Original Research Article

Effect of Tramadol Hydrocholoride on the Biomarkers of Liver and Kidney function and Oxidative stress in Male wistar Albino Rats.

ABSTRACT

Background:
Tramadol is an opioid-like analgesic agent, used parenterally and orally for the treatment of moderate to severe pain. Because it is inexpensive and is not classified as control drug by the government, the authors attempt to establish the possible effect of tramadol (TM) hydrochloride on the biomarkers for liver and kidney function along with oxidative stress in male wistar albino rats was performed to ascertain its likely translational relationship in humans.

Methods: 32 male wistar albino rats of 16 weeks old weighing 180g-200g were purchased from National Vertinary Research Institutes (NVRI), Vom, Plateau state, Nigeria, used as parallel study design and divided into 4 groups of 8 animals each. Group 1; the normal control (NC) administered saline solution only. Group 2 ,3 and 4 received oral dose of 0.1, 0.2 and 0.4 mg/kg/b.wt tramadol hydrochloride (HCl) suspended in saline solution as low, mid and high dose respectively. All the animals were fed the same diets throughout the period of (12) weeks from October 2022-December 2022. The rats were allowed to fast overnight then blood samples were collected and analysed at Department of Vertinary Medicine Laboratory, University of Nigeria, NSUKKA, Enugu State Nigeria for the biochemical markers of liver function, kidney function and oxidative stress.

Results: The result obtained for liver function, showed a significant ($p<0.05$) increase in ALT in group 4, AST in group 3 and 4, ALP in group 4, T. Bilirubin in group 3, D. bilirubin in group 4 when compared to the control group. The kidney function result showed significant ($p<0.05$) increase in Na^+ in group 3, Cl^- in group 2 and HCO_3 significantly ($p<0.05$) increased across all the test groups compared to the control, Urea increased significantly ($p<0.05$) in group 4, Creatinine concentration was high across all the test groups when compared to the control. Changes in antioxidant enzyme of catalase activity is significantly ($p<0.05$) reduced across the test groups compared to group 1. Similarly, the activities of superoxide dismutase decreased significantly ($p<0.05$) across all the test groups compared to the control group.

Conclusion: This result revealed that tramadol HCl at high concentration can interfere with the liver and kidney functions and antioxidant enzyme activity, thus we conclude that taking tramadol HCl could affect the liver and kidney function and cause oxidative stress.

Keywords: Tramdol Hydrochloride, oxidative stress, biochemical markers.

INTRODUCTION

Drugs are natural or synthetic substances used for medical purposes. However, the irrational use of some of these drugs may lead to transient or chronic dependency [1]. Drug abuse has become a major social problem of the modern world as it is very common and involves lifetime exposure of about 46% of the general population [2]. Addiction is a chronic disease characterized by compulsive drug seeking and uses [3]. Drug abuse cause and contribute to the deaths of millions of people every year by worsening comorbid psychiatric symptoms, such as depression, and physical illness, such as cirrhosis of the liver, while also aiding in the spread of infectious diseases such as human immunodeficiency virus (HIV), hepatitis B and hepatitis C. Drug abuse is also linked to crime and disability [4]. The abuse liability of naturally occurring opiates (e.g., morphine, codeine) and synthetic opioids (e.g.Tramadol, heroin, oxycodone, and buprenorphine) is well known [5] [6].

Tramadol is a centrally acting synthetic opioid analgesic agent, used parenterally and orally for the treatment of moderate to severe pain. The mechanism of its analgesic action is complex. Most reports suggest that the analgesic activity and other clinical effects of tramadol are a result of opioid and non-opioid mechanisms. Tramadol binds to the μ-opioid receptor, although much more weakly than morphine. It also inhibits the neuronal reuptake of norepinephrine and serotonin as do the antidepressant drugs such as amitriptyline and desimpramine [7] [8] [9] [10]. 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 [11] the liver is responsible for the metabolism of tramadol. Therefore, may cause hepatotoxicity during chronic administration [12].

Further, biotransformation results in inactive metabolites, which are excreted by kidneys [13] [14]. Metabolites of the drugs that are excreted from kidneys may also cause a cellular damage leading to a kidney dysfunction and may have a higher activity and/or a greater toxicity than the original drug [15].

Tramadol overdose may lead to a Central Nervous System (CNS) depression, nausea and vomiting, tachycardia, seizures, coma, respiratory depression and cardiovascular collapse [16] [17]. Oxidative stress is a phenomenon which is related to the development of many pathological conditions. Pathologies where reactive oxidative species (ROS) were identified as causal factors include cardiovascular disease, diabetes, rheumatoid arthritis, cancer, and neurodegenerative disorders [18]. This research was conducted to assess effect of tramadol hydrochloride on the biochemical markers of liver and kidney function, oxidative stress in male wistar albino rats.

