Current Research in Pharmacology and Drug Discovery 2 (2021) 100049

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Mitochondrial dysfunction and oxidative stress are involved in the mechanism of tramadol-induced renal injury

Khadijah Mousavi [a,b], Ram Kumar Manthari [c], Asma Najibi [a,b], Zhipeng Jia [d], Mohammad Mehdi Ommati [a,e,**], Reza Heidari [a,*]

[a] Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Department of Biotechnology, GITAM Institute of Science, Gandhi Institute of Technology and Management, Visakhapatnam, 530045, Andhra Pradesh, India
[d] College of Animal Sciences, Shanxi Agricultural University, Shanxi, Taigu, China
[e] College of Life Sciences, Shanxi Agricultural University, Shanxi, Taigu, China

ARTICLE INFO

Keywords:
ATP
Bioenergetics
Cytotoxicity
Drug-induced mitochondrial impairment
Mitochondria

ABSTRACT

Tramadol (TMDL) is an opioid analgesic widely administered for the management of moderate to severe pain. On the other hand, TMDL is commonly abused in many countries because of its availability and cheap cost. Renal injury is related to high dose or chronic administration of TMDL. No precise mechanism for TMDL-induced renal damage has been identified so far. The current study aimed to evaluate the potential role of oxidative stress and mitochondrial impairment in the pathogenesis of TMDL-induced renal injury. For this purpose, rats were treated with TMDL (40 and 80 mg/kg, i.p, 28 consecutive days). A significant increase in serum Cr and BUN was detected in TMDL groups. On the other hand, TMDL (80 mg/kg) caused a substantial increase in urine glucose, ALP, protein, and γ-GT levels. Moreover, urine Cr was significantly decreased in TMDL-treated rats (40 and 80 mg/kg). Renal histopathological alterations included inflammation, necrosis, and tubular degeneration in the kidney of TMDL-treated animals. Reactive oxygen species (ROS) formation, increased oxidized glutathione (GSSG), lipid peroxidation, and protein carbonylation was increased, whereas total antioxidant capacity and reduced glutathione levels were considerably decreased in TMDL groups. Significant mitochondrial impairment was also detected in the form of mitochondrial depolarization, adenosine-tri-phosphate (ATP) depletion, mitochondrial permeabilization, lipid peroxidation, and decreased mitochondrial dehydrogenase activity in the kidney of TMDL (80 mg/kg)-treated animals. These data suggest mitochondrial impairment and oxidative stress as mechanisms involved in the pathogenesis of TMDL-induced renal injury.

1. Introduction

Tramadol (TMDL) is an opioid agonist and monoamine neurotransmitter reuptake inhibitor (de Kretser et al.; Subedi et al., 2019). TMDL is used against moderate to severe pain and has analgesic efficacy similar to morphine or alfentanil (Subedi et al., 2019). TMDL and its metabolites are mainly eliminated via the kidney (Scott and Perry, 2000). On the other hand, there is evidence of the toxic effects of TMDL on the kidney in high dose or long-term use (Afshari and Ghooshkhanehee, 2009; Borrego Utiel et al., 2018; Elmanama et al., 2015; Sarret et al., 2008). It has been found that TMDL-induced renal injury is associated with a significant increase in inflammatory cell aggregation and tubular atrophy in human cases (Borrego Utiel et al., 2018). Another exciting finding in TMDL-intoxicated patients is the disturbances in serum electrolytes (Le Berre et al., 2007; Sarret et al., 2008). Cases of hyponatremia have been reported in human TMDL-induced renal injury (Le Berre et al., 2007; Sarret et al., 2008). Unfortunately, there is no specific mechanism(s) for TMDL-induced nephrotoxicity so far.

Renal tissue contains many mitochondria, that their proper function guarantees appropriate renal vital functions such as chemicals absorbance (Heidari, 2019). A plethora of evidence shows that many drugs' adverse effects in the kidney are mediated through mitochondrial impairment and oxidative stress (Emadi et al., 2019; Jamshidzadeh et al., 2015; Ommati et al., 2020h). On the other hand, evidence such as serum

* Corresponding author. P. O. Box 1583, 71345, Roknabad, Karafarin St., Shiraz, Fars, Iran.
** Corresponding author. 030801, Tailuo St, Taigu, China.
E-mail addresses: mehdi_ommati@sxau.edu.cn, mehdi_ommati@outlook.com (M.M. Ommati), rheidari@sums.ac.ir (R. Heidari).

https://doi.org/10.1016/j.crphar.2021.100049
Received 2 July 2021; Received in revised form 14 August 2021; Accepted 31 August 2021

electrolytes disturbances in TMDL toxicity proposes that mitochondrial impairment could be involved in this complication.

It has been well-established that oxidative stress and mitochondrial impairment are tightly related events (Brookes et al., 2004). Mitochondria are crucial intracellular sources of reactive oxygen species (ROS) (Brookes et al., 2004). Therefore, the impaired mitochondrial function could enhance ROS formation and oxidative stress (Brookes et al., 2004). On the other hand, excess ROS could significantly impair mitochondrial function and energy metabolism (Brookes et al., 2004; Duann and Lin, 2017). Although the mechanism of TMDL-induced renal injury is far from clear, some evidence indicates the role of oxidative stress in this complication (Ali et al., 2020; Barbosa et al., 2020; Sheweita et al., 2018). However, the source of TMDL-induced ROS formation is not understood. Excitingly, the evidence of TMDL-induced oxidative stress also has been mentioned in other organs (Ali et al., 2020; Mehdizadeh et al., 2017; Mohamed and Mahmoud, 2019; Zhuo et al., 2012). For example, oxidative stress is a crucial mechanism involved in TMDL neurotoxicity (Mehdizadeh et al., 2017; Mohamed and Mahmoud, 2019; Zhuo et al., 2012). The possibility of mitochondrial impairment and oxidative stress in the kidney of TMDL-treated animals has been evaluated in the current study.

The current investigation aimed to evaluate the role of oxidative stress and mitochondrial impairment in an animal model. As TMDL is a widely used analgesic agent, the data could help develop new preventive/therapeutic strategies against this complication.

2. Materials and methods

2.1. Chemicals and reagents

Glutathione (GSH), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), trichloroacetic acid, malondialdehyde, 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 2,4,6-tripyridyl-s-triazine, ferric chloride hexahydrate ($FeCl_3.6H_2O$), D-mannitol, thiobarbituric acid, 3-(N-morpholino) propane sulfonic acid, fatty acid-free bovine serum albumin fraction V, coomassie brilliant blue, Rhodamine123, dinitrophenylhydrazine (DNPH), dithiothreitol (DTT), ethylene glycol-bis(β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid (EGTA), Ethylenediaminetetraacetic acid (EDTA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tramadol, bovine serum albumin (BSA), and sucrose were obtained from Sigma (Sigma-Aldrich, St. Louis, MO). Kits for assessing biomarkers of renal injury were obtained from Parsazmoon® (Tehran, Iran). High-performance liquid chromatography (HPLC) grade methanol, potassium chloride (KCl), 3-(N-morpholino) propanesulfonic acid (MOPS), iodoacetic acid, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), acetonitrile HPLC grade, meta-phosphoric acid, dinitro fluoro benzene, n-butanol, and 2-amino-2-hydroxymethyl-propane-1,3-diol-hydrochloride (Tris-HCl), were purchased from Merck (Darmstadt, Germany).

2.2. Animals

Male Sprague-Dawley rats (n = 18) weighing 200–250 g were prepared from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were stored in a controlled environment (24 ± 1 °C, ≈ 50% relative humidity, and a 12-h dark/light cycle). Rats had free admission to tap water and a standard rodents pellet chow diet (RoyanFeed®, Isfahan, Iran). Shiraz University's ethical committee approved laboratory animal use of Medical Sciences, Shiraz, Iran (Code: IR.SUMS.REC.1397.095).

2.3. Experimental setup

Rats were randomly allotted into three experimental groups (n = 6/group): **A**) Control (Vehicle-treated; 2.5 mL/kg normal saline) **B**) tramadol (40 mg/kg/day, i.p, for 28 consecutive days), and **C**) tramadol (80 mg/kg/day, i.p, for 28 consecutive days). At day 29, animals were deeply anesthetized (thiopental 80 mg/kg, i.p) and blood and kidney samples were collected.

2.4. Serum and urine biochemistry

Blood samples (5 mL) were collected from abdominal aorta serum and transferred to gel and clot-activator tubes (Improvacuter®; Guangzhou, China). Samples were centrifuged (3000 g, 10 min, 4 °C) to prepare serum. Urine samples (200 μL) were collected during animals handling. Samples were diluted with cold normal saline (200 μL), centrifuged (120000 g, 10 min, 4 °C), and the clear supernatant was used for urinalysis. A Mindray BS-200® auto-analyzer (Guangzhou, China) and standard kits for calcium (Ca^{2+}), glucose, phosphate, total protein, blood urea nitrogen (BUN), creatinine (Cr), alkaline phosphatase (ALP), and γ-glutamyl transferase (γ-GT) were used to assess serum and urine biochemistry (Heidari et al., 2019d; Jamshidzadeh et al., 2015). Serum and urine sodium and potassium level were evaluated by a flame photometer (BWB XP, UK).

