

Some Marker Enzymes and Histological Alteration on the Administration of Tramadol Hydrochloride on Rat Liver

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Abstract

The ease of availability and potential tendency to abuse tramadol hydrochloride prompted this research. Making use of biochemical and histological techniques, concentrations of marker enzymes were monitored along with alterations in the liver architecture of wistar albino rats in graded doses of tramadol hydrochloride for 28 days. Specifically, levels of gammaglutamyltransferase (γ -GT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Statistical evaluation of the results at a $p < 0.05$ elucidated significantly elevated values of these enzymes when compared with controls that were not on tramadol hydrochloride. The variations were time and dose dependent. Histological evaluation presented various degrees of inflammatory cells of the liver, cytolysis in hepatocytes and some portal tract hypertrophy. These findings support the need for caution in administration of tramadol particularly for long term administration.

Keywords

Marker Enzymes, Histological, Alteration, Tramadol

1. Introduction

Tramadol hydrochloride is a racemic mixture of two enantiomers that have two complementary mechanisms of action. Its use as pain reliever has earlier been investigated [1] and [2]. It is a synthetic opioids which has similar effects as codein. The mechanism of action of tradmadol hydrochloride radiates around binding to μ -opiate receptors in the central nervous systems which cause inhibition of the ascending pain pathways by altering the perception of and response to pains inhibiting the re-uptake of norepinephrine and serotonin that modifies

the ascending pathways.

Tramadol has been used in the treatment of pains such as low back pain, osteoarthritis and migraine. It also plays a role in the treatment of opiate withdrawal and premature ejaculation. The metabolism of tramadol occurs in the liver by the cytochrome P450 enzyme system and its byproducts are excreted through the kidneys. Tramadol absorption rate is high reaching 95% - 100% and the bioavailability increases to 100% when used in multiple doses. Tramadol hydrochloride is completely absorbed in the upper part of the small intestine, the plasma concentration of tramadol varies with its form, for example the absorption of capsules represents several hours but five hours for tablet [3]. Tramadol is widely distributed in the body through the lungs, spleen, liver, kidney and brain. The adverse effects of tramadol mostly noticed include: constipation, dizziness, headache, vomiting, hallucination, convulsion, serotonin syndrome and hyper sensitivity reaction.

Tramadol has a major role in the treatment of opiate withdrawal [4] and premature ejaculation [5]. Tramadol which is sold under the brand name ultram among others is named chemically as (\pm) cis-2 ((dimethyl amino) methyl)-1-(3-methoxyphenyl) cyclohexanol hydrochloride with a molecular weight of 299.8. The molecular formula is $C_{16}H_{25}NO_2$. Tramadol has two chiral centers in the cyclohexane ring, thus, four different stereoisomers exist designated as (1R, 2R), (1S, 2S), (1R, 2S), (1S, 2R). *Ab initio* tramadol was synthesized by Grunenthal GmbH (Germany) in 1962 through coupling of the corresponding cyclohexane with 3-methoxyphenyl magnesium bromide in a Grignard reaction [6]. In the recent past, the chemical synthesis of tramadol and two of its metabolites has been described by the same coupling reaction using organo lithium derivatives [7].

Tramadol is structurally similar with codeine as both have a 3-methoxy group on the phenyl ring and share o-demethylation—as a metabolic step, yielding metabolites with stronger μ -opioid agonist activity than the parent compound. The dimethyl amino methyl moiety of tramadol is structurally similar to the methylated ring nitrogen of morphine and codeine and forms an essential portion of the pharmacophore that interacts with μ -opioid receptor and monoamine transporters. N-demethylation yields metabolites that lack significant analgesic activity [8] and [9] [10]. It has been noted that the analgesic mechanism of action of tramadol includes both non-opioid components (noradrenergic and serotonergic components) [11]. Previous animal studies have not revealed a carcinogenic effect of tramadol as reproductive and developmental toxicity studies were negative [12]. However, [13] and [14] have reported few cases of fatal poisoning due to tramadol. This has been attributed to more frequent intoxication with co-ingestion of other drugs or alcohol. Meta analysis carried out in 2006 showed efficacy of tramadol in the treatment of neuropathetic pain [15]. Public health problems related to misuse, abuse and dependence of tramadol have been a source of concern in several countries [16].

