

EFFECTS OF CAMEL'S MILK SUPPLEMENTATION ON ADULT MALE ALBINO RATS SUBJECTED TO TRAMADOL-INDUCED NEPHROTOXICITY

By

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ABSTRACT

Background: Tramadol is a centrally acting analgesic that is commonly prescribed for moderate and severe pain. Camel's milk is different from other ruminant milk as it is low in cholesterol, sugar and protein but high in minerals and vitamins. It also contains a high concentration of insulin and immunoglobulins.

Objective: This study aimed to demonstrate the biochemical, molecular and histological, changes induced by tramadol on the rat's kidney, and evaluate the potential role of camel's milk in the attenuation of these changes.

Materials and Methods: Forty adult male albino rats were equally divided into 4 groups: Control group, tramadol hydrochloride-treated group, tramadol hydrochloride/camel's milk group, and recovery group received tramadol hydrochloride only for 4 weeks and not subjected to any others procedure for 2 weeks. By the end of the experimental period, blood samples were collected to measure serum creatinine, urea and uric acid. Both kidneys of each rat were dissected out carefully. The right kidney was used for measurement of malondialdehyde (MDA), glutathione peroxidase (GPx) and gene expression of B-cell lymphoma 2 (Bcl-2), and Bcl-2-associated X (Bax) proteins. The left kidney was preserved for histological examination. The histological study of different experimental groups was done using hematoxylin and eosin (H & E), Masson trichrome and periodic acid Schiff (PAS) stain followed by morphometric and statistical studies. Also, electron microscopic study was done.

Results: Administration of tramadol induced significant increase in plasma concentrations of urea, creatinine and uric acid as well as significant increase in MDA, as well as a significant decrease in GPx concentration. Also, the gene expression of pro-apoptotic Bax protein was significantly increased after tramadol administration. On the other hand, the gene expression of anti-apoptotic protein Bcl-2 decreased significantly compared to control group. Moreover, examination of the renal cortex of tramadol-treated group demonstrated atrophied glomerulus with collapsed tuft, wide Bowman's space, degenerated tubules, cellular infiltration and hemorrhage. Furthermore, the collagen fibers increased as well as the basement membrane thickness of the renal corpuscles. In contrast, both renal structure and function were preserved in rats treated concomitantly with both tramadol and camel milk. However, the kidney function and structure was improved to some extent at the end of recovery period of group 4.

Conclusion: Camel's milk improves tramadol-induced changes in the renal function and structure of the rat's kidney.

Key words: Tramadol, camel's milk, renal histology, kidney function, Bax, Bcl-2, gene expression and oxidative stress.

INTRODUCTION

Tramadol is a centrally acting synthetic analgesic agent, used for the treatment of

moderate to severe pain (**Vazzana et al., 2015**). It is a highly effective analgesic with low addictiveness and limited side

effects. Therefore, it has wide ranging clinical applications including the treatment of post-operative pain, cancer and musculoskeletal pain (**Nossaman et al., 2010**). Tramadol also has a specific role in the treatment of opiate's withdrawal (**Threlkeld et al., 2006**) and premature ejaculation (**Kirby et al., 2015**).

The most frequent adverse effects of tramadol include constipation, nausea, dizziness, headache, somnolence and vomiting. However, serious side effects of tramadol have been reported especially with large doses and long-term usage including central nervous system depression, seizures, coma, respiratory depression and cardiovascular collapse (**Chandrasekaran et al., 2007**). Moreover, a certain degree of tolerance and withdrawal symptoms to the drug after chronic ingestion may occur. This psychic and physical dependence increases tramadol abuse among teens in most countries (**Barbera et al., 2013**). Also, very few fatalities have been reported (**McKeon et al., 2011**).

Tramadol is metabolized in the liver by the cytochrome P450 into O-desmethyl-tramadol, which itself is an active substance and 2 to 4 times more potent than tramadol (**Lassen et al., 2015**). Tramadol and its metabolites are excreted via the kidneys with a mean elimination half-life of about 5 hours. This elimination is prolonged to about 6-9 hours in the elderly and in patients with renal or hepatic impairment. Consequently, the kidney is considered to be the primary target organ for tramadol toxicity (**Khodeary et al., 2010**).

Camel's milk is considered to have medicinal properties since ancient times (**Yagil et al., 1984**). It is different from other ruminant milks in having low contents of cholesterol, sugar and proteins as well as high contents of minerals (sodium, potassium, iron, copper, zinc and magnesium), vitamins (C, B₂, A and E), insulin and immunoglobulins (**Korhonen and Pihlanto 2001**). Furthermore, camel's milk can be stored at room temperature for longer period than milk from other animals (**Omer and Eltinay 2009**). It has no allergic properties and it can be consumed by lactase deficient persons or those with weak immune systems (**Al-Hashem, 2009**).

Camel's milk has been deeply studied for its special properties because of higher hepato-protective, insulin-like and anti-bacterial activities (**Khan and Alzohairy, 2011**). In addition, several studies reported the antitoxic effects of camel's milk against cadmium chloride (**Al-Hashem et al. 2009 and Dallak, 2009**), carbon tetrachloride (CCl₄) (**Khan and Alzohairy 2011**), cisplatin (**Afifi, 2010**) and paracetamol (**Al-Fartosi et al. 2011**).

The present study was performed to evaluate the possible biochemical, molecular and histological changes induced in kidney tissue and function due to long-term administration of tramadol. Moreover, to investigate the therapeutic effects of camel's milk on those changes and finally to compare the therapeutic effects of camel's milk with the spontaneous reversibility of the tramadol's toxic effects that may occur during the period of recovery.

MATERIALS AND METHODS

Tramadol hydrochloride: Tramadol hydrochloride (TH) tablets (each tablet contains 225 mg of tramadol hydrochloride) were obtained from October Pharma Co. (Giza, Egypt). The tablets were grounded and dissolved in normal saline just before administration.

Camel's milk: Fresh untreated camel's milk samples were collected daily from El-Hamed camel's milk farm (Giza, Egypt). Milk was collected from camels by hand milking and kept in cool sterile screw bottles until transported to the laboratory.

Animals and experimental design: Forty adult male albino rats of local strain, weighing 140–160 gm, were used in this study. The animals were housed in plastic cages (35 x 30 x 35 per 5 rats) in the animal house in the Faculty of Medicine for Girls, Al-Azhar University. They were kept at room temperature (~25°C) under a day/night rhythm with free access to food and water. After seven days of acclimatization the rats were randomized into 4 equal groups as follows:

- **Group 1 (Control group):** Each rat received 1ml normal saline (0.9%) orally for 4 weeks.
- **Group 2 (Tramadol group):** Each rat received 100 mg/kg/day of tramadol hydrochloride in a volume of 1 ml normal saline orally through gastric tube for 4 weeks. The dose was calculated according to **Paget and Barnes (1964)**.
- **Group 3 (Tramadol/Camel's milk):** Each rat received 100 mg/kg/day of tramadol hydrochloride together with 20 ml/rat/day of camel's milk by oral

gavage for 4 weeks (**Afifi, 2010 and Salwa & Lina, 2010**).

- **Group 4 (Recovery group):** The rats in this group received tramadol hydrochloride for 4 weeks. They were not subjected to any procedure for 2 weeks, then killed to study the effects of recovery from tramadol toxicity.

Sample collection and biochemical assays: The animals were anesthetized at the end of the experiments and blood samples were obtained from the orbital sinus of overnight fasted rats. Blood was immediately centrifuged at 3000 rpm for 20 minutes. Sera were separated and stored at -80°C.

Both kidneys of each rat were dissected out carefully. The right kidney was divided into two parts; one part was wrapped with aluminum foil and kept frozen at -80°C (for measurement of MDA content) and GPx level, and the other part was kept in liquid nitrogen (for determination of Bax and Bcl-2 gene expression). The left kidney was preserved for histopathological examination.