2.5. Reactive oxygen species (ROS) formation

Reactive oxygen species (ROS) were estimated in rat kidneys using 2′, 7′-dichlorofluorescein diacetate (DCF-DA) as a fluorescent probe (Heidari et al., 2018b, 2019c; Heidari and Niknahad, 2019). For this purpose, 200 mg of the kidney tissue was homogenized in 5 mL of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4). Then, 100 μL of the resulted tissue homogenate was added to 1 mL of Tris-HCl buffer containing 10 μM of DCF-DA (Heidari et al., 2019a; Heidari et al., 2018f; Jamshidzadeh et al., 2016). Samples were incubated in the dark (10 min, 37 °C). Finally, the fluorescence intensity was assessed (FLUOstar Omega® multifunctional fluorimeter; $\lambda_{excit} = 485$ nm and $\lambda_{emiss} = 525$ nm) (Heidari et al., 2018b; Heidari and Niknahad, 2019; Ommati et al., 2017).

2.6. Protein carbonylation

The extent of oxidative stress-induced protein damage (protein carbonylation) in the rat kidney was assessed based on the DNPH reagent (Heidari et al., 2014; Zhang et al., 2004). For this purpose, samples of the kidney tissue (200 mg) were homogenized in 5 mL of phosphate buffer (pH = 7.5, containing 0.1%. v: v of triton X-100). Samples were centrifuged (700 *g*, 10 min, 4 °C). Then, the supernatant was treated with 1500 μL of DNPH solution (10 mM DNPH dissolved in HCl). Afterward, samples were incubated at room temperature (in the dark, 1 h, vortexing every 10 min) (Heidari et al., 2014; Ommati et al., 2019b; Zhang et al., 2004). Then, trichloroacetic acid (500 μL of 20% w: v in distilled water) was added, and samples were centrifuged (17000 *g*, 5 min, 4 °C). The supernatant was discarded, and the pellet was washed five times with ethanol: ethyl acetate (1 mL of 1:1 v: v). Then, the residues were re-dissolved in a 6 M guanidine chloride solution (pH = 2.3). Finally, samples were centrifuged (17000 *g*, 1 min, 4 °C), and absorbance was assessed at λ = 370 nm (EPOCH® plate reader, BioTek®, USA) (Heidari et al., 2013b, 2014).

2.7. Lipid peroxidation

Lipid peroxidation in the kidney of TMDL-treated rats was assessed using the thiobarbituric acid reactive substances (TBARS) test (Heidari et al., 2017; Heidari and Niknahad, 2019; Shafiekhani et al., 2019). Briefly, renal tissue (200 mg) was homogenized in 5 mL of 40 mM Tris-HCl buffer (pH = 7.4). Tissue homogenate was treated with 4 mL of TBARS assay reagent (a mixture of 1 mL of thiobarbituric acid (TBA) 0.375% w: v, 1 mL of 50% w: v of trichloroacetic acid (TCA), and 3 mL of meta-phosphoric acid 1% w: v, pH = 2; adjusted with HCl) (Heidari et al., 2015, 2016a; Heidari and Niknahad, 2019). Samples were mixed well (1 min) and heated (100 °C water bath, 45 min). Afterward, 2 mL of n-butanol was added. Samples were mixed well again and centrifuged

(10000 *g*, 20 min, 4 °C). Finally, the absorbance of the pink-colored supernatant (n-butanol phase) was assessed (λ = 532 nm, EPOCH® plate reader, BioTek®, USA) (Ahmadi et al., 2021; Heidari et al., 2018g; Heidari and Niknahad, 2019; Niknahad et al., 2017a).

2.8. Total antioxidant capacity of the kidney tissue

Renal tissue antioxidant capacity in TMDL-treated rats was assessed base on the ferric reducing antioxidant power (FRAP) assay (Heidari and Niknahad, 2019; Jamshidzadeh et al., 2017a, 2017b). The FRAP assay reagent was composed of 10 mL of acetate buffer (300 mM, pH = 3.6), 1 mL of TPTZ (10 mM in 40 mM HCl), and 1 mL of ferric chloride hexahydrate (20 mM, $FeCl_3.6H_2O$ in distilled water). Tissue was homogenized in 40 mM ice-cooled (4 °C) Tris-HCl buffer containing 5 mM DTT and 200 mM sucrose (pH = 7.4). Afterward, 100 μL of the tissue homogenate was added to the FRAP reagent (900 μL) (Ommati et al., 2019d). Samples were incubated for 5 min at 37 °C (in the dark). Finally, sample absorbance was measured (λ = 595 nm, EPOCH® plate reader, BioTek®, USA) (Abdoli et al., 2020; Heidari and Niknahad, 2019; Ommati et al., 2020b).

2.9. Kidney histopathology

Renal samples were fixed in10% v: v buffered formalin. Tissue samples were embedded in paraffin blocks, and 5-μm-thick slices were prepared by a microtome and stained with hematoxylin and eosin (H&E). A pathologist blindly analyzed samples.

2.10. Kidney mitochondria isolation

The differential centrifugation method was applied to isolate kidney mitochondria (Abdoli et al., 2021b; Niknahad et al., 2020; Ommati et al., 2021b). Rat's kidney was excised and minced in an ice-cold buffer (70 mM mannitol, 2 mM HEPES, 220 mM sucrose, 0.5 mM EGTA, and 0.1% BSA; pH = 7.4). Minced tissue was homogenized in the mentioned buffer (10 vol buffer/1 g tissue). At the first round of centrifugation (10 min, 1000 *g*, 4 °C), samples were centrifuged, and the supernatant was collected. Then, the supernatant was centrifuged (10 min, 10000 *g*, 4 °C) to pellet the mitochondrial fraction. The second centrifugation round was repeated at least four times to increase mitochondrial yield. Finally, the pellet was resuspended (5 mL/g tissue) in the incubation buffer (0.32 M sucrose, 1 mM EDTA, 2 mM HEPES, and 0.5 mM EGTA, pH = 7.4) and used for further studies.

2.11. Mitochondrial swelling

Analysis of mitochondrial permeabilization was estimated *by alterations in the ultraviolet (UV) absorbance of* mitochondrial suspension (0.5 mg protein/ml) at λ = 540 nm (constant temperature of 30 °C) (Ahmadi et al., 2018; Caro et al., 2012; Heidari et al., 2018d; Niknahad et al., 2016). Ca^{2+} (50 μM) was used to induced mitochondrial permeabilization. *T*he absorbance was measured during 30 min of incubation (EPOCH plate reader, Bio-Tek®, USA).

2.12. Mitochondrial dehydrogenases activity

The 3-(4, 5-dimethylthiazol-2-yl)-2, the 5-diphenyltetrazolium bromide (MTT) assay used to determine dehydrogenases activity in isolated renal mitochondria (Heidari et al., 2016b, 2018c; Niknahad et al., 2015a; Ommati et al., 2018). For this purpose, a mitochondrial suspension (1 mg protein/mL) was incubated with 40 μL of MTT solution (0.4% w: v in incubation buffer) and incubated at 37 °C (30 min, in the dark) (Akram et al., 2017; Heidari et al., 2019b; Ommati et al., 2020g). Then, samples were centrifuged (10000 g, 10 min), and the product of formazan crystals were dissolved in dimethyl sulfoxide (1000 μL). Finally, the absorbance was measured at λ = 570 nm (EPOCH® plate reader, BioTek® Instruments, USA) (Eftekhari et al., 2018; Heidari et al., 2018a; Ommati et al., 2019c).

2.13. Mitochondrial depolarization

Mitochondria depolarization in isolated kidney mitochondria preparations was assessed by the rhodamine 123 as a fluorescent probe (Heidari et al., 2012, 2013a; Heidari et al., 2018h). For this purpose, the mitochondrial fractions (1 mg protein/mL) were incubated with rhodamine 123 (10 μM) and incubated for 15 min (in the dark) (Jamshidzadeh et al., 2017c). Afterward, samples were centrifuged (15000 *g*, 1 min, 4 °C), and the fluorescence intensity of the supernatant was assessed (FLUOstar Omega® multifunctional plate reader, $\lambda_{excitation} = 485$ nm and $\lambda_{emission} = 525$ nm) (Heidari et al., 2018e; Niknahad et al., 2016; Ommati et al., 2019a, 2020c).

2.14. Mitochondrial adenosine-tri-phosphate (ATP) level

Mitochondrial ATP level in TMDL-treated rats was assessed using an HPLC method (Seifi et al., 2018, 2020). For this purpose, ice-cooled perchloric acid (200 mM) was added to freshly isolated mitochondria (1 mg protein/ml) and mixed well. Then, samples were centrifuged (10, 000 *g*, 20 min, 4 °C), and the supernatant was treated with 100 μL of ice-cooled potassium hydroxide (KOH) solution (1 M). Then samples (50 μL) were injected into the HPLC system composed of a C-18 column (μ-Bondapak, 25 cm) and a UV detector set at λ = 254 nm (Ommati et al., 2020d; Volonté et al., 2004). The mobile phase was composed of 215 mM potassium hydrogen phosphate mono-basic (KH_2PO_4), 2.3 mM tertiary butyl ammonium hydroxide, 0.4% v: v of KOH (1 M) and 4% v: v acetonitrile. The flow rate of 1 mL/min.