Tramadol hydrochloride has been reported on parenchymatous toxicity [17]. [18] elucidated the effects of acute sub-lethal dose of tramadol on α 2-Adrenergic receptors and liver histopathology in rats. [19] reported on tramadol-induced biochemical toxicity. A work of [20] buttressed similar effects with elevated transaminases. In this work, we set out to determine the effect of tramadol use at various concentration overtimes.

2. Materials and Methods

2.1. Animal

A total of fifty-five (55) Wistar albino male rats each weighing between 160 - 435 grams were obtained from the animal house in the department of Pharmacology, Faculty of Basic Medical Science, Niger Delta University, Amassoma, Bayelsa State, Nigeria. All animals were maintained at standard condition of 2-hours light/darkness, humidity and temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in the Department of Medical Laboratory Science, Animal house, Niger Delta University. The animals were divided into 10 cages of 5 animals each and fed with standard Chow (Pfizer feeds, Plc, Nigeria) and water *ad libitum*.

The drug tramadol (Tramadol HCl) 50 mg capsules, B.P. was a product of Zim Laboratories Ltd., India).

The animals were grouped into 5 groups of 11 rats each. Group 1 served as control and was given only water throughout the study. Group 2, 3, 4 and 5 were administered oral doses of tramadol hydrochloride at 200, 300, 400 and 500 mg/kg body weight respectively for 28 days.

2.2. Sample Collection

Blood samples were collected on the 5th, 10th, 15th, 20th and 28th day after administration through the jugular vein with the aid of a syringe. Samples were dispensed into plain tubes and allowed to clot. The clotted samples were spun and serum was used for the analysis.

2.3. Histology

The liver was harvested in 10% formal saline for histological examination. The samples were sliced to facilitate complete fixative penetration. They were then processed with paraffin infiltration and embedded in paraffin. The formalin-fixed-paraffin embedded tissue blocks were cut with a rotary microtome (Leitz) into 4 - 6 μm thick and were placed on positively charged glass slide. They were dried and stained with haematoxylin/eosin.

2.4. Enzyme Assay

Gamma-glutamyltransferase (γ -GT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed enzymatically with Randox Kits (A product of Randox Laboratories, UK).

2.5. Statistical Analysis

Values obtained from the various analyses were evaluated statistically by two-way Analysis of Variance (ANOVA) followed by Turkey's post-hoc test.

3. Result

The biochemical assay of liver enzymes in serum alanine aminotransferase (ALT) aspartate aminotransferase (AST) and gamma glutamyl transferase (γ -GT) were evaluated in Wistar.

Albino rats after the treatment of tramadol hydrochloride at a dose of 200, 300, 400 and 500 mg/kg/b.w for 5, 10, 15, 20 and 28 days. The liver serum enzymes activity were recorded for ALT as shown in **Table 1**. The result revealed a significant increase ($p < 0.05$) in serum ALT in treated Wistar albino rats compared to control. There is a gradual significant increase ($p < 0.05$) in serum ALT in all treated groups with different dosage compared to control (see **Table 1**). The maximum increase in ALT (138.50 ± 9.19) was recorded in groups that was orally administered with 500 mg/kg/b.w tramadol hydrochloride. There was a significant decrease ($p < 0.05$) in serum ALT in all treated albino rat groups as the duration of drug administration increases.

Values are given as Mean \pm SD for each group. Superscript * indicate significant difference ($p < 0.05$) compared to Group 1 (Control). P: statistical level of significance was determined by two-way Analysis of Variance (ANOVA) followed by Turkey's post-hoc test.

Values are given as Mean \pm SD for each group. Superscript * indicate significant difference ($p < 0.05$) compared to Group 1 (Control). P: statistical level of significance was determined by two-way Analysis of Variance (ANOVA) followed by Turkey's post-hoc test. The serum AST activity significantly increased ($p < 0.05$) in experimental groups compared to control (**Table 2**). There is a significant increase ($p < 0.05$) in serum AST in all experimental groups with different dosage compared to control. The maximum increase in AST (290 ± 2.83) was recorded in group 5 with the administration of 500 mg/kg/b.w tramadol hydrochloride (**Table 2**). There is a significant decrease ($p < 0.05$) in serum AST in all groups as the duration of drug administration increases.

The result for assay of γ -GT revealed a significant increase ($p < 0.05$) in the experimental groups compared to control (**Table 3**). There was a gradual significant increase ($p < 0.05$) in serum γ GT in experimental groups with different dosage compared to control. The maximum increase in γ GT (208 ± 28.61) was recorded in group 5 with the administration of 500 mg/kg/b.w tramadol hydrochloride (**Table 3, Figure 1**). There was a significant decrease ($p < 0.05$) in serum GGT in experimental groups as the duration of drug administration increases (**Table 3**).

