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Effects of Tramadol on Histopathological and Biochemical Parameters in Mice (*Mus musculus)* Model

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Abstract: The present study was conducted to determine the histopathological and biochemical changes in liver due to injection of tramadol in albino mice (*Mus musculus*). For this purpose, forty albino mice (25-30g) were divided into four groups, each group carried ten mice (three experimental and control group). Experimental groups (B, C and D) were injected tramadol intramuscularly equal to 12.5 mg, 25mg and 50 mg/kg body weight/day respectively for fourteen days. Biochemical analysis indicated that the levels of serum aminotransferase (ALT, AST) significantly ($P < 0.05$) increased than the control group. Similarly creatinine and blood urea nitrogen (BUN) were also increased significantly ($P <0.05$) in the experimental groups than control. The histopathological studies indicated the necrosis, vacuolization, central vein dilation, hemorrhage, cytolysis and complete cell membrane degeneration in hepatocytes in the treated groups. Therefore it is recommended that tramadol should be taken only with the prescription of doctor and self medication of this medicine may be hazardous.

Key words: Tissue · Liver · Necrosis · AST · Creatinine

INTRODUCTION

Opioids are used as analgesics and considered effective for the treatment of acute cancer and non-cancer chronic pain [1-3]. Tramadol hydrochloride (2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol) is a synthetic opioid analgesic of the aminocyclohexanol [4]. Tramadol is a centrally acting analgesic drug, widely used in the treatment of pain. Its efficiency and potency ranges between weak opioids and morphine [5-7].

Tramadol metabolism occurs in liver by the cytochrome p450 enzyme system and by-products are excreted through kidneys. Its biotransformation occurs in the liver, firstly by the phase I reactions (mainly O- and N-demethylation) and secondly by the phase II reactions (mainly conjugation of O- and N-demethylated compounds), in turn eleven and twelve metabolites are produced respectively [8, 9]. The main metabolite is O-desmethyltramadol (M 1) produced by the activity of Sparteine oxygenase (CYP2D6) enzyme which is pharmacologically active metabolite [10, 11]. It has marvelous analgesic activity and shows higher affinity for μ-opioid receptors [12, 13]. (+) M 1 has 300-400 times greater affinity for μ-opioid receptors than tramadol itself [14], whereas (-) M 1 mainly inhibits noradrenalin reuptake and has weaker opioid and á$_{2}$- adrenoceptor compound. The metabolism of tramadol to M1 is very slow in man and it is not more than 25% in serum concencentration than tramadol [15, 16]. O, N-didesmethyltramadol (M5, has weaker analgesic activity than M 1), while other metabolites are pharmacologically inactive. CYP2D6 enzyme (sparteine oxygenase) of cytochrome P450 in the liver is inhibited by quinidine, a selective inhibitor of this enzyme [4].

The absorption of tramadol is 95-100% and the bioavailability is 70% and bioavailability of tramadol is more than morphine (15-65%). When tramadol is used in multiple doses, the bioavailability increased to 100%. The complete absorption of tramadol takes place in the upper part of small intestine. The plasma concentration of tramdol vary with its form, for instance the use of

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capsules represent peak plasma concentration after two hours but five hours for tablets [17, 18]. It is widely distributed throughout the body of the animal, especially in the lungs, spleen, liver, kidneys and brain [19]. Binding to plasma proteins is in the region of 20%. Typical plasma levels of tramadol in humans, following the administration of recommended doses, are of the order of 10µ M [20]. Tramadol crosses the placenta and in lactating mothers only 0.1% of the dose passes into breast milk [21].

There is an extreme dearth of literature with reference to Tramadol influence on animal body in Pakistan. However some studies are available on other antibiotics (22, 23). Hence the present study was designed to highlight the impact of tramadol on biochemical changes, their effects on kidney functions and histopathology of liver in animals in district Gujrat, Pakistan.

