

Parenchymatous Toxicity of Tramadol: Histopathological and Biochemical Study

Essam Hafez M^{1*}, Sahar Issa Y² and Safaa Abdel Rahman M³

¹Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Minia University, Egypt

²Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Alexandria University, Egypt

³Central Laboratory, Alexandria, Egypt

Abstract

Objective: The present study was designed to highlight the toxic impact of tramadol on both biochemical and histopathological aspects in rats' liver, kidney and thyroid gland.

Methods: The study was performed on fifty healthy adult male albino rats divided into five groups with ten rats each. (Four experimental and control groups). Five rats (negative control) were kept in a quite non stressful environment, provided with food ad libitum and free access to water. Normal saline (1 ml) was given intramuscularly as placebo in the positive control group (n = 5). Experimental groups (II, III, IV and V) were injected with tramadol intramuscularly equivalent to 12.5 mg, 25mg, 50 mg and 300 mg/kg body weight/day respectively for two weeks.

Results: The levels of alanine aminotransferase (ALT), Cardiac treponin I (CTnI), Prothrombin time (PT) and partial thromboplastin time (PTT) in all tramadol treated groups showed significant elevation when compared to control. As regards thyroid function tests (T3, T4, and TSH) showed no significant laboratory difference between all studied groups. There was hepatic and renal histopathological changes in tramadol treated rats whose severity varied with doses of tramadol given. Histopathological changes in thyroid tissues were only seen in group treated with tramadol 50 mg/kg and in acute toxicity group.

Conclusion and recommendation: Toxic effects of tramadol on parenchymatous organs as liver, kidney and thyroid gland should be kept in mind and taken cautiously in patient complaining from hepatorenal affection or thyroid diseases.

Keywords: Tramadol; Hepatic; Renal; Thyroid; Biochemical; Histopathology

Introduction

Opioids are used as analgesics and considered effective for the treatment of acute cancer and non-cancer chronic pain [1]. Analgesics are among the most popular drugs which are being abused [2]. Tramadol is a synthetic, centrally acting analgesic, available in Europe since 1977 and in the United States since 1995 for the treatment of pain syndromes previously amenable only to the opiate analogues [3]. It has dual mode of action. Its analgesic efficacy is attributed to its partial affinity for the mu- opiate receptor and its inhibition of norepinephrine and serotonin reuptake [4]. Tramadol is converted in the liver to O-desmethyl-tramadol by cytochrome P 450 which itself is an active substance and is two to four times more potent than tramadol. Further, biotransformation results in inactive metabolites, which are excreted through kidneys [5].

Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction [6]. High-dose glucocorticoids, high-dose dopamine and potent opioids (e.g. tramadol) inhibit thyroid stimulating hormone (TSH) release and therefore may decrease the TSH concentration [7].

Aim of the Work

The present study was designed to highlight the toxic impact of tramadol on both biochemical and histopathological aspects on rats' liver, kidney and thyroid gland.

Materials and Methods

Animals

Fifty healthy adult male albino rats weighting about 150-170 grams were obtained from the animal house in faculty of science Minia University. All animals were allowed free access to distilled water and laboratory chow ad libitum. To avoid stress of isolation or overcrowdings, 6 rats were housed per cage. They were left freely wandering in their cage for two weeks with 12 hour dark to light cycle for acclimatization before starting the experiment. Experimental procedures were performed in accordance with the guide of the care and use of laboratory animals approved by the committee of Minia University, the fewest number of animals estimated to obtain valid results were used and painful procedures were conducted with appropriate sedation to avoid pain and stress.

Drug

Tramadol HCl 100 mg/ 2ml /ampoule, Alexandria Company for pharmaceuticals. All doses of tramadol were delivered in a volume of 1 ml

*Corresponding author: Essam Mahmoud Hafez, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Minia University, Egypt, Tel: +00966-545-990-785; E-mail: essamtox@yahoo.com

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normal saline. At the end of each experimental period and under ether anesthesia all animals were sacrificed after 24 hours of the last dose.

Experimental design

Group I: Control group IA (negative control) (5 rats): were kept in a quite non stressful environment, provided with food ad libitum and free access to water for two weeks.

