AMELIORATIVE EFFECT OF NIGELLA SATIVA ON TRAMADOL-INDUCED TESTICULAR TOXICITY IN ADULT RATS

BY

Rania Saad Mohamed Ramadan[1], Mohamed AbdEl Moneim M. AbdEl Rahman[1], Reham Talaat Eldemerdash Ibrahim[1] and Doaa Mohammed Yousef[1]

[1]Department of Human Anatomy and Embryology Faculty of Medicine, Zagazig University, Egypt

ABSTRACT

Background: Tramadol abuse is a major health problem in Egypt. It promotes oxidative stress via lowering the antioxidant levels. Nigella sativa (NS) possesses strong antioxidant properties. **Objectives:** the aim of the study was to assess the testicular toxicity after oral administration of tramadol, testicular recovery after tramadol withdrawal and the potential ameliorative effect of NS on tramadol-induced testicular impairment. **Material and Methods:** Sixty-six adult male rats were allocated into 4 groups: control group (divided into 3 subgroups orally received normal saline, corn oil, and NS dissolved in corn oil respectively), tramadol-treated group (40mg/kg/day for 30 days), recovery group left to recover for 2 weeks after 30 days of tramadol administration, and NS-co treated group administered NS (1ml/kg/day orally) along with tramadol. At the time of sacrifice, venous blood samples were obtained for assaying serum testosterone levels and the testes were processed for histological, morphometric and immunohistochemical examination. **Results:** Tramadol treatment resulted in deterioration of the testicular microarchitecture, intense expression of iNOS immunoreactivity, and statistically significant decreases in the tubular diameters, seminiferous epithelial heights, and serum testosterone levels. The recovery group revealed partial improvement of all the parameters tested. Co administration of NS significantly improved the detrimental testicular effects of tramadol; this improvement was clearer than that resulted from tramadol withdrawal, proving the protective role of NS. **Conclusion:** NS has a significant role in protection against tramadol-induced testicular damage. **Recommendations:** Tramadol should be administered only when indicated with appropriate dose monitoring. NS supplementation is also advisable in patients on tramadol therapy.

Keywords: *Tramadol, Nigella sativa, Testis, Inducible nitric oxide synthase*.

Corresponding author: Dr. Doaa M. Yousef
E-mail addresses: doaayousefmam@gmail.com

INTRODUCTION

Infertility is a public health issue affecting nearly one of six couples in reproductive age. Male factor infertility accounts for approximately 50% of these cases ***(Sharma et al., 2013; Otasevic et al., 2020)***. Male infertility has also been proposed as a predictor of future health since it is linked to a higher risk of cardiac illnesses, hypogonadism, cancer, and even death ***(Kasman et al., 2020)***. The underlying causes of around 30% of male infertility cases are unknown (idiopathic infertility) ***(Duca et al., 2019)***. Oxidative stress has been documented as a crucial factor for idiopathic male infertility ***(Bisht et al., 2017)***.

Tramadol is an opioid analgesic effectively prescribed for management of moderate to severe pain ***(Preuss et al., 2021)***. It modifies pain both centrally, through μ-opioid receptor agonistic action and serotonin and norepinephrine reuptake inhibition, and locally by increasing nitric oxide (NO) level ***(Dal et al., 2006; Beakley et al., 2015)***. In addition to pain management, tramadol is considered an effective therapy for premature ejaculation ***(Hamidi-Madani et al., 2018)***. Tramadol abuse is a serious health and public challenge facing many countries worldwide. The alleged usages of tramadol; as a mood enhancer, to intensify sexual pleasure or to boost energy during work,

contributed significantly to its massive use ***(INCB, 2018)***. Tramadol-dependent patients are more likely to have abnormal semen parameters, decreased serum testosterone levels and erectile dysfunction ***(Soliman et al., 2021)***. ***Koohsari et al. (2020)*** confirmed the role of oxidative stress and NO overproduction on tramadol-induced testicular toxicity.

Nitric oxide is a free nitrogen radical that regulates diverse physiological processes. NO is formed from L-arginine through nitric oxide synthase (NOS). There are three isoforms of NOS: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Both eNOS and nNOS are constitutive enzymes that are produced in normal physiological processes ***(Peluffo et al., 2009)***. In contrast, iNOS is only expressed when the cell is induced by cytokines and/or bacterial toxins ***(Sharma et al., 2007)***. Excess NO production from iNOS overexpression can result in cellular dysfunction via lipid, protein and DNA damages ***(Cinelli et al., 2020)***. NO overproduction negatively impacts several sperm parameters, testosterone secretion and erectile function ***(Otasevic et al., 2020).***

Nigella sativa (NS) is a medicinal plant traditionally used to treat many illnesses including eczema, fever, diabetes, bronchitis, and influenza ***(Khazdair et al., 2021)***. It has a variety of pharmacological properties as antioxidant, anti-inflammatory, immuno modulatory, anti-cancer, anti-hypertensive and anti-diabetic activities ***(Ahmad et al., 2021a)***. Most of the biological activity of NS has been attributed to its major active component; thymoquinone; which acts a free radical scavenger and modulates the adverse consequences of oxidative stress **(*Sultan et al., 2021)***. ***Kolahdooz et al. (2014)*** reported that administration of NS for two months significantly improved semen volume and pH as well as total sperm number, vitality and morphology in infertile men.

Thus, this study was designed to assess testicular toxicity after oral administration of tramadol, testicular recovery after tramadol withdrawal and to evaluate the potential ameliorative effect of NS on tramadol-induced testicular toxicity.

MATERIAL AND METHODS

- **Material:**

- Chemicals:

Tramadol hydrochloride (Tramundin 150 mg tablets) was obtained from Medical Union Pharmaceuticals (MUP), Cairo, Egypt. The tablets were crushed, dissolved in saline, and given orally at a daily dose of 40mg/kg for 30 days. The therapeutic human dose of tramadol ranges from 50 to 100 mg every 4 to 6 hours, with a maximum daily dose of 400 mg ***(WHO, 2018)***. Based on body surface area, the human equivalent dose (HED) was calculated using the equation: HED (mg/kg) = the rat dose (mg/kg) × (6/37) **(*Reagan-Shaw et al., 2008)***. Accordingly, the applied tramadol dose (40 mg/kg) for rats equals 454 mg for an adult man weighing 70 kg, therefore it could be considered as a model of tramadol abuse. Nigella sativa oil was bought from Kahira Pharmaceuticals & Chemical Industries Company, Zagazig, Egypt; dissolved in corn oil and orally administered for 30 days at a dose of 1ml/kg/day ***(Elkhateeb et al., 2015)***. All other chemicals were commercial preparations of the highest available degree of purity.

- Animals:

I. This study was achieved in the animal house, Faculty of Medicine, Zagazig University, on sixty-six adult male albino rats (150-250 gm). The rats were kept for 15 days before being experimented to be acclimatized to the new environmental conditions. Throughout the experiment, they were housed at standard laboratory environment (23±3°C, 12 h light/12 h dark) and allowed standard diet. The animals were handled according to the standard guide for the care and use of laboratory animals ***(NRC, 2011)***. The Institutional Review Board of the Faculty of Medicine, Zagazig University,

Egypt (ZU-IRB-2947/26-6-2016) approved the study protocol.

