Toxicity of Liver and Kidney Induced by Different Concentrations of Tramadol in Young and Adult Mice

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Abstract:
It is thought that Tramadol TM, a synthetic opioid analgesic agent, is low in abuse and has no side effects if its total daily dose doesn’t exceed 400mg. This work aims to study the pathohistological and physiological toxicity effect of tramadol in different concentrations, equivalent to less than 400 mg, on liver and kidney. Forty-eight male Balb/c mice were used in this study and divided into two age groups (n=24 mice); the first was an adult aged 8-9 weeks and the other was young aged 4-5 weeks. Every age group was divided into three subgroups (n=8 mice). The first group consumed a high concentration of TM, 40 mg TM/kg body weight daily which equivalent about 421.2 mg TM/65kg body weight. The second group was consumed a low concentration of TM, 20 mg/kg daily. The last group consumed only water ad libitum and served as a control group. After one month, the mice were sacrificed. Blood samples were collected to separate the serum and used to determine; kidney function tests (S.Cr and B.U) and liver function tests (GOT, GPT, and ALP). Kidney and liver were collected and put in 10% formalin for pathohistological study. GPT, ALP, B.Ur and S.Cr levels were significantly higher in the young and adult mice consumed TM compared to control while there was no different in GOT level. Comparable results were found in the pathohistological study of kidney and liver section. The infiltration of inflammatory cells and its aggregation were detected within the kidney and liver tissue of adult and young mice group that consumed TM. These changes were severing in the mice consumed TM high concentration compared to lower concentration. As a conclusion, Tramadol could have dangerous side effects even when its total dose doesn’t exceed 400mg daily. These side effects didn’t interact with age.

Key words: Tramadol, liver function, kidney function, mice, and histopathology.


Introduction:
One of the synthetic opioid analgesic agents is tramadol which used intravenously and orally to treat mild to severe pain. Its Dwelling work mechanism is complicated (1). Many research reported that tramadol mechanisms, non-opioid and opioid caused sedative and its other clinical effects (1-3). TM is associated with μ-opioid receptors, although it is much weaker compared to morphine (1). It also prevents nerve uptake of serotonin and norepinephrine exactly like action of anti-depressants for example; amitriptyline and desipramine (4). It is metabolized in the liver and excreted by the kidneys. Biomagnification takes place in the liver and lades to O-desmethyltramadol. This substance is more effective about 2 to 4 times compared to tramadol itself (5-7). In addition, biotransformation produces inactive products, also excreted by the kidneys (8, 9). TM has a dose-based analgesic effect located between the efficacy of codeine and morphine, about 10 to 20% of standard morphine (10). So the dose of tramadol must be regulated depending on the severity of the pain. Total daily dose must not override 400 mg in therapeutic levels of blood between 0.1 to 0.8mg/l (11). The side effects associated with medication are an earnest problem for patients and providers of health care (12). It is predestined about 10% of the drugs associated with undesirable intense side effects (13). Almost every drug is associated with liver toxicity due to the primary
role of the liver in drug metabolism (14). Drug Metabolites excreted from kidneys may cause renal cellular damage (15). It is thought that TM is low in abuse and has no side effects, such as drug addiction only the overdose of TM may result in depression of central nervous system and respiratory, vomiting and nausea, irregular heartbeat, coma, seizures, and heart and vascular collapse (16). A long term consumption of tramadol for the administration of pain, as long its use as a possible alternative for individuals seeking the behavior of drug, is dialectical. Nevertheless, the effects of tramadol at a cellular level aren’t clearly understood (17, 18). This work aimed to study the toxicity effect of tramadol in different concentrations, equivalent to less than 400 mg, on liver and kidney pathohistologically and physiologically.

Materials and Methods

Forty eight Balb/c male mice, obtained from the preventive research center/ Baghdad/ Iraq, were used in this study. Twenty four mice were adult aged 8-9 weeks and the other twenty four mice were young aged 4-5 weeks. Every age group was divided to three groups (n=8 mice). The first group was consumed a high concentration of TM, 40 mg TM/ kg body weight daily which equivalent about 421.2 mg TM/ 65kg body weight according to Aghili (19). The second group was consumed a low concentration of TM, 20 mg/kg daily. The last group consumed only water ad libitum and served as control group. After one month of consuming, the mice were sacrificed by cervical dislocation. Blood samples were collected from the eyes in sterile tubes. The kidneys and liver were collected and placed in a 10% formalin solution and processed through standard procedures. Tissue sections containing paraffin were stained with hematoxylin and eosin and examined by light microscopy (20). Blood samples were centrifuged 10min at 10000 rpm. The serum was collected and used to determine; kidney function tests (serum creatinine and blood urea by the creatinine and urea kit from linear chemicals, S.L.U., Spain), and liver function tests (serum glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, and Alkaine phosphate by the GOT, GPT, and ALP kit from linear chemicals, S.L.U., Spain). Results were expressed as mean ± standard error (M±SE). These data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher's test for multiple comparisons, using Statview version5.0. Differences were considered significant when p<0.05

Results

Concerning with the histological study of organs in Adult and young groups are the same, so the figures were shows the tissues sections of organs in mice consumed higher concentration of TM (H) and in mice consumed lower concentration of TM (L). Figure 1 show the effects of TM in the liver, where the infiltration of inflammatory cells and aggregation around the blood vessels were detected especially in the liver of H mice group. In the kidney sections, the infiltration of inflammatory cells and aggregation around the blood vessels or in the parenchyma of the kidney were detected and also necrosis were noted, but these changes were also more severe in H mice group (Fig. 2).
Figure 1: liver sections of control group (C), mice consumed TM high concentration (H), and mice consumed TM low concentration (L). (H&E stain 400 xs).