Control group IB (positive control) (5 rats): Each animal received 1ml/day normal saline 0.9% intramuscularly. They were kept throughout the experiment under the same conditions for two weeks.

Group II: (10 rats): Each animal received 12.5 mg/kg/day of Tramadol hydrochloride intramuscularly for two weeks.

Group III: (10 rats): Each animal received 25 mg/kg/day of Tramadol hydrochloride intramuscularly for two weeks.

Group IV: Each animal received 50 mg/kg/day of Tramadol hydrochloride intramuscularly for two weeks

Group V: (Tramadol acute toxicity group 10 rats): Each animal received a single dose of LD50 of tramadol hydrochloride 300mg / kg body weight (b wt) intramuscularly (Matthiesen et al., 1998) [8].

Biochemical assay

Blood samples (3 ml) were collected from tail veins of all animals and centrifuged for 10 minutes at 5000 rpm to harvest the clear serum where Liver function tests; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) and renal function tests; serum creatinine (SC) and blood urea nitrogen (BUN), creatinine kinase (CK) were measured by enzyme linked immunosorbent assay (ELISA) were manufactured by Ranbaxy Diagnostics Ltd. (UK). Prothrombin time (PT), partial thromboplastin time PTT) were measured in seconds. Hematocrit percentage, total protein (TP), glucose level, platelet count were measured for all groups. The kits for thyroid function tests T3, T4 and TSH determination were from Calbiotech, 10461 Austine drim vallg CA 91978, USA and the assay was according to Liewendahl, 1990 Kits. [9]

Histopathological studies

After rats scarification and decapitation The right lobe of the liver and right kidney and Thyroid tissues of all rats were excised, fixed in buffered 10% formalin solution for 24 hours and embedded in paraffin wax, then sectioned and stained with Haematoxylin and Eosin stain (H&E) for histological examination using light microscope .

Results

As shown in (Table 1); there was highly significant elevation ($p < 0.01$) of alanine aminotransferase (ALT) in all groups that received tramadol. Also, there was a significant increase ($p < 0.05$) in aspartate aminotransferase (AST) level in group V when compared all other groups. Regarding alkaline phosphatase (ALP), there was only highly significant increase in group III & IV and also significant increase in Group II. There was highly significant increase in cardiac treponin I (CTnl), Prothrombin time (PT) and partial thromboplastin time (PTT) in Group V and significant increase in these parameters in group II, III, IV when compared to control.

A significant difference in blood urea nitrogen (BUN) and creatine kinase (CK) biochemistry parameters was detected between the control group and all other groups indicating toxicity induced by tramadol

administration was detected. There was no significant difference detected between all studied groups as regard serum creatinine (SC), total protein (TP), glucose level, hematocrit %, and platelet count, as shown in (Table 1).

As regards thyroid function tests (T3, T4, TSH) there was no significant laboratory difference between all studied groups as shown in (Table 2).

Histopathological Results

Macroscopic appearance

- Group I: Both control groups "A" and "B" showed no gross abnormality.

- Group II: There was no abnormality seen as compared to control groups.

- Group III: There was adhesion of serosal surface of the liver.

- Group IV: There was fibrinous peritonitis observed in all treated animals characterized by adhesion of serosal surface of the liver, intestine in comparison to control group.

- Group V: There was no abnormality could be as seen in the control groups, apart from congestion in all internal organs.

Microscopic appearance

Control Group I (A &B): Normal H&E for all studied tissues in control groups.

Regarding the liver tissue; mild central vein congestion was detected in group II (Figure 1) while parenchymal changes in the form of mild few foci of hepatic necrosis associated with mononuclear inflammatory cell aggregation and mild central vein congestion were seen in group III, (Figure 2), whereas in group IV there is Marked hepatocellular necrosis, with visible inflammatory infiltrate. In addition there is mild central vein congestion, moderate bile duct hyperplasia and dilation of sinusoidal spaces (Figure 3a). Hyaline droplet degeneration was detected in the hepatocytes in four rats (Figure 3b). Extensive peri-hepatitis is associated with macrophages with brownish injected granules and giant cells, representing tramadol related cholestatic hepatitis plus bile plugs in hepatocytes and canaliculi was seen in two rats (Figure 3c). In group V there was Marked hepatocellular necrosis in zonal, centrilobular pattern; marked inflammatory infiltrate, severe congestion of portal vein and degeneration of hepatocytes (Figure 4a,4b).