- Experimental design:

Drug treatment consisted of daily oral doses of 1ml through gastric tube, at the same time daily for 30 days. The rats were randomly allocated into four groups. **Group 1 (control)**, comprised 33 animals, equally distributed into three subgroup 1a, 1b and 1c that administered normal saline (solvent of tramadol), corn oil (solvent of NS), and NS dissolved in corn oil respectively. **Group 2 (tramadol-treated)** included 11 animal received tramadol (40mg/kg/day) for 30 days. **Group 3 (recovery)** included 11 rats that were left for recovery for 2 weeks, without any supplementary treatment, after 30 days of tramadol treatment (40mg/kg/day). **Group 4 (NS-cotreated)** comprised 11 rats administered tramadol (40mg/kg/day) along with NS (1ml/kg/day). At the time of sacrifice, blood samples were obtained from the retro-orbital plexuses for assaying serum testosterone levels, the animals were sacrificed, and the testes were dissected out for histological examination, morphometric study and immunohistochemical determination of iNOS.

- **Methods:**

I. Biochemical study:

The collected blood samples were centrifuged for 15 minutes (at 3000 r.p.m) to separate the serum. The resulting supernatants were stored at -20^{O}C till performing hormonal analysis. Serum testosterone was assessed using testosterone rat/mouse ELISA (enzyme-linked immunosorbent assay) kit (IBL-America, Minneapolis, Minnesota, USA; Catalog No. IB79174), according to the manufacturer's instructions.

II. Histopathological study:

Testicular specimens were fixed in Bouin fixative for 24 hours, then processed to paraffin blocks and 5µm thick sections were prepared for staining with haematoxylin-Eosin (H&E) and Mallory's trichrome stains ***(Bancroft and Gamble, 2008).***

III. Immunohistochemical study:

Immunohistochemical iNOS staining was performed on paraffin sections using a Streptavidin system with antibody against inducible nitric oxide synthase (iNOS) marker for oxidative stress. The kits were delivered from DAKO life trade, Egypt (code no M 618 for iNOS, clone 608) ***(Kiernan, 2008)***.

IV- Morphometric study:

The following parameters were calculated on routine H&E sections at x100 magnification, using MedCalc image analysis software 4.3.2 (MedCalc Software bvba, Belgium) at Histology Department, Faculty of Medicine, Zagazig University: the mean seminiferous tubular diameter/unit area and the mean epithelial height/unit area (unit area = microscopic field). Non-overlapping five images were randomly taken in the slides of different five rats in each group. The values obtained by these techniques were transformed to percentages and means ± standard deviations (SD) were then calculated. Data were collected, tabulated, and analyzed according to standardized statistical methods.

STATISTICAL ANALYSIS

The results were presented as means ± SD. All statistical analyses were carried out using one-way ANOVA (analysis of variance test) and Kruskal-Wallis tests in SPSS software (SPSS Inc., Chicago, IL, USA; version 18). P value < 0.05 was considered significant and P value < 0.001 was highly significant.

RESULTS

The current results revealed no significant differences among the control subgroups (1a,1b and 1c), therefore they were considered as one group and the average was used as a control to be compared with other groups.

I. Biochemical results:

Tramadol-treated rats revealed highly significant decreases in serum testosterone levels as compared to the controls (P<0.001). The recovery group revealed significant

improvement of serum testosterone levels as compared to tramadol-treated group (P=0.03); however, testosterone levels were still significantly lower than the control group (P=0.019).

NS-cotreated group exhibited highly significant improvement of the serum testosterone levels in comparison to tramadol-treated group (P<0.001), with no significant differences between NS-cotreated group and the control group **(Table 1)**.

Table (1): The mean values of serum testosterone levels in the different groups using analysis of variance test.

	Testosterone: (ng/ml) Mean ± SD	P_0	P_1	P_2
Group 1	2.66 ± 0.46	-		
Group 2	0.81 ± 0.41	<0.001**		
Group 3	1.43 ± 0.62	0.019*	0.03*	
Group 4	2.25 ± 0.63	0.47	<0.001**	0.04*

*F = 7.1; *****: significant (<0.05); ******: highly significant (<0.001)*

***P_0:** compares between group 1 (control) and other groups; **P_1:** compares between group 2 (tramadol-treated) and other groups; **P_2:** compares between group 3 (recovery) and group 4 (NS-cotreated).*

II. Histopathological results:

a-Haematoxylin and Eosin Stain:

The testicular parenchyma of the control group was organized into closely packed, regular seminiferous tubules surrounded by narrow interstitial spaces revealing groups of Leydig cells and blood capillaries. Each tubule was composed of an inner layer of stratified germinal epithelium featuring spermatogenic cells in different developmental stages and supporting Sertoli cells, and an outer layer of flattened myoid cells separated by a basement membrane. The spermatogenic cells included basally located spermatogonia, larger primary spermatocytes with large round dark nuclei, and small spermatids occupying almost half of the diameter of the tubules. They were arranged in that order from the basement membrane to the lumina of the tubules that revealed aggregations of sperms. Sertoli cells appeared pyramidal with triangular pale nuclei and distinct nucleoli **(Figs. 1a, 2a)**. Testicular sections of tramadol-treated group exhibited distorted shrunken seminiferous tubules with irregular contours. They were widely spaced with interstitial deposition of highly acidophilic vacuolated exudate. Sloughed and disorganized germinal epithelia, significant germ cell reduction, vacuolations, darkly stained nuclei and multinucleated giant cells were noticed in numerous tubules. Some tubular lumina showed exfoliated germ cells; others appeared empty wide. Additionally, hyaline material was deposited within some tubules **(Figs. 1b, 1c, 2b, 2c).**

The seminiferous tubules of the recovery group incompletely restored their normal architecture with partial improvement in the germinal epithelial arrangement and relatively narrow interstitial spaces as compared to tramadol-treated group. Some tubules were still distorted with sloughed germinal epithelium, reduced disorganized epithelial lining and vacuolations, while others displayed sperm aggregations in their lumina **(Figs. 1d, 2d).**

Testicular tissue slides of NS-cotreated group showed that most of the seminiferous tubules almost restored their normal morphology with relatively narrow interstitial spaces as compared to tramadol-treated group. However, less organized and detached germinal epithelia

were still detected in some tubules **(Figs. 1e, 2e).**

b- Mallory's trichrome stain:

Normal collagen distribution was detected in the testicular tissue of the control rats **(Fig. 3a)**, whereas tramadol-treated rats revealed increased collagen fiber deposition in the capsule and around blood vessels **(Fig. 3b)**. On the other hand, the recovery group displayed increased collagen fiber deposition only around the blood vessels **(Fig. 3c)**, while NS-cotreated group exhibited normal collagen fiber distribution **(Fig. 3d)**.

