

PAPER

Moringa oleifera leaves extract modulates toxicity, sperms alterations, oxidative stress, and testicular damage induced by tramadol in male rats

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Abstract

Tramadol is a synthetic opioid analgesic used for moderate-to-severe pain structurally related to codeine and morphine, where their analgesic mechanism is a result of opioid and non-opioid mechanisms. This study was designed to evaluate the effects of *Moringa oleifera* leaves extract (MLE) on tramadol-induced testicular toxicity, sperm changes, testicular damage, and oxidative stress in male rats. Forty male albino rats were divided into four groups and treated for 4 weeks (group 1, as control; group 2, MLE; group 3, tramadol; group 4, MLE + tramadol). The relative body weight, relative testes weight, serum total testosterone, luteinizing hormone, follicle-stimulating hormone, sperm counts, vitality, total sperm motility, catalase, and superoxide dismutase activities were significantly decreased in tramadol-treated group when compared with the control group. In contrast, sperm abnormality, immotile sperm percent, testicular injury, and TBARS concentration in testes were significantly increased in the tramadol-treated group. In addition, histopathological examination for the tramadol-treated group has shown incomplete spermatogenesis, moderate degeneration in some seminiferous tubules with a significant decrease in the number of spermatogenic cells and depletion of Leydig cells. The administration of MLE with tramadol ameliorates the testicular toxicity, injury, sperm count, abnormalities, and oxidative stress induced by tramadol.

Key words: tramadol; *Moringa oleifera* leaves extract; testicular toxicity; oxidative stress; spermatogenesis

Introduction

Tramadol (Amadol) is prescribed for moderate-to-severe pain and it a synthetic opioid analgesic that structurally related to codeine and morphine, where their analgesic mechanism is a result of opioids and non-opioid mechanisms [1, 2]. It inhibits the neuronal reuptake of norepinephrine and serotonin as do the antidepressant drugs [3]. Also, it has many adverse effects as

life-threatening ones such as loss of consciousness, cardiovascular failure, pruritus, nausea, somnolence, headache, dizziness, sweating, constipation, seizures, and serotonin syndrome [4]. Tramadol is well known under the trade names amadol, ultracet, tramacet, altram, tramapap, and acetaminophen [5, 6].

People in all continents have used thousands of indigenous plants for the treatment of various ailments dating back to

Received: 13 November 2019; Revised: 31 December 2019; Accepted: 18 February 2020

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prehistory. *Moringa oleifera* is a tree used in traditional medicine worldwide as having value both as a preventative and treatment agent of several health conditions, including the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological, hepatic, and renal disorders [7].

Moringa leaves can be a good source of natural antioxidants, and it has strong antiradical activity. Caffeic, p-coumaric and ferulic acids are the dominant phenolic constituents of *M. oleifera* leave extract [8, 9]. Therefore, the present study was conducted to examine the possible modifying effects of *M. oleifera* leaves extract (MLE) against testicular toxicity, sperm alternations, oxidative stress, and testicular damage induced by tramadol in male rats.

Materials and Methods

Chemicals and reagents

Tramadol or Amadol tablets. Tramadol, commercially available capsules, each contains 50-mg tramadol hydrochloride (Amadol, manufactured by ADWIA Co. S.A.E., 10th of Ramadan City, Egypt); the dose of tramadol was according to Atici et al. [10].

Moringa oleifera leaves extract. The leaves of *M. oleifera* were collected from Elgharbya Governorate, Egypt and dried at 35–40°C in van air circulating oven till dryness. The dried leaves were crushed to obtain the powdered form. The derived aqueous extract tested in this study was prepared in our laboratory by mixing 1-g dried and powdered leaves of *M. oleifera* with 10-ml boiling water for 5 min. The mixture was then filtered twice through a 2- μ m pore sterile filter paper into a sterile tube. The aqueous extract stock solution (100 mg/ml) was freshly prepared for each set of experiments and stored at 4°C for up to 5 days according to Pal et al. [11].

