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Effects of Tramadol on Histopathological and Biochemical Parameters in Male Rabbits

Obed Khalid Ali[1], AL- Jmor Sara Ahmed[1], Al-Graibawi Mawlood. A. A.[2, *]

[1]Department of Medical Laboratory Technology, Al. Mustafa University College, Baghdad, Iraq
[2]Zoonotic diseases unit, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Email address

algraibawi_57@yahoo.com (Al-Graibawi M. A. A.), afaf_a.rahman@yahoo.com (Al-Graibawi M. A. A.)

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Abstract

The present study was designed to investigate the effects of different doses of tramadol hydrochloride (TH) on histopathological and biochemical changes in male rabbits. Fifteen healthy males local breed rabbits weighting 1.6 - 1.8 kg were distributed randomly and equally into three groups, housed under laboratory condition at the same room with natural light 1 dark cycle at 23 $\pm$3 ºC temperature, and given free access to commercial balanced diet and water *ad libitum* all over the experimental period. Group T_1injected intramuscularly(i.m) with tramadol 10 mg/kg b.w for 15 days, while the T_2 group injected i.m with tramadol 15mg/kg b.w for 15 days and the 3rd groups T_3 is control inoculated with distilled water 1/M at the same period. Blood samples were collected for biochemical analysis of creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). After sacrificed the animals, liver and kidney samples were obtained and fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H&E) for histopathological changes. The histopathological examination showed cellular degeneration characterized by vacuolization in the endothelium cells of renal tubules of the kidney and degeneration and pyknotic of the hepatocyte nuclei in the liver of the tramadole administrated rabbits only. The biochemical assay revealed that administration of tramadol for 15 days increased serum creatinine, ALT, AST, and ALP in treated rabbits compared to the control. The maximum increases was recorded in group T2, administrated i.m with 15mg/kg tramadol. In conclusion the tramadol side effects should be avoided during long term therapy especially in large doses.

Keywords

Tramadol Hydrochloride, Rabbits, Creatinine, ALT, AST, ALP

1. Introduction

Tramadol hydrochloride (TH) is a synthetic opioid with effects similar to those of codeine. It is analgesic of significant and has wide ranging application mostly in the treatment of moderate to severe pain including the treatment of fibromyalgia, cancer and musculoskeletal pain (Lehman, 1997).The TH also used for treatment pain in cases of low back, osteoarthritis, migraine (Babul etal., 2004). Tramadol hydrochloride have specific role in the treatment of opiate withdrawal (Threlkeld et al., 2006) and premature ejaculation (Salem etal., 2008).TH act by binding to μ- opiate receptors in the CNS causing inhibition of ascending pain path ways, altering the perception of and response to pain, also inhibit the reuptake of norepinephrine and serotonin, which also modifies the ascending pain path ways (Raffa, 2008). Tramadol hydrochloride pharmacokinetic , rabid and complete absorbed after adminstration in different form, tablet, capsules, drops, injection, suppository and well distributed as protein binding (Dayeretal., 1997).TH metabolism extensively hepatic via demethylation, glucuronidationandsulfation, the active metabolite by Cyp2D6 isoenzyme of P450 the half life of elimination is about 6h.and excretion as metabolite during urine (Dayeretal., 1997; Singhal etal.,1998).The most frequent adverse effects of TH include constipation, nausea, dizziness, headache, somnolence and vomiting (Stitiketal., 2006).The most serious adverse reaction confusion, hallucinations, convulsions, serotonin syndrome and hypersensitivity reaction (ADRAC, 2003). Also cause disposal of cells damaged by toxicants (Feldmann, 2006). The other dosage toxicity symptoms

include CNS and respiratory depression Lethargy- Coma, cardiac arrest and death (Feldmann, 2006).Tramadol hydrochloride is metabolized in the liver and excreted by the kidney; the role of liver and kidney in drug metabolism predisposes them to toxic injury leading to impaired liver and kidney functions. The increases of ALT and AST and creatinine parameters were observed in response to tramadol inoculation in adult male albino rats.(Elkhateeb, et al. 2015).The aims of this study were to investigate the effects of different doses of tramadol on histopathological changes of liver and kidney and biochemical values of serum creatinine, ALT, AST, and ALP in male rabbits.