- **Determination of kidney functions:** Serum creatinine, blood urea nitrogen (BUN) and serum uric acid were determined enzymatically using commercially available kits (Bioclin, Santa Coloma, Spain).
- **Measurement of MDA (Wills, 1987).**
- **Measurement of GPx (Ellman, 1959).**
- **Detection of Bax and Bcl-2 gene expression with real time-polymerase chain reaction (RT-PCR):**
- 1. **Ribonucleic acid (RNA) extraction:** Total RNA was extracted from frozen

tissue samples using the RNeasy Mini Kit (Qiagen Inc) following the manufacturer's protocol. The extracted RNA was confirmed in agarose gel electrophoresis stained with ethidium bromide and visualized by ultra violet transilluminator (Figure 1).

2. **Real-time quantitative PCR:** Real-time RT-PCR for quantitative assessment of mRNA expression was performed on step one plus (Applied Biosystems, USA). The level of expression of each target gene was normalized relative to the expression of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA in that sample (Pfaffl, 2001).

Histological study:

1. **For the light microscopic examination:** Each kidney was fixed in 10% formalin solution for 48-55h, dehydrated in graded alcohol series, embedded in paraffin wax then thin sections of 5µm thickness were obtained. Sections were stained with H&E for routine histological examination. Others were stained with Masson trichrome stain to reveal collagen, PAS stain for basement membrane and morphometric analysis (Bancroft and Gamble, 2002).
2. **For the electron microscopic study:** The specimens were immediately fixed in 5% glutaraldehyde in 0.1bM sodium cacodylate buffer, at 0-4°C and pH 7.3 for 4-24 hours (Robinson *et al.*, 1987). The specimens were washed for 1.5 hour with 3 changes of the same buffer. Then fixed in 1% Osmium tetroxide in the same

cacodylate buffer for 2 hours. Ultra-thin sections were then cut and examined under a Jeol 100s (Japan) transmission electron microscope (William and Carter, 1996).

Quantitative morphometric analysis:

The area percentage of collagen fibers was measured in Masson trichrome stained sections. The measurements were obtained using computer-based image analysis soft-ware (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 5 non-overlapping fields for each group × 400 magnification.

Statistical analysis: Data were expressed as means ± standard deviation (SD). Statistical comparison between different groups were done using one way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test to judge the difference between various groups. All calculations were performed using the SPSS 16.0 software package. Significance was accepted at $P < 0.05$.

RESULTS

Biochemical findings:

The tramadol group showed signs of nephrotoxicity as manifested by a significant increase in plasma concentrations of urea, creatinine, uric acid and MDA, as well as a significant decrease in GPx concentration compared to control group.

Treatment with camel's milk resulted in improvement in kidney function as manifested by a significant decrease in plasma concentrations of urea, creatinine, uric acid and MDA, together with a significant increase in GPx concentration

compared to tramadol group. However, the level of GPx was still significantly lower than control group. Also, levels of urea, creatinine and MDA were still significantly higher than control group while uric acid was returned to normal level.

After the recovery period, the parameters of kidney function improved as manifested by a significant decrease in plasma concentrations of urea, creatinine, uric acid and MDA, together with a significant increase in GPx concentration. However, urea, uric acid and MDA were still significantly higher than tramadol/camel's milk group and control group. No significant changes were noticed between recovery group and tramadol/camel's milk group regarding to creatinine level.

Gene expression of apoptotic factors in kidney tissue:

The expression of Bax gene significantly increased after tramadol administration. However, it significantly decreased in tramadol/camel's milk and recovery groups compared to tramadol group but still significantly higher than control group. The reduction in expression of Bax gene was more obvious in camel's milk group. On the other hand, the gene expression of Bcl-2 decreased significantly in tramadol group, and showed significant increase in both tramadol/camel's milk and recovery groups compared to control group with no significant difference between both groups (Table 1 and Figure 1).

Table (1): Plasma levels of urea, creatinine, uric acid, MDA and GPx as well as Bax and Bcl-2 gene expression in various groups at the end of the treatment period (Mean \pm SD).

Parameters \ Groups	Control	Tramadol	Tramadol/ camel's milk	Recovery
Urea (mg/dl)	36.82 \pm 7.55	99.38 \pm 1.43 ^a	53.96 \pm 1.15 ^{ab}	81.66 \pm 1.22 ^{abc}
Creatinine (mg/dl)	1.02 \pm 0.12	5.13 \pm 2.30 ^a	2.65 \pm 1.28 ^{ab}	3.34 \pm 1.26 ^{ab}
Uric acid (mg/dl)	3.81 \pm 0.99	7.80 \pm 2.35 ^a	4.65 \pm 0.80 ^b	6.27 \pm 1.75 ^{abc}
MDA (nmol/mg protein)	1.23 \pm 0.23	12.64 \pm 1.34 ^a	5.14 \pm 1.10 ^{ab}	9.56 \pm 0.98 ^{abc}
GPx (nmol/mg protein)	57.62 \pm 3.77	20.58 \pm 3.16 ^a	46.08 \pm 4.53 ^{ab}	37.72 \pm 3.43 ^{abc}
Bax (relative expression)	1.06 \pm 0.13	11.12 \pm 2.99 ^a	4.01 \pm 1.46 ^{ab}	6.89 \pm 1.40 ^{abc}
Bcl-2 (relative expression)	1.04 \pm 0.10	0.28 \pm 0.15 ^a	0.64 \pm 0.27 ^{ab}	0.55 \pm 0.14 ^{ab}

(a) Significant values versus control group.

(b) Significant values versus tramadol group.

(c) Significant values versus tramadol/camel's milk group.

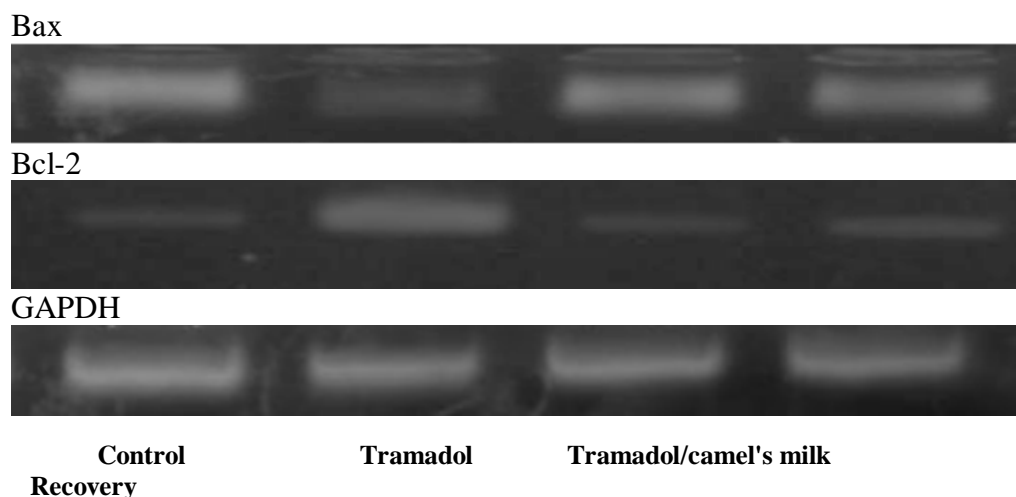


Figure (1): Bromide-stained gel electrophoresis of Bax and Bcl-2 gene expression in different groups relative to the expression of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene.