Values are given as Mean \pm SD for each group. Superscript * indicate significant difference ($p < 0.05$) compared to Group 1 (Control). P: statistical level of significance was determined by two-way Analysis of Variance (ANOVA) followed by Turkey's post-hoc test.

Table 1. Effects of different doses of tramadol hydrochloride on ALT values in Wistar albino rats.

Time of Drug Administration (Days)	Groups				
	Group 1 (Control)	Group 2 (200 mg/kg/b.w.)	Group 3 (300 mg/kg/b.w.)	Group 4 (400 mg/kg/b.w.)	Group 5 (500 mg/kg/b.w.)
5	83.00 ± 14.4	87.00 ± 7.07*	93.00 ± 9.90*	104.00 ± 5.66*	138.50 ± 9.19*
10	43.00 ± 1.41	60.00 ± 15.56*	64.00 ± 2.83*	72.00 ± 25.46*	76.00 ± 5.66*
15	29.00 ± 8.49	59.00 ± 5.66*	62.00 ± 2.83*	65.00 ± 1.41*	72.00 ± 18.39*
20	43.00 ± 1.41	60.00 ± 28.3*	64.00 ± 5.66*	76.00 ± 4.24*	78.00 ± 2.83*
28	34.00 ± 2.83	75.00 ± 7.07*	64.00 ± 7.07*	63.00 ± 1.41*	73.00 ± 2.83*

Table 2. Effect of different doses of tramadol hydrochloride on AST values in Wistar albino rats.

Time of Drug Administration (Days)	Groups				
	Group 1 (Control)	Group 2 (200 mg/kg/b.w.)	Group 3 (300 mg/kg/b.w.)	Group 4 (400 mg/kg/b.w.)	Group 5 (500 mg/kg/b.w.)
5	118.00 ± 22.63	120.00 ± 8.49*	189.00 ± 16.9*	278.00 ± 19.8*	290.00 ± 2.83*
10	90.00 ± 2.83	159.00 ± 22.63*	160.00 ± 9.90*	189.00 ± 12.7*	196.00 ± 5.66*
15	59.00 ± 14.14	152.00 ± 26.87*	159.00 ± 7.07*	176.00 ± 5.66*	234.00 ± 19.8*
20	59.00 ± 4.24	147.00 ± 7.07*	156.00 ± 11.3*	176.00 ± 14.1*	186.00 ± 5.66*
28	76.00 ± 16.97	147.00 ± 18.39*	152.00 ± 4.24*	131.00 ± 2.83*	182.00 ± 8.49*

Table 3. Effect of different doses of tramadol hydrochloride on γ GT values in Wistar albino rats

Time of Drug Administration (Days)	Groups				
	Group 1 (Control)	Group 2 (200 mg/kg/b.w.)	Group 3 (300 mg/kg/b.w.)	Group 4 (400 mg/kg/b.w.)	Group 5 (500 mg/kg/b.w.)
5	46.32 ± 2.38	41.50 ± 1.84*	39.20 ± 2.54*	41.17 ± 1.26*	43.14 ± 0.03*
10	42.20 ± 3.11	34.74 ± 1.75*	127.38 ± 24.3*	173.70 ± 33.23*	208.44 ± 28.61*
15	46.32 ± 2.38	138.98 ± 24.82*	104.22 ± 14.45*	146.30 ± 27.58*	181.06 ± 15.84*
20	57.90 ± 12.30	92.64 ± 20.03*	104.14 ± 21.21*	140.30 ± 27.86*	169.48 ± 14.42*
28	28.95 ± 24.56	40.53 ± 3.96*	52.11 ± 4.26*	63.69 ± 4.45*	75.27 ± 2.59*

The result also revealed a significant increase ($p < 0.05$) in serum AST/ALT ratio in all treated Wistar albino rats in group 4 (400 mg/kg/b.w.) and group 5 (500 mg/kg/b.w.) as compared to control (**Table 4**). The maximum increase in AST/ALT ratio (3.30 ± 0.57) was recorded in group 5 with the administration of 500 mg/kg/b.w. of tramadol of hydrochloride.