For renal tissues in group II (Figure 5) there was a picture of drug induced interstitial nephritis. Multifocal cortical low to moderate numbers of lymphocytes, macrophages, and plasma cells surrounding degenerated and necrotic proximal convoluted tubules (PCT) in group III (Figure 6). In group IV and V there was multi-focal tubular epithelial degeneration, attenuation, and necrosis in the renal cortex as shown in (Figure 7,8).

Regarding thyroid tissues in group II, and III (Figure 9,10) Sections of thyroid showed no significant histopathological changes after administration of tramadol. In group IV thyroid tissue showed follicular hypertrophy and hyperplasia with crowding of follicular epithelium nuclei and minimal protrusion into the follicular lumen (Figure 11), but in group V there was mild congestion as shown in (Figure 12).

Test	Control Group	Group II	Group III	Group IV	Group V	Reference Interval
Hematocrit (%)	35.3 ± 1.2	37 ± 1.4	37.2 ± 1.6	39.4 ± 1.5	35.1 ± 1.5	32-52
TP (g/dl)	6.6 ± 0.29	7.1 ± 0.35	7 ± 0.51	7.4 ± 0.75	7.1 ± 0.32	6.1-8.1
Glucose (mg/dl)	82.5 ± 4.9	87.3 ± 5.6	86.4 ± 5.1	85.7 ± 5.4	84.3 ± 5.1	70-105
AST (U/L)	16.66 ± 3.57	40 ± 5.16	44.2 ± 5.7	48 ± 5.25	38 ± 5.77*	0-35
ALT (U/L)	11.20 ± 3.43	79.66 ± 28.63**	81.69 ± 29.5**	94.61 ± 38.47**	68.66 ± 24.69**	7-56
ALP (U/L)	36.20 ± 9.54	180.33 ± 33.3*	185.23 ± 43.8**	189.37 ± 43.7**	48.33 ± 33.30	30-120
GGT (U/L)	26.73 ± 4.02611	45.33 ± 12.31	48.41 ± 11.29	50.4 ± 15.34	38.31 ± 12.5 1	0-42
cTnl (µg/L)	3.4 ± 1.6	18 ± 4.7*	19 ± 7.4*	19.1 ± 5.4*	24 ± 1.8**	0-10
Platelets (x10 ³ /µl)	126.5 ± 15.5	128.3 ± 15.8	124.31 ± 17.1	121.3 ± 14.4	126.3 ± 17.6	100-600
PT (sec)	8.1 ± 1.2	13.2 ± 1.7*	14.1 ± 1.5*	14.2 ± 1.4*	16.24 ± 1.9**	8-10
PTT (sec)	35.5 ± 3.2	60.6 ± 4.7*	64.6 ± 5.3*	65 ± 5.1*	71.3 ± 2.1**	32-42
BUN(mg/dl)	21.3 ± 1.1	17.1 ± 1.6*	16.4 ± 1.4*	18.1 ± 1.9*	22.1 ± 1.3*	12-26
SC(mg/dl)	1.4 ± 0.08	1.7 ± 0.16	1.8 ± 0.19	1.8 ± 0.24	1.5 ± 0.14	1.2-2
CK (U/L)	250 ± 18.2	443.3 ± 38.4**	448.5 ± 39.6**	467.3 ± 42.1**	371.3 ± 34.9**	0-350

*: Significant at <0.05 **: Highly significant at p< 0.01

Table 1: Effect of Tramadol administration on biochemical laboratory findings in adult male rats.

