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Research Article

Effects of tramadol administration on some biochemical parameters in Wister albino rats under non-induction of pain

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Abstract

The present study investigated the effect of tramadol under non-induction of pain on some biochemical parameters in albino rats. A total of 24 albino rats were randomly divided into four groups of 6 members each. Group 1 received pellet and was allowed free access to water. This was the control. Groups 2, 3 and 4 received 50, 100 and 200 mg/Kg body weight of tramadol respectively. All the administrations were via oral intubation and were done once daily for 30 days. Neurotransmitters, glutamine dehydrogenase, glutaminase, and oxidative stress markers were determined in the brain homogenate using standard methods. Liver function markers, kidney function parameters and pancreatic markers were equally determined in the serum using standard methods. The results showed that levels of serotonin, dopamine, reduced glutathione, albumin and activities of catalase, super oxide dismutase, glutathione transferase, glutathione peroxidase, glutaminase and lipase were significantly ($p<0.05$) lowered in rats administered tramadol only in dose dependent manner when compared with the control. The results also revealed that administration of different doses of tramadol caused significant ($p<0.05$) increase in the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, α-amylase, glutamine dehydrogenase and levels of total bilirubin, uric acid, urea, creatinine and malondialdehyde in dose dependent manner when compared with control. These results generally revealed that intake of tramadol under non-induction of pain caused significant alterations of some biochemical markers, which could lead to health consequences.

Keywords: Albino rats; Biochemical parameters; Tramadol; Alutamine dehydrogenase.

Introduction

Drug abuse or drug dependence (as preferred by the World Health Organization (WHO)), is defined as a state of psychic or physical dependence, or both on a drug, following its administration on a periodic or continuous basis [1]. Drug abuse constitutes the use of any substance under international control outside therapeutic indications, in excessive dose, or over an unjustified period of time [2]. Tramadol is a centrally acting 'atypical' opioid analgesic, producing a synergistic analgesic effect provided by a μ-opioid receptor affinity coupled with inhibitions of synaptic re-uptake of monoamine neurotransmitters such as 5-hydroxytryptamine (5-HT) and norepinephrine

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[3]. More so, it is a potent analgesic medication prescribed worldwide for treatment of acute and chronic pains [4, 5]. However, its analgesic potency is claimed to be about one tenth that of morphine [6].

The opioid analgesic potency of tramadol is influenced by an individual's genetics factor as poor metabolizer have experienced little conversion to the more active analgesic metabolite (M1), whereas individuals with a high metabolic rate have experienced greatest analgesic effects. The toxic effects of tramadol could be due to the generation of more than one metabolites that are associated with the neuro-, hepatic- and the nephrotoxicity especially after long-term therapy [7].

Being one of the most grossly abused drugs, there is little information on tramadol's associated side effect in non-pain induced condition. Hence, to close this gap, there is need to investigate tramadol administration on some biochemical parameters in albino rats under non-induction of pain. Data generated from this study would go a long way to increase the volume of information about the hepato-, nephro- and neurotoxicity of tramadol under non-pain induced conditions.

Materials and methods

Equipment used

The equipment used include stopper florescence flask, kjeldalic flask , electronic weighing balance, crucible, desiccators , spectrophotometer, electric oven, gas column chromatography, refrigerator, soxhlet, murfle furnace, calibrated precision pipettes, absorbent material, vortex mixer, and centrifuge.

Biological Materials

Albino rats were the only biological material used for this study.

Collection and Authentication of Biological Materials

The albino rats were purchased from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. They were allowed to undergo acclimatization for one week before the commencement of experiment in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Experimental Design

A total of 24 albino rats were randomly divided into four groups of six members each. Group 1 rats received pellet and allowed free access to water. This was the control sample. Groups 2, 3 and 4 received 50, 100 and 200 mg/Kg body weight of tramadol respectively. All the administrations were via oral intubation and were done once daily. After 30 days administrations, the rats were anaesthetized, sacrificed and the samples kept in the appropriate tubes for subsequent analyses [8, 9].