Values are given as Mean \pm SD for each group. Superscript * indicate significant difference ($p < 0.05$) compared to Group 1 (Control). P: statistical level of significance was determined by two-way Analysis of Variance (ANOVA) followed by Turkeys' post-hoc test.

Table 4. Effect of different doses of tramadol hydrochloride on AST/ALT ratio in Wistar albino rats.

Time of Drug Administration (Days)	Groups				
	Group 1 (Control)	Group 2 (200 mg/kg/b.w.)	Group 3 (300 mg/kg/b.w.)	Group 4 (400 mg/kg/b.w.)	Group 5 (500 mg/kg/b.w.)
5	140 ± 0.00	1.35 ± 0.21	2.05 ± 0.40	2.65 ± 0.35*	2.10 ± 0.14*
10	2.10 ± 0.14	2.70 ± 0.28*	2.50 ± 0.41	2.85 ± 1.20*	2.60 ± 0.14*
15	2.20 ± 1.13	2.55 ± 0.21	2.60 ± 0.28	2.70 ± 0.14*	3.30 ± 0.57*
20	1.35 ± 0.07	2.45 ± 0.07*	2.45 ± 0.35*	2.35 ± 0.07*	2.40 ± 0.14*
28	2.30 ± 0.70	2.00 ± 0.42	2.35 ± 0.21*	2.10 ± 0.14*	2.50 ± 0.14*

The various dosage of tramadol hydrochloride (200, 300, 400 and 500 mg/kg/b.w.) in the 28th day of drug administration had different morphological histopathological effect on the liver. Its effect increased as the concentration of the doses rises. The characteristics features of the liver damage in the Wistar albino rats were observed in zone 3 of the hepatic parenchyma of liver lobule (centri-lobular area) that surround the central vein.

The examination of liver specimens taken from the control showed normal hepatocytes, central vein and blood vessels. The Wistar albino rat liver in group 2 revealed vacuolation and congestion of the central vein with inflammatory cells (**Figure 2**).

The histological changes in the Wistar albino rat liver in group 3 revealed acidophilic body, central vein congestion and migration of inflammatory cells within the central vein and blood vessels.

Similar to those in group 3, the liver of the Wistar albino rat in group 4 showed cytolysis and portal tract hypertrophy with macrophages. The tramadol hydrochloride treated group 5 showed centrilobular necrosis and migration of inflammatory cells.

4. Discussion

Tramadol hydrochloride is a centrally acting analgesic drug that is prescribed for moderate to severe pains [21]. Nowadays tramadol hydrochloride abuse is becoming more popular among youths and adults in most countries worldwide.

In the present study, biochemical analysis of the liver enzymes revealed significant increase in serum AST, ALT and γ -GT levels in tramadol treated Wistar albino rat groups when compared with control group. These results were in tandem with the findings of [22], who reported increase in AST and ALT activities on mice after administration of different doses of tramadol hydrochloride for fourteen days. In this work, we have doubled the duration of time. [17] had earlier reported an increase in AST, ALT and γ -GT activities on albino rats when studying the parenchymatous toxicity of tramadol hydrochloride on the liver for two weeks.

Similar findings was also reported with the application of morphine like agent

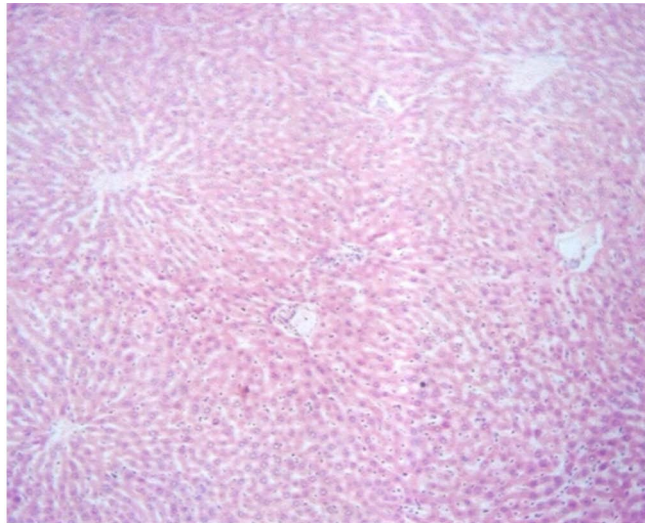


Figure 1. Section of the liver of Wistar albino rats (Control group) showing normal hepatocytes, central vein and blood vessels.