Animal groups. The experiments were performed on 40 male albino rats weighing 180 ± 10 g and 12-week old. The rats were kept in the laboratory for 2 weeks before the experimental work and maintained on a standard rodent diet and water available *ad libitum*. The study protocol was approved (No. 1435-123) by the Scientific Research Ethics Committee of the Institute of Graduate Studies and Research, Alexandria University, Egypt.

Experimental design. Rats were equally divided into four equal groups ($n = 10$) and treated for 4 weeks. Group 1, control in which healthy untreated rats; group 2, MLE in which rats receive MLE orally (100 mg/kg BW/day) according to Pal et al. [11]; group 3, Tramadol in which rats receive tramadol orally (40 mg/Kg BW/day); and group 4, treated tramadol plus MLE include rats that receive orally tramadol and MLE.

At the end of the experimental period, animals were anesthetized by intraperitoneal injection with sodium pentobarbital (240 mg/ml) by a 22–25 gauge needle then subjected to a complete necropsy according to Al Suleimani et al. [12]. Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes and wait for 30 min at room temperature to clot. Blood serum was separated by centrifugation at 4000 rpm for 10 min. The collected serum was stored at -20°C until analysis.

Sexual hormones. The levels of total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were assayed by using the ADVIA Centaur XP system (two-site

sandwich immunoassay using direct chemiluminometric technology; Vidas, France).

Sperms morphometric analysis. Testes and epididymis were carefully removed, cleaned from adhering connective tissue in cold saline and weighed. Sampling for the sperm was collected from the caudal epididymis after carefully removed from each testis, put it in 5 ml of Hanks' balanced salt solution then incubated at room temperature for 15 min. The epididymis was prepared for fertility evaluation to estimate the sperm count, spermatozoa motility parameters, and sperm morphology by computer-assisted semen analysis (CASA System; Germany) with Olympus microscope (Olympus, Tokyo, Japan). A total of 200 spermatozoa from each rat were examined and individually scored normal or abnormal, according to the strict sperm morphology criteria that were used according to the method of Tousson et al. [13] and Eldaim et al. [14].

Tissue preparation. Testes tissues were immediately removed, washed, weighed, and cutting into two different parts—the first part was used for histological examination and the second part was homogenized (10%, w/v)—separately, in ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at $10\,000 \times g$ for 20 min at 4°C and then uses supernatant for different enzyme assays.

Activities of antioxidant enzymes. Thiobarbituric acid-reactive substances (TBARS) were measured in testes homogenate using the method of Oyouni et al. [15]. The basis of the thiobarbituric acid (TBA) methods is the reaction of Malondialdehyde (MDA) with TBA at low pH and high temperature to form a colored complex, the MDA-TBA complex, with an absorption maximum at 532 nm that can be measured by visible absorption spectrophotometry. Furthermore, the activities of CAT (EC 1.11.1.6) and SOD (EC 1.15.1.1) in the testes homogenates were measured using colorimetric kits (Biodiagnostic, Egypt) and using the specific standard for each parameter according to the manufacturer instructions.

Histopathological examination. Testis was fixed with a 10% buffer neutral formalin solution for 48 h and then processed for paraffin sectioning. Sections of 5 μ m were stained with hematoxylin and eosin for histopathological examination according to Tousson [16].

Statistical analysis

Data were expressed as mean values \pm SE, and statistical analysis was performed using one-way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 21 software package (SPSS® Inc., USA).

Results

Changes in weight

Table 1 showed that relative body weight (RBW) and relative testes weight (RTW) showed a significant ($P < 0.05$) decrease in the tramadol group (G3) as compared with the control (G1) and MLE (G2) groups, whereas relative Epididymis weights (REW) showed no changes in the tramadol group as compared with the control and MLE groups. On the other hand, RBW and RTW improved after the addition of MLE to the tramadol (G4).

Table 1: Changes in the RBW (g/100 g), RTW (g/100 g BW), and REW (g/100 g BW) in different groups

	Control	MLF	Tramadol	Tramadol + MLF
RBW	17.9 ± 1.29 [#]	18.5 ± 0.89 [#]	14.3 ± 1.05 [*]	15.5 ± 0.89 [#]
RTW	1.08 ± 0.09 [#]	1.09 ± 0.045 [#]	0.98 ± 0.059 [*]	1.02 ± 0.06 [#]
REW	0.330 ± 0.030	0.334 ± 0.032	0.328 ± 0.025	0.330 ± 0.024

Data are expressed as mean ± SE of 10 observations. Significant difference from the control group at * $P < 0.05$. Significant difference from the tramadol group at [#] $P < 0.05$. Relative organ weight = organ weight/bodyweight × 100.