III-Immunohistochemical results:

Examination of iNOS immunohistochemical-stained sections of the control group exhibited negative immunoreaction in the germinal epithelium and Leydig cells **(Fig. 4a)**. Tramadol-treated group revealed intense expression of iNOS immunoreactivity in the germinal epithelium and Leydig cells **(Fig. 4b)**, whereas moderate and mild immunoreaction were observed in the recovery and NS-cotreated groups respectively **(Fig. 4c, 4d).**

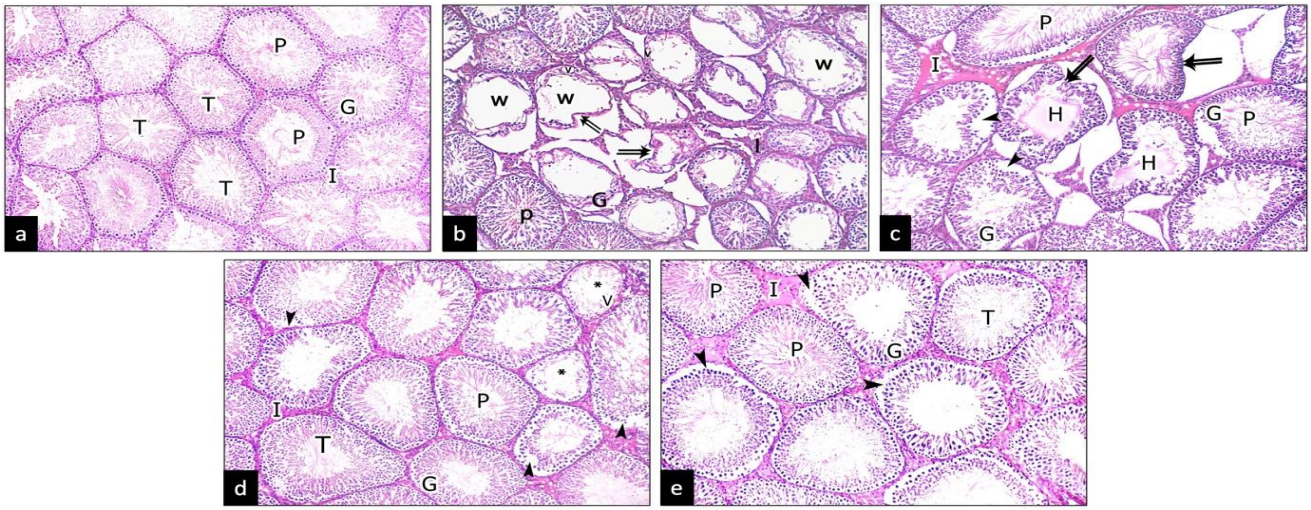

Figure (1). *Photomicrographs of testicular sections.* ***(a)*** *the control group displaying closely packed seminiferous tubules (T) lined by stratified germinal epithelium (G) and separated by narrow interstitial spaces (I). Sperm aggregations (P) are observed in the tubular lumina.* ***(b)*** *tramadol-treated group showing distorted seminiferous tubules with irregular contours (double arrows), apparent germ cell depletion (G), vacuolations (V), empty wide lumina (W) and relatively wide interstitium (I). Sperm aggregations (P) are noticed in few tubules.* ***(c)*** *tramadol-treated group showing irregular contours (double arrows) of the tubules, areas of sloughing (arrowhead) of the germinal epithelium (G), and relatively wide interstitial tissue revealing acidophilic vacuolated exudate (I). Some tubular lumina show deposition of hyaline material (H); others reveal aggregations of sperms (P).* ***(d)*** *The recovery group showing apparently normal seminiferous tubules (T) lined by stratified germinal epithelium (G) formed of several layers with sperm aggregations (P) in the tubular lumina. Few tubules appear distorted (asterisk) with reduced disorganized germinal epithelium and vacuolations (V). Areas of sloughing (arrowheads) and relatively narrow interstitial space (I) are also noticable.* ***(e)*** *NS-cotreated group showing tubules (T) lined by stratified germinal epithelium (G) with sperm aggregations (P) in their lumina. Areas of sloughing (arrowheads) and relatively narrow interstitial space (I) are also observed.* ***[H&E x 100]***

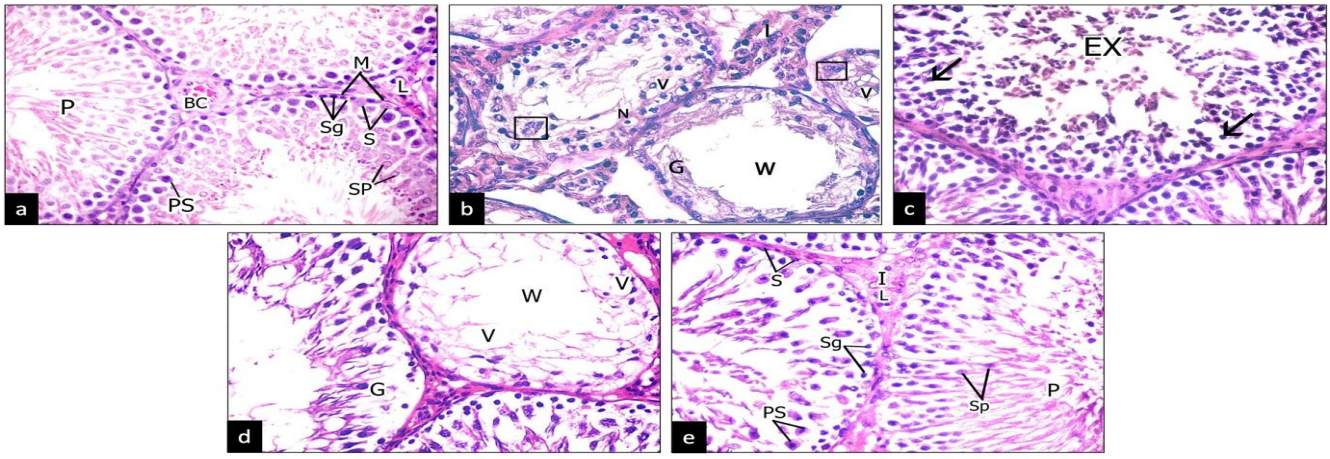

Figure (2). *Photomicrographs of testicular sections.* ***(a)*** *the control group displaying different spermatogenic cells: spermatogonia (Sg), primary spermatocytes (PS), spermatids (SP) and sperms (P), as well as Sertoli cells (S). Each tubule is ensheathed by a single layer of flattened myoid cells (M). The interstitial space shows Leydig cells (L) and blood capillaries (BC)* ***(b)*** *tramadol-treated-group showing markedly reduced and disorganized germinal epithelium (G), numerous vacuolations (V), multinucleated giant cells (square), darkly stained nuclei (N), empty wide lumen (W) and relatively wide interstitium (I)* ***(c)*** *tramadol-treated-group showing marked exfoliation of germ cells toward the lumen (EX) and darkly stained nuclei (arrows)* ***(d)*** *the recovery group showing reduced disorganized germinal epithelium (G), vacuolations (V) and wide empty lumen (W).* ***(e)*** *NS-cotreated group showing one tubule with well organized germ cell layers and aggregation of sperms (P) in the lumen, while the other is less organized. Spermatogonia (Sg), primary spermatocytes (PS), spermatids (Sp) and Sertoli cells (S) are detected. Leydig cells (L) are seen in the interstitial tissue (I)* ***[H&E x 400]****.*