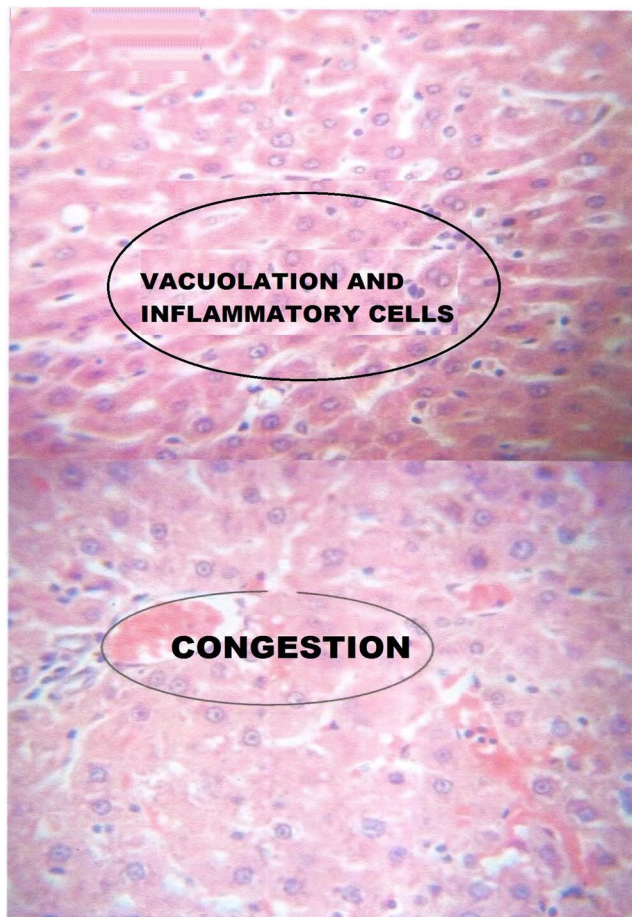


Figure 2. Sections of liver of Wistar albino rats administered with 200 mg/kg/b.w of tramadol hydrochloride showing vacuolation and congestion of the central vein with inflammatory cells.

Levo-alpha-acetylmethadol HCL (LAAM) and morphine for long time in rats [23]. The liver enzymes are normally found in blood circulation in small amount due to hepatic repair and growth. The serum AST, ALT, and γ -GT activities in the blood circulation significantly increase ($p < 0.05$) in experimental groups administered tramadol hydrochloride and the maximum elevation occurs in group 5 administered with 500 mg/kg/b.w. which was the highest dosage. The increase in the liver enzymes especially in group 5 could be accounted for due to alteration, malfunctioning and damage of the parenchymatous tissue of the liver.

In this study, it was also revealed that the higher the dosage of tramadol hydrochloride administered, the higher the secretion of the liver enzymes in circulation and at the same time indicated the severity of the liver damage. This is also in agreement with the findings of [20] who reported the effects of tramadol on histopathological and biochemical parameters in male rabbits.

We further observed a significant decrease ($p < 0.05$) in serum liver enzymes (AST, ALT and γ -GT) in all treated albino rat groups as the duration of drug administration increases. Our attention was not drawn to previous work on this aspect which necessitated the need for further research. It is our view that the decrease in the liver enzymes as the duration of drug administration proceeds may be due to severe or terminal liver failure that resulted to the inability of the liver cells to produce enzymes. The severe liver failure may be due to prolonged