Changes in sexual hormones

Figure 1 showed a significant ($P < 0.05$) decrease in testosterone, LH and FSH in the tramadol group (G3), while there is an improvement in sexual hormones at the administration of MLE with the tramadol (G4) when compared with control (G1) and MLE (G2) groups.

Morphometric alterations of sperms

Table 2 displays that sperm counts and sperm motility significantly ($P < 0.05$) decreased in the tramadol group (G3), whereas the administration of MLE with the tramadol (G4) showed a significant ($P < 0.05$) increase when compared with control (G1) and MLE (G2) groups. Sperm abnormality exhibited a significant ($P < 0.05$) increase in the tramadol group (G3); meanwhile, the administration of MLE with the tramadol (G4) showed an amelioration in the sperm abnormality when compared with control (G1) and MLE (G2) groups.

Epididymal progressive and non-progressive motility showed a significant ($P < 0.05$) decrease in the tramadol group (G3), while the administration of MLE with the tramadol (G4) showed a significant ($P < 0.05$) increase when compared with control (G1) and MLE (G2) groups. In contrast, the percent of immotile sperm showed a significant ($P < 0.05$) increase in the tramadol group (G3), while it showed an improvement in the percent after the administration of MLE with the tramadol (G4) when compared with control (G1) and MLE (G2) groups (Table 2).

The percentage of sperm with head defect (no head), sperm abnormalities, and other head defects such as double head, amorphous, banana shape, hookless head, and pinhead sperm was significantly ($P < 0.05$) increased in the tramadol group (G3); in contrast, the administration of MLE with the tramadol (G4) showed an amelioration in sperm head abnormalities when compared with control (G1) and MLE (G2) groups (Fig. 2).

Oxidative stress markers

The results showed a significant ($P < 0.05$) increase in TBARS concentration in testes of male rats in the tramadol group (G3), while the administration of MLE with the tramadol (G4) showed an amelioration in TBARS concentration as compared with control (G1) and MLE (G2) groups (Table 3).

Antioxidant enzyme activities

As shown in Table 3, catalase (CAT) and superoxide dismutase (SOD) activities were significantly ($P < 0.05$) decreased in the tramadol group (G3); On the other hand, amelioration in SOD and CAT activities was observed in the administration of MLE with the tramadol (G4) as compared with control (G1) and MLE (G2) groups.

