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Effect of *Nigella sativa Linn* oil on tramadol-induced hepato- and nephrotoxicity in adult male albino rats

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ABSTRACT

The present study was carried out to evaluate the role of *Nigella sativa Linn* (*NsL*) oil against subacute tramadol-induced hepatotoxicity, nephrotoxicity as well as oxidative stress in adult male albino rats. Sixty adult male albino rats were divided into four groups. Group I: control group; 30 rats equally subdivided into: Ia; −ve control group, Ib; +ve control group received saline, Ic; +ve control group received corn oil. Group II: 10 rats received *NsL* oil; 1 mg/kg in 1 ml corn oil/day, group III: 10 rats received tramadol; 30 mg/kg/day, group IV: 10 rats received tramadol + *NsL* oil in the previous doses. Treatments were given by gavage for 30 days. Then rats were sacrificed and specimens from the livers and kidneys were taken for biochemical and histopathological study. Biochemical data showed elevated liver enzymes; alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyltransferase (GGT), bilirubin as well as urea and creatinine in tramadol group. A significant increase in hepatic and renal malondialdehyde (MDA) and a decrease in glutathione peroxidase (GPx) levels were also noticed. Histological analysis of the liver showed vacuolated hepatocyte cytoplasm indicating hydropic degeneration with binucleated cells, apoptotic nuclei, congested central veins, cellular infiltration and hemorrhage. Kidney sections revealed atrophied glomeruli with collapsed tufts and wide Bowman's space, degenerated tubules, hemorrhage and mononuclear cellular infiltration. There was also an increase in area % of collagen fibers in both organs. Concomitant use of *NsL* oil with tramadol induced partial improvement in the hepato- and nephrotoxic effects. In conclusion, this study suggested that concomitant use of *NsL* oil with tramadol proved to be capable of ameliorating tramadol-induced hepato- and nephrotoxicity which might be due to its antioxidant potential.

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1. Introduction

Tramadol is a synthetic centrally acting analgesic with effects similar to those of codeine and 10 times less than morphine [1]. Tramadol has a wide range of applications mostly in the treatment of moderate to severe, acute or chronic pain [2]. Tramadol appears to be an ideal analgesic during and after day case surgery and in patients with acute ureteric spasm [3]. Studies have shown tramadol to be effective in both acute myocardial infarction and

Abbreviations: *NsL*, *Nigella sativa Linn*; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma glutamyltransferase; GPx, glutathione peroxidase; MDA, malondialdehyde; ANOVA, analysis of variance.

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unstable angina [4]. Tramadol has also been used in treatment of cancer pain, moderate to severe musculoskeletal pain, rheumatoid arthritis, restless legs syndrome, motor neuron disease, fibromyalgia, diabetic neuropathy and post-herpetic neuralgia [5,6].

Tramadol is metabolized mainly in the liver by cytochrome P450 (CYP_2D_6), cytochrome $P450_3A$ (CYP_3A4) and cytochrome P450 isozyme (CYP2B6), being O- and N-demethylated to five different metabolites, followed by conjugation with glucuronic acid and sulphate [48].

Tramadol toxic effects should be kept in mind during long-term therapy especially in large doses [7]. Wide-spread use of tramadol makes associated hepatotoxicity a clinically and economically important problem [8].

Tramadol and its metabolites are excreted via kidneys, consequently the kidney is considered to be the primary target organ for tramadol toxicity [8].

Turrens [9] stated that oxidative stress is an expression used to describe various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen species and limited antioxidant defenses. The effects of reactive oxygen species are wide-ranging, but three reactions are particularly relevant to cell injuries which are lipid peroxidation of membranes, oxidative modification of proteins and lesions in DNA [10].

Nigella sativa Linn (*NsL*) oil is an annual herbaceous plant belonging to the Ranunculaceae family growing in countries bordering the Mediterranean Sea, Pakistan, India and Iran [11]. *NsL* oil seed was found to contain more than 30% fixed oil, and 0.4–0.45% volatile oil. The fixed oil is composed mainly of unsaturated fattyacids. Thymoquinone is the major active component of the volatile oil [12].