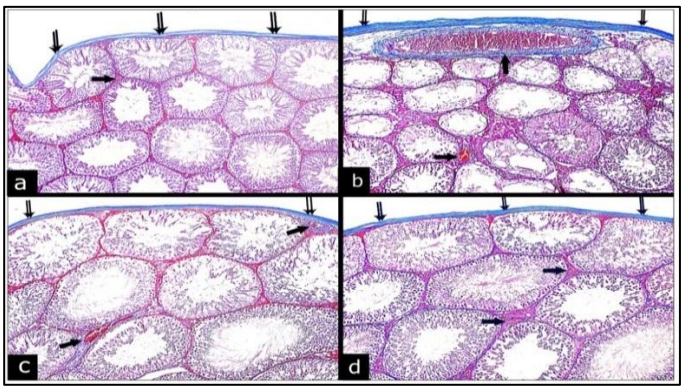

Figure (3). *Photomicrographs of testicular sections.* ***(a)*** *The control group exhibiting normal collagen fiber distribution in the capsule (double arrows) and around the blood vessels (thick arrow).* ***(b)*** *tramadol-treated group shows excessive collagen fiber deposition in the capsule (double arrows) and around the blood vessels (thick arrow).* ***(c)*** *the recovery group shows normal distribution of collagen fibers in the capsule (double arrows) and increased distribution around the blood vessels (thick arrow).* ***(d)*** *NS-cotreated group shows normal collagen fiber distribution in the capsule (double arrows) and around the blood vessels (thick arrow).* ***[Mallory's trichrome x100]***

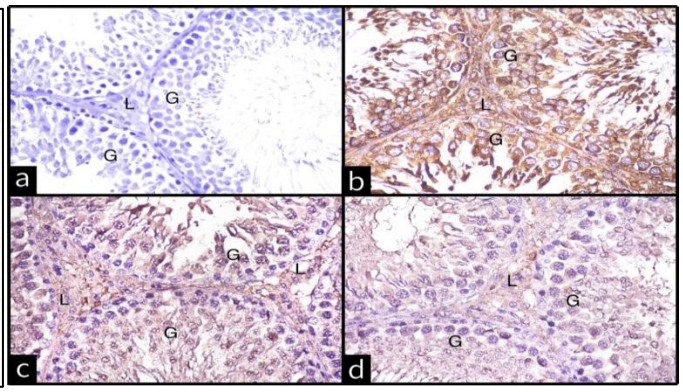

Figure (4). *Photomicrographs of testicular sections.* ***(a)*** *The control group exhibits negative iNOS immunoreactivity in the germinal epithelium (G) and Leydig cells (L).* ***(b)*** *tramadol-treated group showing intense expression of iNOS immunoreactivity in the germinal epithelium (G) and Leydig cells (L).* ***(c)*** *the recovery group exhibiting moderate iNOS expression in the germinal epithelium (G) and Leydig cells (L).* ***(d)*** *NS-cotreated group showing mild iNOS expression in the germinal epithelium (G) and Leydig cells (L).* ***[Anti- iNOS immunostaining x 400]***

IV- Morphometric results:

a. The Diameter of the Seminiferous Tubules

In comparison to the controls, tramadol-treated rats revealed highly significant decreases in the tubular diameters (P<0.001). On the other hand, the tubular diameters of both the recovery and NS-cotreated groups displayed high statistically significant increases when compared with tramadol-treated group and no significant differences when compared with the control group **(Table 2)**.

b. The Height of the Seminiferous Epithelium:

Tramadol-treated group exhibited statistically significant decreases in the seminiferous epithelial heights as compared to the control (P=0.003). In comparison to tramadol-treated group, significant increases were detected in the epithelial heights of the recovery group (P=0.036) and NS-cotreated group (P=0.007). No significant differences in the epithelial heights were detected between the control group and both the recovery and NS-cotreated groups **(Table 3)**.

Table (2). The mean values of the diameters of the seminiferous tubules in the different groups using analysis of variance test

	Diameter (µm) Mean ± SD	P_0	P_1	P_2
Group1	553.5±37.9	-		
Group 2	374.7±45.1	<0.001**		
Group 3	536.6±50.5	0.36	<0.001**	
Group 4	549.4±50.97	0.22	<0.001**	0.54

*F = 41.3; ******: highly significant (<0.001)*

***P_0:** compares between group 1 (control) and other groups; **P_1:** compares between group 2 (tramadol-treated) and other groups; **P_2:** compares between group 3 (recovery) and group 4 (NS-cotreated).*

Table (3). The mean values of the epithelial heights of the seminiferous tubules in the different groups using analysis of variance test

	Height of epithelium (µm) Mean ± SD	P_0	P_1	P_2
Group1	111.9±14.96	-		
Group 2	89.2±16.9	0.003*		
Group 3	105.1±16.3	0.32	0.036*	
Group 4	107.4±10.96	0.43	0.007*	0.69

*F = 4.78; *****: significant (<0.05)*

***P_0:** compares between group 1 (control) and other groups; **P_1:** compares between group 2 (tramadol-treated) and other groups; **P_2:** compares between group 3 (recovery) and group 4 (NS-cotreated).*

DISCUSSION

Tramadol abuse has become a public health crisis in different parts of the world ***(UNODC, 2019)***. Tramadol abuse patients are more likely to have decreased libido, erectile dysfunction, and anorgasmia ***(Bassiony et al., 2019)***. Moreover, tramadol abuse is associated with decreased sperm count, vitality and normal forms, lower free testosterone levels and hyperprolactinemia ***(Bassiony et al., 2020)***.

In the current investigation, tramadol-treated rats displayed highly significant reduction in serum testosterone levels compared with the controls. This finding is in consistence with the results of ***Udefa et al. (2020); Aprioku et al. (2021)*** *and* ***Adelakun et al. (2022)*** who explained that by tramadol's direct influence on the hypothalamic–pituitary axis causing suppression of both FSH and LH secretion. Testosterone is crucial for maintenance of spermatogenesis. Testosterone suppression has three main effects on fertility: a) the blood-testis barrier is disrupted, exposing postmeiotic germ cells to toxic agents, b) round spermatids prematurely detach from Sertoli cells due to a cell adhesion defect, thereby further progression of round to elongated spermatids is arrested, and c) mature spermids fail to spermiate and undergo phagocytosis ***(Meng et al., 2010; Walker, 2011)***. Accordingly, suppression of testosterone secretion could be implicated in impairing testicular structure and function in tramadol-treated rats.