MATERIALS AND METHODS

The present study was conducted to evaluate the effect of Tramal® 100 ((+) cis-2- [(dimethylamino) methyl]-1-(3-methoxyph-enyl) cyclohexanol hydrochloride) injection (Tramadol HCl ph. Eur.), manufactured by Grünethal GmbH, Germany on liver tissues and biochemical changes were assessed, in the histopathology laboratory, Department of Zoology, University of Gujrat, Gujrat, Pakistan.

Swiss albino mice (*Mus musculus*) were collected from Veterinary Research Institute (VRI) Lahore weighing between 25-30 grams. The animals were divided into four groups (n= 10 each group). The mice were kept in a quite non-stressful environment, provided with food *ad libitum* (a frequent food availability) and free access to water. A total of four groups of albino mice, of which three experimental groups B, C and D were administered 12.5 mg/kg, 25 mg/kg and 50 mg/kg tramadol intramuscularly for fourteen days respectively and one control group. Both experimental and control groups of mice were dissected after fourteen days tramadol treatment, blood and liver tissues were collected.

The biochemical parameters were studied by Blood was collected into glass vials with rubber caps, labeled and centrifuged at 4000 g for 10 minutes. Sera were separated and kept at -20°C until analysis. Serum enzymes ALT (Alanine Aminotransferase) AST (Aspartate Aminotransferase) [24], serum creatinine [25] and blood urea nitrogen (BUN) [26] (were determined by chemistry analyzer (Biolab 200) by kinetic and fixed endpoint method using commercially available kits (Human GmbH-65205 Wiesbaden-Germany), at 37°C. Absorbance. The average absorbance per minute for ALT, AST was determined as: $\Delta A \times 1746$= ALT or AST activity at U/L at 340nm. The collected suitable pieces of liver tissues were fixed in Bouine's fixative, labeled and kept in glass vials. Later on removed from Bouine's fluid, washed with 70% alcohol, till removal of Bouine's solution i.e. complete discoloration. The tissues were placed in the 90% alcohol for dehydration and then preserved in absolute alcohol. The serial sections of 7µ tissues were made with the help of Microtome. Hematoxylene and eosin stained slides were prepared using standard protocol. The slides were examined under the light Microscope (40X) for histological variations [27].

Statistical Analysis: The data were expressed as means ± standard errors. The data of different treatments were compared using Analysis of Variance (ANOVA) in SPSS (Version: 16). When the F-test was found significant different ($p< 0.05$), then means of treatments were compared using LSD (Least Significance Difference) test.

RESULTS AND DISCUSSIONS

Biochemical Analysis: The biochemical parameters viz. Serum enzymes ALT (alanine aminotransferase) AST (aspartate aminotransferase) serum creatinine and blood urea nitrogen (BUN) were recorded in the albino mice after the treatment of tramadol. The serum ALT activity significantly increased ($p< 0.05$) in experimental groups administrated with different doses of Tramadol (12.5mg/kg, 25mg/kg and 50mg/kg) compared to control group. However maximum increase in ALT (202.8 ± 5.8 U/L) was recorded with the administration of 25mg/kg tramadol (Table 1). The increase in the level 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 [28]. Furthermore a significant elevated level of ALT has been found in rats receiving morphine and tramadol for longtime compared to control group [29]. Similar results has also been documented in the rats treated with morphine-like agent levo-alpha-acetylmethadol HCl (LAAM) and in chronic heroin users [30,31].

Aspartate aminotransferase (AST) found in liver, heart, skeletal muscle, kidney, brain and red blood cell. It helps in the catabolism of amino acids, ultimately entered in the citric acid cycle. Furthermore acute viral or ischemic or toxic liver injury is also responsible for increased level of AST along with ALT. However both chronic hepatitis and cirrhotic patients may have aminotransferase levels within the reference range [32]. The present study revealed the significantly higher

Table 1: Effect of different treatments of tramadol on selected Haemotological parameters.

