

Pharmacological Evaluation of *Moringa Oleifera* in Mitigating Tramadol-Induced Nephropathy in Mice Models

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ABSTRACT

Tramadol, a broadly in recent years, is an effective analgesic agent for the treatment of moderate to acute pain. Its metabolites are excreted by the kidney which may cause nephrotoxicity. *Moringa oleifera* leaves are commonly used to provide herbal and plant-derived medicinal products especially in developing nations. The present study was carried out to determine the biochemical and histopathological changes in the kidney of tramadol-treated albino mice and to evaluate the possible protective role of *Moringa oleifera* leaves against tramadol-induced nephrotoxicity. Twenty adult albino mice were divided into four groups. Control group (group i) received daily intraperitoneal injection of normal saline only, group ii received oral dose of *Moringa oleifera* leaves extract (20 mg/kg/bw) for three weeks, group iii received daily intraperitoneal dose of tramadol (0.3 mg/kg/bw) for the same period, group iv, received daily oral dose of *Moringa oleifera* leaves extract, (20 mg/kg/bw) three hours before injecting intraperitoneal dose of tramadol (0.3 mg/kg/bw), for the same period. Blood samples were withdrawn at the end of the experiment for kidney function tests and specimens from the kidney were processed for histological study. No significant differences in the mean values of the kidney function tests were noticed between *Moringa oleifera* group and control group. However, there was highly significant increase in the mean values of serum, urea and creatinine in tramadol-treated group as compared to the control group. Although tramadol + *Moringa oleifera* group revealed significant difference in the mean values of urea and creatinine when compared with tramadol-treated group. So, *Moringa oleifera* leaves extract have been shown to attenuate the renal dysfunction, improve the renal architecture, with nearly normalization of serum urea and creatinine levels which indicate improvement of renal function. In conclusion, in the light of biochemical results and histological findings, co-administration of *Moringa oleifera* leaves lessened the negative effects of tramadol-induced nephrotoxicity; possibly by its antioxidant action. Further investigation of these promising protective effects of *Moringa oleifera* leaves against tramadol-induced renal injury may have considerable impact on developing an adjunct therapy aiming to improve the therapeutic index of some nephrotoxic drugs.

Keywords: Nephrotoxicity- Kidney- *Moringa oleifera* leaves- Histopathology.

INTRODUCTION

Tramadol is a narcotic-like analgesic, a synthetic opiate, commonly prescribed for moderate to severe pain^{1,2}. It is converted in the liver to O-desmethyl-tramadol, which itself is an active substance and 2 to 4 times more potent than tramadol^{3,4}. The mechanism of its analgesic action is complex. Most reports suggest that the analgesic activity and other clinical effects of tramadol are a result of opioid and non-opioid mechanisms. Tramadol binds to the μ opioid receptor, although much more weakly than morphine. It also inhibits the neuronal reuptake of norepinephrine and serotonin as do the antidepressant drugs such as amitriptyline and desimpramine⁵⁻⁷. Recently has been demonstrated that the tramadol binds to the μ opioid receptor, although much more weakly than morphine. It also inhibits the neuronal reuptake of norepinephrine and serotonin, which explains the reasons for the relief of pain⁶. Tramadol is metabolized in the liver by the cytochrome P-450 and then excreted through the kidneys. The central role of kidney is excretion of **Author for Correspondence: a.albrakati@tu.edu.sa* tramadol, which increases the risks of cellular damage and then the kidney dysfunction. Almost certainly, metabolites of the drugs that are excreted from kidneys may cause nephrotoxicity leading to a kidney dysfunction and may