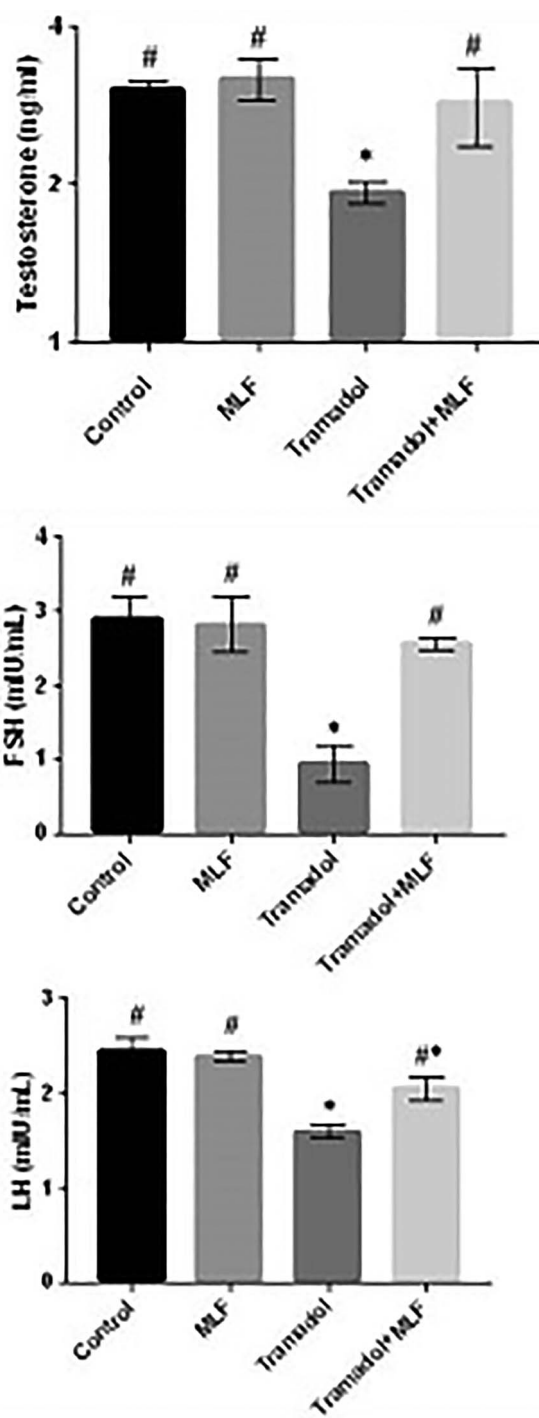


Figure 1: Changes in the testosterone, FSH, and LH levels in different groups under study. Data are expressed as mean ± SE of 10 observations. Significant difference from the control group at * $P < 0.05$. Significant difference from the Tramadol group at [#] $P < 0.05$.

Effect of MLE on testes histopathology

Testes sections in control (G1) and MLE (G2) groups showed normal seminiferous tubules lining spermatocytes and spermatogenesis (Fig. 3A and B). However, the testis section in the tramadol group (G3) revealed moderate morphological changes as incomplete of spermatogenesis, moderate degeneration in some seminiferous tubules with a significant decrease in the

Table 2: Changes in sperm counts (number/g epididymis wt.) $\times 10$ [6], vitality, abnormality, total sperm motility, progressive motility, non-progressive, and immotile sperms percent in the different groups

	Control	MLF	Tramadol	Tramadol + MLF
Sperm counts (million/ml)	130.9 \pm 10.3 [#]	138.5 \pm 11.5 [#]	83.1 \pm 8.3*	140.0 \pm 10.6 [#]
Vitality (%)	66.4 \pm 1.50 [#]	69.1 \pm 2.10 [#]	56.7 \pm 1.40*	66.0 \pm 1.50 [#]
Sperm abnormality (%)	32.42 \pm 1.39 [#]	28.55 \pm 1.05 [#]	45.50 \pm 1.92*	37.71 \pm 2.06 ^{#*}
Total motility (PR + NP)	68.12 \pm 3.59 [#]	70.45 \pm 4.48 [#]	60.55 \pm 4.08*	64.30 \pm 4.25 ^{#*}
Progressive motility (PR)	41.60 \pm 3.41 [#]	43.23 \pm 4.37 [#]	32.31 \pm 4.75*	38.05 \pm 2.15 ^{#*}
Non-progressive (NP)	26.40 \pm 1.55 [#]	27.22 \pm 1.07 [#]	24.24 \pm 2.33*	26.25 \pm 1.62 [#]
Immotile (IM)	31.88 \pm 0.97 [#]	29.55 \pm 1.91 [#]	42.45 \pm 2.27*	35.70 \pm 2.29 ^{#*}

Data are expressed as mean \pm SE of 10 observations. Significant difference from the control group at *P < 0.05. Significant difference from the Tramadol group at [#]P < 0.05.