- Infiltration and aggregation of inflammatory cells around blood vessels.

Figure 2: Kidney sections of control group (C), mice consumed TM high concentration (H), and mice consumed TM low concentration (L). (H&E stain 400 xs).

- Infiltration and aggregation of inflammatory cells.
- Necrosis.
While there was no different in GOT level, GPT and ALP levels were significant higher in the young and adult mice consumed TM compared to control which was more significant higher in the mice consumed Thigh concentration compared to lower concentration (fig. 3). Comparable results were found in the Blood urea (B.Ur) and serum creatinin (S.Cr) which were significant higher in the young and adult mice consumed TM compared to control. (fig. 4).

**Figure 3:** GPT (A), GOT (B), and ALP (C) levels in the young and adults mice consumed TM high concentration and low concentration and in control group.

*significantly difference between treated groups and control, # significantly difference between high and low group

**Figure 4:** B.Ur (A) and S.Cr (B) levels in the young and adults mice consumed TM high concentration and low concentration and in control group

*significantly difference between treated groups and control, # significantly difference between high and low group

**Discussion**

Analgesics are the most over the counter medicines worldwide. Tramadol hydrochloride, an artificial match of codeine, is a central analgesic (1). After tramadol introduced in the 1970s, it acquired considerable attention because it is described as mild to severe pain (6). Tramadol is now more common among adolescents in most countries around the world as an alternative to drugs because of the difficulty of obtaining it (21). Therefore, this study was carried out to investigate the

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pathological and physiological features of chronic toxic effects of tramadol hydrochloride in kidney and liver function on adult and young male mice.

In this work, there was many histopathological changes in the liver section of mice consumed TM which have also significant high level of GPT and ALP. Our findings can be demonstrated by the fact that the liver is responsible for tramadol metabolism and excretion (22). Liver mainly metabolizes tramadol hydrochloride by removing the methyl from O and N, followed by conjunction with glucuronic acid and sulfate. O-desmethyltramadol is the active metabolite which shows a higher affinity for µ-opioid receptors and has a lower affinity for original drugs (22). Many studies have shown that the tramadol effects on hepatic anatomy. These findings suggest that tramadol may result in some liver tissue changes (23-25). The changes of liver histopathological were supported by the results of liver function indicators. EL-Gaafarawi (24) recorded a marked increase in GPT and GOT levels in rats after taking tramadol. Adikwu (25) have also reported a significant increase in GOT, GPT, and ALP in rats consumed tramadol. GOT and GPT tests are extremely useful for liver damage or injury measures from different types of disease (26). GPT is primarily found in the liver, but the GOT is naturally present in a variety of tissues including the kidneys, liver, muscles, heart, and brain. GOT enter the blood and elevated when each of these tissues are damaged while GPT enter bloodstream and elevated only when liver is damage. So GPT test is more specific in the liver disease than (26). These could explain the absence of any change in GOT level in mice consumed tramadol in this study.

Current study shows many pathohistological changes in the kidney section of mice consumed TM. Tramadol toxicokinetics process could explain these changes. Where 30% of the drug is remain unchanged while the remainder is changed to active metabolites by the liver. Both unchanged and active metabolites excreted by kidney which might result in cellular damage cause dysfunction of kidney (27) especially the active metabolites. The pathohistological outcome of kidney was supported by the results of renal function indicators in this study. In clinical cases, S.Cr level is widely used to estimate the rate of glomerular filtration as a pointer to evaluate renal function (28). B.Ur is an amportant clinical parameter for assessing the renal toxicity of vital aliens (29). Evaluation of S.Cr and B.Ur levels can be correlated with the functional state of the kidney (30). Observations in the current study showed increased levels of S.Cr and B.Ur in mice consumed TM. This result is also consistent with the results of Atici (18), EL-Gaafarawi (24), and Adikwu (25). These results are a sign of renal toxicity that may cause a decrease in glomerular filtration rate resulting in the accumulation of S.Cr and B.Ur in the blood (25).

The above observations can be confirmed by Wu's (6) suggestions that kidneys and liver are responsible for tramadol metabolism and excretion, which might result in renal and liver toxicity. As conclusion, Tramadol could cause liver and renal toxicity even when its total dose doesn’t exceed 400mg daily. This toxicity didn’t interaction with the age.

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