MATERIALS AND METHODS

Experimental animals:

Thirty –two male wistar albino rats of 16 weeks old weighing 180-250g were used as a parallel study design. Rats were housed in separated metal cages and kept at constant environmental condition of temperature (22±1°C) and humidity throughout the period of experiment. The rats were fed on constant diet and fresh, clean drinking water was supplied *adlibitum.* All rats were acclimatized for a period of two weeks prior to the beginning of the study.

Materials and reagent:

Tramadol Hydrochloride 50mg, Diethyl ether P-nitrophenyl phosphate, buffer solution for ALP containing 0.5mmol magnesium chloride, 2,4-nitrophenyl hydrazine, buffer solution containing 100mmol/L phosphate buffer.

Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into four groups placed in individual cages and classified as follows:
Group I (Control normal group): Eight normal non-medicated rats served as control for all experimental groups, and received 1 ml (0.9% NaCl) oral doses of saline solution for 12 weeks.
Group II (Low dose): received a daily oral dose of tramadol HCl suspended in saline solution equivalent to 0.1 mg/kg/b.wt for twelve weeks.
Group III (Mid dose): received oral dose of tramadol HCl suspended in saline solution at doses of 0.2mg/kg/b.wt for 12 weeks.

Group IV (High dose): received a daily oral dose of tramadol HCl suspended in saline solution equivalent to 0.4 mg/kg/b.wt for twelve weeks.

Animal Sacrifice and Blood Sample Collection:

After the treatment period, the rats were allowed to fast overnight and sacrificed under mild euthanasia with pentobarbital. Blood samples were collected by cardiac perforation into plain, and bottles, for chemistry assay. The blood in the plain bottles was allowed to clot and the serum separated at 3500 rpm for 15 min was used for evaluation of liver and kidney functions, with antioxidant enzyme activity.

Biochemical analysis:
Biochemical analysis was carried out to determine liver function (serum concentrations of AST, ALT, ALP, conjugated and total bilirubin), Kidney function (Urea, Creatinine and electrolytes) using Automated Biochemical Analyzer. The antioxidant activity of Superoxide dismutase (SOD) was estimated according to the method by Misra and Fridovich, [19] and the Catalase (CAT) was estimated according to the method by Aebi H. [20].

Statistical analysis
One-way analysis of variance was used in analyzing the results using the Predictive Analytics Software (International Business Machines (IBM), United States) Statistics 18 package. All the results were expressed as mean ± standard error and $P < 0.05$ was taken to be significant.

Results and Discussion

Effect of Tramadol hydrochloride on Liver Function.
As shown in table-1 above, the results showed a significant ($p<0.05$) increase in ALT in group 4 compared to group 1, AST significantly ($p<0.05$) increased in group 3 and 4, ALP significantly ($p<0.05$) increased in group 4. T. Bilirubin significantly ($p<0.05$) increased in group 3. Direct bilirubin significantly ($p<0.05$) increased in group 4. The total protein showed no significant ($p<0.05$) changes compared to the control.

Table -1 Effect of Tramadol hydrochloride on Liver Function.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	T. Billirubin (mg/dl)	D. Billirubin (mg/dl)	T. Protien (mg/dl)
1	35.00±1.15[a]	18.75±0.95[a]	60.75±1.50[a]	82.50±1.73[a]	0.43±0.08[a]	6.50±0.25[a]
2	36.00±1.63[a]	19.25±0.50[a]	61.50±2.08[a]	84.00±0.81[a]	0.51±0.02[a]	6.60±0.16[a]
3	35.50±0.57[a]	23.00±0.81[b]	61.00±0.81[a]	87.00±0.81[b]	0.57±0.01[a]	6.50±0.24[a]
4	37.00±0.81[b]	24.00±0.81[b]	64.00±0.81[b]	83.00±0.81[a]	0.60±0.03[b]	6.55±0.04[a]

Results are presented in Mean ± SD, (N = 8), mean values with different letters as superscripts are considered at $p < 0.05$. Group 1= control, group 2= Low dose, group 3= Mid dose, group 4= High dose

Effect of Tramadol hydrochloride on Kidney Function

Table- 2 is a presentation of the results of kidney function parameters in albino rats administered Tramadol hydrochloride which shows a significant ($p<0.05$) increase in sodium ion in group 3, no significant ($p<0.05$) changes were observed for potassium ion in the test groups compared to the control. Chloride ion increased significantly($p<0.05$) in group 2. The concentration of bicarbonate ion increased significantly($p<0.05$) across all the test groups compared to the control, the concentration of urea increased significantly($p<0.05$) in group 4 compared to the control. Creatinine concentration was significantly ($p<0.05$) higher across all the test groups compared to the control.