Many medicinal properties have been attributed to *NsL* oil seed extract and/or its oil, including antihistaminic, anti-hypertensive, analgesic, anti-inflammatory, hypoglycemic, antibacterial, antifungal, antitumour as well as protective effects against hepatotoxicity and nephrotoxicity [11,13].

In addition, *NsL* oil has clear antioxidant properties and can be used against oxidative stress by increasing antioxidant enzymes activities and decreasing oxidant enzymes activities [14].

Therefore, the aim of this work was to evaluate the role of *NsL* oil against subacute tramadol-induced hepatotoxicity, nephrotoxicity as well as oxidative stress in adult male albino rats.

2. Materials and methods

2.1. Material

2.1.1. Chemicals

Tramadol tablets, each contains 225 mg tramadol hydrochloride obtained from October Pharma Company (Giza, Egypt). *Nigella sativa Linn* oil (*NsL* oil) 2oz/60 ml – 100% pure, undiluted – obtained from El-Captain Company (Cairo, Egypt). Saline (500 ml) solution; 0.9% NaCl (solvent of tramadol) and corn oil (solvent of *NsL* oil) were also obtained.

2.1.2. Kits

Kits of alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyltransferase (GGT), serum bilirubin, creatinine and urea, glutathione peroxidase (GPX) and malondialdehyde (MDA) were purchased from Biodiagnostic Co. (Giza, Egypt).

The study was carried out on 60 adult male albino rats, their weights ranging from 250 to 300 g for each. They were obtained from the Animal House of the Faculty of Veterinary Medicine, Zagazig University. The study had been conducted in the Animal House of the Faculty of Medicine, Zagazig University in accordance with the guidance of ethical committee for research on laboratory animals [15]. The study protocol was approved by the Institutional Review Board of the faculty of Medicine, Zagazig University, Egypt (ZU-IRB-910).

Experimental design: The rats were randomly divided into four groups as follow:

Group I (control group): 30 rats, equally subdivided into three subgroups:
Subgroup Ia (negative control group): Rats received only regular diet and tap water for 30 days to measure the basic parameters.
Subgroup Ib (positive control group) with saline: Each rat received 1 ml normal saline/day, orally for 30 days.
Subgroup Ic (positive control group) with corn oil: Each rat received 1 ml corn oil/day, orally for 30 days.
Group II (*NsL* oil group): 10 rats received *NsL* oil (1 ml/kg in 1 ml corn oil/day), orally for 30 days (each rat received 0.2 ml (about 315 mg), each 1 ml in 1 ml corn oil [16].
Group III (tramadol-treated group): 10 rats treated with tramadol (30 mg/kg/day), orally for 30 days. This dose is 1/10 of LD50. LD 50 is 286–300 mg/kg [17,18].
Group IV (tramadol + *NsL* oil-treated group): 10 rats treated with tramadol along with NsL oil in the previous doses.

At the time of sacrifice, rats were anaesthetized with ether and then blood samples were collected from the retro-orbital plexuses. The liver and kidneys of each rat were dissected out carefully. Each specimen was divided into two parts; one part was wrapped with aluminum foil and kept frozen at −80 °C till they were used for measurement of MDA content and GPx level and the other part was preserved for histopathological examination.

3. Methods

3.1. Biochemical analysis

3.1.1. Assessment of liver and kidney function tests

The blood samples were used for estimation of serum alanine transaminase (ALT) as described by Reitman and Frankel [19], aspartate transaminase (AST) according to Thomas [20], serum gamma glutamyltransferase (GGT) according to Gendler [49], total serum bilirubin as described by Mallay and Evelyn [21] and urea and

creatinine according to Orsonneau et al. [22] and Fossati et al. [23], consequently.