2. Materials and Methods

2.1. Experimental Animals and Dosing

The drug tramadol HCl (Trabilin Ampoule) 100mg/2ml manufactured by Mepha company purchased from Iraqi pharmacy. Fifteen adult male rabbits were used in this study; they were purchased from local markets. Rabbits were left in the animal's house for 2 week before experimentation to adapt to laboratory condition under the following conditions natural light 1 dark cycle at 23 $\pm$ 3 ^{O}C temperature, and given free access to commercial balanced diet and water *ad libitum* all over the experimental period. They divided randomly and equally into three groups as follow: The T1 and T2 groups were inoculated i.m with tramadol 10 mg / kg b.w. and 15 mg / kg b.w. respectively, for 15 days. Control group T3, inoculated i.m with distill water. Biochemical measurements were carried out. Control and treated rabbits were sacrificed at the end of experiment.

2.2. Measurement of Biochemical Blood Indices

Blood samples were collected from control and tramadol treated rabbits before and at the end of experiment in dry centrifuge tubes to determine the enzymes activities on serum samples. The activities of serum AST and ALT were determined according to the method of Reitman and Frankel, (1957). The measurement of serum ALP was based on the method of Bessey et al.(1946) and Perry et al.(1983). Serum creatinine was measured according to the methods described previously (Bartels et al., (1972).

2.3. Histopathological Procedure

The animals were sacrificed post 3 days withdrawal of tramadol , one centimeter cubes from kidneys and liver were taken and fixed in 10% buffered formalin, dehydrated in ascending concentrations of ethanol and cleared in xylene followed by embedding in paraffin. Sections (5 μm) were prepared from each tissue block and stained with hematoxylin-eosin stain (H&E) for histological examinationas described previously (luna, 1968).

3. Statistical Analysis

Statistical significance on the dose 15mg/kg was taken at P< 0.05 by using SPSS analysis system and applying the LSD all data were presented as mean $\pm$ SD and compare between the different doses.

Ethics: This study was approved by the Ethical and Research Committee of the Department of Medical Laboratory Technology - Al. Mustafa University College

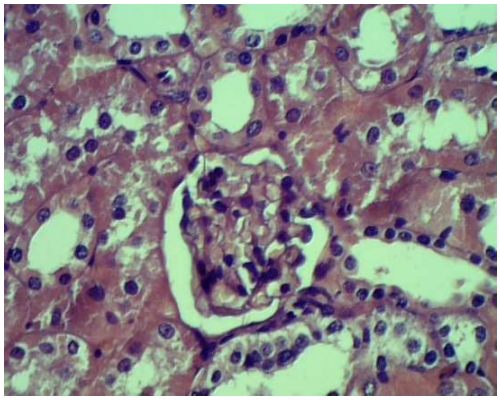

Fig. 1. *Kidney of male rabbit (control group) showing normal tissues (X40 H&E).*

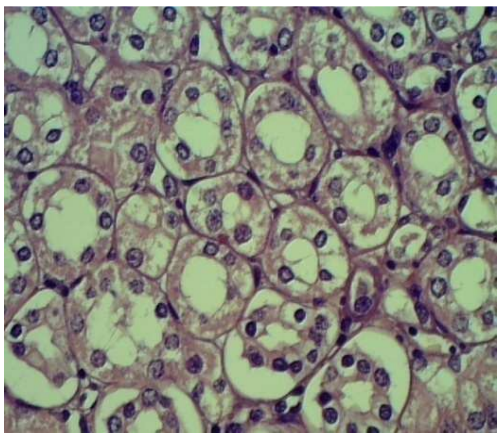

Fig. 2. *Kidney of male rabbit (control group) showing normal tissues (X40 H&E).*