2.15. Renal tissue and isolated mitochondrial glutathione content

GSH and GSSG content in renal mitochondria preparations isolated from TMDL-treated rats was assessed based on an HPLC protocol (Meeks and Harrison, 1991; Siavashpour et al., 2020; Truong et al., 2006). The HPLC apparatus consisted of an amine column (NH_2, 25 cm Bischoff chromatography, Leonberg, Germany) and a UV detector (set at λ = 252) (Meeks and Harrison, 1991). A gradient method using buffer A (Acetate buffer: Water; 1: 4 v: v) and buffer B (Methanol: Water; 4: 1 v: v) were used as mobile phases. The gradient method involved a regular increase of buffer B to 95% in 30 min. The flow rate was 1 mL/min (Meeks and Harrison, 1991; Niknahad et al., 2017b). For sample preparation, 5 mL of tissue homogenate (200 mg in 40 mM Tris-HCl buffer, pH = 7.4; 4 °C) or 1 mL of mitochondria preparations (1 mg protein/ml, 4 °C) were treated with 200 μL of TCA (50% w: v). Samples were mixed well and incubated on ice (10 min, 4 °C) (Mohammadi et al., 2020; Ommati et al., 2020a; Ommati et al., 2020f). Afterward, the incubated specimens were mixed gently and centrifuged (17000 g, 30 min, 4 °C). The supernatant (1000 μL) was collected in 5 mL tubes, and 300 μL of the sodium hydroxide (NaOH) and sodium carbonate ($NaHCO_3$) (2 M: 2 M) was added. Then, iodoacetic acid (100 μL of 1.5% w: v in HPLC grade water) was added and incubated in the dark (1 h, 4 °C). Afterward, 2, 4-dinitrofluorobenzene (500 μL; 1.5% v: v dissolved in HPLC grade ethanol) was added and mixed. Samples were incubated in the dark (25 °C, 24 h). After 24 h of incubation, samples were centrifuged (17000 *g*, 30 min), filtered, and injected (50 μL) into the mentioned HPLC system (Meeks and Harrison, 1991; Truong et al., 2006).

2.16. Lipid peroxidation in liver mitochondria

TBARS assay was also used to assess lipid peroxidation in isolated mitochondria preparations (Caro et al., 2012). However, previous studies mentioned that sucrose (used in mitochondria isolation procedure) interacts with the TBARS test (Caro et al., 2012). Therefore, isolated kidney mitochondria were washed to remove sucrose. For this purpose, 1 mL

(10 mg protein/ml) of isolated mitochondria was suspended in an ice-cold buffer of MOPS-KCl (50 mM MOPS, 100 mM KCl, pH = 7.4). Samples were centrifuged (10,000 g, 20 min, 4 °C). Then, the supernatant was discarded, and the pellet was re-suspended in fresh MOPS–KCl buffer. Afterward, the mitochondrial suspension was mixed with twice its volume of 15% TCA, 0.375% TBA, 0.24 N HCl. Samples were heated for 15 min at a 100 °C water bath (Caro et al., 2012; Caro and Cederbaum, 2001). After centrifugation (15000 g, 1 min), the absorbance of the supernatant was measured (λ = 532 nm) with an Epoch plate reader (BioTek Instruments, USA) (Caro et al., 2012; Niknahad et al., 2015b).

2.17. Statistical analysis

Data are characterized as the mean ± SD (n = 6). Data comparison was accomplished by one-way analysis of variance (ANOVA) test with Tukey's test as a *post hoc*. A $P < 0.05$ was considered as a statistically significant difference.

3. Results

A significant increase in serum BUN and Cr were evident in TMDL-treated animals (Table 1). On the other hand, serum phosphate and K^+ were decreased in the high dose (80 mg/kg) of the TMDL group (Table 1). No significant changes in serum Ca^{2+}, Na^+, glucose, and total protein were detected in TMDL-treated animals compared to the control animals. Urinalysis of TMDL-treated rats revealed significant proteinuria in 80 mg/kg dose of TMDL (Table 2). Moreover, urine ALP and γ-GT were increased in the high dose of TMDL-challenged rats (Table 2). Urine Cr levels were decreased in both 40 mg/kg and 80 mg/kg doses of TMDL (Table 2). No significant changes in urine protein, glucose, ALP, and γ-GT were detected when animals were treated with 40 mg/kg of TMDL (Table 2).

The evaluation of animals' weight gain revealed a significant decrease in rats' bodyweight treated with TMDL 80 mg/kg (Fig. 1). Moreover, the kidney weight index was significantly decreased in TMDL 80 mg/kg group (Fig. 1). No significant changes in animals' bodyweight gain or renal weight index were detected when rats were treated with 40 mg/kg of TMDL (Fig. 1). It is noteworthy to mention that no mortality was seen in TMDL-treated animals in the current study.

Biomarkers of oxidative stress were assessed in the kidney of TMDL-treated rats (Fig. 2). Significant ROS formation, protein carbonylation, and lipid peroxidation were detected in the renal tissue of rats treated with 40 and 80 mg/kg doses of TMDL (Fig. 2). On the other hand, markers such as increased GSSG, depleted tissue antioxidant capacity, and decreased GSH levels were evident in TMDL 80 mg/kg group (Fig. 2).

Evaluating effects of TMDL treatment on renal mitochondrial indices revealed significant mitochondrial depolarization, decreased mitochondrial dehydrogenases activity, mitochondrial permeabilization, increased lipid peroxidation, and depleted ATP stores in TMDL 80 mg/kg group (Fig. 3). No significant changes in mitochondrial indices of functionality were detected when rats were treated with a 40 mg/kg dose of TMDL (Fig. 3).

Renal tissue histopathological alterations included tubular degeneration, and inflammation was detected in TMDL-treated animals (Fig. 4 and Table 3). Moreover, mild kidney tissue necrosis was seen in TMDL 80 mg/kg group (Fig. 4 and Table 3).

Table 1
Serum biochemical measurements in tramadol (TMDL)-treated rats.

	Control	TMDL 40 mg/kg	TMDL 80 mg/kg
Ca^{2+} (mg/dl)	5.3 ± 0.30	5.04 ± 0.3	5.14 ± 0.32
K^+ (mmol/l)	5.6 ± 0.40	4.9 ± 0.40	3.3 ± 0.40[a]
Na^+ (mmol/l)	84.0 ± 4.00	77 ± 9.00	77.0 ± 5.00
Glucose (mg/dl)	112.0 ± 9.00	105 ± 4.00	102.0 ± 6.00
Phosphate (mg/dl)	3.24 ± 0.50	2.73 ± 0.46	2.1 ± 0.08[a]
Total protein (mg/dl)	7.13 ± 0.44	7.38 ± 0.53	6.97 ± 0.22
BUN (mg/dl)	45.2 ± 3.00	60.0 ± 3.80[a]	76 ± 8.80[a]
Cr (mg/dl)	0.26 ± 0.03	0.67 ± 0.05[a]	0.91 ± 0.03[a]

Data are given as mean ± SD (n = 6). Ca^{2+}: Calcium, K^+: potassium, Na^+: Sodium, BUN: Blood urea nitrogen, and Cr: Creatinine.
[a] Indicates significantly different as compared with the control group ($P < 0.05$).

Table 2
Urinalysis of tramadol (TMDL)-treated animals.

	Control	TMDL 40 mg/kg	TMDL 80 mg/kg
Protein (mg/dl)	0.46 ± 0.09	0.55 ± 0.20	0.86 ± 0.30[a]
ALP (U/l)	2244 ± 134	2345 ± 280	3033 ± 489[a]
γ-GT (U/l)	2552 ± 317	2655 ± 512	3312 ± 355[a]
Glucose (mg/dl)	80.0 ± 4.00	78.0 ± 6.00	88.0 ± 13.00
Cr (mg/dl)	0.14 ± 0.02	0.048 ± 0.014[a]	0.021 ± 0.008[a]

Data are given as mean ± SD (n = 6).
ALP: alkaline phosphatase; γ-GT: γ-glutamyl transferase; Cr: Creatinine.
[a] Indicates significantly different as compared with the control group ($P < 0.05$).

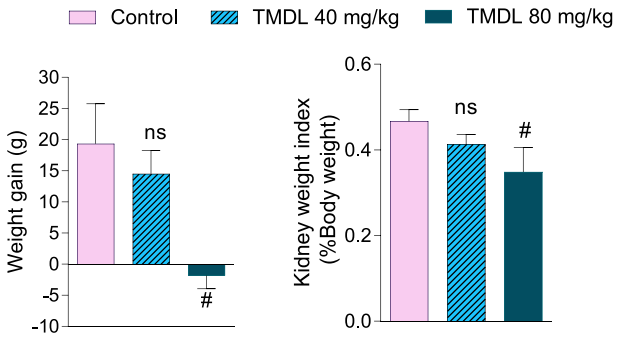

Fig. 1. Animals bodyweight gain and kidney weight index in the control and tramadol (TMDL)-treated rats. Data are given as mean ± SD (n = 6).
# Indicates significantly different as compared with the control group ($P < 0.05$).
ns: not significant as compared with the control group.