3.1.2. Preparation of liver and kidney homogenate

Liver and kidney tissues were homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer pH (7.4). Aliquots of homogenates were used to determine lipid peroxidation and glutathione peroxidase. GPx activity was measured by the method of Paglia and Valentine [24]. Malondialdehyde (MDA) was assayed according to the method proposed by Ohkawa et al. [25].

3.1.3. Histopathological examination

Liver and kidney specimens (1 cm thick) of different groups were fixed in 10% buffered formalin solution and processed to obtain paraffin sections of 5 μm thickness. The sections were stained with hematoxylin and eosin and Masson's trichrome stains [26].

3.1.4. Quantitative morphometric analysis

The area percentage of collagen fibers was measured in Masson's trichrome-stained sections. The measurements were obtained using computer-based image analysis software (Leica Qwin 500; Imaging Systems, Cambridge, UK) at the image analyzing unit of the Pathology department, Faculty of Dentistry, Cairo University – Egypt. Measurements were performed in 10 non-overlapping fields for each rat of four randomly chosen rats of each group at 400×.

3.2. Statistical analysis

Data were represented as means ± SD. The differences were compared for statistical significance by ANOVA and post hoc Tukey's tests. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using a software (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Biochemical results

Statistical comparison between negative and positive control groups as regard liver and kidney function tests and oxidative stress markers revealed no significant difference ($p > 0.05$) by ANOVA test (Table 1). So, the negative control group was used for comparison with the other groups of the study. No significant differences in the mean values of the liver and kidney function tests were noticed between *NsL* oil group and negative control group. However, there was a highly significant increase in the mean values of serum ALT, AST, GGT, total bilirubin, urea and creatinine in tramadol-treated group as compared to the negative control group ($p < 0.001$). Although tramadol + *NsL* oil group revealed a significant decrease in the mean values of serum ALT, AST, GGT, total bilirubin, urea and creatinine when compared with tramadol-treated group ($p < 0.001$), the values of the above mentioned parameters were still higher than those of the negative control group (Table 2).

As regard the mean values of the oxidative stress markers (MDA and GPx) in the liver and kidney tissues, non-significant differences were noticed between *NsL* oil group and negative control group. On the other hand, a significant increase in the MDA level was observed in tramadol-treated group when compared to the negative control. *NsL* oil group showed a significant decrease in the MDA level in comparison to the tramadol group. The results of tramadol + *NsL* oil group revealed a significant decrease in the mean values of liver and kidney MDA level when compared with that of tramadol-treated group ($p < 0.001$), but still non-significantly higher than the negative control group. In addition, rats treated with tramadol showed a highly significant decrease in the GPx level when compared to the negative control group ($p < 0.001$). In *NsL* oil group, a significant increase in GPx level was noticed in comparison to the tramadol group ($p < 0.001$). As regarding tramadol + *NsL* oil group, the results revealed a significant increase in the mean values of liver and kidney GPx level when compared with that of tramadol-treated group ($p < 0.001$), but still non significantly lower than the negative control group (Table 3).

4.2. Histopathological results

4.2.1. The liver

Examination of liver specimens taken from the control groups and *NsL* oil group showed normal liver architecture with trabeculae of normal hepatocytes running radiantly from central veins, and separated by sinusoids. Hepatocytes revealed acidophilic stippled cytoplasm and vesicular nuclei. The portal areas contained branches of the

Table 1
Comparison among negative control, positive control (saline) and positive control (corn oil) groups as regard the mean values of serum ALT (IU/L), AST (IU/L), GGT (IU/L), total bilirubin (mg/dl), urea (mg/dl) and creatinine (mg/dl), using ANOVA test.