Test	Control Group	Group II	Group III	Group IV	Group V	Reference Interval
T3(nmol/L)	0.98 ± 0.12	0.93 ± 0.17	1.30 ± 0.04	0.97 ± 0.110	1.29 ± 0.31	0.92–2.78
T4(nmol/L)	65.1 ± 2.3	41.70 ± 2.77	64.69 ± 1.71	61.19 ± 3.74	63.5 ± 1.5	64–144
TSH(mIU/mL)	0.7 ± 0.4	0.52 ± 0.01	0.65 ± 0.01	0.55 ± 0.01	1.5 ± 0.01	0.5–3.9

*: Significant at <0.05 **: Highly significant at p< 0.01

Table 2: Effect of Tramadol administration on thyroid functions in adult male rats

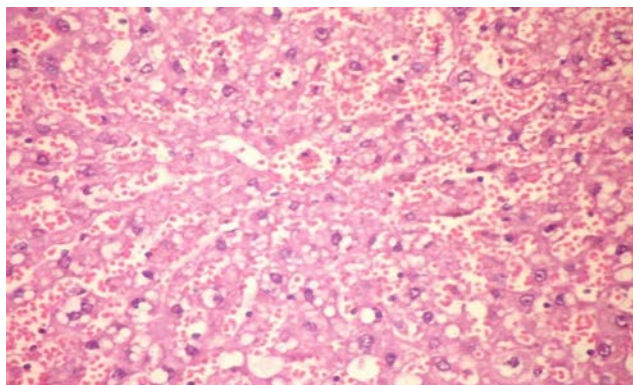


Figure 1: Liver showing mild central vein congestion.in group (II) animals injected with tramadol 12.5 mg/kg intramuscularly for two weeks.

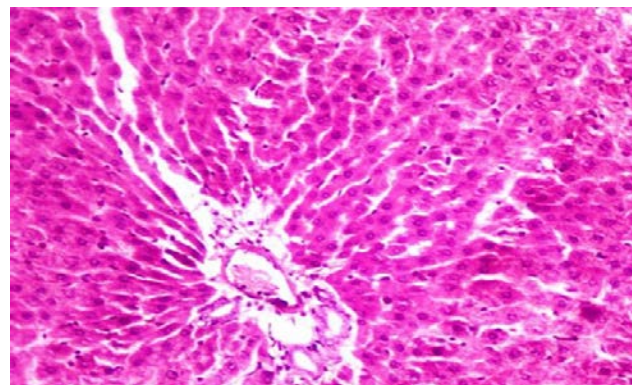


Figure 3a: Marked hepatocellular necrosis, with visible inflammatory infiltrate. In addition there is mild central vein congestion, moderate bile duct hyperplasia and dilation of sinusoidal spaces after administration of Tramadol 50 mg/kg intramuscularly for two weeks (group IV).

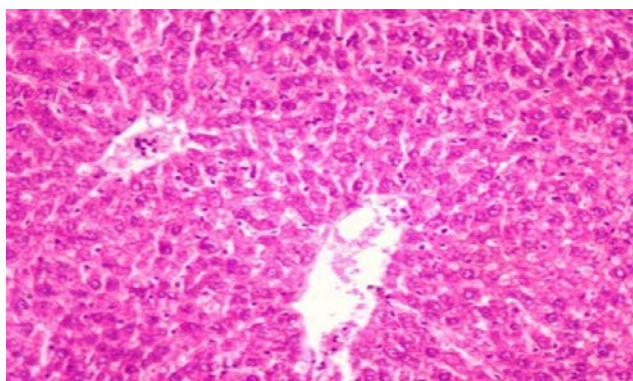


Figure 2: Liver showing mild central vein congestion and few foci of hepatic necrosis associated with mononuclear inflammatory cell aggregation after administration of Tramadol 25 mg/kg intramuscularly for two weeks (group III).