The current histological results of tramadol-treated group strongly confirm the biochemical findings. Testicular tissue damage was evidenced by sloughed and disorganized germinal epithelial lining with marked germ cell depletion, vacuolations, pyknotic nuclei and multinucleated giant cells, in addition to interstitial deposition of highly acidophilic vacuolated exudate. Similar results were detected by ***Minisy et al. (2020)*** and **A*hmad et al. (2021b)***. Conversely, ***Udefa et al. (2020)*** observed no histological lesions other than decreased sperm population in the testicular tissue of rats received tramadol (20 mg/kg for 4 weeks). Differences in the daily tramadol dose might have been responsible for such controversy.

Manivannan et al. (2009) elucidated germ cell sloughing on the basis that impaired Sertoli cells cause decrease in seminiferous tubular fluid secretion, resulting in apical shedding and germ cell death. According to ***El-Sherif and El-Mehi (2015)***, germ cell vacuolations might be due to metabolic disturbances in these cells or due to sloughing of the germ cells and loss of their exact biological environment.

Multinucleated giant cells detected in the testicular tissue of tramadol-treated rats might have been resulted from widening of the intercellular bridges between adjacent spermatids resulting in fusion of two or more cells ***(Ghoneim et al., 2014)***. Additionally, accumulation of acidophilic hyaline material in the interstitial tissue and in the lumina of the tubules could be attributed to increased microvascular permeability induced by excess NO resulting from iNOS activation ***(Salama et al., 2003; Hauser et al., 2008)***.

Increased collagen fiber deposition depicted in the testicular tissue of tramadol-treated group in the current research might be owing to disrupted collagen metabolism which could be linked to oxidative stress ***(Minisy et al., 2020)***. In addition to serving a supportive function, collagen acts as a pivotal organizer of lipid metabolism, ion transport and genetic expression. Accordingly, altered collagen fiber distribution may promote cellular dysfunction ***(Surazynski et al., 2008)***. It is noteworthy that increased extracellular matrix thickness is

associated with male infertility ***(Adam et al., 2012).***

Confirming the present histopathological results, morphometric examination of tramadol-treated group exhibited statistically significant decreases in the mean values of the tubular diameters and the epithelial heights. These results are in agreement with the findings of ***Adelakun et al. (2022)***. In contrast, ***Ahmed and Kurkar (2014)*** reported insignificant differences in the aforementioned parameters in tramadol-treated rats as compared to the control. Variations in dosing schedules and regimens may account for this contradiction. In the current work and that of ***Adelakun et al. (2022)***, tramadol (40 mg/kg/d) was administered orally for 4 weeks and 8 weeks respectively, while in the study by ***Ahmed and Kurkar (2014)***, tramadol (40 mg/kg) was injected subcutaneously three times weekly for 8 weeks.

In the current investigation, tramadol administration resulted in intense expression of iNOS immunoreactivity in the germinal epithelium and Leydig cells. Increased expression of iNOS mediates the production of a large amount of NO. Excess NO reacts with superoxide anion and forms potent oxidant peroxynitrite responsible for cell damage by nitrating cellular macromolecules. In addition, excess NO diminishes intracellular glutathione and increases the vulnerability to oxidative stress ***(Xu et al., 2014)***. Oxidative stress results from disturbed balance between reactive oxygen species (ROS) generation and the defensive antioxidant system ***(Juan et al., 2021)***. Generally, ROS can cause damage to various cellular components including: a) lipid peroxidation of polyunsaturated fatty acids (e.g. membrane phospholipids), b) damage of nucleic acids and c) oxidation of proteins ***(Hawkins and Davies, 2019; Ito et al., 2019; Yan and Zaher, 2019)***. Testes, being rich in polyunsaturated fatty acids, are particularly vulnerable to oxidative damage ***(Lenzi et al., 2002)***. Accordingly, Oxidative stress could be implicated as a main mechanism of tramadol-induced testicular tissue damage.

In the current research, the recovery group displayed partial improvements in serum testosterone levels, cellular damage, morphometric findings and iNOS immunoreactivity as compared to tramadol-treated group, however all these parameters did not completely return to normal. These finding are supported by ***Nna and Osim (2017)*** and ***Ibrahim and Salah-Eldin (2019)***. On the other hand, ***Aprioku et al. (2021)*** stated that the toxic testicular effects of therapeutic doses of tramadol are reversible at lower doses (1.25 and 2.5 mg/kg/day for 30 days), but persistent at higher doses (5 mg/kg/day for 30 days) following 30 days of drug withdrawal. Additionally, ***Azari et al. (2014)*** reported that the testes restored their normal structure and function following recovery period of 12 weeks after tramadol administration of 10 and 20 mg/kg, 3 times a week, for 6 weeks. These variations might be due to differences in the experimental design. Taken together, reversibility of tramadol-induced testicular alterations depends on dosage and duration of administration as well as period of drug withdrawal.

In the current study, coadministration of NS ameliorated the detrimental testicular effects of tramadol, as evidenced by marked improvement in the testicular architecture and morphometric parameters, decreased iNOS immunoreactivity, and increased serum testosterone levels as compared to tramadol-treated rats. In accordance with these findings, ***Elkhateeb et al. (2015)*** stated that concomitant administration of NS with tramadol resulted in amelioration of tramadol's hepatic and renal toxicities. They ascribed this to the antioxidant effect of NS and its ability to counteract the increased lipid peroxidation. Furthermore, NS alleviated

tramadol-induced ultrastructural changes in the rat brain ***(Omar, 2016).***

Additionally, ***Awadalla (2012); Assi et al. (2017)*** *and* ***Mosbah et al. (2018)*** mentioned that NS mitigated cisplatin, lead acetate and acetamiprid induced oxidative and structural testicular tissue damage. Moreover, ***Abd-Elkareem et al. (2021)*** illustrated the protective role of NS against structural and functional testicular damage associated with monosodium glutamate intake through enhancing redox homeostasis and restoring the hormonal balance of hypothalamic–pituitary axis.

In accordance with the findings of the present investigation, ***Elshama et al. (2013); Hussein et al. (2014)*** and ***Mohamed et al. (2015)*** stated that concomitant administration of NS along with colchicine, aluminium chloride and noise resulted in marked decrease in collagen fibers in testicular tissues, Moreover, ***Kanter (2011); Fouad and Jresat (2015)*** and ***Erol et al. (2017)*** reported that thymoquinone, one of NS most prominent phytochemical ingredients, decreased testicular iNOS expression after exposure to toluene, cadmium and ischemia – reperfusion injury respectively. The restoration of serum testosterone levels in NS-cotreated rats could be attributed to the ability of NS to increase the testicular steroidogenic enzymes activities ***(Akintunde et al., 2019)***. NS is a rich source of unsaturated fatty acids which induce the activity of 3β- and 17 β-hydroxysteroid dehydrogenases; the key regulatory enzymes in the biosynthesis of testosterone ***(de Catalfo et al., 2009; Alrashidi et al., 2020).***

CONCLUSION

It can be concluded that coadministration of NS oil along with tramadol ameliorated the deleterious testicular toxic effects of tramadol. This improvement was clearer than that resulted from tramadol withdrawal, proving the testicular protective role of NS.