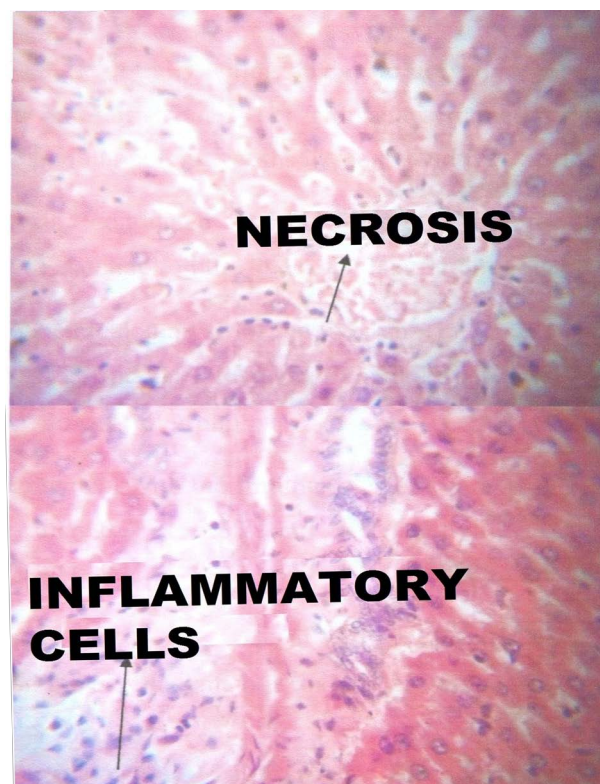


Figure 3. Section of the liver of Wistar albino rats administered with 500/mg/kg/b.w tramadol hydrochloride showing centrilobular necrosis and migration of inflammatory cells.

administration of tramadol hydrochloride which is contrary to the normal dosage and timing of the drug that was supposed to be seven (7) days of administration.

Histological evidence support this assertion when compared with the normal liver features (Figure 3) as histological examination of the liver tissue in group 2 displayed vacuolation and congestion of the central vein with inflammatory cells (Figure 2). Group 3 and group 4 displayed central vein congestion, migration of inflammatory cells within central vein and blood vessels, cytolysis and portal tract hypertrophy with macrophages, while group 5 also showed centrilobular necrosis and migration of inflammatory cells when both groups are compared to control (see Figure 4).

This result is largely in agreement with the findings of [24], who reported that tramadol administration in adult male rats for one month was accompanied by hepatic congestion, hemorrhage and necrosis. Similar findings was also reported by [25], who revealed loss of architecture, congested central veins, expanded portal area with edema and inflammatory reaction in rats treated with tramadol.

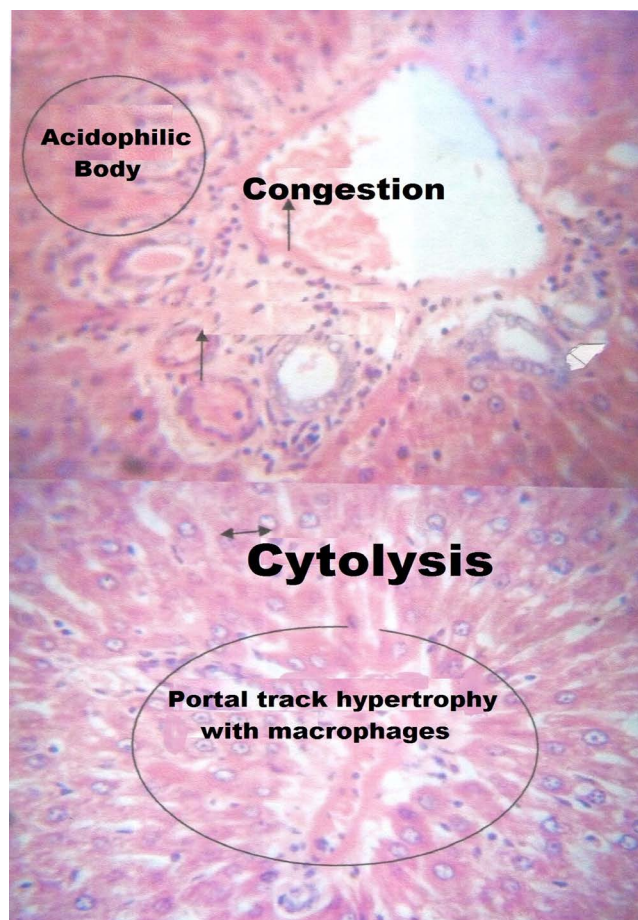


Figure 4. Section of the liver of Wistar albino rats administered with 300/400 mg/kg/b.w of tramadol hydrochloride showing homogenous pink structure (acidophilic body), central vein congestion and migration of inflammatory cells within the central vein and blood vessels.

In addition, our results further epitomize the work of [26], who emphasized that treatment of tramadol is more harmful to the liver and causes a serious cellular toxicity and a liver failure. The liver histopathological changes in this study proved the toxic effect of tramadol hydrochloride on the liver of Wistar albino rats. The hepatic histopathological results in this study pointed out the risk of increased hepatic damage due to long time abuse of tramadol.

5. Conclusion

In conclusion, tramadol hydrochloride treatment in Wistar albino male rats has a toxic effect on the structure and function of hepatic tissue. Therefore, it is suggested that due to the toxic effect of tramadol hydrochloride as shown in this study, the absolute need to exercise caution in its administration and use is strongly advised.

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