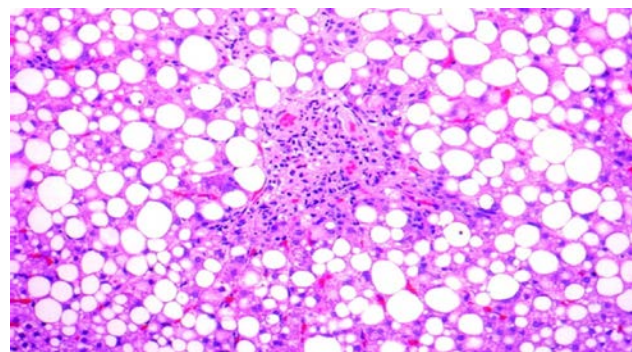


Figure 3b: Hyaline droplet degeneration in the hepatocytes in 4 rats after administration of Tramadol 50 mg/kg intramuscularly for two weeks (group IV).

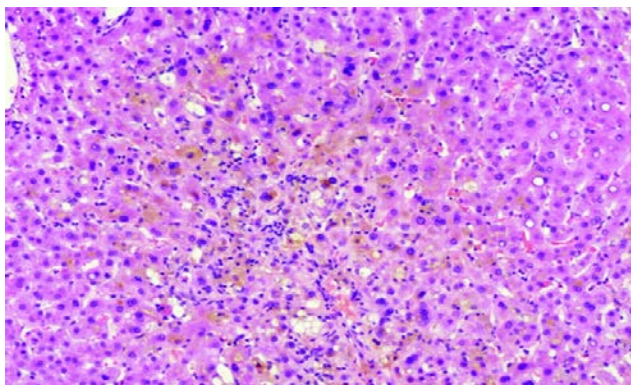


Figure 3c: Extensive peri-hepatitis is associated with macrophages with brownish injected granules and giant cells, representing Tramadol-related cholestatic hepatitis: has features similar to acute hepatitis, plus bile plugs in hepatocytes and canaliculi in 2 rats after administration of Tramadol 50 mg/kg intramuscularly for two weeks (group IV).

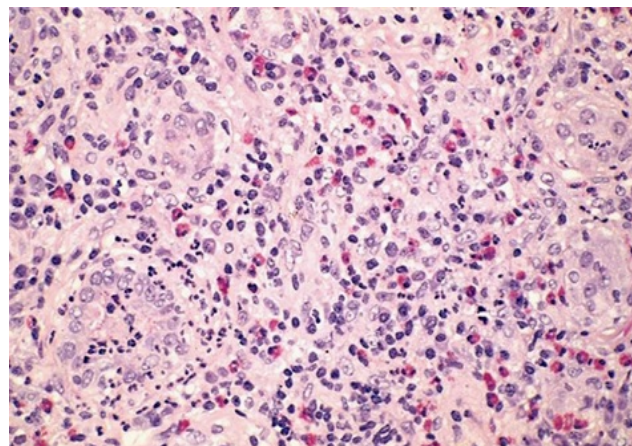


Figure 5: Kidney, showing drug induced interstitial nephritis in animals injected with tramadol 12.5 mg/kg intramuscularly for two weeks (group II).

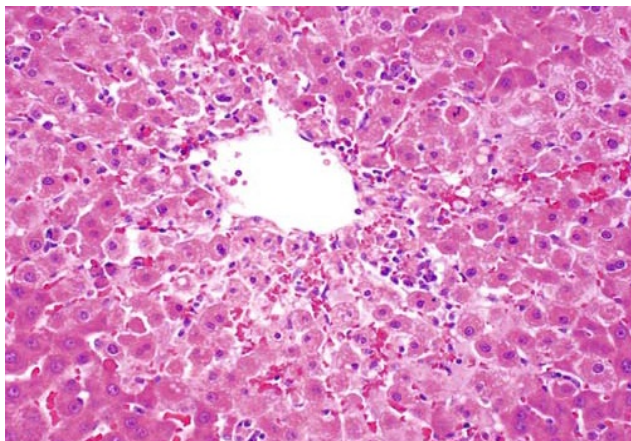


Figure 4a: Marked hepatocellular necrosis in zonal, centrilobular pattern; inflammatory infiltrate is marked after administration of a single dose of LD50 (300mg/kg B Wt) of tramadol intramuscularly (group V).