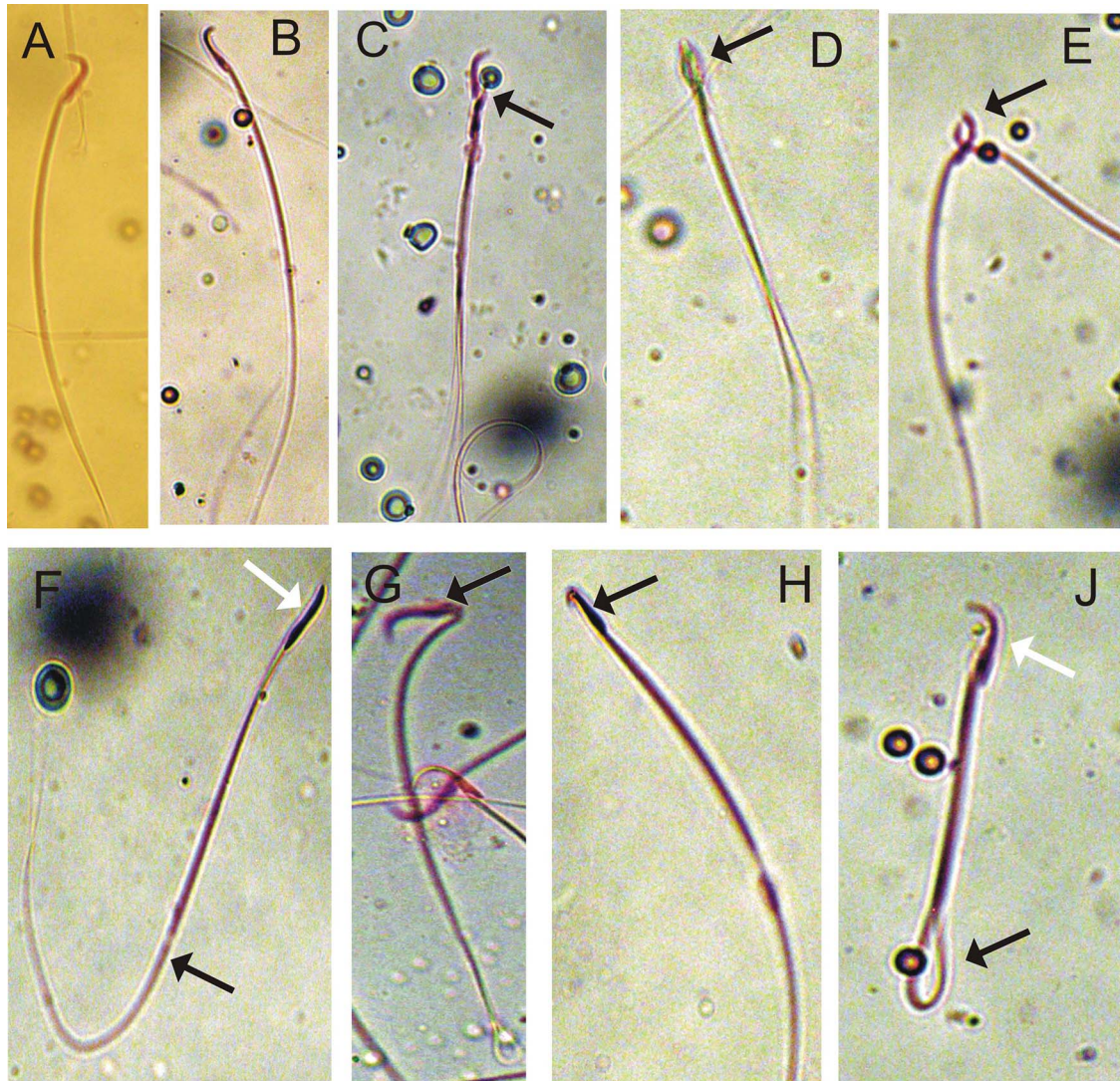


Figure 2: Microphotographs illustrating morphologically normal sperm (A and B) and various sperm defects (C–H) were observed. C, double head; D, amorphous head; E and F, fused heads; G, bent head; H, hook head; and J, coiled tail.

number of spermatogenic cells with a moderate decrease in sperm, and depletion and little numbers of Leydig cells (Fig. 3C). Testes in rats with the administration of MLE with the tramadol (G4) showed the normal structure of seminiferous tubules with the normal distribution of the spermatogenic cells and an increase in the sperm numbers (Fig. 3D).