Histological results:

H&E:

In H&E stained sections, the control group showed renal corpuscles with glomeruli, surrounded by visceral and partial layers of Bowman's capsules which were separated by Bowman's spaces. Proximal and distal convoluted tubules were separated by peritubular capillaries (**figure 2**). Tramadol treated group showed shrunken renal corpuscles with collapsed tufts and wide Bowman's spaces. There were vacuolations, degenerations and sloughings of the tubular cells. The tubular nuclei showed shrinkages with chromatin condensations, fragmentations and loss of nuclei in some cells (**figure 3a**). Mononuclear cellular infiltration (**figure 3b**) and hemorrhage were also noticed in some areas (**Figure 3c**). Co-administration of camel's milk along with tramadol revealed partial improvement in the structure of the kidney nearly similar to control group. However,

there were slightly wide Bowman's spaces, some vacuolated tubular cells and mild congestion of peritubular capillaries (**Figure 4**). The recovery group showed nearly the same results of tramadol/camel's milk group (**Figure 5**).

Using Masson's trichrome stain, the control group showed normal distribution of collagen fibers among the glomerular capillaries, around the renal corpuscles and tubules (**Figure 6a**). The collagen fibers increased in the tramadol group (**Figure 6b**). Co-administration of tramadol and camel's milk showed decreased amount of collagen fibers in kidney sections of rats of this group when compared with that of tramadol-treated rats (**Figure 6c**). Collagen fibers also decreased after stopping treatment with tramadol for two weeks (**Figure 6d**).

In periodic acid Schiff (PAS) Staining, the control rats revealed a normal positive PAS reaction in the renal corpuscles and in the basement membrane of the tubules (**Fig. 7**). In tramadol- treated group, there

was a strong positive PAS reaction in the basement membrane surrounding renal corpuscles and glomerular capillaries together with focal loss of basement membrane around some tubules (**Fig.8**). In tramadol-camel milk treated group, a positive PAS reaction was observed along the brush border and basement membrane of proximal tubules and basement membrane of distal tubules (**Fig.9**). In rats, which stopped treatment with tramadol for two weeks, a positive PAS reaction was observed in the regenerated brush border of the proximal tubular cells, the basement membrane of both proximal and distal tubules and the basement membranes surrounding the glomerular capillaries (**Fig.10**).

Electron microscopic results:

Normal ultrastructure of renal corpuscles and renal tubules were seen in control groups (**Fig.11 a, b & c**). In tramadol-treated rats, the renal corpuscles revealed widening of the capsular spaces, dilation and congestion of the glomerular capillaries with fusion of the secondary foot processes of the podocytes in certain areas with apparent thickening of basement membranes together with loss of trilaminar appearance in some areas (**Figure 12a**). The proximal tubular cells revealed reduction of cell sizes, increased deepened and dilated basal infoldings, and many lysosomes with decreased apical microvilli. In addition, small sized pyknotic nuclei with irregular nuclear envelopes, margination of heterochromatins, and reduction of mitochondrial

number and size were seen in cells as well (**Figure 12b**).

In tramadol/ camel milk-treated rats, regular distribution of the secondary foot processes on normal capillary basement membrane with narrow capsular spaces were noticed in the renal corpuscles nearly similar to control group (**Figure 13a**). The proximal tubular cells had rounded nuclei with prominent nucleoli. The cell cytoplasm contained many elongated mitochondria and few vacuoles and lysosomes. The luminal surface of the cells had long densely packed microvilli (**Figure 13**).

In recovery group, some podocytes returned to normal structures with normal basement membrane thickening while others were still showed fused foot processes and thickened basement membrane (**Figure 14a**). The proximal tubular cells revealed rounded euchromatic nuclei with electron dense nucleoli, numerous elongated mitochondriae, few lysosomes, many pinocytotic vesicles and long microvilli (**Figure 14b**).

Morphometric results:

There was a significant increase in area % of collagen fibers in sections of kidney of tramadol group when compared to the control group. A significant decrease in area % of collagen fibers was found in the kidney of tramadol / camel's milk group in comparison to tramadol group. Also, a significant decrease in area % of collagen fibers was found in the kidney of recovery group compared to tramadol/camel's milk (**Table 2**).

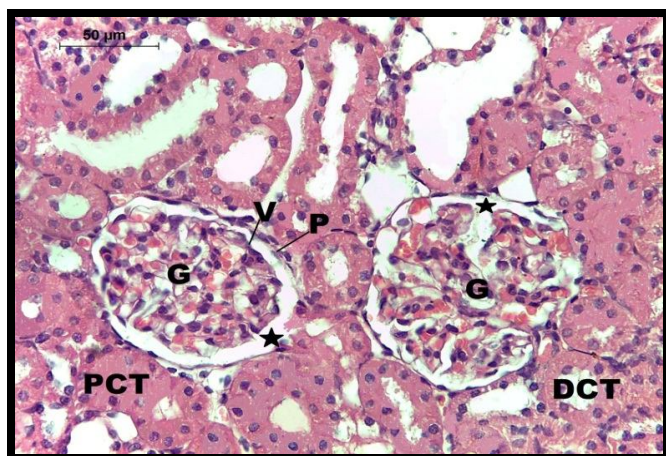


Figure (2): Section of the renal cortex from the control group (G1) showing a renal corpuscles consisting of glomerulus (G) surrounded by visceral (V) and parietal (P) layers of Bowman's capsules which were separated by Bowman's space (☆), proximal (PCT) and distal convoluted tubules (DCT) were seen (H&E x400).

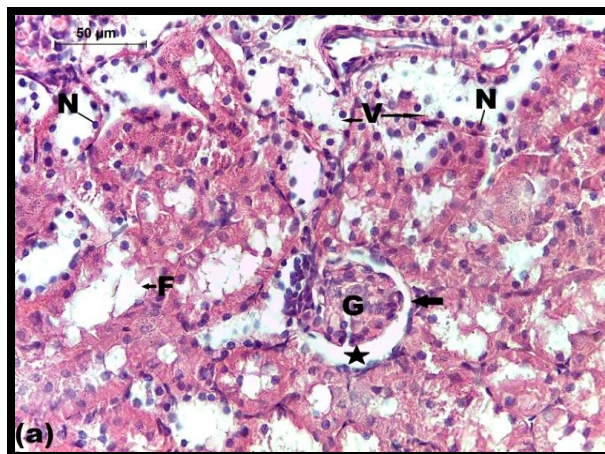


Figure (3a): Section of the renal cortex from tramadol-treated group showing shrunken glomeruli (G) with marked widening of Bowman's space (asterisk). Many tubular cells appeared with darkly stained nuclei (N), while others had vacuolated degenerated sloughed cells (V), with fragmentation and loss of nuclei (F) (H&E x400).

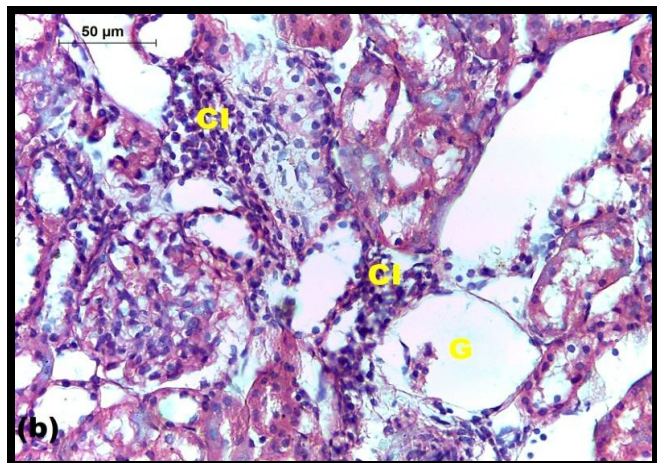


Figure (3b): Section of the renal cortex from tramadol-treated group showing cellular infiltration (CI) and degenerated glomerulus (G) (H&E x400).