4. Discussion

Tramadol (TMDL) is an opioid analgesic widely administered for moderate to severe pain (Subedi et al., 2019). However, several adverse effects, including renal injury, are related to chronic TMDL use or overdose (Afshari and Ghooshkhanehee, 2009; Borrego Utiel et al., 2018; Elmanama et al., 2015; Sarret et al., 2008). To date, there have not been studies dedicated to the precise mechanism for TMDL-induced renal injury. In the current study, a significant increase in oxidative stress markers and mitochondrial dysfunction was evident in TMDL-treated animals' kidneys. These data indicate that oxidative stress and mitochondrial impairment play a crucial role in the pathogenesis of TMDL-induced renal injury.

Several human cases of TMDL-induced renal injury have been reported (Afshari and Ghooshkhanehee, 2009; Borrego Utiel et al., 2018). Borrego et al. reported significant proteinuria, albuminuria, increased Cr levels, and the presence of β-macroglobulin in the urine of 71-year-old women taking TMDL (200 mg/day, prolonged-release formulation) (Borrego Utiel et al., 2018). Marked tubule-interstitial inflammation and tubular atrophy were dominant histopathological alterations in this patient (Borrego Utiel et al., 2018). Borrego et al. reported that prednisolone (3 × 500 mg bolus i.v dose) in addition to oral prednisone (1 mg/kg/day, 10 days) significantly alleviated TMDL-induced renal injury (Borrego Utiel et al., 2018). These data could indicate that the infiltration of inflammatory cells in the kidney of TMDL-treated patients

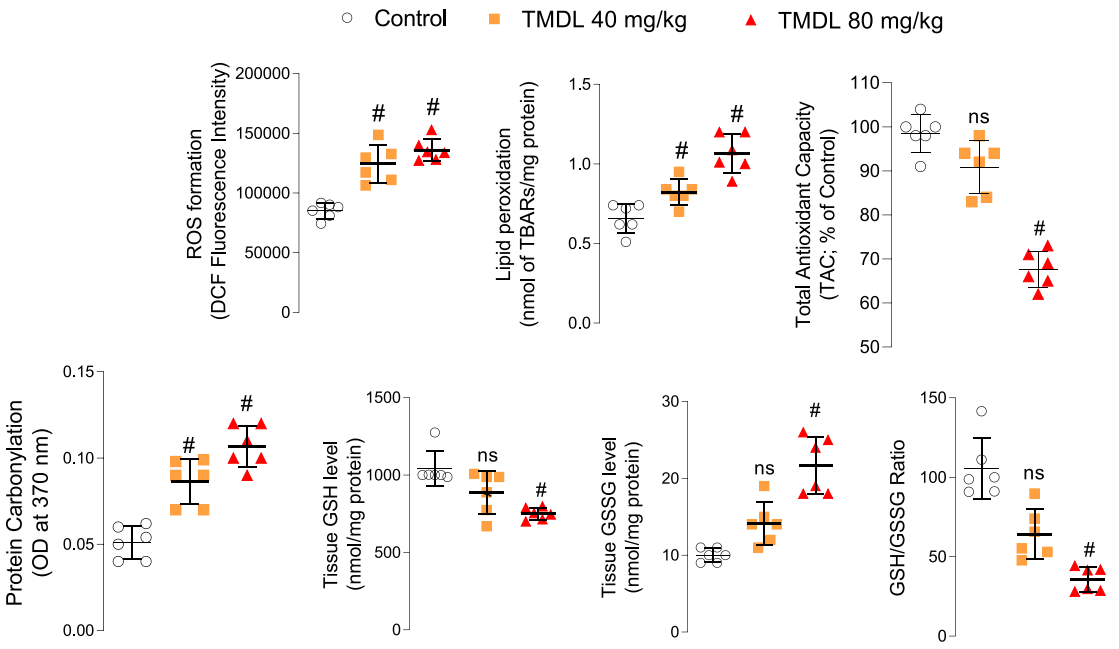

Fig. 2. Biomarkers of oxidative stress in the kidney tissue of tramadol (TMDL)-treated rats. Data are given as mean ± SD (n = 6). ROS: Reactive oxygen species; TBARS: Thiobarbituric acid reactive substances; TAC: Total antioxidant capacity; OD: Optical density; GSH: Reduced glutathione; GSSG: Oxidized glutathione.
# Indicates significantly different as compared with the control group (P < 0.05).
ns: not significant as compared with the control group.

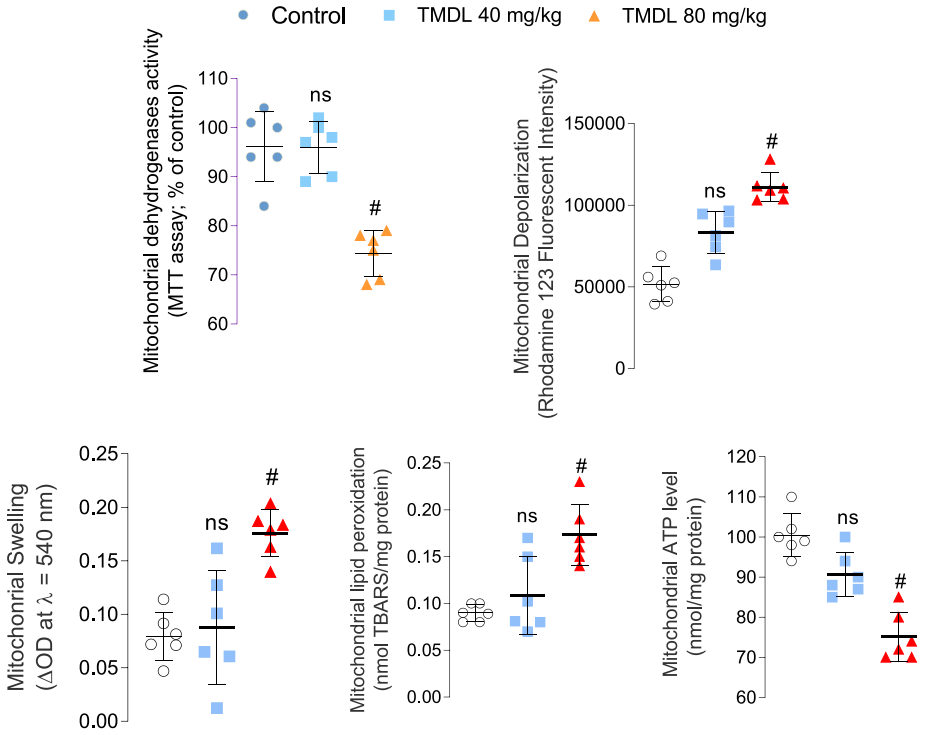

Fig. 3. Effect of tramadol (TMDL) on kidney mitochondrial indices. MTT: methyl tetrazolium; TBAR: Thiobarbituric acid reactive substances; ATP: Adenosine triphosphate.
Data are given as mean ± SD (n = 6).
# Indicates significantly different as compared with the control group (P < 0.05).
ns: not significant as compared with the control group.

could play a significant role in its mechanism of renal injury. Inflammatory cells could act as a critical source of ROS production via the action of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Touyz et al., 2019). Therefore, these cells could serve as a source of ROS in the kidney during TMDL toxicity. Based on these data, anti-inflammatory agents (*e.g.*, corticosteroids such as prednisolone) could be a good option for alleviating TMDL-induced kidney injury in human cases.

Several antioxidants with renoprotective background have been tested against renal disease or xenobiotics-induced kidney injury

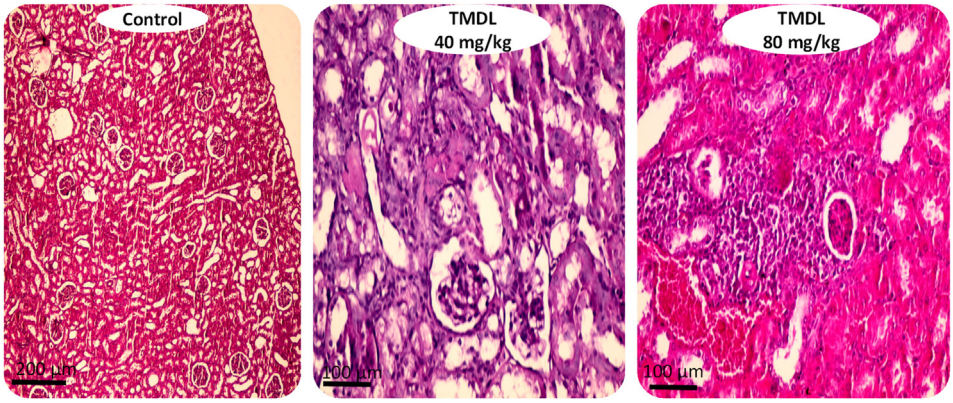

Fig. 4. Kidney histopathological assessments in tramadol (TMDL)-treated animals. Significant histopathological changes, including inflammatory cell infiltration, necrosis, and hemorrhage, were detected in the tramadol 80 mg/kg treated group. Scores of renal histopathological alterations are represented in Table 3.

Table 3
Scores of renal tissue histopathological alterations in tramadol (TMDL)-treated rats.