RECOMMENDATIONS

Tramadol should be administered only when indicated with appropriate dose monitoring and under medical supervision. Regular monitoring of testosterone is highly recommended during long term tramadol therapy to avoid its adverse effects on male fertility. NS supplementation is also advisable in patients on tramadol therapy as it mitigates tramadol-induced testicular damage. Further studies with larger doses of NS and longer durations of recovery are also recommended.

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REFERENCES

1. **Abd-Elkareem, M., Soliman, M., Abd El-Rahman, M. A. M., and Khalil, N. S. A. (2021):** Antioxidant and cytoprotective effects of Nigella sativa L. seeds on the testis of monosodium glutamate challenged rats. *Sci. Rep., 11:13519.* https://doi.org/10.1038/s41598-021-92977-4
2. **Adam, M., Urbanski, H. F., Garyfallou, V. T., Welsch, U., Köhn, F. M., Ullrich Schwarzer, J., Strauss, L., Poutanen, M., and Mayerhofer, A. (2012):** High levels of the extracellular matrix proteoglycan decorin are associated with inhibition of testicular function. *Int. J. Androl., 35(4): 550–561.* https://doi.org/10.1111/j.1365-2605 .2011 .01225.x
3. **Adelakun, S. A., Ukwenya, V. O., and Akintunde, O. W. (2022):** Vitamin B_{12} ameliorate Tramadol-induced oxidative stress, endocrine imbalance, apoptosis and NO/iNOS/NF-κB expression in Sprague Dawley rats through regulatory mechanism in the pituitary-gonadal axis. *Tissue & cell, 74: 101697.* https://doi.org/10.1016/j.tice.2021.101697

4. **Ahmad, M. F., Ahmad, F. A., Ashraf, S. A., Saad, H. H., Wahab, S., Khan, M. I., Ali, M., Mohan, S., Hakeem, K. R., and Athar, M. T. (2021a):** An updated knowledge of Black seed (Nigella sativa Linn.): Review of phytochemical constituents and pharmacological properties. *J. Herbal med., 25: 100404.* https://doi.org/10.1016/j.hermed.2020.100404
5. **Ahmad, R. M., AL-Hubaity, A. Y., and Alazow, N. S. (2021b):** The Role of Vitamin C against Structural Changes in Testis of Male Albino Rats Induced by Tramadol and its Withdrawal. *Coll. Basic Edu. Res. J., 17(2): 1865-1878.* doi: 10.33899/berj.2021.168581.
6. **Ahmed, M. A., and Kurkar, A. (2014):** Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress. *Clin. .Exp. Pharmacol. Physiol., 41(4): 317–323.* https://doi.org/10.1111/1440-1681.12213
7. **Akintunde, J. K., Irondi, A. E., Ajani, E. O., and Olayemi, T. V. (2019):** Diet formulated with black seed flour activates regulatory androgenic enzymes and neuroendocrine hormones in leachate-exposed reproductive damage of rat testes. *J. Nutr. Int. Metab., 15:70-77.*
8. **Alrashidi, M., Derawi, D., Salimon, J., and Yusoff, M. F. (2020):** An investigation of physicochemical properties of Nigella sativa L. Seed oil from Al-Qassim by different extraction methods. *J. King Saud U. Sci., 32(8): 3337-3342.*
9. **Aprioku, J., Okpe, B., and Ben, D. (2021):** Therapeutic levels of short-term tramadol administration negatively affect testis function in rats. *Asi. Pac. J. Repr., 10 (1): 29–35.*
10. **Assi, M. A., Hezmee, M. N. M., Abba, Y., Rajion, M. A., Wahid, H., and Yusof, M. S. M. (2017):** Assessment of therapeutic effects of Nigella sativa against chronic lead acetate-induced reproductive dysfunction in male Sprague-Dawley rats. Comparative Clinical Pathol., 26 (1): 87-97.
11. **Awadalla, E. A. (2012):** Ameliorative effect of the crude oil of the Nigella sativa on oxidative stress induced in rat testes by cisplatin treatment. *Biomed. Prev. Nutr., 2(4): 265-268.*
12. **Azari, O., Emadi, L., Kheirandish, R., Bafti, H.S., Reza, M., Nejad, E., and Faroghi, F. (2014):** The effects of long-term administration of tramadol on epididymal sperm quality and testicular tissue in mice. *Ir. J. Vet. Sur., 9 (1): 23- 30.*
13. **Bancroft, J.D. and Gamble, A. (2008):** Theory and practice of histological techniques. 6th Edition, *Churchill Livingstone/ Elsevier Health Sci.. pp: 165- 175.*
14. **Bassiony, M. M., Youssef, U. M., and El-Gohari, H. (2020):** Free Testosterone and Prolactin Levels and Sperm Morphology and Function Among Male Patients with Tramadol Abuse: A Case-Control Study. *J. Clin. Psychopharmacol., 40(4): 405–408.*
15. **Bassiony, M. M., Youssef, U. M., El-Gohari, H., Abdelghani, M., and Hassan, M.S. (2019):** Sexual dysfunction among male patients with tramadol abuse: a case-control study. *Int. J. Sex Health, 31:109–114.*
16. **Beakley, B. D., Kaye, A. M., and Kaye, A. D. (2015):**Tramadol, Pharmacology, Side Effects, and Serotonin Syndrome: A Review. *Pain Physician, 18(4): 395–400.*
17. **Bisht, S., Faiq, M., Tolahunase, M., and Dada, R. (2017):** Oxidative stress and male infertility. *Nature rev.. Urol., 14(8): 470–485.*
18. **Cinelli, M. A., Do, H. T., Miley, G. P., andSilverman, R. B. (2020):** Inducible nitric oxide synthase: Regulation, structure, and inhibition. *Med. Res. Rev., 40(1): 158–189.* https://doi.org/10.1002/med.21599
19. **Dal, D., Salman, M. A., Salman, A. E., Iskit, A. B., and Aypar, U. (2006):** The involvement of nitric oxide on the analgesic effect of tramadol. *Eur. J. Anaesthesiol., 23: 175–177.*
20. **de Catalfo, G. E. H., de Alaniz, M. J., andMarra, C. A. (2009):** Influence of commercial dietary oils on lipid composition

and testosterone production in interstitial cells isolated from rat testis. *Lipids, 44(4): 345.*