have a higher activity and/or a greater toxicity than the original drug^{8,9}. The increases of creatinine, and urea parameter were observed in response to tramadol inoculation in adult male albino rats¹⁰. Various studies to find out new effective medicines, without side effects, for treating liver and other organs diseases are still on-going. Natural remedies, mainly from traditional plants are found to be both effective and safe alternatives for the treatment of hepatotoxicity and nephrotoxicity. Extracts from plant sources have also been investigated for hepato-protective, nephroprotective and antioxidant effects against organs damage^{11,12}. *Moringa oleifera* leaves is one of the herbal plants with a wide range of medicinal applicability¹³. *Moringa* is an important tropical crop that is used as human food, medicine¹⁴. The leaves are a source of protein, β -carotene, vitamins (A, B, C, E, riboflavin), nicotinic acid, folic acid, pyridoxine, amino acids, minerals, various phenolic^{15,16}, with a known powerful antioxidant property¹⁷. But, the anti-toxic nature of *Moringa oleifera* leaves against tramadol induced liver and kidney injury in mice has not yet been demonstrated. All parts of this plant are applied in traditional medicine for the treatment of human diseases, whereby the leaves are rich in protein, carotenoids, ascorbic acid and iron¹⁸. The biological activities that is hepatoprotective¹⁹, hypocholesterolemia²⁰, antifungal²¹, antioxidant¹⁸, and anti-tumor²² have been

documented. Its leaves are also used as nutritional supplement and growth promoters due to the significant presence of protein, Se, P, Ca, β -carotene and α -tocopherol^{18,23}. The protective effects of *Moringa oleifera* leaves in kidney against gentamicin-induced nephrotoxicity in experimental animals have been shown clearly the protective *Moringa oleifera* leaves against the nephrotoxicity²⁴.

Aim of the work

Recently, an increasingly dangerous effects of tramadol abuse has been massively demonstrated in a worldwide. Therefore, the aim of the current study was designed to examine the biochemical and histopathological effects of tramadol on the kidney and the possible beneficial effect of *Moringa oleifera* leaves extract on tramadol-induced renal functional and structural abnormalities via biochemical and histopathological analysis.

MATERIALS AND METHODS

Experimental animals

Twenty adult albino mice (*Mus musculus*) approximately 4-5 months old and each weight from 40-60 gm were obtained from the animal house of faculty of medicine, King Abdel-Aziz University, Jeddah were used in the present investigation. Animals were housed in specially designed cages and were kept in the laboratory under the same standard conditions for at least one week for acclimatization before experimentation. The animals were provided with free access to pellets and allowed to drink tap water ad libitum throughout the duration of the experiment. *Dose and treatment*

Only the therapeutic dose of tramadol was used in the present study, according to the manufacture. The therapeutic dose of tramadol was estimated to be 40 mg/kg/day. Tramadol were diluted with saline (0.9% Na Cl) to obtain the concentration used. Experimental animals received a single daily intraperitoneal injection of tramadol (40 mg/kg/day) for one week, two weeks and three weeks. *Plant materials*

Samples of *Moringa oleifera* leaves were obtained from Egyptian Society of *Moringa*– National Research Center.

Preparation of extract

The leaves of the plant were cleaned thoroughly. They were then dried in room temperature & crushed into coarse powder. About 20 g of powder was taken and soaked separately in 100 ml of water and chloroform by keeping it in a Shaker for 3 days. It was filtered through cheese cloth and reduced to 10% of its original volume (organic solvent). Then, using a rotary evaporator, the filtrate was concentrated in vacuum, while aqueous extract was dried using water bath²⁵.

The extract was dissolved in normal saline and adjusted according the required dose and administered with intragastrical tube according to the animal's body weight.

Animal groups

Animals were divided into four (4) groups, with five animals in each group, as follows:

Group i- Five mice used as control group, received intraperitoneal injecting of normal saline only for three weeks.

Group ii- Five mice, received daily oral dose of *Moringa oleifera* leaves extract, (20 mg/kg/bw) for three weeks. Group iii- Five mice, received daily intraperitoneal dose of tramadol (0.3 mg/kg/bw) for three weeks. Group iv- Five mice, received daily oral dose of *Moringa oleifera* leaves extract, (20 mg/kg/bw) three hours before injecting intraperitoneal dose of tramadol (0.3 mg/kg/bw), for three weeks. *Biochemical studies*

At the end of the experiment, animals were fasten for 12 hours. then anaesthetized with ether and blood samples (2ml) were withdrawn by cardiac puncture²⁶.

Statistical analysis

The data were expressed as means \pm SD from 5 animals per group. The differences between the groups were compared for statistical significance by using the student 't' test. $p < 0.05$ was taken as significant.