	Control	TMDL 40 mg/kg	TMDL 80 mg/kg
Inflammation	–	++	+++
Tubular degeneration	–	+	++
Necrosis	–	–	+

- Indicates no significant histopathological changes+$^{++}$, and $^{+++}$: Indicate mild, moderate, and severe histopathological changes, respectively.

(Farombi and Ekor, 2006; Ommati et al., 2021a; Vazin et al., 2020). As oxidative stress and its related complications play a role in TMDL-induced renal injury, the use of clinically applicable molecules (*e.g.*, N-acetylcysteine) might also mitigate this complication. Some studies administered antioxidants such as curcumin, gallic acid, and *Nigella sativa* against TMDL-induced hepatic and renal injury (Elkhateeb et al., 2015; Sheweita et al., 2018). Therefore, antioxidants with a high potency of the mitochondrial protective effect are recommended in future investigations.

TMDL-induced mitochondrial impairment in the renal tissue was an exciting finding in the current study (Fig. 3). Oxidative stress and mitochondrial impairment are two tightly related phenomena (Brookes et al., 2004). Mitochondria are the primary sources of intracellular ROS formation (Brookes et al., 2004). On the other hand, significant ROS formation and oxidative stress could impair mitochondrial function (Brookes et al., 2004). Therefore, mitochondria could act as an essential source of ROS formation in the kidney of TMDL-treated animals. We might be concluded that a part of enhanced ROS formation and the increase in the markers of oxidative stress in the renal tissue of TMDL-treated animals could be associated with the effects of this drug on kidney mitochondria. However, the precise mechanism(s) of TMDL-induced mitochondrial injury in the kidney need further studies to be revealed.

Excitingly, some studies mentioned the role of mitochondrial dysfunction in other adverse effects induced by TMDL (Mehdizadeh et al., 2017). Mehdizadeh et al. reported significant mitochondrial impairment caused by TMDL in the brain of rats treated by this drug (Mehdizadeh et al., 2017). In another study conducted by Zhuo and colleagues, TMDL significantly decreased mitochondrial activity and energy metabolism in the brain of a zebrafish model (Zhuo et al., 2012). All these data could indicate the importance of mitochondrial impairment in the mechanism of cytotoxicity induced by TMDL.

Kidneys are high-energy consuming organs and contain a considerable number of mitochondria (Heidari, 2019). Enough energy (ATP) production in the kidney guarantees vital processes such as chemicals reabsorption in renal tubules (Duann and Lin, 2017; Emma et al., 2016; Heidari, 2019). The reabsorption of many compounds such as glucose, amino acids, several ions, and phosphate is rigorously dependent on ATP availability (Heidari, 2019). As observed in the current study, serum levels of ions such as K^+ as well as phosphate levels were significantly decreased in TMDL-treated animals (hypophosphatemia; Table 1). On the other hand, significant proteinuria, in addition to increased urine levels of γ-GT and ALP, was detected in TMDL-treated rats (Table 2). These data could indicate significant tubular injury induced by TMDL. Interestingly, it has been reported that TMDL caused significant hyponatremia (Le Berre et al., 2007; Sarret et al., 2008). This complication could be associated with impaired reabsorption of ions and chemicals due to mitochondrial impairment and energy crisis in the kidney during TMDL exposure. Therefore, monitoring serum electrolytes and applying appropriate clinical intervention is essential in human cases of TMDL-induced renal injury. Moreover, some researchers mentioned the promising role of targeting mitochondria in renal disease (Eirin et al., 2017). Hence, the administration of mitochondria protecting agents could serve as a viable therapeutic intervention in this complication. Several agents with positive effects on the cellular mitochondria have been identified (Abdoli et al., 2021a; Mitchell et al., 2011; Mohammadi et al., 2019; Ommati et al., 2019a, 2019c; Szeto, 2017). Some of these agents s such as NAC are readily administered in clinical settings (Aparicio-Trejo et al., 2019). On the other hand, researchers are working on mitochondria-targeted antioxidants (Oyewole and Birch-Machin, 2015; Sheu et al., 2006). These agents might finally find an application against TMDL-induced renal injury in clinical settings.

Altogether, the data obtained from the current study indicate oxidative stress and mitochondrial impairment as mechanisms involved in the pathogenesis of TMDL-induced renal injury. Further studies are required to develop preventive/therapeutic strategies based on these data.

Credit author statement

Khadijeh Mousavi, Dr. Mohammad Mehdi Ommati, Dr. Ram Kumar Manthari, Zhipeng Jia, and Asma Najibi were involved in data collection, experimental setup, and data visualization. Dr. Reza heidari and Dr. Mohammad Mehdi Ommati were engaged in study concepts, Formal analysis, and data visualization. All authors were involved in manuscript draft preparation and revision. All authors read and approved the draft(s) as well as the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This investigation was financially supported by Shiraz University of Medical Sciences (Grant number: 13629 by Dr. Reza Heidari). This study was also supported by outstanding doctors volunteering to work in Shanxi Province, Science and Technology Office, and Shanxi Government Scholarship for an international research assistant (No. K271999031 and No. 2018YJ33; by Dr. M. Mehdi Ommati).

References

Abdoli, N., Sadeghian, I., Azarpira, N., Ommati, M.M., Heidari, R., 2021a. Taurine mitigates bile duct obstruction-associated cholemic nephropathy: effect on oxidative stress and mitochondrial parameters. Clin. Exp. Hepatol. 7 (1), 30–40. https://doi.org/10.5114/ceh.2021.104675.

Abdoli, N., Sadeghian, I., Azarpira, N., Ommati, M.M., Heidari, R., 2021b. Taurine mitigates bile duct obstruction-associated cholemic nephropathy: effect on oxidative stress and mitochondrial parameters. Clin. Exp. Hepatol. 7 (1), 30–40. https://doi.org/10.5114/ceh.2021.104675.

Abdoli, N., Sadeghian, I., Mousavi, K., Azarpira, N., Ommati, M.M., Heidari, R., 2020. Suppression of cirrhosis-related renal injury by N-acetyl cysteine. Curr Res Pharmacol Drug Discov 1, 30–38. https://doi.org/10.1016/j.crphar.2020.100006.

Afshari, R., Ghooshkhanehee, H., 2009. Tramadol overdose induced seizure, dramatic rise of CPK, and acute renal failure. J. Pakistan Med. Assoc. 59 (3), 178.

Ahmadi, A., Niknahad, H., Li, H., Mobasheri, A., Manthari, R.K., Azarpira, N., Mousavi, K., Khalvati, B., Zhao, Y., Sun, J., Zong, Y., Ommati, M.M., Heidari, R., 2021. The inhibition of NFκB signaling and inflammatory response as a strategy for blunting bile acid-induced hepatic and renal toxicity. Toxicol. Lett. 349, 12–29. https://doi.org/10.1016/j.toxlet.2021.05.012.

Ahmadi, N., Ghanbarinejad, V., Ommati, M.M., Jamshidzadeh, A., Heidari, R., 2018. Taurine prevents mitochondrial membrane permeabilization and swelling upon interaction with manganese: implication in the treatment of cirrhosis-associated central nervous system complications. J. Biochem. Mol. Toxicol. 32 (11), e22216. https://doi.org/10.1002/jbt.22216.

Akram, J., Hossein, N., Reza, H., Maryam, A., Forouzan, K., Mohammad Reza, A., Omid, F., 2017. Propylthiouracil-induced mitochondrial dysfunction in liver and its relevance to drug-induced hepatotoxicity. Pharmaceut. Sci. 23 (2), 95–102. https://doi.org/10.15171/PS.2017.15.

Ali, H.A., Afifi, M., Saber, T.M., Makki, A.A., Keshta, A.T., Baeshen, M., Al-Farga, A., 2020. Neurotoxic, hepatotoxic and nephrotoxic effects of tramadol administration in rats. J. Mol. Neurosci. 70 (12), 1934–1942. https://doi.org/10.1007/s12031-020-01592-x.

Aparicio-Trejo, O.E., Reyes-Fermín, L.M., Briones-Herrera, A., Tapia, E., León-Contreras, J.C., Hernández-Pando, R., Sánchez-Lozada, L.G., Pedraza-Chaverri, J., 2019. Protective effects of N-acetyl-cysteine in mitochondria bioenergetics, oxidative stress, dynamics and S-glutathionylation alterations in acute kidney damage induced by folic acid. Free Radic. Biol. Med. 130, 379–396. https://doi.org/10.1016/j.freeradbiomed.2018.11.005.

Barbosa, J., Faria, J., Garcez, F., Leal, S., Afonso, L.P., Nascimento, A.V., Moreira, R., Queirós, O., Carvalho, F., Dinis-Oliveira, R.J., 2020. Repeated administration of clinical doses of tramadol and tapentadol causes hepato- and nephrotoxic effects in wistar rats. Pharmaceuticals 13 (7). https://doi.org/10.3390/ph13070149.

Borrego Utiel, F.J., Luque Barona, R., Pérez Del Barrio, P., Borrego Hinojosa, J., Ramírez Tortosa, C., 2018. Acute kidney injury due to granulomatous interstitial nephritis induced by tramadol administration. Nefrologia 38 (2), 227–228. https://doi.org/10.1016/j.nefro.2017.05.005.