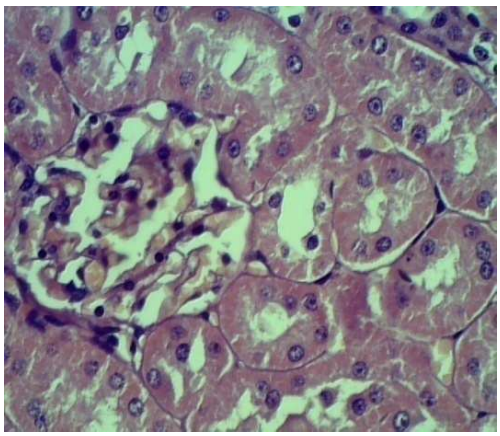

Fig. 3. *Kidney of male rabbits (tramadol group) showed cellular degeneration characterized by and vacuolization (x40 H&E).*

4. Results

Histopathological examination of the kidney showed a normal histological structure in control rabbits (Figure 1,2) in contrast the tramadole administrated rabbits showed cellular degeneration characterized by vacuolization in the endothelium cells of renal tubules of the kidney (Figure 3,4).

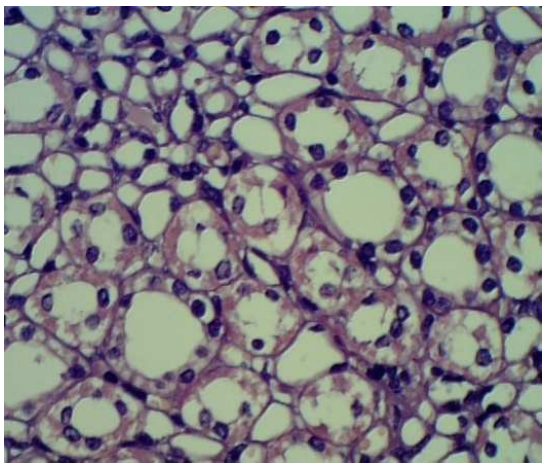

Fig. 4. *Kidney ofmale rabbits (tramadol group) showed cellular degeneration characterized by and vacuolization (x40 H&E).*

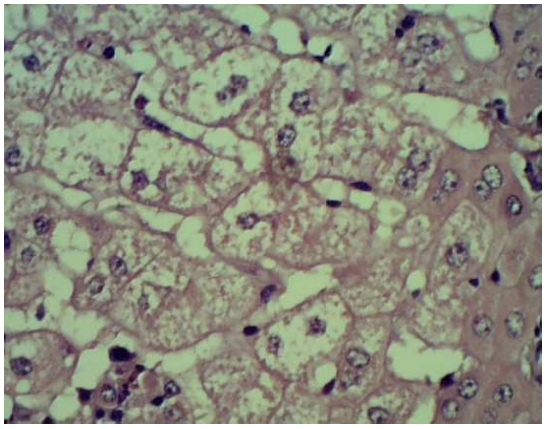

Fig. 5. *Liver of male rabbits (control group) showing normal heptocytes (X40 H&E).*

Histopathological examination of the liver showed no histopathological abnormalities in the control group (Figure 5). The pathologic findings of the liver were fatty degeneration and pyknotic of the hepatocyte nuclei in tramadol group (Figure 6).

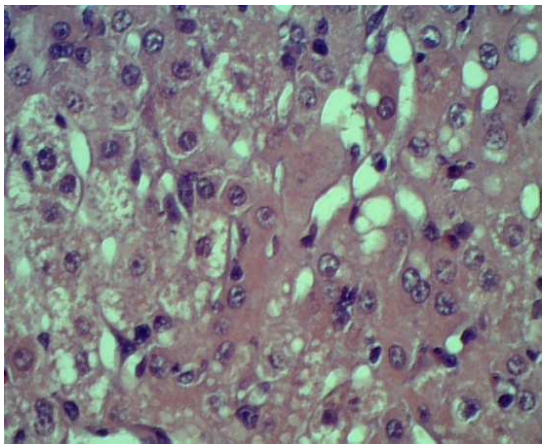

Fig. 6. *Liver of male rabbits injected with 15mg/kg b.w. of tramadol showing fatty degeneration and pyknotic of the hepatocyte nuclei (X40 H&E).*