Histopathological preparation

The kidneys of each rat were quickly dissected out carefully, and cut to small pieces and then fixed in 10% neutral buffered formalin fixative fluid. Following fixation, specimens were dehydrated, embedded then sectioned with thickness of 5 microns and then mounted on the clean slides without using any adhesives medium. For histopathological examination sections were stained with Ehrlich Hematoxylin and Eosin²⁷. The cytoplasm appeared pink and nuclei acquire a blue color.

RESULTS

Biochemical results

Statistical comparisons regard kidney function tests between control groups and *Moringa oleifera*, tramadol, and tramadol + *Moringa oleifera* groups using ANOVA test were done (Table 1). No significant differences in the mean values of the kidney function tests were noticed between *Moringa oleifera* group and control group. However, there was a highly significant increase in the mean values of serum, urea and creatinine in tramadol-treated group as compared to the control group ($p < 0.001$). Although tramadol + *Moringa oleifera* group revealed significant difference in the mean values of urea and creatinine when compared with tramadol-treated group ($p < 0.001$).

Histopathological results

The histological examinations of the kidney of control group showed normal glomeruli, containing a tuft of glomerular capillaries surrounded by Bowman's capsule which are separated by narrow Bowman's space proximal and distal convoluted tubules were seen lined with cuboidal epithelium with eosinophilic cytoplasm and central rounded nuclei (Figure 1).

Histological sections of the kidney of mice treated with extract of *Moringa oleifera* leaves (20 mg/kg) for three weeks did not differ from the control group (normal histoarchitecture) (Figure 2).

While histological sections of treated mice with tramadol alone for three weeks showed atrophied glomerulus with collapsed tuft, wide Bowman's space, degenerated tubules with widening of its lumen and cellular infiltration. Hemorrhage and mononuclear cellular infiltration were also seen in the mesangium and convoluted tubules (Figures 3&4)

Co-administration of *Moringa oleifera* leaves extract 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 (Figure.5).

DISCUSSION

Tramadol is available worldwide as a synthetic centrally acting analgesic for treatment of moderate to severe, acuter chronic pain². Tramadol hydrochloride is one of the synthetic opiate; widely used opioid in recent years as an effective analgesic agent for the treatment of acute or chronic pain¹. It is metabolised in the liver and excreted by the kidneys, it may cause hepatotoxicity and nephrotoxicity during its metabolism. The central role of liver and kidney in detoxification and drug metabolism increases the risks of toxic injury⁴. So, the role of the liver and the kidneys in tramadol metabolism and excretion predisposes them to toxic injury²⁸. Tramadol and its metabolites are excreted via kidneys, consequently the kidney is considered the primary target organ for tramadol toxicity²⁹.

The results of our study revealed that administration of tramadol alone produced renal damage as evidenced from the elevated levels of serum urea and creatinine. Our results are in good agreement with those previously reported^{30,31}. As, laboratory evaluation of serum blood creatinine is considered "standard fare" in the determination of renal functions³². So, elevation of urea and creatinine level in these rum is taken as the index of nephrotoxicity³³.

Metabolites of tramadol may have a higher activity and/ or a greater toxicity than the original drug. These metabolites, excreted via kidneys, may also cause a cellular damage and, thus, a kidney dysfunction⁹. The liver and kidney are responsible for the tramadol metabolism and excretion and the high risk of hepatotoxicity and nephrotoxicity⁴. Thirty percent of the drug is excreted through the kidneys in an unchanged manner.

The result of the present work is in accordance with Aitic et al.,³⁴, who reported that the increase in BUN and creatinine levels in rats with long-term tramadol receiving; others studies reached similar results^{35,36}. So, the significant increase detected in our study in serum urea and creatinine, as compared to the control group is considered as a sign of impairment of the renal functions in tramadol-treated mice. Similar assumption was reached by ElGaafarawi³⁷. Our biochemical results supported the previous theory as assessment of oxidative stress markers revealed a significant increase in the renal malondialdehyde level and a decrease in the glutathione peroxidase level in tramadol-treated group when compared to the control³⁸.

