidative stress: Role of nitric oxide and oxidative stress. *Clin Exp Pharmacol Physiol.* 41(4):317-23.
- Aversa A, Mazzilli F, Rossi T, Delfino M, Isidori AM. and Fabbri A. (2000) Effect of sildenafil (Viagra) administration on seminal parameters and post-ejaculatory refractory time in normal males. *Hum. Reprod.* 15 (1):131-4.
- Bliesener N, Albrecht S, Schwager A, Weckbecker K, Lichtermann D., and Klingmuller D. (2005). Plasma testosterone and sexual function in men receiving buprenorphine maintenance for opioid dependence. *J Clin Endocrinol Metab.* 90(1):203-6.
- Boolell, M., Allen, M. J., Ballard, S. A., Gepi-Attee, S., Muirhead, G. J., Naylor, A. M. and Gingell, C. (1996). Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *International journal of impotence research*, 8(2), 47-52.
- Burger M, Sikka SC, Bivalacqua TJ, Lamb DJ. and Hellstrom WJ. (2000). The effect of sildenafil on human sperm motion and function from normal and infertile men. *Int J Impot Res.* 12 (4):229-34.
- Conti CR, Pepine CJ. and Sweeney M. (1999). Efficacy and safety of sildenafil citrate in the treatment of erectile dysfunction in patients with ischemic heart disease. *Am J Cardiol.* 83(5A):29-34
- Daubin, C., Quentin, C., Goullé, J. P., Guillotin, D., Lehoux, P., Lepage, O. and Charbonneau, P. (2007). Refractory shock and asystole related to tramadol overdose. *Clinical toxicology*, 45(8), 961-964.
- Dott, H.M., Foster, G.C., (1972). A technique for studying the morphology of mammalian spermatozoa which are eosinophilic in a differential "Live/Dead" stain. *J. Erprod. Ferti.* 29, 443-445.

- Fisch JD, Behr B. and Conti M. (1998). Enhancement of motility and acrosome reaction in human spermatozoa: differential activation by type-specific phosphodiesterase inhibitors. *Hum Reprod.* 13(5), 1248–54.
- Fraser, L. (2004). Structural damage to nuclear DNA in mammalian spermatozoa: its evaluation techniques and relationship with male infertility. *Polish J. Vet. Sci.*, 7: 311-321.
- Hughes, G. E. and Cresswell, M. J. (1996). A new introduction to modal logic. Psychology Press.
- Jarrar BM. (2011). Histological alteration in the testicular tissue induced by sildenafil overdoses. *Drugs Metab Lett.* 5 (2): 99-103.
- Khalifa, M.A.S. (1977). Genetical studies on semen characteristics of cock. Ph.D. Faculty of Agricultural Cairo University.
- Marmor MF, and Kessler R. (1999). Sildenafil (Viagra) and ophthalmology. *Surv Ophthalmol.* 44(2):153-62.
- Moncada S. and Higgs A. (1993). The L-arginine-nitric oxide pathway. *N Engl J Med.* 329(27):2002-12
- Morales A, Gingell C, Collins M, Wicker PA, and Osterloh IH. (1998). Clinical safety of oral sildenafil citrate (VIAGRA) in the treatment of erectile dysfunction. *Int J Impot Res.* 10(2):69-74.
- Nna, V. U.; Udefa, A. L.; Ofutet, E. O. and Osim, E. E. (2017). Testicular and Epididymal Histology of Rats Chronically Administered High Doses of Phosphodiesterase-5 Inhibitors and Tramadol. *Niger. J. Physiol. Sci.* 32: 55-61.
- Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, and Vaught JL. (1992). Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. *J Pharmacol Exp Ther.* 260(1):275-85.
- Reuhl J, Bachl M, Schneider M, Lutz F, and Bratzke H. (2001). Morphometric assessment of testicular changes in drug related fatalities. *Forensic Sci Int.* 115(3):171-81.
- Soad, S.A, Abd-EL-Rahim, M.I and Hoda, F.S (1996). Semen characteristics, blood constituents, testicular histology of bucks and reproductive performance of doses of New Zealand Whit rabbits fed barley gains as a substitute for the pelleted complete ration. *Egyptian Journal of rabbit science*, 1(1),73.
- Sorge RE, and Stewart J. (2006). The effects of long-term chronic buprenorphine treatment on the locomotor and nucleus accumbent dopamine response to acute heroin and cocaine in rats. *Pharmacol Biochem Behav.* 84(2):300-5.
- Spiller HA, Gorman SE, Villalobos D., Benson, B. E., Ruskosky, D. R., Stancavage, M. M., and Anderson, D. L. (1997). Prospective multicenter evaluation of tramadol exposure. *J Toxicol Clin Toxicol.* 35(4):361-4.
- Yamamoto Y, Yamamoto K, Hayase T, Abiru H, Shiota K, and Mori C. (2002). Methamphetamine induces apoptosis in seminiferous tubules in male mice testis. *Toxicol Appl Pharmacol.* 178 (3):155–60.
- Zavos PM, and Zarmakoupis-Zavos, PN. (2000). The use of sildenafil citrate for treatment of male infertility in males with erectile dysfunction. *Obstet Gynecol.* 95:549-550.

## التأثيرات الجزيئية والسمية الجينية لسيترات السيلدينافيل والترامادول على كفاءة الخصية والحيوانات المنوية لدى ذكور الفئران: دور موت الخلايا المبرمج

عماد عبدالبديع التهامي ، عبد الوهاب محمد حسن ، هادي سالم الجارحي و احمد محمد سراج الدين

قسم الوراثة والهندسة الوراثية كلية الزراعة جامعة بنها

### الملخص

يستخدم الرجال الأبوبية عن طريق الفم مثل الترامادول وسيلدينافيل بشكل متكرر هذه الأيام لعلاج مشاكل الانتصاب وتحقيق المستوى المناسب من الفعالية للأداء الجنسي. من خلال هذه الدراسة تم تقييم تأثير تناول الترامادول وسيترات السيلدينافيل على كفاءة الخصية والحيوانات المنوية لدى ذكور الفئران. تم تقسيم 40 فأر إلى 4 مجموعات: المجموعة الطبيعية الغير معاملة ، المجموعة المعاملة بالتزامادول ، المجموعة المعاملة بسيترات السيلدينافيل ، المجموعة المعاملة بمزيج من الترامادول وسيترات السيلدينافيل، كل مجموعة تضم عشرة فئران. في نهاية التجربة تم تقييم القياسات البيوكيميائية والنسجية وتقييم السمية الجينية من خلال (comet assay). أظهرت المعاملات انخفاضاً ملحوظاً في عدد الحيوانات المنوية وزيادة في الأشكال الشاذة مقارنةً بالمجموعة الطبيعية. أظهر التشريح المرضي للخصية انخفاضاً في كمية الخلايا المولدة للحيوانات المنوية بالإضافة إلى زيادة في النسيج الخلالي بين الأنابيب في المجموعة المعاملة بالتزامادول ، وفقدان غالبية الخلايا المولدة للحيوانات المنوية ، والتغيرات التنكسية ، وتعطل الأنابيب المنوية ، وتورم الأنسجة الخلالية ، أكثر من مجموعة سيترات السيلدينافيل. تسبب الجمع بين العقارين في أكبر ضعف في جميع المعاملات المقاسة. لذلك ، يوصى بالحد من الاستخدام المتزامن لكلا الدوائين لتقليل التأثير السام المحتمل على كفاءة الخصية والحيوانات المنوية ، وخاصة الترامادول.