Biochemical analysis of creatinine, ALT, ASTand ALP

The biochemical parameters of serum creatinine, ALP, ALT and AST, were recorded in male rabbits before and after administration of tramadol at a dose of 10 and 15 mg/Kg b.wt. daily for 15 days. The results revealed a significant increase in serum creatinine, ALT, AST, and ALP in treated rabbits compared to the control (Table 1). The maximum increase in creatinine was recorded in group T2, administrated i.m with 15mg/kg tramadol (1.5 ± 0.0.5 mg/kg) (Table 1, Figure, 7). Highest increase in the levels of ALP (149.5 $\pm$ 50) in the rabbits of group T2 receiving 15mg/kg of tramadol compared to the rabbits of group T1 and controls (Table 1, Figure, 8), the increase in ALT (458 ± 5.8 U/L) was recorded in group T2 (Table1, Figure, 9), and Similarly a increase in the level of AST (790 ± 20 U/L) was found in group T2 (Table 1, Figure, 10).

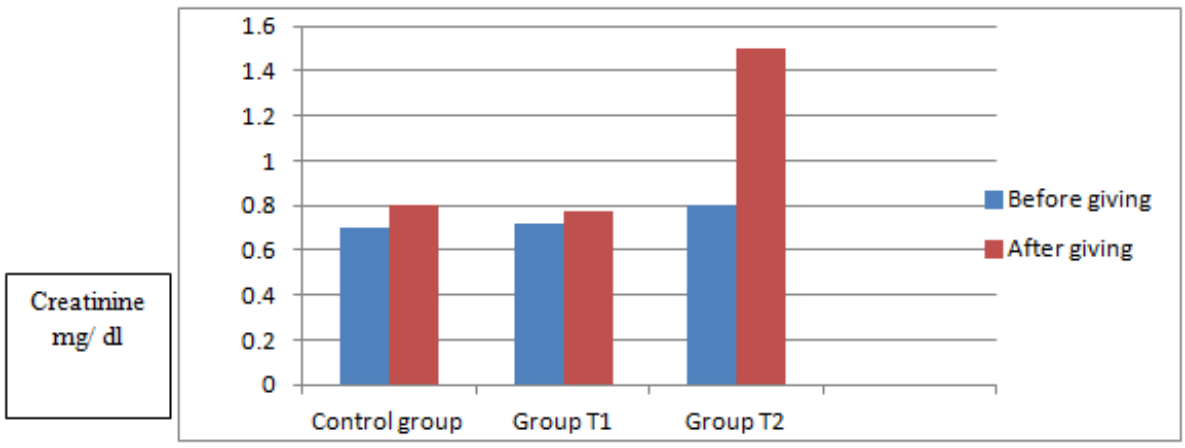

Fig. 7. *Creatinine value in control and tramadol treated rabbit groups.*

Table 1. *Effects of different doses of tramadolon Creatinine , ALT, AST, and ALP parameters in male rabbits.*

Enzymes	Control group,		Group T1 (10m g/kg/ b.w)		Group T2 (15 mg/kg/ b.w)	
	Before giving pbs	After giving pbs	Before giving tramadol	After giving tramadol	Before giving tramadol	After giving tramadol
Creatinine mg/ dl	0.9 ± 0.1	0.8 ± 0.2	0.7 ± 0.3	0.75 ±0.2	0.8 ± 0.5	1.5 ± 0.5
ALP µg/L	66 ± 13	79 ± 6	53 ± 16	79 ± 12	79 ± 11	149.5 ± 50
ALT µg/L	149 ± 1	150 ± 1	107 ± 24	150 ± 11	134 ± 16	458 ± 58
AST µg/L	150 ± 2	151 ± 1	149 ± 9	585 ± 14	151± 14	970 ±20