Assessment of oxidative stress parameters

Determination of Catalase Activity (CAT)

CAT was determined according to method reported by Aebi [10]. 2.5 ml of phosphate buffer and 2 ml of hydrogen peroxide were added to the test tube. Thereafter, 0.5 ml of the sample was added. To 1 ml portion of the reaction mixture, 2 ml of dichromate acetic acid reagent was added. Absorbance was read at 240 nm against the bank at a minute interval. The CAT was calculated by using the formula;

$$\text{Catalase concentration (U/L)} = \frac{0.23 \times \log \text{Absorbance 1/Absorbance 2}}{0.00693}$$

Determination of Super Oxide Dismutase (SOD) Activity

SOD was assayed by the method reported by Marklund and Marklund [11]. 0.2 ml of the sample was introduced into 2.5 ml of 0.05 phosphate buffer. At pH of 7.8, 0.3 ml of newly prepared adrenaline solution was added to the reaction mixture followed by quick mixing by inversion of the cuvette. The increase in absorbance was taken every 30 seconds for 3 minutes at 480 nm against blank. Blank contained 0.3 ml of adrenaline and 2.5 ml buffer. SOD activity was measured by determining the inhibition of auto oxidant of adrenalins as shown below;

$$\% \text{ Inhibition} = \frac{(\text{O. D ref} - \text{O. D test}) \times 100}{\text{O. D ref}}$$

Determination of Glutathione-S-Transferase (GST) Activity

GST activity of tissues was measured spectrophotometrically by the method reported by Habig et al. [12]. Three wells were prepared and designated as non-enzymatic well, sample well and control well. 170 µL of assay buffer and 20 µL of glutathione were added to non- enzymatic wells. 150 µL of assay buffer, 20 µL of glutathione and 20 µL of the reconstituted GST-control were added to control wells. 150 µL of assay buffer, 20 µL of glutathione and 20 µL of sample were added to sample wells. Finally, 10 µL of 1-chloro-2,4-dinitrobenzene (CDNB) was added to all the wells. The well plate was carefully shaken for a few seconds to mix properly and the absorbance was read once every minute at 340 nm using a plate reader to obtain at least 5 time points. GST activity was calculated using;

$$\text{GST Activity} = \frac{\Delta A340/\text{min}}{0.00503\mu/\text{m}} \times \frac{0.2mL}{0.02mL} \times Sample\ dilution$$

$$\Delta A340/\text{min} = \frac{\text{A340 (Time 2)} - \text{A340 (Time 1)}}{\text{Time 2} - \text{Time 1}}$$

Where

ΔA340/min = Change in absorbance at 340 nm per minute

Determination of Glutathione Peroxidase (GPx) Activity

GPx activity was estimated using the method reported by Flohe and Gunzler [13]. The reaction mixtures were prepared by putting in a test tube, 14.0 ml of distilled water, 2.0 ml of 5% pyrogallol solution, 1.0 ml of 0.147 M H_2O_2 solution and 2.0 ml of 0.1 M phosphate buffer (pH 6.0). The mixture was then equilibrated at 20^{o}C for about 5 minutes, after which there was the addition of 1.0 ml of the sample solution and the solution was mixed. This was followed by the addition of 1.0 ml of 2.0M H_2SO_4 to stop the reaction after exactly 20 seconds. The optical density of the resulting solution was measured at 420 nm against a blank. The activity of peroxidase was calculated using the formula;

$$\text{Peroxidase Actvity} = \frac{\Delta OD \times df}{0.117 \times Vs}, \text{where:} \quad \Delta OD = (ODtest - ODblank)$$

Where:

Vs = sample volume

0.117 = optical density at 420 nm corresponding to 1 mg % pyrogallol in ether.

df = dilution factor

Determination of Reduced Glutathione (GSH) Level

GSH level was determined by the method reported by Jollow et al. [14]. 1 ml of the sample was added to 4.0 % sulfo-salicyclic acid and the mixture centrifuged at 3,000 rpm for 15 minutes at 2^{o}C. The samples were introduced to 4.5 ml of Ellman reagent and absorbance was measured at 412 nm. The blank was prepared by addition of 0.5 ml of 4 % sulfo-salicyclic acid to 4.5 ml of Ellman reagent while absorbance was measured at 412 nm. GSH level was calculated by using the formula;

$$\text{GSH Level} = \frac{\text{Asample - Ablank}}{\text{Astandard - Ablank}} \text{X Cstandard}$$