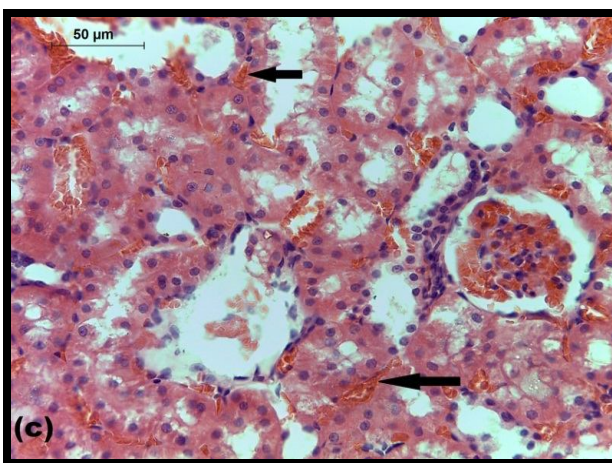


Figure (3c): Section of the renal cortex from tramadol-treated group showing congested dilated peritubular blood vessels (arrows) within interstitial hemorrhage (H&E x400).

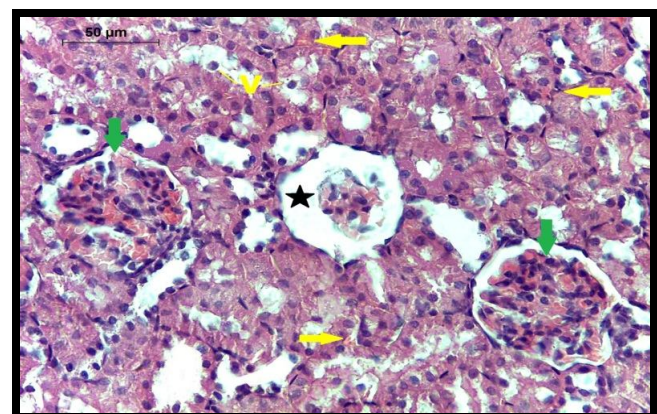


Figure (4): Section of the renal cortex from tramadol and camel's milk-treated group, showing some normal renal corpuscles (green arrows), while others had wide Bowman's space (☆), some vacuolated tubular cells (V) and mild congestion of peritubular capillaries (arrows) (H&E x400).

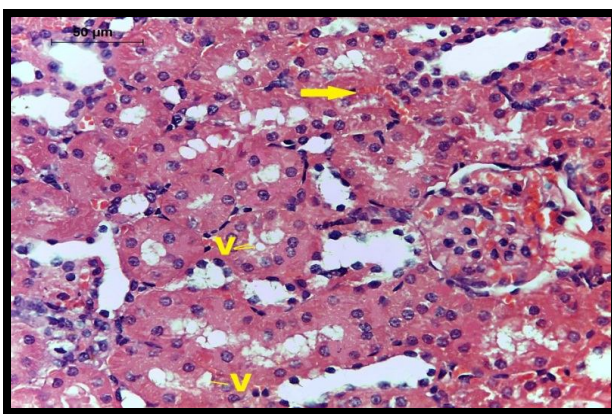


Figure (5): Section of the renal cortex from recovery group showing PCT and DCT tubules with normal structure, and some tubules appeared with vacuoles (V) and congestion (arrow) (H&E x400).

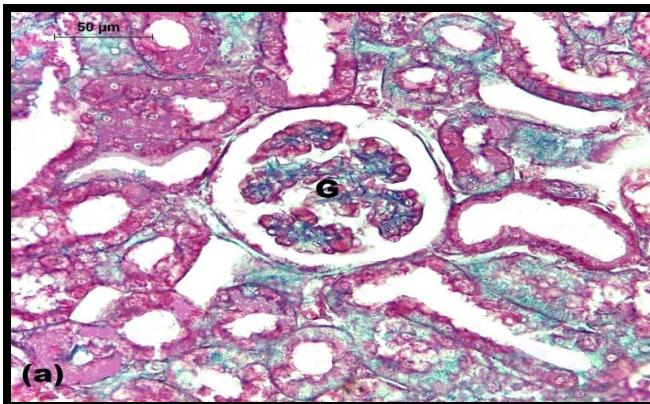


Figure (6a): Section of the renal cortex from the control group showing a renal corpuscles showing normal distribution of collagen fibers among the glomerular capillaries (G) and around the renal corpuscles and tubules (Masson's trichrome x400)

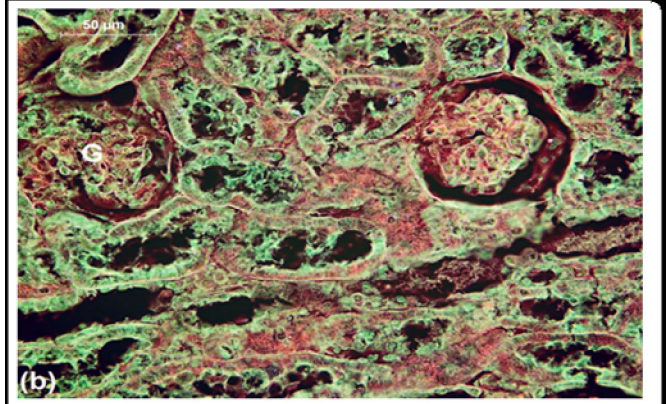


Figure (6b): Section of the renal cortex from tramadol treated group showing increase amount of collagen fibers in the interstitium around the renal corpuscles and among the glomerular capillaries (G) (Masson's trichrome x400).

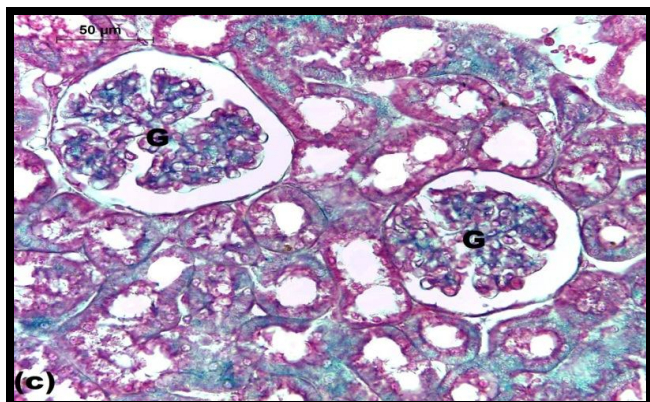


Figure (6c): Section of the renal cortex from tramadol and camel's milk-treated group showing mild distribution of collagen fibers among the glomerular capillaries (G) and around the renal corpuscles and tubules (Masson's trichrome x 400).

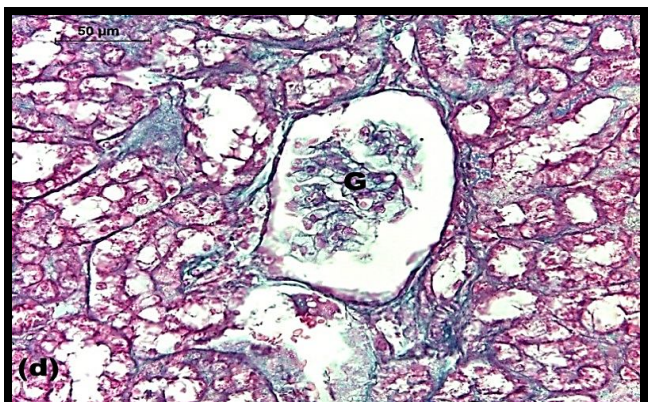


Figure (6d): Section of the renal cortex from recovery group showing mild distribution of collagen fibers among the glomerular capillaries (G) and around the renal corpuscles and tubules (Masson's trichrome x400).