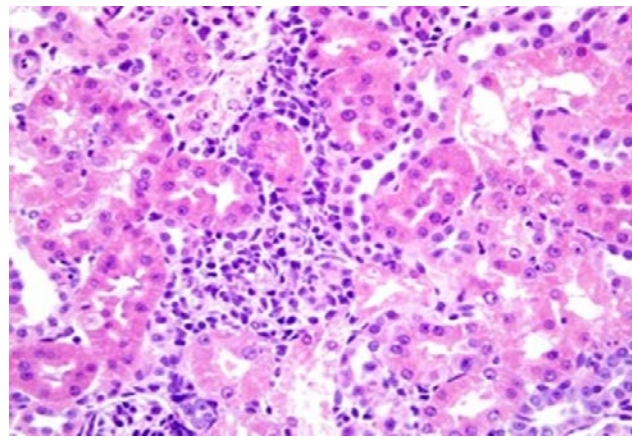


Figure 6: Kidney showing multifocal cortical low to moderate numbers of lymphocytes, macrophages, and plasma cells surrounding degenerated and necrotic proximal convoluted tubules (PCT) after administration of tramadol 25 mg/kg intramuscularly for two weeks (group III).

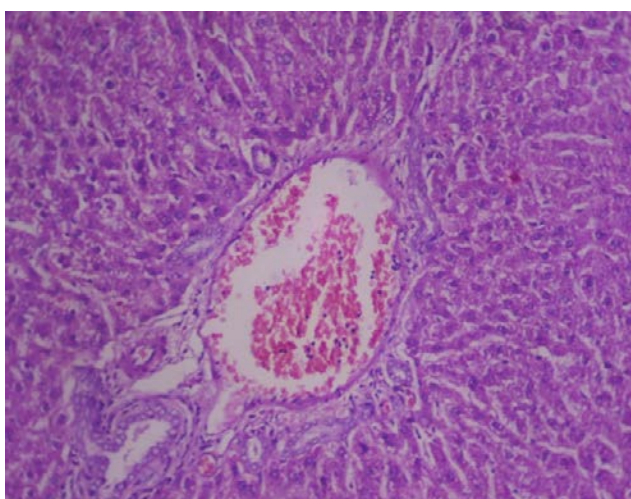


Figure 4b: Severe congestion of portal vein and degeneration of hepatocytes marked after administration of a single dose of LD50 (300mg/kg B Wt) of tramadol intramuscularly (group V).

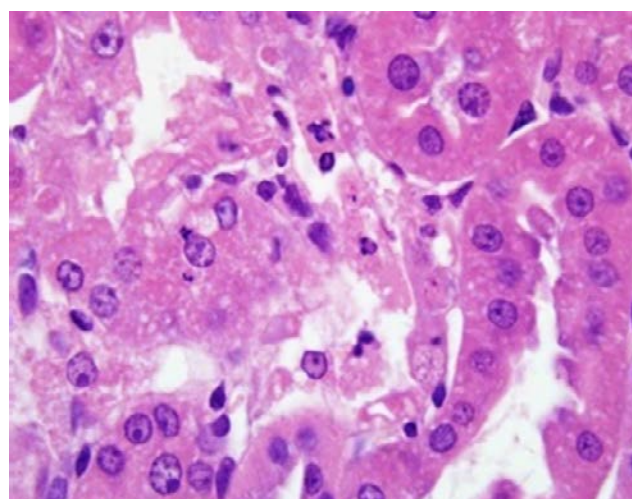


Figure 7: kidney showing multi-focal tubular epithelial degeneration, attenuation, and necrosis in the renal cortex after administration of Tramadol 50mg/kg intramuscularly for fourteen days two weeks (group IV).

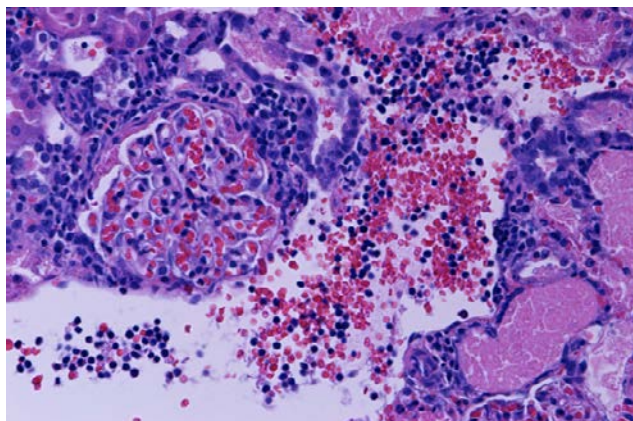


Figure 8: Tramadol treated rat. Haematoxylin and Eosin. Multifocally in the renal cortex there is tubular epithelial degeneration, attenuation, and necrosis after administration of a single dose of LD50 (300mg/kg B Wt) of tramadol intramuscularly (group V).