Parameters	Groups			*F*	*P*
	Negative control *N* = 10	Positive control saline (1 ml/kg) *N* = 10	Positive control corn oil (1 ml/kg) *N* = 10		
	X ± SD				
ALT (IU/L)	50.32 ± 3.78	48.46 ± 2.1	49.26 ± 1.34	0.759	0.49#
AST (IU/L)	157.18 ± 3.17	157.53 ± 1.92	158.42 ± 1.21	0.481	0.63#
GGT (IU/L)	48.52 ± 2.93	49.07 ± 2.43	49.63 ± 1.26	0.343	0.72#
Total serum bilirubin (mg/dl)	0.36 ± 0.11	0.37 ± 0.08	0.35 ± 0.14	0.079	0.924#
Urea (mg/dl)	42.86 ± 1.70	42.19 ± 1.19	41.57 ± 1.27	1.255	0.313#
Creatinine (mg/dl)	0.89 ± 0.05	0.86 ± 0.04	0.85 ± 0.04	1.542	0.25#

N: number of rats in each group.
$P > 0.05$ (non-significant).

Table 2
Comparison of the mean values of liver function tests [serum ALT (IU/L), AST (IU/L) & GGT (IU/L), total bilirubin] and kidney function tests [serum urea &creatinine] of negative control, *NsL* oil, tramadol, and tramadol + *NsL* oil groups using ANOVA test and post hoc Tukey's test.

Parameters	Groups					
	Negative control N = 10	*NsL* oil group N = 10	Tramadol group N = 10	Tramadol + *NsL* oil group N = 10	*F*	*P*
			X ± SD			
ALT (IU/L)	50.32 ± 3.78	38.05 ± 16.72	145.01 ± 5.37[a]	94.64 ± 6.1[a,b]	157.068	0.001[**]
AST (IU/L)	157.18 ± 3.17	151.35 ± 1.47	194.03 ± 15.7[a]	167.77 ± 2.9[b]	32.058	0.001[**]
GGT (IU/L)	48.52 ± 2.93	41.72 ± 1.54	78.94 ± 5.22[a]	60.19 ± 5.49[b]	93.202	0.001[**]
Total Serum Bilirubin (mg/dl)	0.36 ± 0.11	0.38 ± 0.19	0.66 ± 0.14[a]	0.41 ± 0.02[b]	14.17	0.001[**]
Urea (mg/dl)	42.86 ± 1.7	39.02 ± 0.96	74.56 ± 5.27[a]	52.41 ± 8.56[b]	58.097	0.001[**]
Creatinine(mg/dl)	0.89 ± 0.05	0.79 ± 0.02	1.81 ± 0.12[a]	1.51 ± 0.13[a]	165.648	0.001[**]

N: number of rats in each group.
[a] Significant difference ($p < 0.05$) when compared with the negative control group.
[b] Significant difference ($p < 0.05$) when compared with tramadol-treated group.
[**] Highly significant ($P < 0.001$).

hepatic artery and bile duct, embedded in connective tissue (Fig. 1a and b). Tramadol-treated group showed vacuolated hepatocytes' cytoplasm indicating hydropic degeneration, with congested central veins. Some hepatocytes were binucleated while others contained apoptotic nuclei. The portal tract revealed proliferated bile ducts, cellular infiltration and fibrosis of blood vessels. Hemorrhage was also observed (Fig. 1c–e). Co-administration of *NsL* oil along with tramadol revealed improved liver architecture. However, there was congestion of central veins which indicate partial improvement (Fig. 1f). Masson's trichrome-stained sections of the negative control group showed few collagen fibers in the portal tract. The collagen fibers were increased in tramadol-treated group. Tramadol + *NsL* oil-treated group showed decreased amount of collagen fibers in comparison to that of tramadol treated group (Fig. 2a and c).

4.2.2. The kidney

Examination of H&E-stained sections of the kidney of the control and *NsL* oil groups showed normal glomeruli, each contained a tuft of glomerular capillaries surrounded by visceral and parietal layers of Bowman's capsule which are separated by narrow Bowman's space. Sections of the proximal and distal convoluted tubules were seen lined with cuboidal epithelium with strongly eosinophilic cytoplasm and vesicular central rounded nuclei (Fig. 3a). Tramadol-treated group showed atrophied glomerulus with collapsed tuft, wide Bowman's space, degenerated tubules, cellular infiltration and haemorrhage. Haemorrhage and mononuclear cellular infiltration were also seen (Fig. 3b). Co-administration of *NsL* oil along with tramadol revealed partial improvement with structure of the kidney nearly similar to control group. However, there were slightly wide Bowman's space, some vacuolated tubular cells and mild congestion of peritubular capillaries (Fig. 3c).