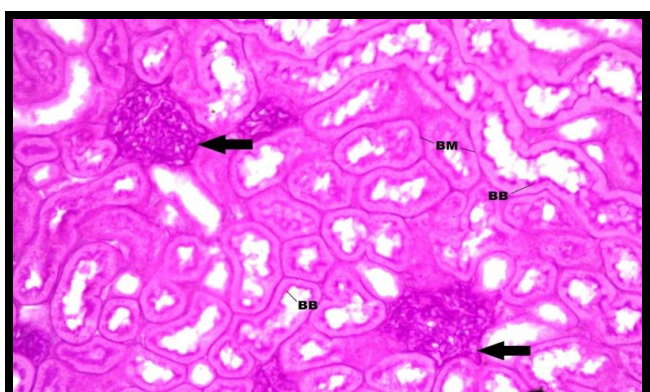


Figure (7): A kidney section in the control group revealed normal positive PAS reaction in glomerular capillaries (arrows) in the basement membrane of the tubules (BM) and in the apical brush border (BB) (PAS x 400).

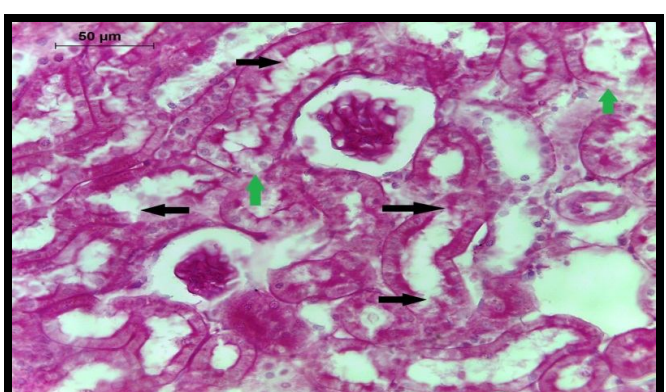


Figure (8): A kidney section in tramadol-treated group showed a decrease in the PAS positive reaction at the apical brush borders (arrows) and the basement membranes (green arrows) (PAS x400).

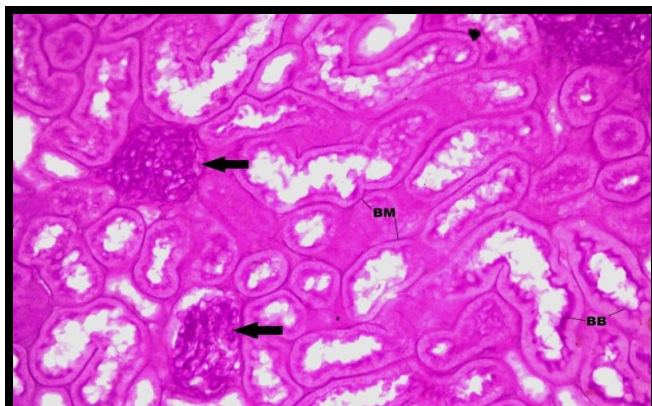


Figure (9): a kidney section in combined tramadol and Camel milk-treated group showing positive PAS reaction along the brush borders (BB), in the basement membranes (BM) tubules and basement membrane surrounding the glomerular capillaries (arrows) (PAS x 400).

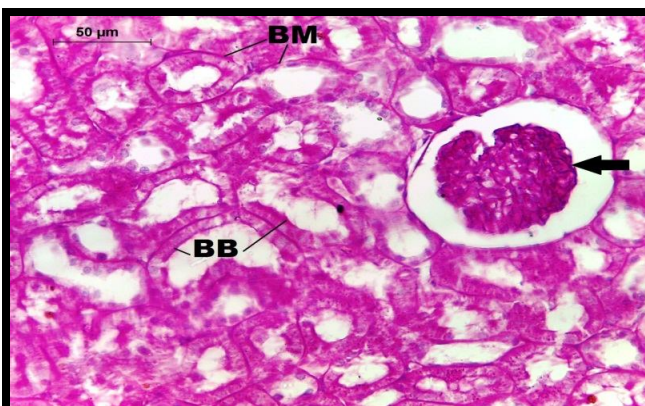


Figure (10): A kidney section in recovery group showing a mild decrease of the positive reaction in the basement membranes (BM), brush borders (BB) of almost tubules and in the basement membrane surrounding glomerular capillaries (arrows) (PAS x400).

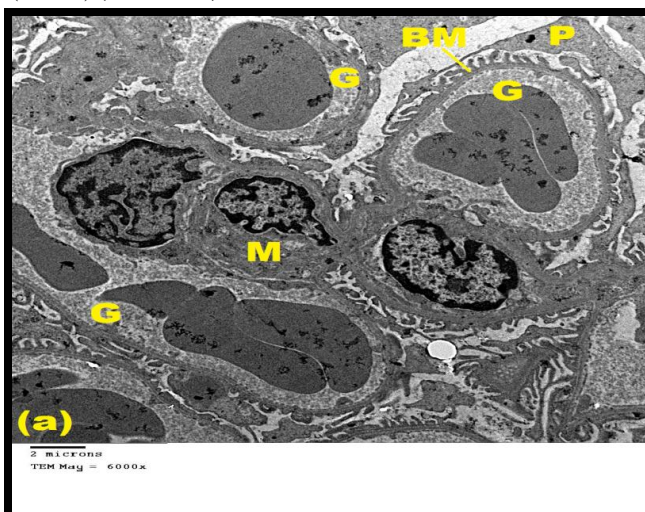


Figure (11a): An electron micrograph of the kidney of control group showed multiple glomerular capillaries (G), podocytes with its processes (P), and mesangial cells (M). The basement membranes (BM) appeared trilaminar with filtration slit in-between the podocyte foot processes (x 6000).

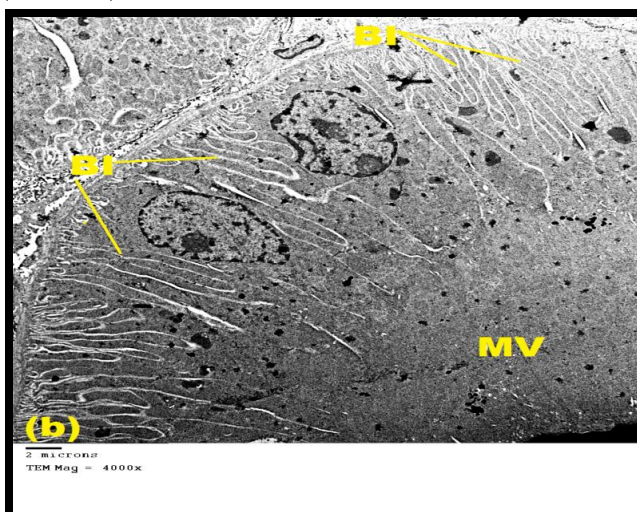


Figure (11b): An electron micrograph of the kidney of control group showing a part of the proximal convoluted tubules with their characteristic microvilli (MV) and basal infoldings (BI) (x 4000).

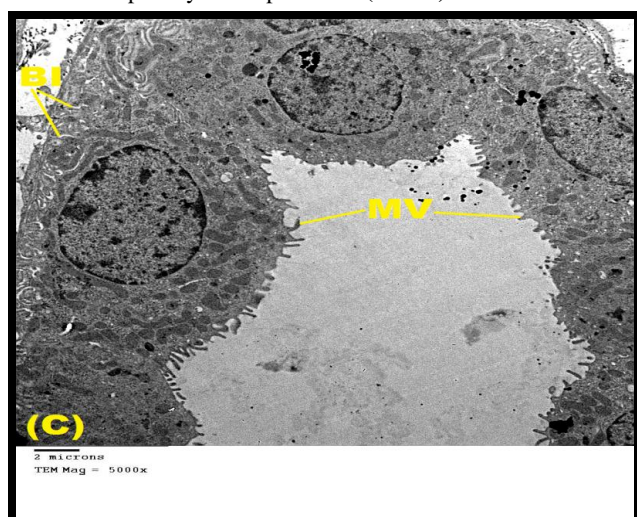


Figure (11c): An electron micrograph of the kidney of control group showing a part of the distal convoluted tubule with short microvilli (MV) and basal infoldings (BI) (x 5000).