Biochemicals	Control	Tramadol(12.5mg/kg)	Tramadol(25mg/kg)	Tramadol(50mg/kg)
ALT(U/L)	34.3 ± 1.1^{d}	92.6 ± 2.6^{b}	202.8 ± 5.8^{a}	85.5 ± 2.1^{c}
AST(U/L)	187 ± 15^{c}	456 ± 18^{a}	664 ± 57^{b}	401 ± 24^{a}
BUN (mg/dl)	26.5 ± 1.2^{b}	51.2 ± 1.7^{a}	50.2 ± 3.4^{a1}	44.5 ± 5.6^{a}
Creatinine (mg/dl)	0.55 ± 0.05^{b}	1.08 ± 0.09^{a}	0.98 ± 0.06^{a}	0.90 ± 0.12^{a}

Mean with different superscript differ significantly (p<0.05)

(p< 0.05) level of AST in all the experimental groups of mice compared to control group and highest level AST (664 ± 57 U/L) was found with the administration of 25mg/kg tramadol (Table 1). A significant increase found in the level of AST in rats treated with tramadol (40 mg/kg and 80 mg /Kg) [33]. Similar findings are also reported with the application of morphine-like agent levo-alpha-acetylmethadol HCl (LAAM) and morphine for longtime in rats [29, 30].

The assessment of serum creatinine and blood urea nitrogen (BUN) is highly important to determine the kidney function in the clinical setting. Moreover the serum creatinine level is not largely affected by extra-renal factors compared to BUN level [34]. It has been documented that increased creatinine level is not only the measure of renal functions but also generation, intake and metabolism of creatinine. Moreover it helps to assess the glomerular filtration rate (GFR). Therefore laboratory evaluation of serum blood creatinine and BUN levels are considered "standard fare" in the determination of renal functions [34]. In the present study, the maximum increase in creatinine (1.08 ± 0.09 mg/dl) and BUN (51.2 ± 1.7 mg/dl) were recorded with tramdol (12.5 mg/kg) injection in the mice, as well as experimental groups were significantly different (p< 0.05) from control group. Increase in BUN and creatinine levels was noticed in rats receiving morphine and long- term use of LAAM [29, 30, 33].

Histopathological Studies: The different level of tramadol doses (12.5 mg/kg, 25mg/kg and 50mg/kg) had varied adverse effect on morphology and histopathology and its effect increased with the rise in dose quantity. The characteristic features of liver damage in mice were observed largely in the perivenular area (also called centrilobular zone). However in control group (A Group) the liver tissue were found normal (Fig. 1).

The size of liver was found normal in group B (12.5 mg/kg), the color was slightly changed, although there was no signs of cirrhosis. However in liver, central vein dilation and vacuolization was observed (Figure 2). Similarly mice treated with morphine and tramadol for long time, but in their studies the histopathological changes were more pronounced in the morphine group than the tramadol [29].

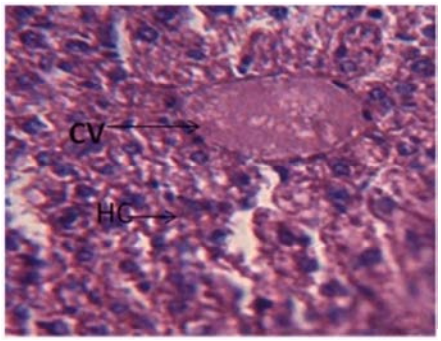

Fig. 1: Cross section of liver of group A (control) showing normal central vein (CV) and hepatocytes (HC) (hematoxylin-eosin × 40 original magnification)

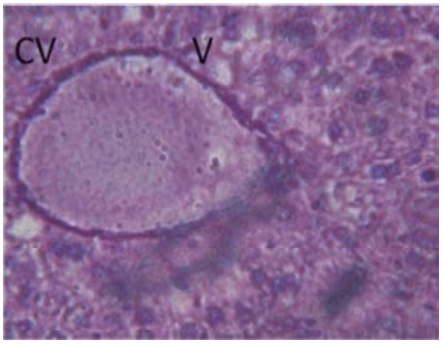

Fig. 2: Cross section of liver showing dilation of central veins(CV) and vacuolization (V) (hematoxylin-eosin × 40 original magnification)