Brookes, P.S., Yoon, Y., Robotham, J.L., Anders, M.W., Sheu, S.-S., 2004. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am. J. Physiol. 287 (4), C817–C833. https://doi.org/10.1152/ajpcell.00139.2004.

Caro, A.A., Adlong, L.W., Crocker, S.J., Gardner, M.W., Luikart, E.F., Gron, L.U., 2012. Effect of garlic-derived organosulfur compounds on mitochondrial function and integrity in isolated mouse liver mitochondria. Toxicol. Lett. 214 (2), 166–174. https://doi.org/10.1016/j.toxlet.2012.08.017.

Caro, A.A., Cederbaum, A.I., 2001. Synergistic toxicity of iron and arachidonic acid in HepG2 cells overexpressing CYP2E1. Mol. Pharmacol. 60 (4), 742–752. PMID: 11562436.

de Kretser, D.M., Loveland, K.L., Meinhardt, A., Simorangkir, D., Wreford, N., 1998. Spermatogenesis. Hum. Reprod. 13 (Suppl. l_1), 1–8. https://doi.org/10.1093/humrep/13.suppl_1.1.

Duann, P., Lin, P.-H., 2017. Mitochondria damage and kidney disease. Adv. Exp. Med. Biol. 982, 529–551. https://doi.org/10.1007/978-3-319-55330-6_27.

Eftekhari, A., Heidari, R., Ahmadian, E., Eghbal, M.A., 2018. Cytoprotective properties of carnosine against isoniazid-induced toxicity in primary cultured rat hepatocytes. Pharmaceut. Sci. 24 (4), 257–263. https://doi.org/10.15171/PS.2018.38.

Eirin, A., Lerman, A., Lerman, L.O., 2017. The emerging role of mitochondrial targeting in kidney disease. Handb. Exp. Pharmacol. 240, 229–250. https://doi.org/10.1007/164_2016_6.

Elkhateeb, A., El Khishin, I., Megahed, O., Mazen, F., 2015. Effect of Nigella sativa Linn oil on tramadol-induced hepato-and nephrotoxicity in adult male albino rats. Toxicol Report 2, 512–519. https://doi.org/10.1016/j.toxrep.2015.03.002.

Elmanama, A.A., Abu Tayyem, N.E., Essawaf, H.N., Hmaid, I.M., 2015. Tramadol-induced liver and kidney toxicity among abusers in Gaza strip, Palestine. Jordan J. Biol. Sci. 8 (2), 133–137.

Emadi, E., Abdoli, N., Ghanbarinejad, V., Mohammadi, H.R., Mousavi Mobarakeh, K., Azarpira, N., Mahboubi, Z., Niknahad, H., Heidari, R., 2019. The potential role of mitochondrial impairment in the pathogenesis of imatinib-induced renal injury. Heliyon 5 (6), e01996. https://doi.org/10.1016/j.heliyon.2019.e01996.

Emma, F., Montini, G., Parikh, S.M., Salviati, L., 2016. Mitochondrial dysfunction in inherited renal disease and acute kidney injury. Nat. Rev. Nephrol. 12 (5), 267–280. https://doi.org/10.1038/nrneph.2015.214.

Farombi, E.O., Ekor, M., 2006. Curcumin attenuates gentamicin-induced renal oxidative damage in rats. Food Chem. Toxicol. 44 (9), 1443–1448. https://doi.org/10.1016/j.fct.2006.05.005.

Heidari, R., 2019. The footprints of mitochondrial impairment and cellular energy crisis in the pathogenesis of xenobiotics-induced nephrotoxicity, serum electrolytes imbalance, and Fanconi's syndrome: a comprehensive review. Toxicology 423, 1–31. https://doi.org/10.1016/j.tox.2019.05.002.

Heidari, R., Abdoli, N., Ommati, M.M., Jamshidzadeh, A., Niknahad, H., 2018a. Mitochondrial impairment induced by chenodeoxycholic acid: the protective effect of taurine and carnosine supplementation. Trends Pharmacol. Sci. 4 (2). https://doi.org/10.1111/tips.v4i2.182.

Heidari, R., Babaei, H., Eghbal, M., 2013a. Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. Drug Chem. Toxicol. 36 (4), 403–411. https://doi.org/10.3109/01480545.2012.749272.

Heidari, R., Babaei, H., Eghbal, M.A., 2012. Ameliorative effects of taurine against methimazole-induced cytotoxicity in isolated rat hepatocytes. Sci. Pharm. 80 (4), 987–999. https://doi.org/10.3797/scipharm.1205-16.

Heidari, R., Babaei, H., Eghbal, M.A., 2013b. Cytoprotective effects of organosulfur compounds against methimazole induced toxicity in isolated rat hepatocytes. Adv. Pharmaceut. Bull. 3 (1), 135–142. https://doi.org/10.5681/apb.2013.023.

Heidari, R., Babaei, H., Eghbal, M.A., 2014. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. Res Pharm Sci 9 (2), 97–105.

Heidari, R., Behnamrad, S., Khodami, Z., Ommati, M.M., Azarpira, N., Vazin, A., 2019a. The nephroprotective properties of taurine in colistin-treated mice is mediated through the regulation of mitochondrial function and mitigation of oxidative stress. Biomed. Pharmacother. 109, 103–111. https://doi.org/10.1016/j.biopha.2018.10.093.

Heidari, R., Esmailie, N., Azarpira, N., Najibi, A., Niknahad, H., 2016a. Effect of thiol-reducing agents and antioxidants on sulfasalazine-induced hepatic injury in normotermic recirculating isolated perfused rat liver. Toxicol Res 32 (2), 133–140. https://doi.org/10.5487/TR.2016.32.2.133.

Heidari, R., Ghanbarinejad, V., Mohammadi, H., Ahmadi, A., Esfandiari, A., Azarpira, N., Niknahad, H., 2018b. Dithiothreitol supplementation mitigates hepatic and renal injury in bile duct ligated mice: potential application in the treatment of cholestasis-associated complications. Biomed. Pharmacother. 99, 1022–1032. https://doi.org/10.1016/j.biopha.2018.01.018.

Heidari, R., Ghanbarinejad, V., Mohammadi, H., Ahmadi, A., Ommati, M.M., Abdoli, N., Aghaei, F., Esfandiari, A., Azarpira, N., Niknahad, H., 2018c. Mitochondria protection as a mechanism underlying the hepatoprotective effects of glycine in cholestatic mice. Biomed. Pharmacother. 97, 1086–1095. https://doi.org/10.1016/j.biopha.2017.10.166.

Heidari, R., Ghanbarinejad, V., Ommati, M.M., Jamshidzadeh, A., Niknahad, H., 2018d. Mitochondria protecting amino acids: application against a wide range of mitochondria-linked complications. PharmaNutrition 6 (4), 180–190. https://doi.org/10.1016/j.phanu.2018.09.001.

Heidari, R., Ghanbarinejad, V., Ommati, M.M., Jamshidzadeh, A., Niknahad, H., 2018e. Regulation of mitochondrial function and energy metabolism: a primary mechanism of cytoprotection provided by carnosine. Trends Pharmacol. Sci. 4 (1).

Heidari, R., Jafari, F., Khodaei, F., Shirazi Yeganeh, B., Niknahad, H., 2018f. Mechanism of valproic acid-induced Fanconi syndrome involves mitochondrial dysfunction and oxidative stress in rat kidney. Nephrology 23 (4), 351–361. https://doi.org/10.1111/nep.13012.

Heidari, R., Jamshidzadeh, A., Ghanbarinejad, V., Ommati, M.M., Niknahad, H., 2018g. Taurine supplementation abates cirrhosis-associated locomotor dysfunction. Clin. Exp. Hepatol. 4 (2), 72–82. https://doi.org/10.5114/ceh.2018.75956.

Heidari, R., Jamshidzadeh, A., Ommati, M.M., Rashidi, E., Khodaei, F., Sadeghi, A., Hosseini, A., Niknahad, H., 2019b. Ammonia-induced mitochondrial impairment is intensified by manganese co-exposure: relevance to the management of subclinical hepatic encephalopathy and cirrhosis-associated brain injury. Clin. Exp. Hepatol. 5 (2), 109–117. https://doi.org/10.5114/ceh.2019.85071.

Heidari, R., Mandegani, L., Ghanbarinejad, V., Siavashpour, A., Ommati, M.M., Azarpira, N., Najibi, A., Niknahad, H., 2019c. Mitochondrial dysfunction as a mechanism involved in the pathogenesis of cirrhosis-associated cholemic nephropathy. Biomed. Pharmacother. 109, 271–280. https://doi.org/10.1016/j.biopha.2018.10.104.

Heidari, R., Moezi, L., Asadi, B., Ommati, M.M., Azarpira, N., 2017. Hepatoprotective effect of boldine in a bile duct ligated rat model of cholestasis/cirrhosis. PharmaNutrition 5 (3), 109–117. https://doi.org/10.1016/j.phanu.2017.07.001.

Heidari, R., Mohammadi, H., Ghanbarinejad, V., Ahmadi, A., Ommati, M.M., Niknahad, H., Jamshidzadeh, A., Azarpira, N., Abdoli, N., 2019d. Proline supplementation mitigates the early stage of liver injury in bile duct ligated rats. J. Basic Clin. Physiol. Pharmacol. 30 (1), 91–101. https://doi.org/10.1515/jbcpp-2017-0221.