The histopathological results in this study were confirmed the toxic effects of tramadol on the kidney after administration of tramadol for successive twenty-one days, in the form of degenerated renal tubules to obstructed lumen and atrophied glomerulus with collapsed tuft and wide Bowman’s space. Our results are in agreement with the findings of Atici, Sebnem *et al.*,³⁴, who observed that the renal tubular vacuolization, mononuclear cell infiltration, focal necrosis and hemorrhage as well as an increase in creatinine levels in rats receiving opioids. These observations can be considered as evidence of renal damage. Similar results were reported by Elkhateeb, *et al.*,¹⁰, who found atrophied glomerulus with collapsed tuft, wide Bowman’s space, degenerated tubules, cellular infiltration and hemorrhage in tramadol treated group¹⁰.

The histopathological evidence of renal damage that observed after administration of tramadol 3 weeks were in agreement with Nehru and Anand³⁹, who reported that the reactive oxygen species generation and lipid peroxidation are responsible for tramadol-induced nephrotoxicity. The previous theory coincides with the results of Elkhateeb *et al.*¹⁰, who revealed that the increase in malondialdehyde level and a decrease glutathione peroxidase level in kidneys of rats receiving tramadol.

Moringa oleifera leaves have gained popularity especially in recent times due to several publications reporting various nutritional and health benefits of the plant⁴⁰; though it is important to note that most investigations on this plant are basic and the reports would require proper trials to evaluate the exact benefits to human health. Paliwal, R. *et al.*⁴¹, who reported that the anti-nephrotoxic effect of *Moringa oleifera* leaves; Ezejindu, DN *et al.*⁴², who reported that the *Moringa oleifera* leaves extract would not produce any deleterious effects on the kidney of experimental animals even in cases of chronic administration. Awodele, *et al.*⁴³, reported that the *Moringa oleifera* leaves consumption to be relatively safe at sub-lethal doses especially with respect to its effects on the kidney and liver tissues Oyagbemi, *et al.*⁴⁴, however suggested that the chronic use could predispose animals to hepatic and kidney damage. The current finding, however, shows that at moderate doses, *Moringa oleifera* leaves extract ingestion is safe and has protective role for the renal tissues.

In the present work, co-administration of *Moringa oleifera* leaves extract 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.

Concomitant use of *Moringa oleifera* leaves extract along with tramadol in the current study, produced partial improvement in the nephrotoxic effects which is in

Table 1: Comparison of the mean values of total kidney function tests [serum urea & creatinine] of control, *Moringa*

oleifera, tramadol, and tramadol + *Moringa oleifera* groups using ANOVA test.

Parameters	<i>Moringa</i> oleifera group	Tramadol group	Tramadol + <i>Moringa</i> oleifera groups	P control group
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Urea (mg/dl)	41.85 ± 1.7	39.05 ± 0.95	73.56 ± 5.26 a	51.41 ± 8.57 b	0.001**
Creatinine (mg/dl)	0.87 ± 0.03 b	0.79 ± 0.02	1.73 ± 0.12 a	1.42 ± 0.13 a	0.001** a

Significant difference ($p < 0.05$) when compared with the control group. b Significant difference ($p < 0.05$) when compared with tramadol-treated group.

** Highly significant ($P < 0.001$).

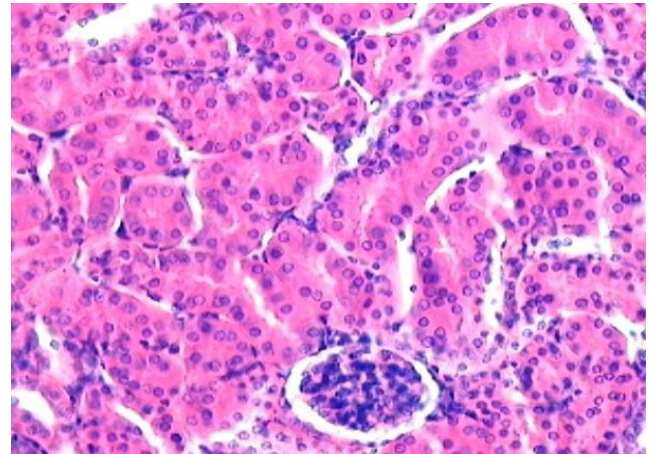
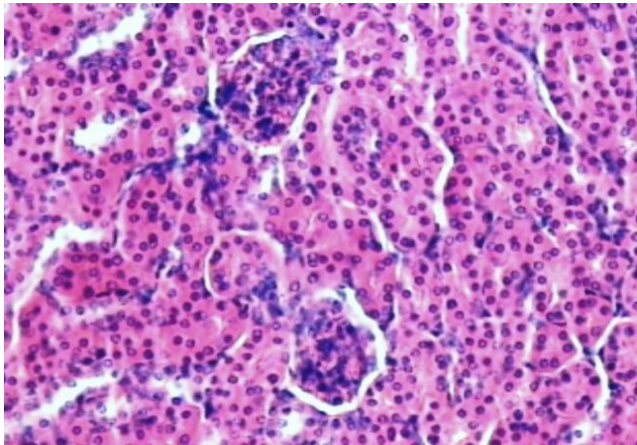