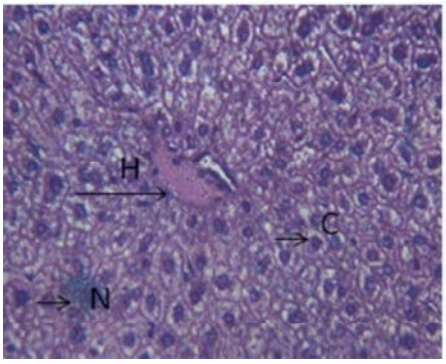

Fig. 3: Cross section of liver showing necrosis (N), hemorrhage (H) and cytolysis (C) (hematoxylin-eosin × 40 original magnification)

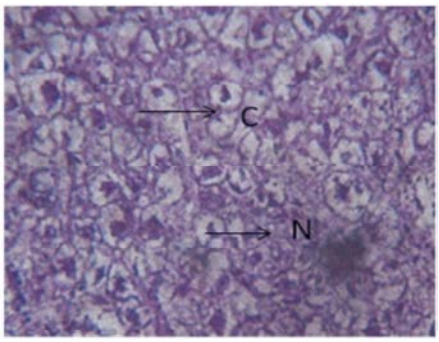

Fig. 4: Cross section of liver showing necrosis (N) and cytolysis (C) (hematoxylin-eosin × 40 original magnification)

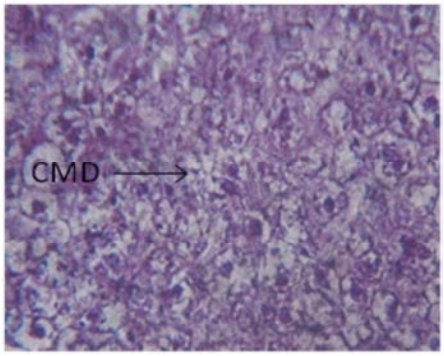

Fig. 5: Cross section of liver showing complete cell membrane degeneration (CMD) of hepatocytes (hematoxylin-eosin × 40 original magnification)

The mice liver in group C (tramadol = 25mg/kg) was found a little enlarged and cirrhotic. Furthermore the histopathological changes in liver were revealed necrosis, hemorrhage and cytolysis (Figure 3). The metabolites produced as result of drug metabolism has little pharmacological activity and can be easily removed from the body [36]. Yet the metabolites may be more toxic than the parent drug [37]. For instance morphine can produce hepatotoxic effects during its metabolism [38] and Necrosis, hemorrhage and cytolysis were also documented in the morphine treated rats [29]. But these histopathaloguical changes (Necrosis, hemorrhage and cytolysis) were not recorded previously in rats and the present study first time recorded these effects of tramadol in mice.

Like the group C, the liver in group D (50mg/kg), also showed slightly enlarged and cirrhotic conditions. Moreover, histopathological changes viz. necrosis and cytolysis were found in mice liver as well as complete cell membrane degeneration of hepatocytes was also recorded in this group (Figures 4, 5). Previously cell death was documented to the isolated hepatocytes of rats when treated with morphine [39] Similar results were demonstrated with the long term treatment of morphine, however they noted only perivenular hydropic degeneration in rats liver with treatment of tramadol [29].

CONCLUSIONS

Previous Surveys and case reports revealed that tramadol drug is safe and have no side effects in case of chronic non-malignant nociceptive and neuropathic pain. Whereas the present study revealed that tramadol has damaging effects on liver tissues, moreover various chemical changes also occurs due to the use of this drug. Therefore it is suggested that tramadol as reported more effective in pain management, yet its toxic effects should be kept in mind.

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