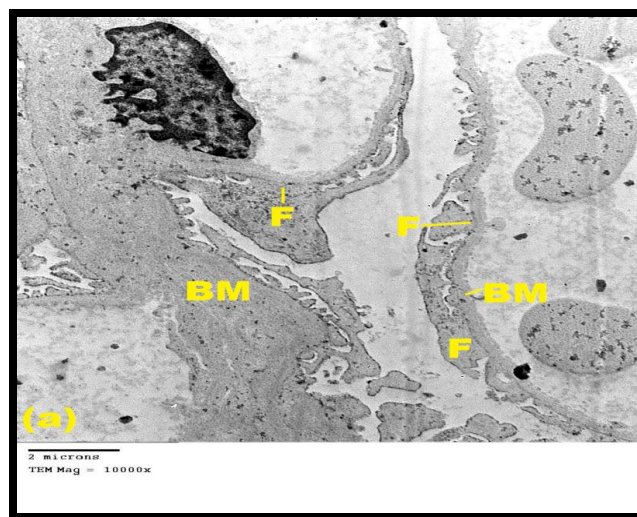


Figure (12a): An electron micrograph of the kidney of tramadol-treated rats showing fusion of the secondary foot processes of the podocytes in certain areas (F) with apparent thickening of basement membrane (BM) together with loss of trilaminar appearance in some areas (x 10000).

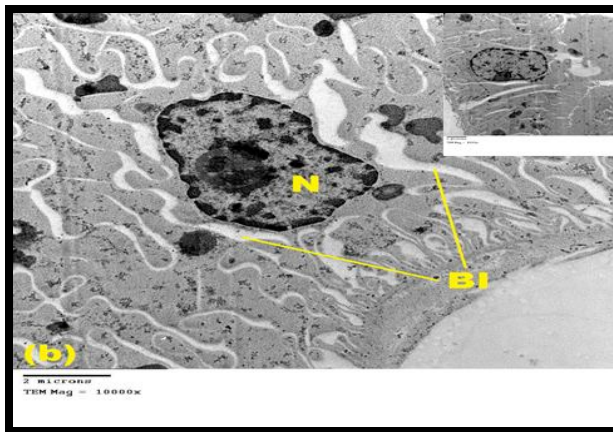


Figure 12b: The proximal tubular cells of the kidney of tramadol-treated rats revealed reduction of cell size, increased deepened and dilated basal infoldings (BI), and many lysosomes with decreased apical microvilli (inset). Small sized pyknotic nucleus with irregular nuclear envelope, margination of heterochromatin (N) is seen as well (x 10000).

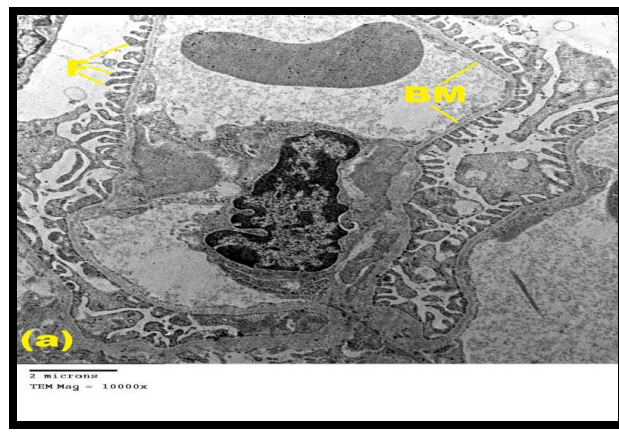


Figure (13a): An electron micrograph in combined tramadol and camel milk-treated rats revealed regular distribution of podocyte secondary foot processes (F) with narrow spaces in-between on normal trilaminar basement membrane (BM) were noticed in the renal corpuscle nearly similar to control group (x10000).

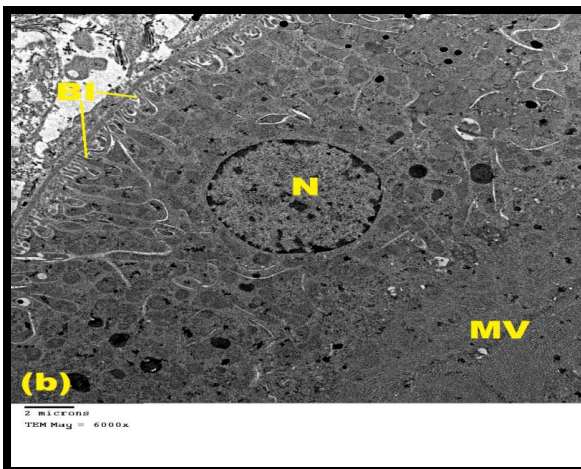


Figure (13b): The proximal tubular cells in combined tramadol and camel milk-treated rats had rounded nuclei with prominent nucleoli (N). The cell cytoplasm contained many elongated mitochondria and a few vacuoles and lysosomes. The luminal surface of the cells had long densely packed microvilli (MV), and the basal surface has nearly normal basal infoldings (BI) (x 6000).

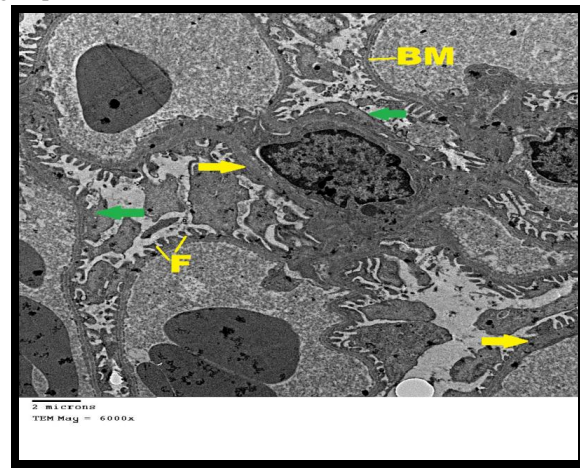


Figure (14a): In recovery group, some podocytes returned to normal structures (F) with normal basement membrane thickening and trilaminar appearance (BM) while others showed fused foot processes (green arrows) and thickened basement membrane (yellow arrows) (x 6000).

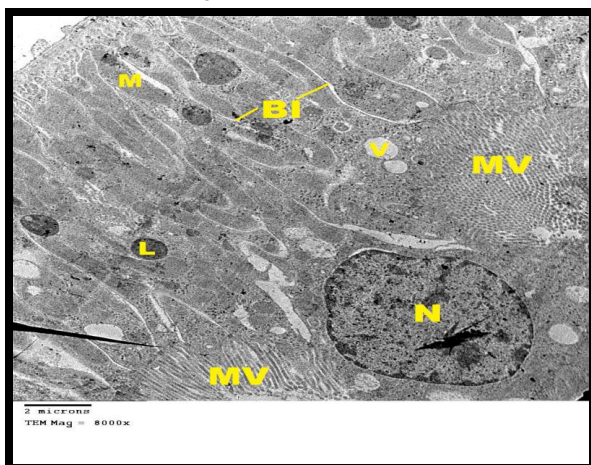


Figure (14b): In recovery group, the proximal tubular cells revealed rounded euchromatic nucleus (N) with electron dense nucleolus, numerous elongated mitochondria (M), few lysosomes (L), many pinocytotic vesicles (V), long microvilli (MV) and normal basal infoldings (BI) were seen (x 8000).