Figure 1: Photomicrograph of the cortex kidney of control (group i) showing a normal structure of glomerulus surrounded by visceral and parietal layers of Bowman's capsule, separated by narrow Bowman's space and normal architecture of proximal and distal tubules, (H&E Stain X 650). Figure 2: Photomicrograph of the cortex of the kidney of group (ii) receiving *Moringa oleifera* leaves extract for 3 weeks, showing nearly normal histological architecture, capsule separated by narrow Bowman's space and normal architecture of proximal and distal tubules, (H&E Stain X 650).

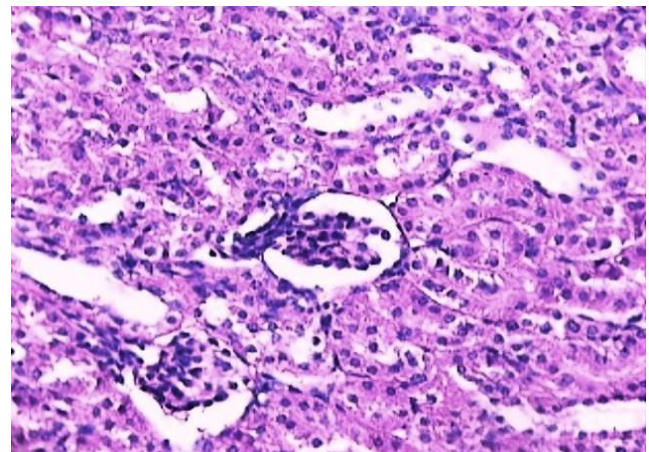
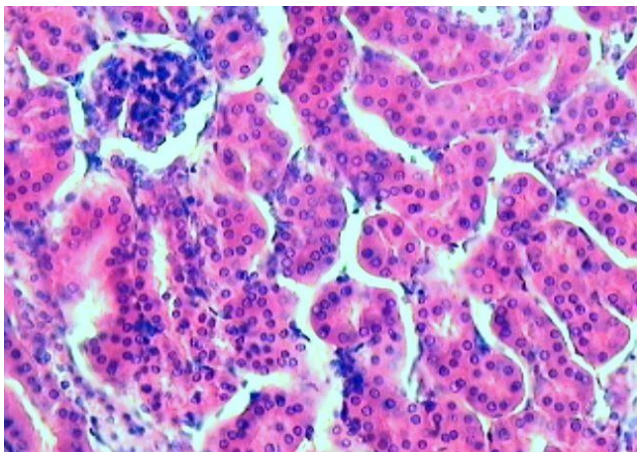


Figure 3: Photomicrograph of the cortex of the kidney of group (iii) receiving dose of tramadol (0.3 mg/kg/bw) for three weeks showing atrophied glomeruli with collapsed tuft and wide Bowman's space and degenerated tubules, cellular.

infiltration, and degenerated tubules. Hemorrhage and mononuclear cellular infiltration were also seen in the mesangium and convoluted tubules. (H&E Stain X 650).

Figure 4: Photomicrograph of the cortex of the kidney of group (iii) receiving dose of tramadol (0.3 mg/kg/bw) for accordance with Fakurazi, S. *et al.*⁴⁵, who assumed that the *Moringa oleifera* leaves extract has together with the antioxidant effect evidenced by significant reduction of malondialdehyde content levels.