21. **Duca, Y., Calogero, A. E., Cannarella, R., Condorelli, R. A., and La Vignera, S. (2019):** Current and emerging medical therapeutic agents for idiopathic male infertility. *Expert opin. Pharmacother., 20(1): 55-67.*
22. **El-Sherif, N.M. and El-Mehi, A.E. (2015):** Effect of Semicarbazide on the testis of juvenile male albino rat. *J. Interdisp. Histopathol., 3 (1): 9- 18.*
23. **Elkhateeb, A., El Khishin, I., Megahed, O., and Mazen. F. (2015):** Effect of Nigella sativa Linn oil on tramadol-induced hepato- and nephrotoxicity in adult male albino rats. *Toxicol. Reports, 2(C): 512–519.*
24. **Elshama, S. S., Shehab, G. M., El-Kenawy, A. E., Osman, H. E. H., and Farag, M. M. (2013):** Role of Nigella Sativa Seeds on modulation testicular toxicity of colchicine repeated use in adult albino rats. *Life Sci. J., 10(4), 1629-1639.*
25. **Erol, B., Sari, U., Amasyali, A.S., Ozkanli, S., Sogut, S., Hanci, V., Efiloglu, O., Danacioglu, Y.O., Engin, P., Yencilek, F., Atis, G., Yildirim, A., Alkoc, A.O, and Caskurlu, T. (2017):** Comparison of combined antioxidants and thymoquinone in the prevention of testis ischemia –reperfusion injury. *Androl., 5 (1): 119- 124.*
26. **Fouad, A.A. and Jresat, I. (2015):** Thymoquinone therapy abrogates toxic effect of cadmium on rat testes. *Androl., 47 (4): 417-426.*
27. **Ghoneim, F. M., Khalaf, H. A., Elsamanoudy, A. Z., and Helaly, A. N. (2014):** 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., 7(11): 7323–7341.*
28. **Hamidi-Madani, A., Motiee, R., Mokhtari, G., Nasseh, H., Esmaeili, S., and Kazemnezhad, E. (2018):** The Efficacy and Safety of On-demand Tramadol and Paroxetine Use in Treatment of Life Long Premature Ejaculation: Randomized Double-blind Placebo-controlled Clinical Trial. *J. Repr. Infer.,19(1): 10–15.*
29. **Hauser, B., Matejovic, M., and Radermacher, P. (2008):** Nitric oxide, leukocytes and microvascular permeability: causality or bystanders?. *Crit. Care 12: 104* https://doi.org/10.1186/cc6214
30. **Hawkins, C. L., and Davies, M. J. (2019):** Detection, identification, and quantification of oxidative protein modifications. *J. Biol. Chem., 294(51): 19683-19708.*
31. **Hussein, O.A., Abou Elghait, A.T., and Ahmed, S.F. (2014):** Effect of nigella sativa oil on aluminum chloride-induced testicular damage in male albino rats: a light and electron microscopic study. *Egy. J. Histol., 37 (4): 741-755.*
32. **Ibrahim, M. A.-L., and Salah-Eldin, A. -E. (2019)**: Chronic Addiction to Tramadol and Withdrawal Effect on the Spermatogenesis and Testicular Tissues in Adult Male Albino Rats. *Pharmacol., 103 (3-4): 202–211.* https://doi.org/10.1159/000496424
33. **International Narcotics Control Board (2018)**: Tramadol, review of the global situation, in Alert7. New York: United Nations Publications.
34. **Ito, F., Sono, Y., and Ito, T. (2019):** Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antiox., 8(3): 72.*
35. **Juan, C. A., Pérezdela Lastra, J.M., Plou, F.J., and Pérez-Lebeña, E. (2021):** The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *Int.. J. Mol. Sci., 22: 4642.* https://doi.org/10.3390/ijms 22094642
36. **Kanter, M. (2011):** Thymoquinone re-establishes spermatogenesis after testicular injury caused by chronic toluene exposure in rats. *Toxicol. Ind. Health, 27 (2): 155- 166.*

37.Kasman, A. M., Del Giudice, F., and Eisenberg, M. L. (2020): New insights to guide patient care: the bidirectional relationship between male infertility and male health. *Fert. Ster., 113(3): 469-477.*

38. Khazdair, M. S., Ghafari, S., and Sadeghi, M. (2021): Possible therapeutic effects of Nigella sativa and its thymoquinone on COVID-19, *Pharmaceut.l Biol., 59:1: 694-701*, DOI: 10.1080/13880209.2021.1931353

39. Kiernan, J. (2008): Histological and histochemical methods. Theory and Practice; 4th edition. Butterworth – Heinmann. Oxford, Boston.

40. Kolahdooz, M., Nasri, S., Modarres, S. Z., Kianbakht, S., and Huseini, H. F. (2014): Effects of Nigella sativa L. seed oil on abnormal semen quality in infertile men: A randomized, double-blind, placebo-controlled *Clin. Trial. Phytomed., 21(6): 901–905.* https://doi-org.sdl.idm.oclc.org /10.1016 /j.phymed.2014.02.006

41. Koohsari, M., Ahangar, N., Mohammadi, E., and Shaki, F. (2020): Ameliorative Effect of Melatonin Against Reproductive Toxicity of Tramadol in Rats via the Regulation of Oxidative Stress, Mitochondrial Dysfunction, and Apoptosis-related Gene Expression Signaling Pathway. *Addic. health, 12 (2): 118–129.*

42. Lenzi, A., Gandini, L., Lombardo, F., Picardo, M., Maresca, V., Panfili, E., Tramer, F., Boitani, C., and Dondero, F. (2002): Polyunsaturated fatty acids of germ cell membranes, glutathione and blutathione-dependent enzyme-PHGPx: from basic to clinic. *Contracep., 65(4): 301-304.*

43. Manivannan, B., Mittal, R., Goyal, S., Ansari, A.S., and Lohiya, N.K. (2009): Sperm characteristics and ultrastructure of testes of rats after long-term treatment with the methanol subfraction of Carica papaya seeds. *As. J. Androl., 11 (5): 583- 599.*

44. Meng, J., Greenlee, A. R., Taub, C. J., and Braun, R. E. (2011): Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. *Biol. Repr., 85(2): 254–260.* https://doi.org/10.1095/biolreprod.110.090621

45. Minisy, F. M., Shawki, H. H., El Omri, A., Massoud, A. A., Omara, E. A., Metwally, F. G., Badawy, M. A., Hassan, N. A., Hassan, N. S., and Oishi, H. (2020): Pomegranate Seeds Extract Possesses a Protective Effect against Tramadol-Induced Testicular Toxicity in Experimental Rats. BioMed Res. Int., 2732958. https://doi.org/10.1155/2020/2732958

46. Mohamed, A.F., Hanafy, S.M., and Mahmoud, A. (2015): The possible protective effect of black seed (nigella sativa) oil on the testes of adult male albino rat exposed to noise. *J. Histol. Histopathol., 2 (1): 21.*

47. Mosbah, R., Djerrou, Z., and Mantovani, A. (2018): Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. *Drug Chem. Toxicol., 41(2): 206-212.*

48. National Research Council (2011): Guide for the Care and Use of Laboratory Animals. 8th ed. *National Academies Press (US), Washington DC, pp. 21-55.* https://doi.org/10.17226/12910.