Heidari, R., Niknahad, H., 2019. The role and study of mitochondrial impairment and oxidative stress in cholestasis. In: Vinken, M. (Ed.), Experimental Cholestasis Research. Springer, New York, NY, pp. 117–132.

Heidari, R., Niknahad, H., Jamshidzadeh, A., Azarpira, N., Bazyari, M., Najibi, A., 2015. Carbonyl traps as potential protective agents against methimazole-induced liver

injury. J. Biochem. Mol. Toxicol. 29 (4), 173–181. https://doi.org/10.1002/jbt.21682.
Heidari, R., Niknahad, H., Sadeghi, A., Mohammadi, H., Ghanbarinejad, V., Ommati, M.M., Hosseini, A., Azarpira, N., Khodaei, F., Farshad, O., Rashidi, E., Siavashpour, A., Najibi, A., Ahmadi, A., Jamshidzadeh, A., 2018h. Betaine treatment protects liver through regulating mitochondrial function and counteracting oxidative stress in acute and chronic animal models of hepatic injury. Biomed. Pharmacother. 103, 75–86. https://doi.org/10.1016/j.biopha.2018.04.010.
Heidari, R., Rasti, M., Shirazi Yeganeh, B., Niknahad, H., Saeedi, A., Najibi, A., 2016b. Sulfasalazine-induced renal and hepatic injury in rats and the protective role of taurine. Bioimpacts 6 (1), 3–8. https://doi.org/10.15171/bi.2016.01.
Jamshidzadeh, A., Abdoli, N., Niknahad, H., Azarpira, N., Mardani, E., Mousavi, S., Abasvali, M., Heidari, R., 2017a. Taurine alleviates brain tissue markers of oxidative stress in a rat model of hepatic encephalopathy. Trends Pharmacol. Sci. 3 (3), 181–192.
Jamshidzadeh, A., Heidari, R., Abazari, F., Ramezani, M., Khodaei, F., Ommati, M.M., Ayarzadeh, M., Firuzi, R., Saeedi, A., Azarpira, N., Najibi, A., 2016. Antimalarial drugs-induced hepatic injury in rats and the protective role of carnosine. Pharmaceut. Sci. 22 (3), 170–180. https://doi.org/10.15171/PS.2016.27.
Jamshidzadeh, A., Heidari, R., Latifpour, Z., Ommati, M.M., Abdoli, N., Mousavi, S., Azarpira, N., Zarei, A., Zarei, M., Asadi, B., Abasvali, M., Yeganeh, Y., Jafari, F., Saeedi, A., Najibi, A., Mardani, E., 2017b. Carnosine ameliorates liver fibrosis and hyperammonemia in cirrhotic rats. Clin Res Hepatol Gastroenterol 41 (4), 424–434. https://doi.org/10.1016/j.clinre.2016.12.010.
Jamshidzadeh, A., Heidari, R., Mohammadi-Samani, S., Azarpira, N., Najbi, A., Jahani, P., Abdoli, N., 2015. A comparison between the nephrotoxic profile of gentamicin and gentamicin nanoparticles in mice. J. Biochem. Mol. Toxicol. 29 (2), 57–62. https://doi.org/10.1002/jbt.21667.
Jamshidzadeh, A., Niknahad, H., Heidari, R., Zarei, M., Ommati, M.M., Khodaei, F., 2017c. Carnosine protects brain mitochondria under hyperammonemic conditions: relevance to hepatic encephalopathy treatment. PharmaNutrition 5 (2), 58–63. https://doi.org/10.1016/j.phanu.2017.02.004.
Le Berre, J.P., Desramé, J., Lecoules, S., Coutant, G., Béchade, D., Algayres, J.P., 2007. Hyponatrémie due au tramadol. Rev. Med. Interne 28 (12), 888–889. https://doi.org/10.1016/j.revmed.2007.06.011.
Meeks, R.G., Harrison, S., 1991. Hepatotoxicology. CRC Press, New York, p. 716, 9780849388101.
Mehdizadeh, H., Pourahmad, J., Taghizadeh, G., Vousooghi, N., Yoonessi, A., Naserzadeh, P., Behzadfar, L., Rouini, M.R., Sharifzadeh, M., 2017. Mitochondrial impairments contribute to spatial learning and memory dysfunction induced by chronic tramadol administration in rat: protective effect of physical exercise. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 79 (Pt B), 426–433. https://doi.org/10.1016/j.pnpbp.2017.07.022.
Mitchell, T., Rotaru, D., Saba, H., Smith, R.A.J., Murphy, M.P., MacMillan-Crow, L.A., 2011. The mitochondria-targeted antioxidant mitoquinone protects against cold storage injury of renal tubular cells and rat kidneys. J. Pharmacol. Exp. Therapeut. 336 (3), 682–692. https://doi.org/10.1124/jpet.110.176743.
Mohamed, H.M., Mahmoud, A.M., 2019. Chronic exposure to the opioid tramadol induces oxidative damage, inflammation and apoptosis, and alters cerebral monoamine neurotransmitters in rats. Biomed. Pharmacother. 110, 239–247. https://doi.org/10.1016/j.biopha.2018.11.141.
Mohammadi, H., Ommati, M.M., Farshad, O., Jamshidzadeh, A., Nikbakht, M.R., Niknahad, H., Heidari, R., 2019. Taurine and isolated mitochondria: a concentration-response study. Trends in Pharmaceutical Sciences 5 (4), 197–206. https://doi.org/10.30476/tips.2020.84851.1037.
Mohammadi, H., Sayad, A., Mohammadi, M., Niknahad, H., Heidari, R., 2020. N-acetyl cysteine treatment preserves mitochondrial indices of functionality in the brain of hyperammonemic mice. Clin. Exp. Hepatol. 6 (2), 106–115. https://doi.org/10.5114/ceh.2020.95814.
Niknahad, A.M., Ommati, M.M., Farshad, O., Moezi, L., Heidari, R., 2020. Manganese-induced nephrotoxicity is mediated through oxidative stress and mitochondrial impairment. J Renal Hepatic Disord 4 (2), 1–10. https://doi.org/10.15586/jrenhep.2020.66.
Niknahad, H., Heidari, R., Alzuhairi, A.M., Najibi, A., 2015. Mitochondrial dysfunction as a mechanism for pioglitazone-induced injury toward HepG2 cell line. Pharmaceut. Sci. 20 (4), 169–174.
Niknahad, H., Heidari, R., Mohammadzadeh, R., Ommati, M.M., Khodaei, F., Azarpira, N., Abdoli, N., Zarei, M., Asadi, B., Rasti, M., Yeganeh, B.S., Taheri, V., Saeedi, A., Najibi, A., 2017a. Sulfasalazine induces mitochondrial dysfunction and renal injury. Ren. Fail. 39 (1), 745–753. https://doi.org/10.1080/0886022X.2017.1399908.
Niknahad, H., Jamshidzadeh, A., Heidari, R., Hosseini, Z., Mobini, K., Khodaei, F., Ommati, M.M., Abdoli, N., Keshavarz, N., Bazyari, M., Najibi, A., 2016. Paradoxical effect of methimazole on liver mitochondria: in vitro and in vivo. Toxicol. Lett. 259, 108–115. https://doi.org/10.1016/j.toxlet.2016.08.003.
Niknahad, H., Jamshidzadeh, A., Heidari, R., Zarei, M., Ommati, M.M., 2017b. Ammonia-induced mitochondrial dysfunction and energy metabolism disturbances in isolated brain and liver mitochondria, and the effect of taurine administration: relevance to hepatic encephalopathy treatment. Clin. Exp. Hepatol. 3 (3), 141–151. https://doi.org/10.5114/ceh.2017.68833.
Ommati, M.M., Amjadinia, A., Mousavi, K., Azarpira, N., Jamshidzadeh, A., Heidari, R., 2021a. N-acetyl cysteine treatment mitigates biomarkers of oxidative stress in different tissues of bile duct ligated rats. Stress 24 (2), 213–228. https://doi.org/10.1080/10253890.2020.1777970.
Ommati, M.M., Attari, H., Siavashpour, A., Shafaghat, M., Azarpira, N., Ghaffari, H., Moezi, L., Heidari, R., 2020a. Mitigation of Cholestasis-Associated Hepatic and Renal Injury by Edaravone Treatment: Evaluation of its Effects on Oxidative Stress and Mitochondrial Function. Liver Res In-Press. https://doi.org/10.1016/j.livres.2020.10.003.
Ommati, M.M., Farshad, O., Azarpira, N., Shafaghat, M., Niknahad, H., Heidari, R., 2021b. Betaine alleviates cholestasis-associated renal injury by mitigating oxidative stress and enhancing mitochondrial function. Biologia 76 (1), 351–365. https://doi.org/10.2478/s11756-020-00576-x.
Ommati, M.M., Farshad, O., Ghanbarinejad, V., Mohammadi, H.R., Khadijeh, M., Negar, A., Zahra, M., Ilkhaninasab, F., Moezi, L., Heidari, R., 2020b. The nephroprotective role of carnosine against ifosfamide-induced renal injury and electrolytes imbalance is mediated via the regulation of mitochondrial function and alleviation of oxidative stress. Drug Res. 70 (1), 49–56. https://doi.org/10.1055/a-1017-5085.
Ommati, M.M., Farshad, O., Jamshidzadeh, A., Heidari, R., 2019a. Taurine enhances skeletal muscle mitochondrial function in a rat model of resistance training. PharmaNutrition 9, 100161. https://doi.org/10.1016/j.phanu.2019.100161.
Ommati, M.M., Farshad, O., Mousavi, K., Jamshidzadeh, A., Azmoon, M., Heidari, S., Azarpira, N., Niknahad, H., Heidari, R., 2020c. Betaine supplementation mitigates intestinal damage and decreases serum bacterial endotoxin in cirrhotic rats. PharmaNutrition 12, 100179. https://doi.org/10.1016/j.phanu.2020.100179.
Ommati, M.M., Farshad, O., Mousavi, K., Khalili, M., Jamshidzadeh, A., Heidari, R., 2020d. Chlorogenic acid supplementation improves skeletal muscle mitochondrial function in a rat model of resistance training. Biologia 75 (8), 1221–1230. https://doi.org/10.2478/s11756-020-00429-7.
Ommati, M.M., Farshad, O., Niknahad, H., Arabnezhad, M.R., Azarpira, N., Mohammadi, H.R., Haghnegahdar, M., Mousavi, K., Akrami, S., Jamshidzadeh, A., Heidari, R., 2019b. Cholestasis-associated reproductive toxicity in male and female rats: the fundamental role of mitochondrial impairment and oxidative stress. Toxicol. Lett. 316, 60–72. https://doi.org/10.1016/j.toxlet.2019.09.009.
Ommati, M.M., Farshad, O., Niknahad, H., Mousavi, K., Moein, M., Azarpira, N., Mohammadi, H., Jamshidzadeh, A., Heidari, R., 2020f. Oral administration of thiol-reducing agents mitigates gut barrier disintegrity and bacterial lipopolysaccharide translocation in a rat model of biliary obstruction. Curr Res Pharmacol Drug Discov 1, 10–18. https://doi.org/10.1016/j.crphar.2020.06.001.
Ommati, M.M., Heidari, R., Ghanbarinejad, V., Abdoli, N., Niknahad, H., 2019c. Taurine treatment provides neuroprotection in a mouse model of manganism. Biol. Trace Elem. Res. 190 (2), 384–395. https://doi.org/10.1007/s12011-018-1552-2.
Ommati, M.M., Heidari, R., Jamshidzadeh, A., Zamiri, M.J., Sun, Z., Sabouri, S., Wang, J., Ahmadi, F., Javanmard, N., Seifi, K., Mousapour, S., Yeganeh, B.S., 2018. Dual effects of sulfasalazine on rat sperm characteristics, spermatogenesis, and steroidogenesis in two experimental models. Toxicol. Lett. 284, 46–55. https://doi.org/10.1016/j.toxlet.2017.11.034.
Ommati, M.M., Jamshidzadeh, A., Heidari, R., Sun, Z., Zamiri, M.J., Khodaei, F., Mousapour, S., Ahmadi, F., Javanmard, N., Shirazi Yeganeh, B., 2019d. Carnosine and histidine supplementation blunt lead-induced reproductive toxicity through antioxidative and mitochondria-dependent mechanisms. Biol. Trace Elem. Res. 187 (1), 151–162. https://doi.org/10.1007/s12011-018-1358-2.
Ommati, M.M., Jamshidzadeh, A., Niknahad, H., Mohammadi, H., Sabouri, S., Heidari, R., Abdoli, N., 2017. N-acetylcysteine treatment blunts liver failure-associated impairment of locomotor activity. PharmaNutrition 5 (4), 141–147. https://doi.org/10.1016/j.phanu.2017.10.003.
Ommati, M.M., Manthari, R.K., Tikka, C., Niu, R., Sun, Z., Sabouri, S., Zamiri, M.J., Ahmadi, H.N., Ghaffari, H., Heidari, R., Wang, J., 2020g. Arsenic-induced autophagic alterations and mitochondrial impairments in HPG-S axis of mature male mice offspring (F1-generation): a persistent toxicity study. Toxicol. Lett. 326, 83–98. https://doi.org/10.1016/j.toxlet.2020.02.013.
Ommati, M.M., Niknahad, H., Farshad, O., Azarpira, N., Heidari, R., 2020h. In vitro and in vivo evidence on the role of mitochondrial impairment as a mechanism of lithium-induced nephrotoxicity. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-020-02302-9.
Oyewole, A.O., Birch-Machin, M.A., 2015. Mitochondria-targeted antioxidants. Faseb. J.: official publication of the Federation of American Societies for Experimental Biology 29 (12), 4766–4771. https://doi.org/10.1096/fj.15-275404.
Sarret, D., Berre, J.-P.L., Zemraoui, N., 2008. Tramadol-induced hyponatremia. Am. J. Kidney Dis. 52 (5), 1026. https://doi.org/10.1053/j.ajkd.2008.08.007.
Scott, L.J., Perry, C.M., 2000. Tramadol. Drugs 60 (1), 139–176. https://doi.org/10.2165/00003495-200060010-00008.
Seifi, K., Rezaei, M., Yansari, A.T., Riazi, G.H., Zamiri, M.J., Heidari, R., 2018. Saturated fatty acids may ameliorate environmental heat stress in broiler birds by affecting mitochondrial energetics and related genes. J. Therm. Biol. 78, 1–9. https://doi.org/10.1016/j.jtherbio.2018.08.018.
Seifi, K., Rezaei, M., Yansari, A.T., Zamiri, M.J., Riazi, G.H., Heidari, R., 2020. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. J. Therm. Biol. 89, 102520. https://doi.org/10.1016/j.jtherbio.2020.102520.
Shafiekhani, M., Ommati, M.M., Azarpira, N., Heidari, R., Salarian, A.A., 2019. Glycine supplementation mitigates lead-induced renal injury in mice. J. Exp. Pharmacol. 11, 15–22. https://doi.org/10.2147/JEP.S190846.
Sheu, S.-S., Nauduri, D., Anders, M.W., 2006. Targeting antioxidants to mitochondria: a new therapeutic direction. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1762 (2), 256–265. https://doi.org/10.1016/j.bbadis.2005.10.007.
Sheweita, S.A., Almasmari, A.A., El-Banna, S.G., 2018. Tramadol-induced hepato- and nephrotoxicity in rats: role of Curcumin and Gallic acid as antioxidants. PloS One 13 (8), e0202110. https://doi.org/10.1371/journal.pone.0202110.
Siavashpour, A., Khalvati, B., Azarpira, N., Mohammadi, H., Niknahad, H., Heidari, R., 2020. Poly (ADP-Ribose) polymerase-1 (PARP-1) overactivity plays a pathogenic role