Our results also showed that treatment with *Moringa oleifera* leaves extract reduced the levels of raised serum urea and creatinine and restored the renal function; suggesting that the contents of *Moringa oleifera* leaves not only protected the integrity of kidney but, at the same time,

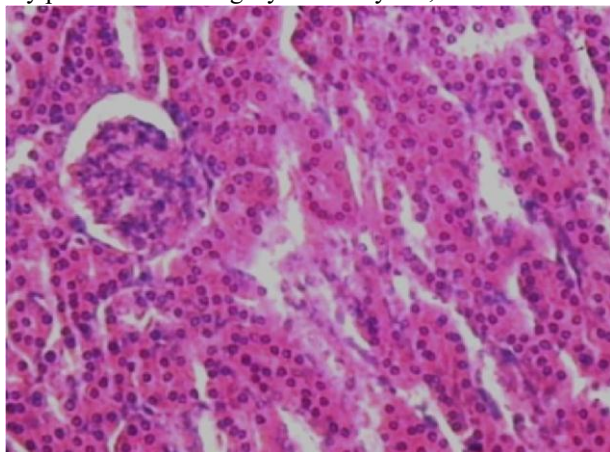


Figure 5: Photomicrograph of the cortex of the kidney of group (iv) receiving *Moringa oleifera* leaves extract (20 mg/kg/bw) three hours before injecting subcutaneous dose of tramadol (0.3 mg/kg/bw), showing partial improvement with structure of the kidney nearly similar to control group with slightly wide Bowman's space, some vacuolated tubular cells and mild congestion of peritubular capillaries, (H&E Stain X 650).

increased its regenerative and reparative capacity. Alterations or normalization in biochemical parameters were correlated with renal histological results. On histological examinations of the kidney of intoxicated mice groups which received *Moringa oleifera* leaves showed nearly complete recovery. The above changes can be considered as an expression of the functional improvement of renal tubules, which may be caused by an accelerated regeneration of tubular cells. So, treatment with *Moringa oleifera* significantly reversed the degenerated changes, hence it may be possible that the mechanism of nephroprotection by *Moringa oleifera* is due to its antioxidant effect. This suggestion was strengthened by recent findings that *Moringa oleifera* leaves possess potent antioxidant properties. These properties may be mediated through direct trapping of the free radicals and also through metal chelation^{46,47}. Also, the protective effect of *Moringa oleifera* leaves extract on the kidney observed in the present work could be explained by the results of the work of

three weeks showing the same changes of the previous micrograph, atrophied glomeruli with marked collapsed tuft and degenerated tubules with widening of its lumen, (H&E Stain X 650).

Fakurazi *et al.*,¹⁷ on rat liver who suggested that the plant extract have some roles in preserving structural integrity of cell membrane, they also suggested that protective effects afforded by *Moringa oleifera* against chemical induced hepatotoxicity and nephrotoxicity is due to its ability to induce phase II detoxification pathway via promoting reduced glutathione conjugation with toxic metabolites generated from CYP450 pathway¹⁷. The further research should investigate the mechanism of protective activities of *Moringa oleifera* leaves and the role of bioactive components of this plant responsible for this action. As assumed by Sokunbi, OA, *et al.*⁴⁸, who reported that the protective effect of *Moringa oleifera* extract, could be attributed to the ability to antagonize the enhanced lipid peroxidation, and in turn stabilize the integrity of the cellular membranes. In view of the above findings, it is suggested that the phytochemical constituents in *Moringa oleifera* could contribute to its antioxidant activity and, thus, nephroprotection⁴⁶.

In conclusion, our results indicated that the use of *Moringa oleifera* leaves extract alleviated the toxic effects of tramadol on the kidney including the histopathological and biochemical changes. The protective effect of *Moringa oleifera* leaves 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 kidney functions. Also, our study revealed that administration of *Moringa oleifera* leaves extract during tramadol treatment would be beneficial. Medicinal preparations that combine tramadol *Moringa oleifera* leaves extract might decrease the toxic tramadol effects.

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