49. Nna, V. U., and Osim, E. E. (2017): Testicular toxicity following separate and combined administration of PDE5 inhibitors and opioid: assessment of recovery following their withdrawal. *Androl., 49(6).* https://doi-org.sdl.idm.oclc.org/10.1111/and.12669

50. Omar, N. M. (2016): Nigella sativa oil alleviates ultrastructural alterations induced by tramadol in rat motor cerebral cortex. *J. Micro. Ultrastr., 4(2): 76–84.* https://doi.org/10.1016/j.jmau.2015.12.001

51. Otasevic, V., Stancic, A., Korac, A., Jankovic, A., and Korac, B. (2020): Reactive oxygen, nitrogen, and sulfur species in human male fertility. A crossroad of cellular signaling and pathology. *BioFactors, 46(2): 206–219.* https://doi-org.sdl.idm. oclc.org/ 10.1002/ biof.1535

52. Peluffo, G., Calcerrada, P., Piacenza, L., Pizzano, N., and Radi, R. (2009): Superoxide-mediated inactivation of nitric oxide and peroxynitrite formation by tobacco smoke in vascular endothelium: studies in cultured cells and smokers. American Journal of Physiology-*Heart Circ. Physiol., 296(6): 1781-1792.*

53. Preuss, C. V., Kalava, A., and King, K. C. (2021): Prescription of Controlled Substances: Benefits and Risks. *In StatPearls. StatPearls Publishing.*

54. Reagan-Shaw, S., Nihal, M., and Ahmad, N. (2008): Dose translation from animal to human studies revisited. *FASEB J., 22(3): 659-661.*

55. Salama, N., Bergh, A., and Damber, J.E. (2003): The changes in testicular vascular permeability during progression of the experimental varicocele. *Eur. Urol., 43:84-91.*

56. Sharma, J. N., Al-Omran, A. and Parvathy, S. S. (2007): Role of nitric oxide in inflammatory diseases. *Inflammopharmacol., 15(6): 252-259.*

57. Sharma, R., Biedenharn, K. R., Fedor, J. M., and Agarwal, A. (2013): Lifestyle factors and reproductive health: taking control of your fertility. *Repr. Biol. Endocrinol., 11: 66.* https://doi.org/10.1186/1477-7827-11-66

58. Soliman, T., Shaher, H., Mohey, A., El-Shaer, W., and Sebaey, A. (2021): Gonadotoxic effect of tramadol administration: A prospective controlled study. *Arab J. Urol., 20(1): 54–60.* https://doi.org/ 10.1080/ 2090598X.2021.2002634

59. Sultan, M. T., Aslam, F., Rasool, B., Imran, M., Ahmad, A. N., Tariq, H.B., Afzal, M.I., Habib-ur-Rehman, Shahbaz, M. and Nadeem, M. (2021): Nigella sativa ameliorates oxidative stress induced adverse effects in rodent modeling studies: Indices of serum chemistry and hematology. Food Science and *Technol., 41(2): 404-411.* https://doi.org/10.1590/fst.01120

60. Surazynski, A., Miltyk, W., Palka, J., and Phang, J. M. (2008): Prolidase-dependent regulation of collagen biosynthesis. *Amino Acids, 35(4): 731–738.* https://doi.org/10.1007/s00726-008-0051-8

61. Udefa, A. L., Beshel, F. N., Nwangwa, J. N., Mkpe, I. D., Ofuru, O. S., Sam-Ekpe, V. G., and Stephen, G. I. (2020): Vitamin E administration does not ameliorate tramadol-associated impairment of testicular function in Wistar rats. *Androl., 52(1): e13454.* https://doi-org.sdl.idm.oclc.org/10.1111/and.13454

62. United Nations Office on Drugs and Crime (2019): World Drug Report 2019, United Nations Publication, No. E.19.XI.8; Available Online: wdr.unodc.org/wdr2019.

63. Walker W. H. (2011): Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis, 1(2): 116–120.* https://doi.org/10.4161/spmg.1.2.16956

64. World Health organization (2018): Critical Review Report: Tramadol. 41st *Expert Committee on Drug Dependence Meeting, Geneva.*

65. Xu, M., Wei, Q., Zheng, K., Mao, D., Zheng, Y., Li, Y., and Shi, F. (2014): Protective effects of big-leaf mulberry and physiological roles of nitric oxide synthases in the testis of mice following water immersion and restraint stress. *Acta Histochemica., 116 (8): 1323-1330.*

66. Yan, L. L., and Zaher, H. S. (2019): How do cells cope with RNA damage and its consequences?. *J. Biol. Chem., 294(41): 15158-15171.*

التأثير التحسيني لحبة البركة على سمية الخصية المحدثة بالترامادول في الجرذان البالغة

رانيا سعد محمد رمضان، محمد عبد المنعم محمود عبد الرحمن، ريهام طلعت الدمرداش إبراهيم، دعاء محمد يوسف
قسم التشريح الآدمي وعلم الأجنة ،
كلية الطب البشري – جامعة الزقازيق – مصر

المقدمة: يمثل تعاطي الترامادول مشكلة صحية كبيرة في مصر. حيث ان الترامادول يسبب الإجهاد التأكسدي عن طريق خفض مستويات مضادات الأكسدة في الجسم. تمتلك حبة البركة خصائص قوية مضادة للأكسدة.
الهدف من البحث: تهدف هذه الدراسة إلى تحديد التغيرات التي قد تحدث في خصية الفئران البيضاء نتيجة التعرض لعقار الترامادول وتقييم استشفائها بعد انسحاب الترامادول وكذلك إمكانية الوقاية أو التقليل من حدة هذه الآثار الضارة باستخدام حبة البركة بالتزامن مع الترامادول.
المواد والطرق المستخدمة: تم تقسيم ستة وستين من ذكور الجرذان البالغة إلى أربع مجموعات: المجموعة الضابطة (تم تقسيمها بالتساوي إلى ٣ مجموعات فرعية تلقت محلول ملحي طبيعي وزيت ذرة وحبة البركة مذابة في زيت الذرة على التوالي) ومجموعة معالجة بالترامادول (٤٠ مجم / كجم / يوم) عن طريق الفم لمدة ٣٠ يومًا ومجموعة الاستشفاء (تركت لمدة أسبوعين بدون أي علاج إضافي بعد تناول عقار الترامادول لمدة ٣٠ يوم والمجموعة المعالجة بحبة البركة (١ مل/ كجم / يوم عن طريق الفم) مع الترامادول. في نهاية التجربة تم الحصول على عينات من الدم لقياس مستويات هرمون التستوستيرون وتمت معالجة الخصيتين للفحص النسيجي والمورفومتري والكيميائي المناعي.
النتائج: أدى العلاج بالترامادول إلى تدهور البنية الدقيقة للخصية وزيادة رد الفعل المناعي للاينوز، إضافة الى انخفاض ذو دلالة احصائية في أقطار الأنيبيبات المنوية وارتفاع النسيج الطلائي ومستويات هرمون التستوستيرون في الدم مقارنة بالمجموعة الضابطة. على الجانب الآخر، أظهرت مجموعة الاستشفاء تحسن جزئي في جميع المقاييس التي تم اختبارها. كما أدى التناول المتزامن لـحبة البركة إلى تحسن كبير في التأثيرات الضارة للترامادول على الخصية؛ كان هذا التحسن أوضح من ذلك الناتج عن انسحاب الترامادول، مما يثبت الدور الوقائي لـحبة البركة.
الاستنتاج: حبة البركة لها دور مهم في الحماية من تلف الخصية الناجم عن الترامادول.
التوصيات: يجب إعطاء الترامادول فقط عند وصفه للعلاج مع مراقبة الجرعة المناسبة. كما يُنصح بتناول حبة البركة في المرضى الذين يخضعون للعلاج بالترامادول.