Table (2): Collagen area % in all experimental groups (Mean \pm SD).

Groups	Mean \pm SD
Control group	6.34 \pm 1.91
Tramadol group	28.57 ^a \pm 7.79
Tramadol/camel's milk group	6.44 ^b \pm 2.62
Recovery group	14.72 ^{abc} \pm 4.5

(a) Significant values versus control group.

(b) Significant values versus tramadol group.

(c) Significant values versus tramadol/camel's milk group.

DISCUSSION

Tramadol has a wide range of applications in treatment of severe, acute or chronic pain (Nossaman *et al.*, 2010). However, its toxic effects should be kept in mind especially in large doses and long-term using (Abdel-Zaher *et al.*, 2011 and Buhari *et al.*, 2012).

The biochemical assay of kidney function demonstrates that administration of tramadol induced significant increase in plasma concentrations of urea, creatinine and uric acid compared to control group. These results were in accordance with Ali *et al.* (2015) who reported that rabbits treated with tramadol for 15 days showed increased serum urea and creatinine levels. Moreover, the results of Elmanama *et al.* (2015) showed an increase in urea, uric acid and creatinine levels in samples obtained from the-more-than-5-years tramadol abuser indicating negative impacts of tramadol on kidney function. Others also reported an increase in urea and creatinine levels in rats with long- term tramadol receiving (Elyazji *et al.*, 2013 and Rukhshanda *et al.*, 2014).

This work also showed significant increase of MDA, as well as a significant decrease in GPx concentration in rats treated with tramadol compared to control group. Toxic effects of tramadol at the cellular level could be induced by augmenting lipid peroxidation due to increased reactive oxygen species (ROS) and also by inhibiting antioxidant enzymes activities (Elkhateeb *et al.*, 2015). In addition, these data were confirmed by previous studies which demonstrated that treatment of rats with morphine and tramadol yielded an increased MDA level, which suggests an

increased lipid peroxidation. They also observed a decrease in the level of reduced glutathione in renal tissue (Nehru & Anand, 2005 and Noori & Mahboobe, 2010).

Moreover, the oxidative stress induced by tramadol in the kidney and liver was reported by Awadalla and Salah-Eldin (2015). They found that administration of tramadol for 20 consecutive days caused a significant reduction in the activities of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), with a significant increase in the level of MDA in tramadol- treated rats compared to control groups in both liver and kidney. 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 oxidative modification of proteins and single- or double- strand DNA breaks (Ghoneim *et al.*, 2014).

The present results showed that the gene expression of pro-apoptotic Bax protein significantly increased after tramadol administration. On the other hand, the gene expression of anti-apoptotic protein Bcl-2 decreased significantly in tramadol-treated rats. Bax and Bcl-2 are intracellular proteins involved in apoptosis signaling, suggesting that tramadol induced damage in a manner of activation of apoptotic cell death pathway in renal tissues (Khodeary *et al.*, 2010).

The pathways responsible for adult tissue homeostasis are governed significantly but not exclusively by Bcl-2–family proteins (Hata *et al.*, 2015). Several Bcl-2–family proteins, both anti-apoptotic and pro-apoptotic, have C-

terminal transmembrane domains that insert in the outer membrane of mitochondria. Pro-apoptotic proteins, such as Bax, induce mitochondrial outer membrane permeabilization (MOMP), allowing the influx of reactive oxygen species into cells, causing lipid peroxidation and membrane damage, which impair normal ion-homeostasis, causing cellular swelling and plasma membrane rupture, as well as rupture of lysosomes and release of hydrolytic enzymes that destroy proteins, nucleic acids, and lipids (**Anilkumar and Prehn, 2015**). Bax enhances the necrotic actions of mitochondria, as MOMP, also releases several proteins that contribute to non-apoptotic cell death including DNase, endonuclease G, and apoptosis-inducing factor; a flavoprotein reported to enter the nucleus and promote genome destruction (**Barclay et al., 2015**).

On the other hand, the anti-apoptotic proteins such as Bcl-2 serve as guardians of the outer membrane and preserve its integrity by opposing Bax. Bcl-2 suppresses apoptosis, necrosis, and autophagy (**Reed, 2008**). Bcl-2 suppresses autophagy by binding the protein Bcln (**Pattinre et al., 2005**) an essential component of the mammalian autophagy system that marks autophagic vesicles for fusion with lysosomes for digestion and recycling of components. The anti-autophagic function of Bcl-2 occurs in the endoplasmic reticulum, where a considerable proportion of anti-apoptotic Bcl-2 and related proteins often resides (**Xu et al., 2005**).

In the current study, the light microscopic examination revealed that tramadol-treated rats showed manifesta-

tions of renal damage in the form of atrophied renal corpuscles with collapsed tuft and widened Bowman's spaces. In the tubules there was vacuolation, degeneration and sloughing of proximal tubular cells. The nuclei showed apoptotic changes as pyknosis, fragmentation and loss of nuclei in some cells. Mononuclear cellular infiltration and hemorrhage were also noticed in some areas. These results were in agreement with **Elkhateeb et al. (2015)** who reported that rats treated with tramadol for 30 days showed renal damage in the form of glomeruli with collapsed tufts and wide Bowman's space, atrophic tubules, sloughing of tubular cells, cellular infiltration, and hemorrhage. **Ezzeldin et al. (2014)** also confirmed that, in tramadol-treated rats, the main histological findings in the kidney samples were vacuolization and swelling of endothelial cells and associated with degeneration in cells lining the tubule and congestion in the tuft of glomeruli at the cortex. Focal degeneration with cystic dilation and renal cast formation in some tubules of the cortico-medullary portion were also observed. The tubular affection, which was observed by the present histological study, could be another explanation for the increased serum urea and creatinine since there is a part of body creatinine and other body toxins excreted via the convoluted tubules (**Marieb, 2006**).

Moreover, examination of Masson trichrome stained sections revealed increased collagen fibers in tramadol-treated group. These results were in agreement with **Elkhateeb et al. (2015)** and **Altindag et al. (2007)** who suggested that the increased collagen fibers in Masson's trichrome-stained sections of

kidneys in tramadol-treated rats occurs due to decreased collagen metabolism that may be related with oxidative stress. Moreover, **Surazynski et al. (2008)** 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.

In addition, the ultrastructural changes of the kidney obtained by administration of tramadol were widening of the capsular space, dilation and congestion of the glomerular capillaries with fusion of the secondary foot processes of the podocytes in certain areas, and apparent thickening of basement membranes together with loss of its trilaminar appearance. Also, the proximal tubular cells revealed reduction of cell size, deepened and dilated basal infoldings, many lysosomes with decreased apical microvilli, small sized pyknotic nuclei with irregular nuclear envelope, margination of heterochromatin, and reduction of mitochondrial number and size. Similar changes were noticed by many researchers on administration of other nephrotoxic drugs as cisplatin (**Nasr, 2013 and Crăciun & Pașca, 2014**)

In the present work, treatment of rats with camel's milk resulted in improvement in kidney functions as manifested by a significant decrease in plasma concentrations of urea, creatinine, uric acid and MDA, together with a significant increase in GPx concentration compared to tramadol group. Moreover, the expression of Bax gene significantly decreased in camel's milk group compared to tramadol group, but still

significantly higher than control group, whereas the gene expression of Bcl-2 showed significant increase.

In the present study, administration of camel's milk improved the histological and ultrastructural changes obtained by administration of tramadol. These results were in agreement with the results of **Wang et al. (2014)** who revealed that camel's milk can reduce kidney injury and suppress cell apoptosis of type 2 diabetes mellitus rats.

