The Role of Vitamin C on the Structural Changes of Male Albino Rats Kidney Induced by Tramadol

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ABSTRACT

Aim of study: The aim of this work is to detect the toxic effects of tramadol on the microscopic structure of the kidneys of adult male albino rats, and the possible protective role of vitamin C against these toxic effects.

Method: Thirty adult albino rats are used in this study. The animals were divided into three equal groups: Group A (control group): Animals were injected daily with normal saline for 4 weeks. Group B (Tramadol treated group) (TGI): Animals were injected daily with tramadol in a dose of 50mg/kg b.wt for 4 weeks. Group C (group treated with tramadol+vitamin C) (TGII): Animals were injected with vitamin C in a dose 100mg/Kg/b.wt half hour prior to tramadol injection in the same dose as in group B for 4 weeks. The injection was given intraperitoneally for all animals. At the end of the experiments, the rats were anaesthetized by ether, then killed. After that kidneys removed, fixed in formaldehyde and processed for microscopic examination.

Result: Tramadol causes shrinkage of renal glomeruli with widening of bowman's space. Renal tubules showed hydropic degeneration and vacuolization of their epithelial cells. Infiltration of mononuclear cells along with Hyaline cast is seen within the degenerated tubules. Giving vitamin C prior to tramadol injection results in improvement in the microscopic structure of kidney mainly in glomeruli and tubules.

Conclusion: Using tramadol caused damage to renal glomeruli and tubules in renal cortex. The addition of vitamin C partially improved the histological structure of kidney.

Keywords: Tramadol, Rats kidney, vitamin C.
INTRODUCTION

Adiction is a growing public and health problem widespread in spite of all hard work to avoid and switch it; painkillers are amid the most common medications which abused. Among the opioids that frequently prescribed include morphine, tramadol, methadone, diamorphine, and codeine.

Tramadol is a opioid pain reliever that acting centrally which is mostly in use for combating pain ranging from moderate to severe. It is analgesic effect has a complex mechanism; as many reports suggested that opioid and non-opioid mechanisms of action is responsible for it's painkilling effects and other medical properties.

The absorption of tramadol is 95-100% happen in small intestine namely in its upper part with bioavailability is 70% and when tramadol used in frequent doses, it is bioavailability amplified reaching to 100%.

The metabolized of tramadol done in the liver via cytochrome p450 enzyme system and its bio transformed products are expelled by the kidneys by urine accordingly; this creates the kidney is a main goal organ of tramadol toxicity particularly in misuse and over dose cases. About 30% of the dose is expelled in the urine as unaffected drug, and 60% of the dosage is eliminated as metabolites while the residual drug is evacuated with the feces.

MATERIAL AND METHODS

In the current study both tramadol hydrochloride and vitamin C ampoules are used and are obtained from local pharmacies in Iraq. Thirty healthy Wister albino male rats weighting about (220-250) gm. and aged three months are used in this study. These animals were kept at controlled room temperature (23-25°C) with a 12 hours light/dark cycle and placed in plastic cages using homogenized wood shaving as bedding for acclimatization for one week before start of the experiment. The animals had allowed access of both water ad libitum and packed optimized food. The animals were divided into three groups.

Group A (control group): are daily injected with distilled water intraperitoneally (I.P).

Group B (tramadol treated group) (TGI): are injected with tramadol in dose 50mg/kg I.P. daily for four weeks.

Group C (treated with tramadol + Vitamin C) (TGII): are injected with 100 mg/kg b.wt. of vitamin C I.P. daily half hour prior to tramadol injection in a dose 50 mg /kg b.wt. I.P. for four weeks.

After finishing of the fourth weeks of experiment, the animals were sacrificed and kidneys were removed and utilized for histopathological examinations, in which 10% neutral buffered formalin was used as fixator for the specimens which then processed to get paraffin sections of 5μm thickness, that stained with Haematoxylin and Eosin to be examined by light microscopic.

RESULT

Group A (Control group): Light microscopic study showed no deviation in histological findings from those seen in other normal tissues, in which kidney sections consist of renal cortex and medulla. In the cortex there is renal corpuscle, proximal convoluted tubules (PCT) along with distal convoluted tubules (DCT). Every renal corpuscle consists of blood capillaries tuft, named as glomerulus bounded by Bowman's capsule. The latter is a double walled cup formed of two layers of simple squamous epithelium, an outer parietal layer and an inner visceral one separated by a capsular space. The PCT is lined by cuboidal epithelium with spherical and basally located nuclei. The DCT is lined by cuboidal epithelium and spherical and apically located nuclei and their cytoplasm is lighter than those of PCT (Fig. 1).
**Group B (TGI):** Shows atrophy and congestion of glomeruli, others showed segmentation and lobulation along with widening of bowman's space (Fig. 2). The most severe changes are observed in the tubules and mainly PCT as some of tubules are dilated, others showed marked hydropic degeneration and cytoplasmic vacuolization of tubular epithelial cells (Fig. 3). Patches of mononuclear cells (MNCs) infiltration are noticed around blood vessels and within the necrotic tubules (Fig. 4). Hyaline cast is seen within the cortex mainly inside the degenerated tubules along with hemorrhage in the interstitium (Fig. 5).

**Group C (TGII):** shows more preserved kidney architecture, most glomeruli regained their normal appearance (Fig. 6) Some glomeruli showed mild atrophy with mild dilatation of bowman's space (Fig. 7). Few tubules showed vacuolation in their epithelial cytoplasm (Fig. 8). Interstitial bleeding is still present but mild (Fig. 9) and little MNCs infiltration in the interstitium of renal cortex (Fig. 10).

