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Effect of tramadol on liver enzymes, oxidative stressm and some antioxidant markers in male rabbits

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***Abstract*---**An overdose of tramadol is a frequent reason for hepatic and renal toxicity and possible death.Hence, this study aimed at investigating the impact of tramadol on serum liver enzyme, oxidative stressm and som antioxidant markers in male rabitts. Methods**:** A total of 20 growing rabbits (7.5 weeks old) reared under high ambient temperature were divided into two equal groups, 10 rabbits each. The first group control administered with normal saline and the second group tramadol-treated rats (50 mg/kg b.w. orally) for 30. Blood samples were withdrawn to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Glutathione (GSH), malondialdehyde (MDA) levels and catalase (CAT), activities were assayed. Results: In tramadol-treated group, significant increases in aspartate aminotransferases, and alanin aminotransferases activities, malondialdehyde (MDA) levels and significant decreases in catalase (CAT), glutathione activities levels were determined compared to the control group. Conclusion: tramadol administration produces noticeable biochemical changes in a dosedependent manner associated with increased liver enzyme and oxidative stress markers and decreased antioxidative activity.

***Keywords*---**Tramadol, malondialdehyde, hepatic toxicity, GSH.

Introduction

Tramadol hydrochloride (tramadol HCl) is one of the centrally acting opioid drugs that is extensively used parenterally and orally as a pain reliever moderate to severe pain caused by cancer, osteoarthritis, and other musculoskeletal

diseases.It is a synthetic analogue of codeine with central effects. It is not an opioid derivative or Non-Steroidal Anti-Inflammatory Drug (NSAID) medication. Actually, tramadol has low affinity for opioid receptors[1-4]. Today, Tramadol has become one of the most widely used drugs globally, Liver is one of the largest organs in the body. It has many important metabolic functions. Liver tissue has a relatively large amount of enzymes activity and alteration of various enzymes in hepatitis[5,6]**.** the liver is responsible for the metabolism of tramadol. Therefore, may cause hepatotoxicity during chronic administration[7-9].

Within the liver, tramadol is metabolized into *O*- and *N*-demethylated five different metabolites *via* the action of cytochrome P450. *O*-desmethyltramadol is the most significant metabolite and has 200 times the μ-opioid receptors affinity of the parent tramadol molecule and its elimination half-life is 9 h[10]. Oxidative and reductive stress are dual dynamic phases experienced by the cells undergoing adaptation towards endogenous or exogenous noxious stimulus. Tramadol and/or its active metabolite may produce excessive release of reactive oxygen species leading to single-strand or double-strand DNA breaks. The accumulation of DNA strand breaks is a well-established stimulus for p53 activation. In addition, oxidative damage of proteins involved in cell cycle regulation or DNA repair may contribute to accumulating DNA damage and finally to activation of p53, which, in turn, mediates either DNA repair or apoptosis[11-14].

Material and Methods

Twenty healthy male rabbits weighting about (1.5–2.3 kg) g with an average age of 75±5 days. Purchased from center for experimental animals, Faculty of Veterinarian Medicine, Tikrit University were used in the study. This study was performed in accordance with the Guide for the ethical care and use of laboratory animals. They were left to acclimatize for 1 week. They were housed at room temperature in metallic cages and were kept under constant healthy environmental and nutritional conditions. Animals were kept under a schedule of diurnal lighting conditions (12 h of darkness and 12 h of light); they were fed on ordinary food and housed under standard laboratory conditions.

The animals were divided into 10 cages of 5 animals each

Group I (positive control group) with saline: Each rabbits received 1 ml normal saline/day, orally for 30 days.
Group II (tramadol-treated group): 10 rabbits treated with tramadol (50 mg/kg/day), orally for 30 days.
Blood samples (5 ml) were collected through retro-orbital puncture ,serum samples were prepared for biochemical assays by centrifuging for 10 min at 3000 rpm. All serum samples were kept at −80°C until the assays were performed. Aspartate aminotransferase (AST), ALP, and alanine aminotransferase (ALT) were measured by using kits from product of Randox Laboratories, UK).Serum SOD, GSH, MDA, G-Px, and catalase levels were measured by spectrophotometric kit,

Statistical evaluation

Data was expressed as mean ± SD. Differences between groups were compared by ANOVA using the SPSS software (version 16). A p-value of less than 0.05 was considered to be statistically significant.