The protective effect of camel milk against tramadol-induced nephrotoxicity could be attributed to its antioxidant properties (**Althnaian et al., 2013**). Camel's milk contains high concentrations of vitamins A, B₂, C and E, trace elements (magnesium, sodium, potassium, iron, zinc and selenium), insulin and immunoglobulins (**Al-Hashem, 2009; Khan & Alzohairy, 2011; Al-Fartosi et al., 2012 and Korish & Arafah, 2013**). These vitamins act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury (**Yousef, 2004**). Magnesium protects the cell against free radical damage and assists in the absorption and metabolism of B vitamins, vitamin C and E (**Martin et al., 2003**). It has been noted that zinc has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system (**Ozdemir & Inanc, 2005 and Santos et al., 2008**). Zinc is an essential component of the oxidant defense system and may be a cofactor in the activity of superoxide dismutase (**Cabrera et al., 2003**). Selenium is a cofactor of glutathione peroxidase in the elimination of peroxide radicals. Selenium also seems to prevent

cancer development (**Cabrera et al., 2003; Hassan et al., 2006 and Salwa and Lina, 2010**). Camel's milk contains high levels of lactoferrin. Lactoferrin is an iron-binding glycoprotein of the transferrin family (**Al-Majali et al., 2007**). This protein has a number of properties such as antibactericidal activity, antiviral, antifungal, anticarcinogenic, antiinflammatory activity and antioxidant properties (**Konuspayeva et al., 2004 and Hasson et al., 2015**).

The study of **Soliman et al. (2015)** evaluated the effects of camel's milk on gene expression of Bax and Bcl-2 proteins that are involved in the regulation of programmed cell death. However, they investigated the antioxidant and antiapoptotic effects of camel's milk against *E. coli* and *S. aureus* hepatic pathogenicity. They reported that administration of camel's milk increases the expression levels of glutathione-S-transferase and superoxide dismutase genes, and decreases the expression of interleukin-6 and apoptosis-associated genes in the liver of the infected rats. Very few studies demonstrated that camel's milk triggers apoptosis in human hepatoma HepG2 and breast cancer MCF7 cell lines (**Korashy et al., 2012 and Hasson et al., 2015**). However, further studies are needed to clarify the underling mechanism.

After the recovery period the kidney structure and function improved as manifested by a significant decrease in plasma concentrations of urea, creatinine, uric acid and MDA, together with a significant increase in GPx concentration. However, urea, uric acid and MDA were still significantly higher than tramadol/

camel's milk and control groups. Moreover, the expression of Bax gene was significantly increased after tramadol administration. However, it significantly decreased in tramadol/camel's milk and recovery groups compared to tramadol group, but, still significantly higher than control group. The reduction in expression of Bax gene was more obvious in tramadol/camel's milk group. On the other hand, the gene expression of Bcl-2 decreased significantly in tramadol group and showed significant increase in both camel's milk and recovery groups with no significant difference between both groups.

In agreement with this study, oxidative stress induced by tramadol on different organs by induction of inflammatory reaction that is effectively reduced after withdrawal period was confirmed by **Rabei (2011)**. Others reported that apoptotic index increased in testicular tissue of rats under tramadol administration than the control group, and decreased in rats under withdrawal (**Ghoneim et al., 2014**). These results coincided with a similar study dealing with Methadone and Buprenorphine opioids which are similar in action to tramadol by (**Heidari et al., 2012**).

In conclusion, the present study illustrated the toxic effects of tramadol on renal tissues, through induction of oxidative stress and through alteration of Bax and Bcl-2 apoptotic pathway. Therefore, the toxic effects of tramadol should be kept in mind, even when prescribed in pain management. Moreover, it can be concluded that camel's milk has a renoprotective potential against tramadol-induced renal

dysfunction through its antioxidant effects and the antiapoptotic mechanisms. However, the improvement of kidney functions and structures were less apparent in the recovery group.

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آثار تناول حليب الإبل على ذكور الجرذان البيضاء البالغين المعرضة للتسمم الكلوي الناجم عن الترامادول

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خلفية البحث: ترامادول هو مسكن للألم يؤثر على الجهاز العصبى المركزى ويعتاد وصفه فى حالات الألم المتوسطة والحادة ويختلف لبن الإبل عن الألبان الأخرى المجتررة فى أنه أقل فى الكوليستيرول والسكر والبروتين، لكنه أعلى فى الأملاح والفيتامينات، ويحتوى أيضا على تركيز عالى من الإنسولين والأجسام المضادة.

الهدف من البحث: توضيح التغيرات الكيميائية والجزيئية والنسجية الناجمة عن عقار الترامادول على كلى الجرذان وتقييم الدور المحتمل للبن الإبل فى تقليل هذه التغيرات.

مواد و طرق البحث: تم تقسيم 40 من ذكور الجرذان البيضاء إلى أربع مجموعات متساوية : الأولى ضابطة، والثانية أعطيت عقار الترامادول هيدروكلورايد، والثالثة أعطيت عقار الترامادول هيدروكلورايد وعولجت بلبن الإبل، والرابعة مجموعة النقاهاة والتي تم إعطائها عقار الترامادول هيدروكلورايد لمدة أربع أسابيع فقط ولم تتعرض لأى تدخل آخر لمدة أسبوعين. فى نهاية مدة التجربة تم جمع عينات الدم لقياس نسبة اليوريا والكرياتينين وحمض اليوريك فى مصل الدم. وقد تم استخراج كلا الكلتيين لكل فأر تم بعناية. واستخدمت الكلية اليمنى لقياس نسبة مالوندايالدهيد والجلوتاثيون بيروكسيديز والتعبير الجينى للباكس وللبي سى ال-2. أما الكلية اليسرى فقد تم الاحتفاظ بها للفحص النسيجى. وقد تم الفحص النسيجى لمجموعات التجربة المختلفة باستخدام صبغة الهيماتوكسلين والإيوسين وصبغة الماسون تراكروم وصبغة البيريديك اسيد شيف، وتم إتباعها بدراسات مورفومترية وإحصائية. وقد تم أيضا عمل فحص نسيجى بواسطة الميكروسكوب الالكترونى.

نتائج البحث: نتج عن إعطاء الترامادول إرتفاعا ذو دلالة إحصائية فى مستوى اليوريا والكرياتينين وحمض اليوريك فى مصل الدم وأيضا فى مالوندايالدهيد، بينما إنخفضت نسبة الجلوتاثيون بيروكسيديز إنخفاضا ذو دلالة إحصائية. وقد ارتفعت نسبة التعبير الجينى للعامل المحفز لموت الخلايا المبرمج (باكس) إرتفاعا ذو دلالة إحصائية بعد اعطاء الترامادول. وعلى الجانب الآخر، فقد انخفضت نسبة التعبير الجينى للعامل المثبط لموت الخلايا المبرمج (البي سى ال-2) إنخفاضا ذو دلالة إحصائية مقارنة بالمجموعة الضابطة. كما أوضح الفحص النسيجى للقشرة الكلوية فى المجموعة التى تم إعطاؤها الترامادول ضموراً فى الكبيبة الكلوية وخصلات الأوعية الدموية بداخلها واتساع فى كبسولة بومان وتحلل فى القنيات الكلوية وتراكم للخلايا المناعية ونزيف. علاوة على ذلك، فقد زادت الاليف الكولاجينية وزاد سمك الغشاء القاعدى فى الجسيمات الخلوية. وعلى النقيض، فقد كانت الوظائف الكلوية و النسيج الكلوى سليمة فى الجرذان المعطاة عقار الترامادول والمعالجة فى نفس الوقت بلبن الإبل، بينما تحسن الوظيفة و التركيب الكلوى إلى حد ما فى نهاية فترة النقاهاة للمجموعة الرابعة.

الاستنتاج: لبن الإبل يحسن التغيرات فى وظائف الكلى والتركيب الكلوى فى الجرذان الناجمة عن إعطاء الترامادول.