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**Figure 1:** Photomicrograph of control group showing glomerulus (black arrow), bowman's space (arrow head), PCT (blue arrow) and DCT (green arrow) (H & E X 400).

**Figure 2:** Photomicrograph of TGI showing glomerular atrophy with segmentation (red arrow) and widening of bowman's space (arrow head)(H & E X 400).

**Figure 3:** Photomicrograph of TGI showing extensive hydropic degeneration (black arrows), with interstitial bleeding (arrow head) (H &E X 400).

**Figure 4:** Photomicrograph of TGI showing MNCs infiltration within the interstitium (black arrows) (H &E X 400).
Figure 5: Photomicrograph of TGI showing hyaline casts within the degenerated tubules (black arrows), extensive vacuolar degeneration (red arrow) and hemorrhage (green arrow) (H&E X 400).

Figure 6: Photograph of kidney rat of TGII showing normal architecture of renal cortex with nearly normal looking glomeruli (black arrows) (H&EX100).

Figure 7: Photomicrograph of TG II showing mild glomerular atrophy (black arrow) with mild dilatation of bowman's space (arrow head) (H & E X 400).

Figure 8: Photomicrograph of TGII showing nearly normal looking renal corpuscle (red arrow) while few tubules showed vacuolar degeneration (green arrow) (H&EX400).

Figure 9: Photomicrograph of TG II showing interstitial bleeding (green arrow) with scattered vacuolar degeneration (red arrows) with (H & E 400).

Figure 10: Photomicrograph of TG II showing mild MNCs infiltration (red arrow) (H & E 400).
DISCUSSION
As the kidney is the main excretory organ for drugs and chemicals, so it is susceptible to toxicity which requests for its continuous clinical assessment throughout treatment with drugs to defense against toxicity.8

In the current study there is many microscopical deviations in the tramadol treated group TGI if compared to the group A (control group), as glomerular atrophy, and widening of bowman's space are in agreements with El-khateeb et al,20159 along with Youssef and Zidan, 2016 10 findings. Some glomeruli in TGI group showed segmentation and lobulation and this run with similar finding reported by Zaghlol et al., 2018.11

The mechanism of expansion of the glomerular chamber and swelling of their lining epithelium and consequently glomerular atrophy result from drop in aerobic metabolism due to decrease in O2 level, as renal cells consume O2 at high rate and in need to aerobic metabolism for ATP generation.12 The cells must rely more on glycolysis in order to maintain such ATP levels, as glycolysis can leads to the accumulation of lactic acid, that causes drop in the intracellular pH which induce cell dysfunction of the Na+/K+ ATPase followed by an influx of sodium and H2O with consequent cell swelling.13

Another important finding of the current study is the degenerative injuries in the renal tubules particularly in PCT. A similar dose i.e. 50mg/kg b.wt produced the same degree of degeneration after treating animal for only two weeks as reported by Hafez et al,2015.14 Also such tubular epithelial injuries induced by tramadol was reported by many studies.9-11 The mechanism of tubular damage of the renal tubules is particularly due to sensitivity of renal tubules to toxic effects, in part since they have high consumption of O2 and weak enzyme systems.15 On the other hand a study done by Atici et al, 200515 indicated that the risk of increased level of lipid peroxidation and renal damage after prolonged use of opioids, particularly morphine in the rat's kidney.

Other observation in the current study is MNCs infiltration in the renal cortex around degenerating tubules giving a picture of interstitial nephritis, this finding is in agreement with previous studies9,10,15, and this finding in addition to the other findings can be reflected as a sign of renal damage as described by Muslim Z, 201816 while is in conflicts with this finding by Zaghlol et al., 2018 11 and Ali et al., 201817 and this might be due to small dose or small dose plus short period, respectively.

The hyaline cast seen within the degenerating tubules is another finding in the present study which ascribed to the solidification of Tamm-Horsfall mucoprotein, that secreted by the renal tubular cells.18

Many researchers proposed that vitamins, mineral selenium, ascorbic acid, quercetin and melatonin fractions in date fruit may be held responsible for renal protecting action.19 However, yet now there is no previous study about the role of vitamin C against nephrotoxicity induced by tramadol. In the current study using of vitamin C, result in mild improvement in many microscopical changes induced by tramadol and showed more preserved renal architecture, with decrease in glomerular atrophy, bowman's space dilatation with degenerated tubules and mild to moderate MNCs infiltration, but congestions of interstitisum and blood vessels is still present.

Many researchers proved the protective effect of vitamin C against histological changes induced by formaldehyde.20,21 Also vitamin C as chemoprotective agent was proved by Al-jammas S.,2011 after he injected rats with vitamin C prior to cisplatineum result in improvements in the microscopical changes induced by cisplatineum.22

The protection produced by vitamin C in renal tissue in concomitant use with tramadol was recognized due to antioxidant property of vitamin C that capable to reduce lipid peroxidation which results in alleviate kidney damage by enrichment of scavenging capability of antioxidant defense organization.23 But such improvement is not bring the renal tissues to complete normality which might be due to short period of treatment with antioxidant vitamin C or might need a larger doses vitamin C.

CONCLUSION
Tramadol had toxic effects on kidney structure as glomerular atrophy, widening bowman's space and degenerating tubules with vacoulation in their epithelial lining, all are improved after the addition of vitamin C.
REFERENCES