in bile acids-induced nephrotoxicity in cholestatic rats. Toxicol. Lett. 330, 144–158. https://doi.org/10.1016/j.toxlet.2020.05.012.

Subedi, M., Bajaj, S., Kumar, M.S., Yc, M., 2019. An overview of tramadol and its usage in pain management and future perspective. Biomed. Pharmacother. 111, 443–451. https://doi.org/10.1016/j.biopha.2018.12.085.

Szeto, H.H., 2017. Pharmacologic approaches to improve mitochondrial function in AKI and CKD. J. Am. Soc. Nephrol. 28 (10), 2856–2865. https://doi.org/10.1681/ASN.2017030247.

Touyz, R.M., Anagnostopoulou, A., Rios, F., Montezano, A.C., Camargo, L.L., 2019. NOX5: molecular biology and pathophysiology. Exp. Physiol. 104 (5), 605–616. https://doi.org/10.1113/EP086204.

Truong, D.H., Eghbal, M.A., Hindmarsh, W., Roth, S.H., O'Brien, P.J., 2006. Molecular mechanisms of hydrogen sulfide toxicity. Drug Metab. Rev. 38 (4), 733–744. https://doi.org/10.1080/03602530600959607.

Vazin, A., Heidari, R., Khodami, Z., 2020. Curcumin supplementation alleviates polymyxin E-induced nephrotoxicity. J. Exp. Pharmacol. 12, 129–136. https://doi.org/10.2147/JEP.S255861.

Volonté, M.G., Yuln, G., Quiroga, P., Consolini, A.E., 2004. Development of an HPLC method for determination of metabolic compounds in myocardial tissue. J. Pharmaceut. Biomed. Anal. 35 (3), 647–653. https://doi.org/10.1016/j.jpba.2004.02.002.

Zhang, Y.-T., Zheng, Q.-S., Pan, J., Zheng, R.-L., 2004. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin. Pharmacol. Toxicol. 95 (2), 53–58. https://doi.org/10.1111/j.1742-7843.2004.950202.x.

Zhuo, H.-Q., Huang, L., Huang, H.-Q., Cai, Z., 2012. Effects of chronic tramadol exposure on the zebrafish brain: a proteomic study. J Proteomics 75 (11), 3351–3364. https://doi.org/10.1016/j.jprot.2012.03.038.