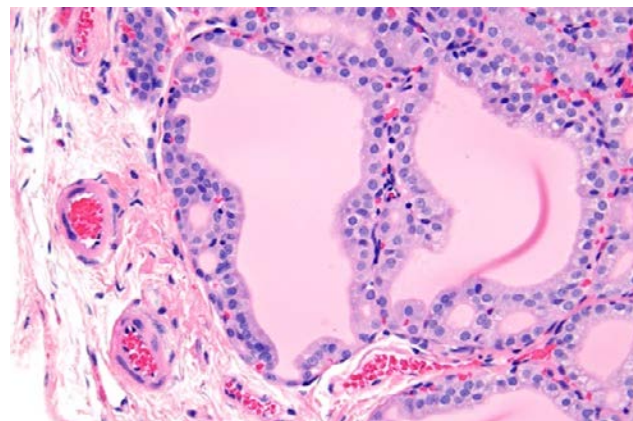


Figure 11: Sections of Thyroid showing follicular hypertrophy and hyperplasia with crowding of follicular epithelium nuclei and minimal protrusion into the follicular lumen after administration of Tramadol 50 mg/kg intramuscularly for two weeks (group IV).

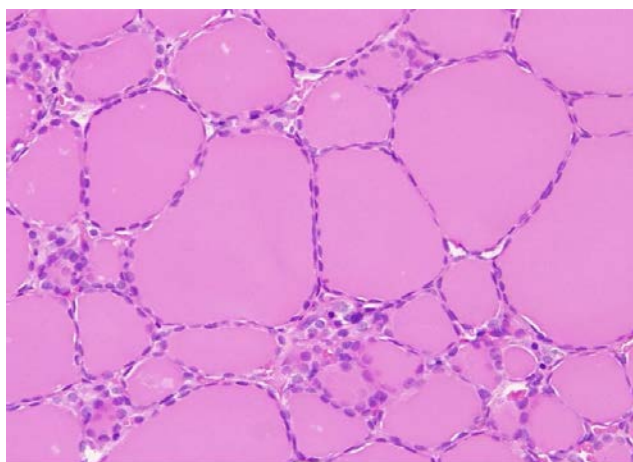


Figure 9: Sections of Thyroid showing no significant histopathological changes as compared to control group after administration of Tramadol 12.5 mg/kg intramuscularly for two weeks (group II).

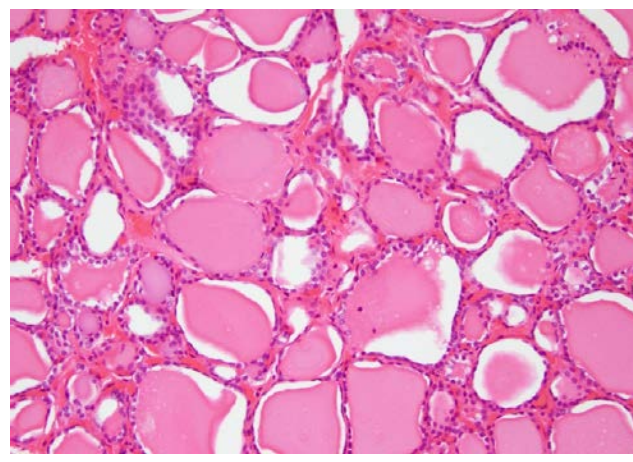


Figure 12: Sections of Thyroid showing no significant histopathological changes apart from mild congestion after administration of a single dose of LD50 (300mg/kg B Wt) of tramadol intramuscularly (group IV).