Using Masson's trichrome stain, the negative control group showed few collagen fibers in the glomerular basement membrane, mesangium and Bowman's capsule (Fig. 4a). The collagen fibers were increased in the tramadol-treated group (Fig. 4b). Co-administration of tramadol and *NsL* oil showed decreased amount of collagen fibers in kidney sections of rats of this group when compared with that of tramadol treated rats (Fig. 4c).

4.3. Morphometric results

Non-significant difference in area % of collagen fibers was detected in sections of liver and kidney of *NsL* oil and negative control groups. There was a significant increase in area % of collagen fibers in sections of liver and kidney of tramadol-treated group when compared to the

Table 3
Comparison of the mean values of oxidative stress markers: MDA (nmol/mg protein), SOD (U/ml) and CAT (U/ml) of the negative control, *NsL* oil, tramadol and tramadol + *NsL* oil groups using ANOVA test and post hoc Tukey's test.

Parameters	Groups				*P*
	Negative control N = 10	*NsL* oil group N = 10	Tramadol group N = 10	Tramadol + *NsL* group N = 10	
MDA (mmol/l)					
Liver	90.86 ± 2.62	79.63 ± 3.63	148.83 ± 3.97[a]	97.74 ± 1.13[b]	0.001[**]
Kidney	89.36 ± 1.13	76.08 ± 3.73	136.27 ± 12.39[a]	94.55 ± 2.45[b]	0.001[**]
GPx (ng/ml)					
Liver	19.59 ± 0.71	25.03 ± 2.66	12.96 ± 1.01[a]	17.23 ± 0.98[b]	0.001[**]
Kidney	21.18 ± 1.55	26.05 ± 1.38	13.67 ± 1.83[a]	17.85 ± 0.35[b]	0.001[**]

N: number of rats in each group.
[a] Significant difference ($p < 0.05$) when compared with the negative control group.
[b] Significant difference ($p < 0.05$) when compared with tramadol group.
[**] $P < 0.001$ highly significant.

Fig. 1. A photomicrograph of sections in rat liver of all studied groups. (a and b) Negative control group showing in (a): sheets of normal hepatocytes (arrow) with acidophilic stippled cytoplasm and vesicular nuclei radiating from central vein (v) and separated by sinusoids (s). (b) Portal area with hepatic artery (A) and bile duct (d), embedded in connective tissue (ct). (c–e) Tramadol-treated group showing in c): hepatocytes with vacuolated cytoplasm (arrow), indicating hydropic degeneration, and congested central veins (v). (d) Some hepatocytes are binucleated (short arrow). The portal tract reveals proliferated bile ducts (d), cellular infiltration (double arrow) and fibrosis of blood vessels (f). (e) Many hepatocytes showing hydropic degeneration (long arrow), apoptotic nuclei (green arrow head) others are binucleated (short arrow). Hemorrhage (hg) is also seen. (f) Tramadol + *NsL* oil-treated group showing improved liver architecture, and hepatocytes showing eosinophilic cytoplasm however there is congested central veins (v) [H&E 400×].