Discussion

Opiate use is known to decrease the levels of male sex hormones, and this lowered hormonal level is thought to be responsible for the diminished fertility of male opiate users [17]. Amadol or tramadol belongs to the same family of codeine, morphine, and oxycodone [5]. The current results revealed a dramatically

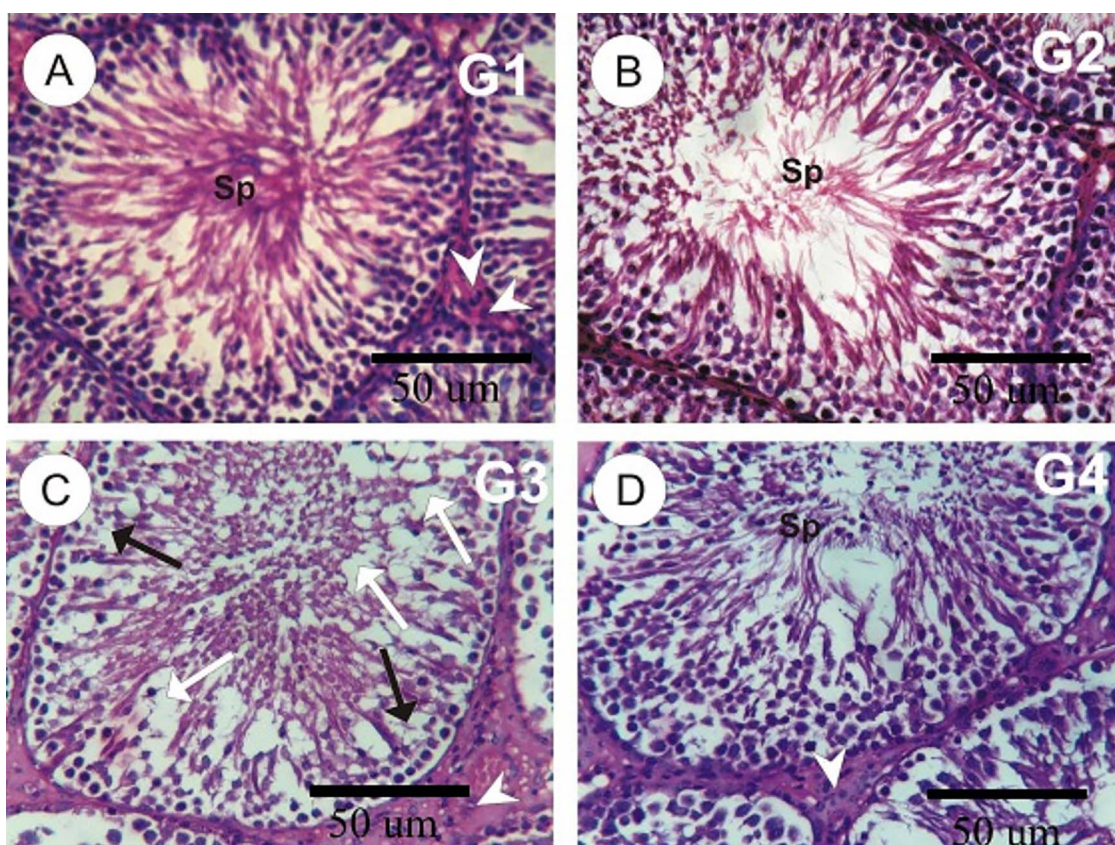


Figure 3: (A–D): photomicrographs of rat testes sections in the different experimental groups. A and B: testes sections in control and MLE rat groups showed normal seminiferous tubules lining spermatocytes and spermatogenesis. C: testis in tramadol group revealed the incomplete of spermatogenesis (black arrows), moderate degeneration (white arrows) in some seminiferous tubules with a decrease in the number of sperms, and depletion and little numbers of Leydig cells (arrowheads). D: testes in tramadol plus MLE showed the normal structure of seminiferous tubules with a normal distribution of the spermatogenic cells.