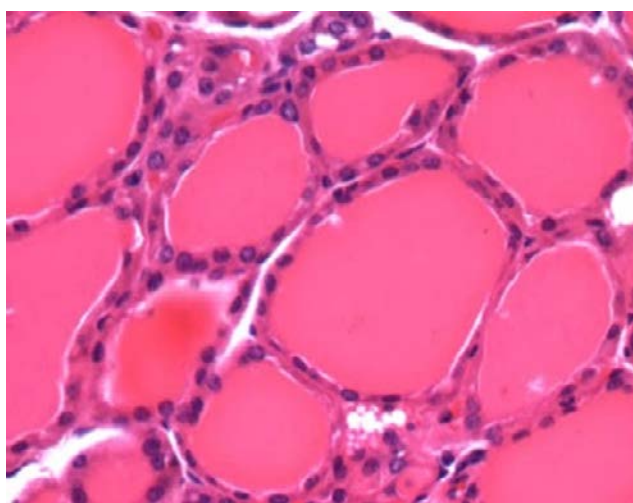


Figure 10: Sections of Thyroid showing no significant histopathological changes after administration of Tramadol after administration of Tramadol 25 mg/kg intramuscularly for two weeks (group III).

Discussion

The central role of liver and kidney in drug metabolism predisposes them to toxic injury. In agreement with our study there was an increase in the level of ALT indicating the malfunctioning and damage of liver tissues due to repeated tramadol use. However, its elevation has also been documented in non-liver injury conditions e.g. muscle injury [10].

Furthermore a significant elevated level of ALT has been found in rats receiving morphine and tramadol for long time compared to control group. Similar results have also been documented in the rats treated with morphine-like agent; levo alpha acetyl methadol HCl (LAAM) and in chronic heroin users [11].

Aspartate aminotransferase (AST) found in liver, heart, skeletal muscle, kidney, brain and red blood cell. Acute viral, ischemic or toxic liver injuries might be responsible for increased level of AST along with ALT. However both chronic hepatitis and cirrhotic patients may have aminotransferase levels within the reference range [12]. In another study performed by Habibian-Dehkordi et al., (2010) [13], short term intravenous administration of tramadol had no effect on ALT, AST, and ALP levels.

Laboratory evaluation of serum blood creatinine and BUN levels are considered as a good marker for the determination of renal functions [14].

The present study revealed that there is significant difference in blood urea nitrogen (BUN) and creatine kinase (CK) between the control group and other groups indicating toxicity induced by tramadol administration which in contrast to results detected by Sebnem [15] who reported the absence of significant differences in BUN levels between the tramadol and non-tramadol treated groups.

But our results were in accordance with other researches confirming the increased BUN levels in rats receiving tramadol [16,17] and morphine [18]. Serum creatinine showed no significant difference between control and tramadol treated groups in accordance to results detected by Sebnem [15].

In the present study the different level of tramadol doses had varied adverse effect on morphology and histopathology of the studied tissues whose severity was directly proportional to increase in the dose given. Thus was in contrast with Tolman, 1998 who stated that the metabolites produced as a result of tramadol metabolism had little pharmacological activity and can be easily removed from the body. Our results were in agreement with Atici and his associates [18], who reported severe centrilobular congestion and focal necrosis in the rat liver of chronic tramadol group pointing out the risk of increased hepatic damage due to long term use of tramadol. Hepatic stellate cells (HSCs) are located in the perisinusoidal space of Disse were first described by Kupffer in 1876 [19]. Activated HSCs proliferate vigorously and secrete a large amount of extra-cellular matrix which contributes to hepatic fibrosis in response to injury [20].

In the present study there were no thyroid significant histopathological changes in group II, and III after administration of Tramadol but in group IV there was follicular hypertrophy and hyperplasia with crowding of follicular epithelium nuclei and minimal protrusion into the follicular lumen in addition to mild congestion seen in group V. To our knowledge tramadol related thyroid histopathological changes were not recorded previously in rats, the results of the current study are not comparable to other researches

Declaration of Interest

The authors report no conflict of interest.

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