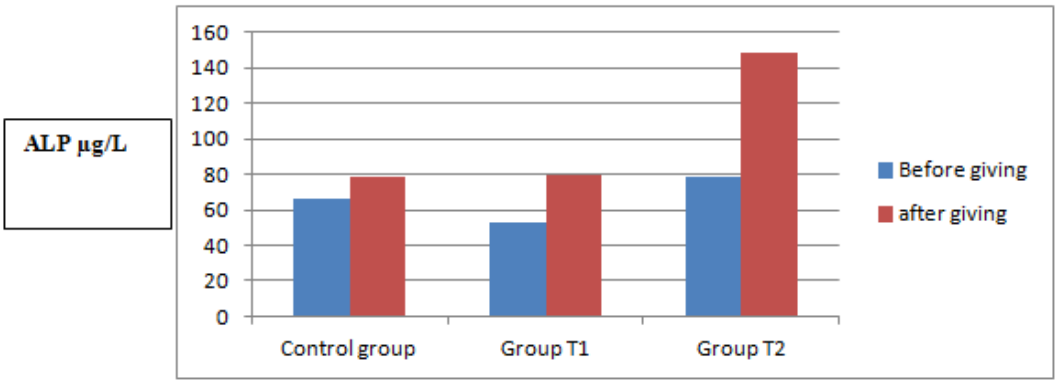

Fig. 8. *Value ofALP in control and tramadol treated rabbit groups.*

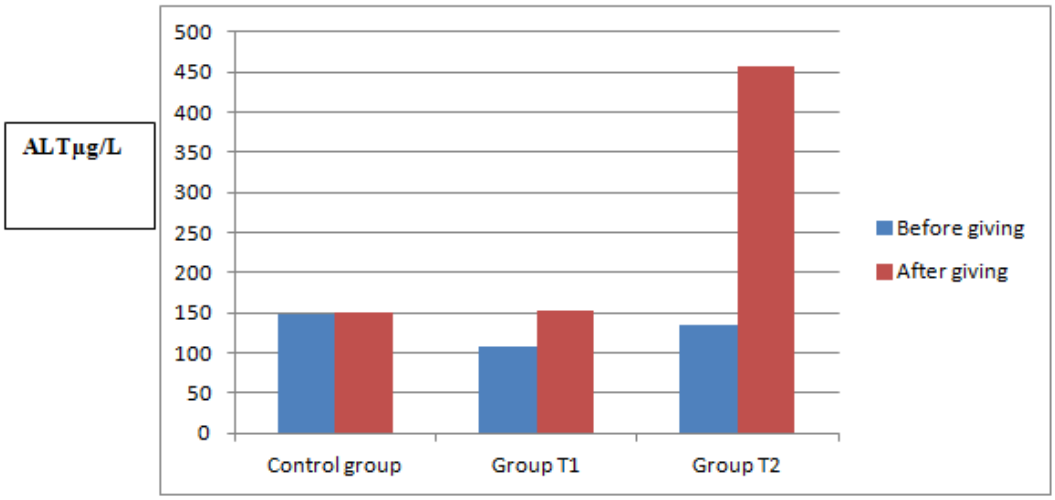

Fig. 9. *Value of ALT in control and tramadol treated rabbit groups.*

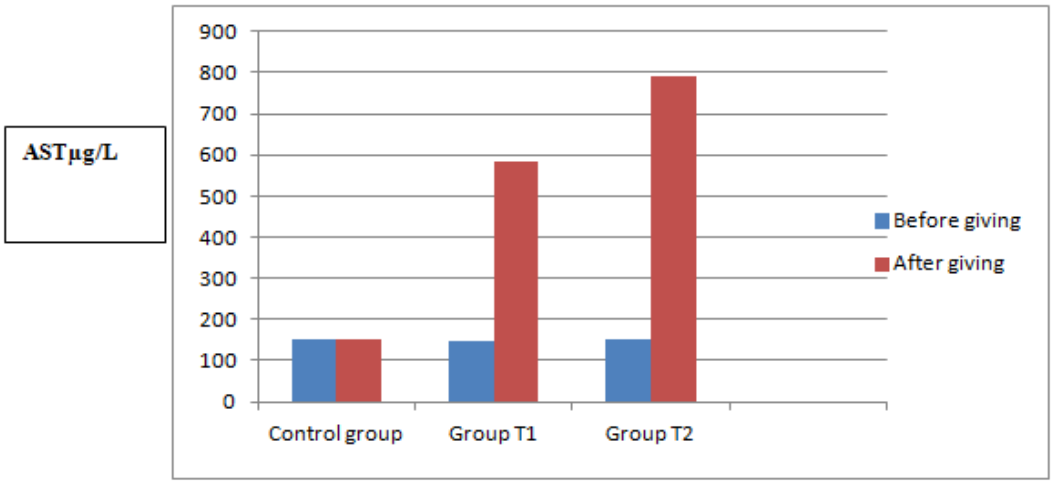

Fig. 10. *Value of AST in control and tramadol treated rabbit groups.*

5. Discussion

Tramadol hydrochloride is one of morphine derivatives; widely used opioid in recent years as an effective analgesic agent for the treatment of acute or chronic pain (Lee *et al* 1993). It is metabolised in the liver and excreted by the kidneys, it may cause hepatotoxicity and nephrotoxicity during its metabolism (Wu et al., 2001).The histopathological examination in the current study showed cellular degeneration characterized by vacuolization in the endothelium cells of renal tubules of the kidney. These results were in agreement with Nehru and Anand(2005), they reported that reactive oxygen species generation and lipid peroxidation are responsible for tramadol-induced nephrotoxicity. Our study revealed degeneration and pyknotic of the hepatocyte nuclei in the liver of the tramadole administrated rabbits which coincided with Loughrey et al. (2003); they mentioned that daily administration of tramadol to adult male rats for one month was accompanied by hepatic congestion, hemorrhage and necrosis. Also, El-Wessemy(2008) noticed loss of architecture, congested central veins, and expanded portal area with edema and inflammatory reaction in rats treated with tramadol.

The biochemical assay revealed that administration of tramadol for 15 days increased serum creatinine, ALP,ALT and AST in treated rabbits compared to the control. The maximum increase in creatinine was recorded in group T3, administrated i.m with 15mg/kg tramadol. These are in accordance with Atici et al. (2005) who reported an increase in creatinine and BUN levels in rats receiving morphine for a month. Serum creatinine concentration is maintained by the balance between its generation and excretion by the kidneys. Levels are affected by factors that influence the generation, glomerular filtration, and tubular secretion of serum creatinine , the assessment of serum creatinine is highly important to determine the kidney function in the clinical setting. Therefore laboratory evaluation of serum blood creatinine is considered "standard fare" in the determination of renal functions (Lyman, 1986).