Table 3: Activities of TBARS (nmol/g protein), CAT (U/mg protein), and SOD (U/mg protein) in testes of male rats

Item	Control	MLF	Tramadol	Tramadol + MLF
TBARS	16.95 ± 1.19 [#]	17.55 ± 1.13 [#]	26.62 ± 1.35 [*]	21.22 ± 1.00 ^{**}
Catalase	61.58 ± 2.99 [#]	62.70 ± 3.02 [#]	48.50 ± 3.11 [*]	51.60 ± 2.43 [*]
SOD	5.48 ± 0.39 [#]	5.33 ± 0.46 [#]	2.83 ± 0.51 [*]	3.06 ± 0.32 ^{**}

Data are expressed as mean ± SE of 10 observations. Significant difference from the control group at ^{*}P < 0.05. Significant difference from the Tramadol group at [#]P < 0.05.

drop in the levels of total testosterone, FSH, and LH hormones in the tramadol group compared with the control and MLE groups. However, rat's administration of MLE with the tramadol showed an amelioration in the levels of total testosterone, FSH, and LH hormones when compared with control and MLE groups. This remarkable reduction of sexual hormones that were found in the current study might be explained by severe damages, which tramadol exerted on Leydig and Sertoli cells by increased generation of free radicals is one of the possible mechanisms involved in opioid-induced Leydig cell degeneration. A moderate decrease of gonadotropin secretion causes atrophy of the testes, as well as a decrease in sperm cell production [18]. Similar results for reduced testosterone have been reported by Abdellatif *et al.* [19]. Also, our results agree with Tennese and Wevrick [20] who reported the decreased levels of LH and testosterone with increased prolactin hormone after morphine and methadone administration. Also, Inass *et al.* [21] who observed a decrease of serum levels of FSH,

LH, and testosterone and the induction of prolactin hormone (PRL) and E2 secretions after cannabis use.

Antioxidants are molecules that are capable of slowing or preventing the oxidation of other molecules, thereby protecting cells from damages caused by exposure to free radicals, including reactive oxygen species, which are produced during oxidation reactions in biological cells. Antioxidants can be phytochemicals; they range from micromolecules such as glutathione and vitamins to macromolecules such as catalase, glutathione, and peroxidase [22]. Tousson *et al.* [8] reported that the crude extract of phenolic compounds was obtained from MLE has strong antiradical activity against DPPH radical. Phenolic acids (derivatives of caffeic, p-coumaric, and ferulic acids) are the dominant phenolic constituents of MLE. Oxidative stress and lipid peroxidation are some of the mechanisms that can lead to liver dysfunction. Therefore, lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury. The toxic effects of opioids at the cellular level may be explained by lipid peroxidation. Catalase activity and SOD activity in the tramadol group showed significantly decrease, while TBARS activity showed a significant increase when compared with control and MLE groups. Lipid peroxidation induced by tramadol can eventually result in dysfunction and structural damage of cells [23]. Therefore, decrease in the activity of catalase may result in a number of fatal effects due to the assimilation of superoxide radical and hydrogen peroxide. Our results agree with AKUNNA *et al.* [24] and Sadek [25] who found that *M. oleifera* leaf extracts have a chemotherapeutic and ameliorative role against

chromium-induced damage in rat testes. Treatment with tramadol and MLE succeeded to modulate these observed abnormalities in testes structure, functions, and sperms resulting from tramadol as indicated by the reduction of enzymes activity and the pronounced improvement of the investigated biochemical, antioxidant, and oxidative stress parameters. Further studies are needed to investigate the impacts of tramadol on human health. In the current study, testicular histopathological results in the tramadol group were supported by sex hormonal dysfunction evident by a highly significant decrease in LH, FSH, and testosterone hormone levels when compared with group control. The present study showed the atrophy of seminiferous tubules with interstitial calcification, focal testicular degeneration with single or multiple layers of vacuolated spermatocytes with a little number of spermatogenesis in tramadol group. This is in agreement with Cajú *et al.* [26] who detected the decrease of Sertoli and Leydig cells in rats exposed to chronic and acute doses of morphine. Our recommendation, with the increasing use of tramadol for pain control, it is important for physicians to be aware of its potential side effects.

Conclusion

MLE has an ameliorative role against tramadol-induced toxicity, fertility potential, changes in sex hormones, oxidative stress, and testicular injury in male rats. *M. oleifera* increases the sperm count, reduced the sperm abnormality, and improved the testes' injury. Hence, these results suggest that MLE can be a reliable therapy for tramadol toxicity and further validate the therapeutic effectiveness of MLE for some diseases.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study did not receive any grant from any funding agency.

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