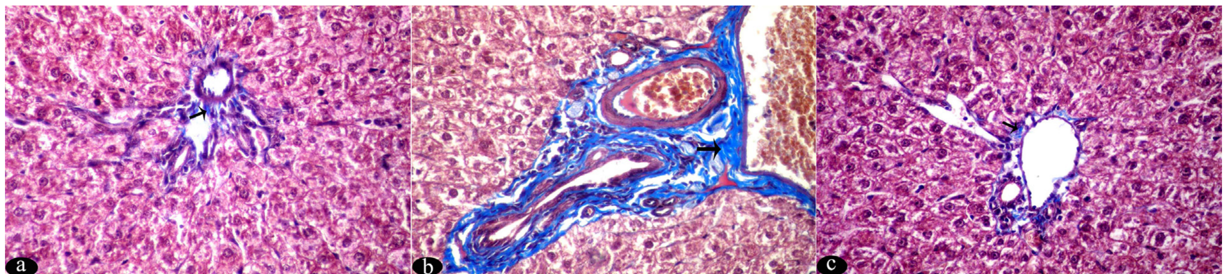

Fig. 2. Sections in rat liver showing distribution of collagen fibers (arrow) in all studied groups. (a) Negative control group showing few collagen fibers in the portal tract. (b) Tramadol-treated group shows increased collagen fibers in portal tract. (c) Tramadol + *NsL* oil-treated group shows decreased amount of collagen fibers in the portal tract [Masson's trichrome 400×].

Table 4
Comparison between the area percentages (area %) of collagen fibers in the different studied groups.

Parameters	Groups			
	Negative control	*NsL* oil Group	Tramadol group	Tramadol + *NsL* oil group
Area %of collagen fibers				
Liver	0.35 ± 0.09	0.48 ± 0.11	3.93 ± 1.23[a]	0.58 ± 0.13[b]
Kidney	7.48 ± 1.6	8.45 ± 1.9	20.3 ± 3.52[a]	12.14 ± 2.33[a,b]

[a] Significant difference ($p < 0.05$) when compared with the negative control group.
[b] Significant difference ($p < 0.05$) when compared with tramadol group.

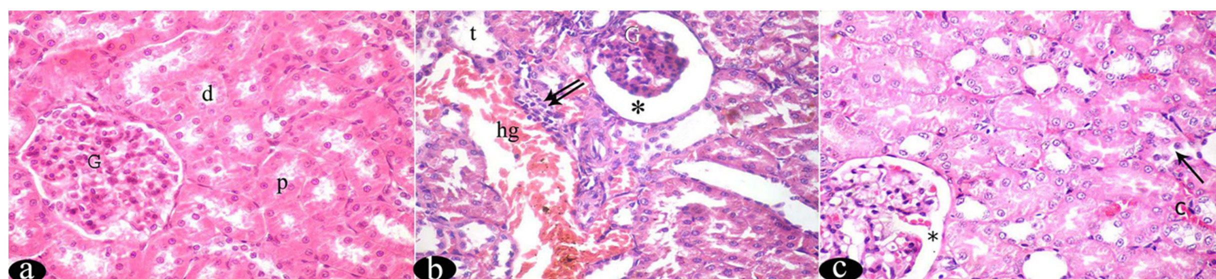

Fig. 3. A photomicrograph of renal sections from all studied groups. (a) Negative control group: showing a glomerulus (G) composed of a tuft of glomerular capillaries, surrounded by visceral and parietal layers of Bowman's capsule, separated by narrow Bowman's space. Proximal (p) and distal (d) convoluted tubules are also seen. (b): Tramadol-treated group showing atrophied glomerulus with collapsed tuft (G), wide Bowman's space (*) and degenerated tubules (t). Cellular infiltration (double arrow) and haemorrage (hg) are also seen. (c) Tramadol + *NsL* oil -treated group showing nearly normal structure of the kidney with slightly wide Bowman's space (*). Some vacuolated tubular cells (arrow) and mild congestion of peritubular capillaries (c) are also seen [H&E 400×].

negative control. However, a significant decrease in area % of collagen fibers was found in the liver and kidney of tramadol + *NsL* oil group in comparison to tramadol-treated group (Table 4).

5. Discussion

Tramadol is available worldwide as a synthetic centrally acting analgesic for treatment of moderate to severe, acute or chronic pain [2]. The role of the liver and the kidneys in tramadol metabolism and excretion predisposes them to toxic injury [18].