The liver enzymes are normally found in circulation in small amounts duo to hepatic repair and growth. The serum ALT activity significantly increased ($p< 0.05$) in experimental group administrated withtramadol of 15mg/kg. The increase in the parameter of ALT indicated the malfunctioning and damage of liver tissues. However, its elevation has also been documented in non-liver injury conditions e.g. muscle injury (Yang, et al 2009) Significantincrease in level of ALT has been found in rats receiving morphine and tramadol for longtime compared to control group (Atici, et al 2005).In this study the significantly higher ($p< 0.05$) level of AST in the experimental groups of rabbits compared to control group and highest level AST (790 ± 20 U/L) was found with the administration of 15mg/kg tramadol ,similarly a significant increase was reported in the level of AST in rats treated with tramadol(Gaafarawi, 2006).

The elevated ALT and AST activities observed in response to tramadol inoculation could be a common sign of impaired liver function (Aldalou, et al. 2014). Similarly a significant increase in the levels of ALP in the rabbits receiving 15mg/kg of tramadol compared to controls. Impaired liberation of hepatic ALP of liver cell origin may be accompanied by acute cell necrosis, so secretion of ALP in the circulation is increased. The results of the present study were in agreement with that of Senay et al., (2003) and Atici, et al. (2005)they reported that the values of ALT, AST, LDH and Blood urea nitrogen (BUN), and creatinine were significantly increased in morphine grouped compared to the control group. Nehru and Anand(2005)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 the current study vacuolization in the endothelium cells of renal tubules of the kidney and degeneration and pyknotic of the hepatocyte nuclei in the liver as well as increase inserum creatinine, ALP, ALT and AST levels in the tramadole administrated rabbits can be considered as evidence of renal and liver damage.

6. Conclusion

The tramadol side effects should be avoided during long term therapy especially in large doses.

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