Table -2 Effect of Tramadol hydrochloride on Kidney Function

Groups	Na^+(mg/dl)	K^+ (mg/dl)	Cl^- (mg/dl)	$HCO3^+$ (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)
1	5.73±0.47[a]	2.18±1.12[a]	2.39±0.25[a]	6.82 ±0.67[a]	62.00 ±0.81[a]	4.80±0.51[a]
2	5.53±0.09[a]	19.25±0.4[a]	3.53±0.08[b]	8.53 ±0.08[b]	62.00 ±1.63[a]	5.30±0.09[b]
3	6.46±0.34[b]	2.15±0.03[a]	2.92±0.62[a]	8.42 ±0.15[b]	61.00 ±2.44[a]	6.15±0.02[c]
4	5.78±0.13[a]	2.90±0.45[a]	3.12±0.00[a]	8.32±0.11[b]	64.75±1.25[b]	6.59±0.20[d]

Results are presented in Mean ± SD, (N = 8), mean values with different letters as superscripts are considered at $p < 0.05$. Group 1= control, group 2= Low dose, group 3= Mid dose, group 4= High dose

Effect of Tramadol hydrochloride on antioxidant enzyme activity

As shown in table 3, the activities of catalase significantly ($p<0.05$) reduced across the test groups compared to group 1. Similarly, the activities of superoxide dismutase decreased significantly ($p<0.05$) across all the test groups compared to the control group.

Table -3 Effect of Tramadol hydrochloride on antioxidant enzyme activity

Groups	CAT (mg/dl)	SOD (mg/dl)
1	0.934 ± 0.35^{a}	11.47 ± 3.63^{a}
2	0.51 ± 0.04^{b}	8.55 ± 0.07^{b}
3	0.59 ± 0.00^{b}	8.92 ± 0.25^{b}
4	0.61 ± 0.02^{b}	9.62 ± 0.00^{a}

Results are presented in Mean ± SD, (N = 8), mean values with different letters as superscripts are considered at $p < 0.05$. Group 1= control, group 2= Low dose, group 3= Mid dose, group 4= High dose

DISCUSSION

Substance abuse can lead to an increased risk of chronic diseases, family breakdown, job loss, reduced longevity, crime and increased violence [21]. Repeated tramadol administration in such patients leads to the accumulation of toxic metabolites in their bodies, increase the risk for pharmacokinetics interactions and or decreases the clearance of tramadol thus increasing its potential for toxicity [22].

The results of ALT, AST and ALP were significantly ($p<0.05$) increased at the high dose of Tramadol compared to the control. These results were comparable with the findings of Youssef and Zidan[21] who reported increased ALT, AST and ALP activities in rats after acute and long-term administration of Tramadol. These data are in agreement with [23], who reported that liver exposes to sever oxidative stress is associated with the elevation of serum liver function tests. This also affirms the report of Bethesda [22] that serum aminotransferase levels can be elevated in a small proportion of patients receiving tramadol, particularly with high doses.

The hydration of the body is maintained by the osmotic gradients of electrolyte, which in turns regulate the hydration and pH, being critical for the muscle and nerve function, and mechanisms such as tubular reabsorption play important roles in keeping the concentrate of various electrolytes under strict control. The results showed significant ($p<0.05$) increased of Na^{+} and HCO_3^{-} upon administration of tramadol indication imbalance of electrolytes compared to control Significant ($p<0.05$) increase in the values of creatinine and urea is an indication of interference by the drug with creatinine metabolism leading to decreased synthesis in a dose-dependent pattern effect. Previous studies have shown the correlation between renal injury and disease with free radicals. These free radicals can lead to oxidative stress as demonstrated by significant ($p<0.05$) decrease in CAT and SOD activities with Tramadol administration compared to control.

Conclusion

Findings from this research showed that intake of Tramadol hydrochloride, at high dose increase liver enzymes activities which are signs of hepatotoxicity. Also, there were signs of nephrotoxicity shown by increased in bicarbonate, urea and creatinine. Furthermore, Tramadol generates oxidative stress as shown by decreased in SOD and CAT activities.

Further research should be done on detection of Tramadol in biological samples in order to reduced rat of abuse of the drug and finding remedy for Tramadol complications such as antioxidants to neutralized the free radicals generated.

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