In the present study, the liver functions were impaired in tramadol-treated group as reflected by elevation of serum ALT, AST, GGT and total bilirubin. Similar results were obtained by Wu et al. [27], Atici et al. [28] and Janssen-Ortho Inc. [8] who reported significant increase in the levels of serum ALT, AST and LDH (lactate dehydrogenase) in rats after long term usage of tramadol.

Serum ALT is relatively specific, affected early by hepatotoxicity and is considered an excellent marker of cellular necrosis, as it is a cytoplasmic enzyme [47]. On the other hand, AST is mainly a mitochondrial enzyme. Although its elevated level in the serum is not specific of the hepatic disorder, AST is used mainly to diagnose and to verify persistent cellular injury with other enzymes like ALT [29].

The histopathological results of the current work supported the toxic effect of tramadol on the liver as tramadol-treated rats showed hydropic degeneration of hepatocytes, apoptotic nuclei and occasional binucleation. There were also congestion of central veins, proliferated bile ducts, cellular infiltration, haemorrhage and fibrosis of blood vessels. These changes coincided with Loughrey et al. [30] who reported that daily administration of tramadol at a dose of (40 mg/kg body weight) to adult male rats for one month was accompanied by hepatic congestion, hemorrhage and necrosis. Also, El-Wessemy [31] reported loss of architecture, congested central veins, expanded portal area with edema and inflammatory reaction in rats treated with tramadol.

For explaining the previous findings, Muhtaseb et al. [32] and Appiah et al. [33] stated that hepatotoxic chemicals cause liver damage through lipid peroxidation and other oxidative damages. In agreement, Abdel-Zaher et al. [34] found that repeated administration of tramadol for several consecutive days to mice resulted in development of oxidative stress in tissues.

Our biochemical results supported the previous theory as assessment of oxidative stress markers revealed a significant increase in the MDA level and a decrease in the GPx level in tramadol-treated group when compared to the control. It was reported that MDA is widely accepted as a sensitive biomarker of lipid peroxidation. It is considered a useful measure of oxidative stress status [35]. Also, GPx enzymes were found to contain a transition metal as a cofactor. The interaction of tramadol with metals of these enzymes may explain the observed inhibition in the activities of these enzymes [36].

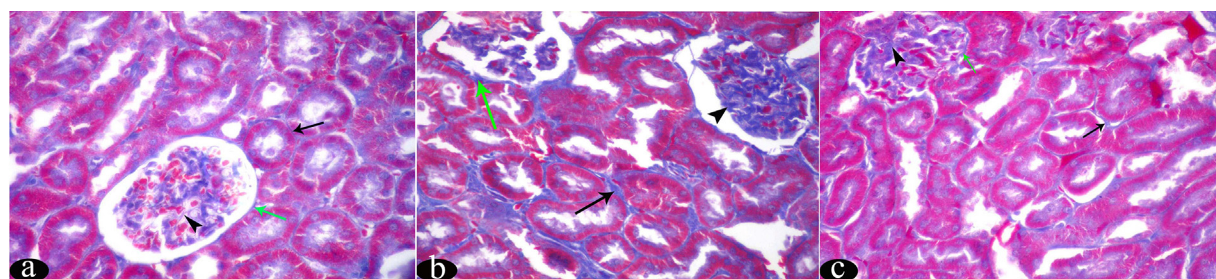

Fig. 4. (a) The negative control group showing few collagen fibers in the glomerular basement membrane (arrow head), mesangium (arrow) and Bowman's capsule (green arrow). (b) Tramadol-treated group showing increased collagen fibers around the glomerular basement membrane (arrow head), mesangium (arrow) and Bowman's space (green arrow). (c) Tramadol + *NsL* oil-treated group showing few collagen fibers in the glomerular basement membrane (arrow head), and nearly normal collagen distribution in both mesangium (arrow) and Bowman's capsule (green arrow) [Masson's trichrome 400]×.