Results

Table 1 shows highly significant increase in the mean values of serum AST, ALT, and ALP level in the tramadol treated groups when compared to the control group(241.80 ± 8.11 vs 86.60 ± 2.91 IU/L: P < 0.01), (273.90 ± 8.65 vs 71.70 ± 2.39 IU/L: P < 0.01), and (191.56±2.99 vs 137.39±0.80: P < 0.01).

Treatment of rabbits with tramadol resulted in decrease ($p < 0.05$) in catalase and glutathione activities when compared to the control group (39.73 ± 0.68 vs 52.09 ± 0.84 K/ml: P < 0.01), and(0.30 ± 0.03 vs0.63 ± 0.05 mg/mg protein: P < 0.01).whereas it markedly increased MDA levels in the serum of treated rabitts compared to control rabbits(9.68 ± 0.87 vs 4.74 ± 0.35 mol/L: P < 0.01). as shown in Table2.

Table 1: Effect of Tramadol administration on AST, ALT, and ALP of rabbits

Parameters	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	86.60 ± 2.91	71.70 ± 2.39	137.39±0.80
Trmadol	241.80 ± 8.11	273.90 ± 8.65	191.56±2.99

Table 2: Effect of Tramadol administration on catalase and glutathione and MDA of rabbits.

Parameters	Catalase (K/ml)	Glutathione (mg/mg protein)	MDA (mol/L)
Control	52.09 ± 0.84	0.63 ± 0.05	4.74 ± 0.35
Trmadol	39.73 ± 0.68	0.30 ± 0.03	9.68 ± 0.87

Discussion

Aspartate aminotransferases are predominantly mitochondrial enzymes. Although an elevated level of AST in the serum is not specific for a hepatic disorder, it is used primarily to diagnose and confirm persistent cellular injury in conjunction with other enzymes such as ALT.The increase in aminotransferase levels may be attributed to Cell damage of hepatocytes which alters their transport function and membrane permeability, leading to the leakage of cytosolic enzymes into the blood refers to the extensive damage of hepatic tissue membranes[15,16].

The lipid peroxidation could be considered as a leading cause of elevated liver enzymes as it leads to leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability and subsequently necrosis with leakage of the cytoplasmic enzymes into the blood[17].

Malondialdehyde is currently the most widely used indicator for the detection of oxidative stress and lipid peroxidation products and is often used to reflect the degree of oxidative stress.The current study also showed that levels of MDA increased after administration of tramadol as repeated-doses for 30 consecutive days, due to generation of excess free radicals more than the available scavenging antioxidant capacity of the body. Due to excessive generation of free radicals, the body synthesizes uric acid in an attempt to mop-up the generated free radicals [18,19]. which are in agreement with the finding of [5, 20-22].

Under normal physiological conditions, the antioxidant enzymes work to maintain the redox balance of the body and neutralize rampaging free radical and thus reducing its capacity to damage. They act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, and synergist[23-25]. At the molecular levels, glutathione system is the mother of all antioxidants, the master detoxifier and maestro of the immune system. It plays an important role in cellular defense against oxidantby scavenging ROS as a cofactor of antioxidant enzymes.It is reacts with free radicals such as singlet oxygen, peroxy radicals, and is converted into GSSG and other disulfides. that showed it preventing nature. GSH depletion may cause an impaired cell defense that may lead to tissue [22,26].

The reduction in NADPH and malic NADPH effects on the state of sustainability of the reduced form of the glutathione which is considered an important antioxidant for the cell and its decrease level in the cell leads to the case of oxidation of unsaturated fatty acids and this leads to the oxidative phosphorylation of fats and increase the MDA that may be a reason for the decreased glutathione[22]. In the present study, there was a significant decrease in GSH activity in the tramadol-treated group compared to the control reflected the formation formation of free radicals and initiation of lipid peroxidation that may be associated with cellular damage[27].

Catalase is a widely spread antioxidant enzyme in animal tissues. It protects tissues from hyperactive hydroxyl radicals through decomposing hydrogen peroxide[28,29]. In our study, Cat level was decreased significantly in treated Group due to formation of free radicals in heart and decreases its ability to detoxify reactive oxygen species.

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