Nehru and Anand [37] postulated that elevation in hepatic (ALT, AST, LDH) indices could be a secondary event following tramadol-induced lipid peroxidation of hepatocyte with the subsequent increase in the leakage of these biomarkers from the liver. Lipid peroxidation of cell membranes leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability, all of which lead to leakage of the enzymes from the liver cells.

In our study, impairment of the renal functions in tramadol-treated rats was indicated by a significant increase in serum urea and creatinine, as compared to the control group. Similar results were obtained by Atici et al. [28] and El-Gaafarawi [38].

Noori and Mahboobe [39] suggested that renal insufficiency associated with tramadol may be due to the decreased glomerular filtration rate or secondary to the increase in reactive oxygen species. On the contrary, Nagaoka et al. [40] reported that tramadol did not change renal blood flow (RBF) despite its increased serum norepinephrine (NE) levels and subsequently increased mean arterial blood pressure (MAP). This was attributed to that the maximal serum NE concentration that tramadol induced was 360 pg/mL, a concentration considered too small to reduce RBF (although it did transiently increase MAP).

Also, a histopathological evidence of renal damage was observed after administration of tramadol 30 days in the form of glomeruli with collapsed tufts and wide Bowman's space, atrophic tubules, sloughing of tubular cells, cellular infiltration, and hemorrhage. These results were in agreement with Nehru and Anand [37] who reported that reactive oxygen species generation and lipid peroxidation are responsible for tramadol-induced nephrotoxicity. The previous theory coincides with our results which revealed an increase in MDA level and a decrease GPx level in kidneys of rats receiving tramadol.

Abdel-Zaher et al. [34] reported that administration of tramadol to normal mice resulted in depletion of GPx and subsequent potentiation of lipid peroxidation in kidney cortical slices resulting in inhibition of protein synthesis, and mitochondrial damage.

Moreover, the results of the current work revealed increased collagen fibers in Masson's trichrome-stained sections of the liver and kidneys of tramadol-treated rats. Surazynski et al. [41] stated that collagen is not only a structural component of extracellular matrix, but it has also been recognized as a ligand for integrin receptors, which play an important role in signaling that regulate ion transport, lipid metabolism, kinase activation and gene expression. Therefore, changes in the quantity, structure and distribution of collagens in tissues may affect cell signaling, metabolism and function. Altindag et al. [42] suggested that the increased collagen fibers occur due to decreased collagen metabolism that may be related with oxidative stress.

In the current study, concomitant use of *NsL* oil along with tramadol produced partial improvement in the hepato- and nephrotoxic effects together with the antioxidant effect evidenced by significant reduction of MDA content and elevation of GPx levels. According to Padhye et al. [13], *NsL* oil can protect against hepatotoxicity by regulating the actions of the ultrastructures of liver cells, and normalizing the values of hepatic enzymes.

Also, the protective effect of *NsL* oil could be attributed to the ability to antagonize the enhanced lipid peroxidation, and in turn stabilize the integrity of the cellular membranes leading to prevention or at least decreasing the leakage of liver enzymes [43,44].

Saleem et al. [45] and Yaman and Balikci [46] reported nephroprotective effect of *NsL* oil against gentamycin-induced nephrotoxicity in rabbits where *NsL* oil lowered the values of serum creatinine and blood urea nitrogen, decreased the tubular necrosis score and prevented the degenerative changes in kidney tissues.

In conclusion, our results indicated that the mixture of corn oil and NsL oil alleviated the toxic effects of tramadol on the liver and kidney including the histopathological and biochemical changes as well as the oxidative damage. The protective effect of *NsL* oil could be due to its antioxidant potential by scavenging the free radicals. So, patients using tramadol for long times should be checked regularly for their liver and kidney functions. Our study revealed that administration of *NsL* oil during tramadol treatment would be beneficial. Medicinal preparations that combine tramadol and *NsL* oil might decrease the toxic tramadol effects. Other studies on dose-dependent effects of *NSL* oil are also recommended.

Conflict of interest

None.

Transparency document

The Transparency document associated with this article can be found in the online version.

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