Determination of Malondialdehyde (MDA) Level

MDA level was estimated by measuring thiobarbituric acid reactive substances (TBARS) by method reported by Ohkawa et al. [15]. 0.1 ml of sample, 0.9 ml of distilled H_2O, 0.5 ml of 25 % TCA reagent and 0.5 ml of 1% TBA reagent in 0.3% NaOH were added to a test tube. The test tube was incubated at 95 ^{0}C for 40 minutes. Thereafter, the test tube was allowed to cool in water and exactly 0.1 ml of 20 % sodium dodecyl sulphate was added to the test tube. The absorbance of the sample was read against the blank reagent at 532 and 600 nm. MDA level was calculated from the formula;

$$\%\text{TBARS} = \frac{\text{A532} - \text{A600}}{0.5208 \times 0.1} \times 100$$

Where A= absorbance

Assessment of liver and kidney function parameters

Determination of Alanine Aminotransferase (ALT) Activity

Two test tubes labeled reagent blank and sample were arranged. 0.1 ml of the sample was added to the sample test tube. Thereafter, 0.5 ml of reagent 1 were added to both blank and sample test tubes followed by the addition of 0.1 ml of distilled water to the reagent blank only. The tubes were mixed and incubated for 30 min at a temperature of 37^{0}C. After that, 0.5 ml of reagent 2 was added to the two test tubes. The test tubes were mixed and allowed to stand for 20 minutes at 20-25^{0}C. 0.5 ml of 0.4 M sodium hydroxide was finally added to the test tubes. The tubes were mixed again and the absorbance

of the sample (Asample) against the reagent blank was read at the wavelength of 546 nm after 5 minutes. The activity of ALT in the serum was obtained from the standard table.

Determination of Aspartate Aminotransferase (AST) Activity

Two tests tubes labeled sample and reagent blank were used. To the sample test tube, 0.1 ml of the sample was added followed by addition of 0.5 ml of the reagent1 to the two test tubes. After that, 0.1 ml of distilled water was added to the reagent blank test tube. The test tubes were mixed properly and incubated for exactly 30 minutes at 37 ^{0}C. After the incubation, 0.5 ml of reagent 2 was added to the two test tubes, mixed and allowed to stand for 20 minutes at 20-25 ^{0}C. Finally, 5.0 ml of 0.4 M sodium hydroxide was added to each of the test tubes, mixed and after 5 minutes, the absorbance of sample (Asample) was read against the reagent blank at the wavelength of 546 nm. AST activity was obtained from the standard table.

Determination of Alkaline Phosphatase (ALP) Activity

0.01 ml and 0.5 ml of the sample and reagent respectively were added into micro cuvette. The mixture was properly mixed and the initial absorbance was read at 405 nm wavelength and the timer was started simultaneously. The absorbance was read again after 1, 2 and 3 minutes to get the changes in absorbance. ALP was calculated using the formula;
ALP (U/L) = 2760 x ΔA nm/minutes

Determination of Total Bilirubin Concentration

Sample blank and sample cuvette were prepared and arranged. 200 µl of reagent 1 was added to the two cuvette followed by addition of 1 drop (50 µl) of the reagent two only to the sample cuvette. After that, 1000 µl and 200 µl of reagent three and sample (serum) respectively were added to the two cuvettes. The cuvette was properly mixed and incubated for 10 minutes at 20-25 0C. Finally, 1000 µl of reagent four was added to both sample blank and sample cuvette, mixed and incubated for a further 5–30 min at 250C and the absorbance of the sample against sample blank was read at the wavelength of 578 nm. Total bilirubin was calculated thus;

Total Bilirubin (mg/dl) = 10.8 x ATB

Determination of Albumin Level (ALB)

Test tubes were prepared and designated as reagent blank, standard and sample test tubes. 0.01 ml of distilled H_2O was added to the reagent blank. 0.01 ml of standard was added to standard test tube followed by the addition of 0.01 ml of serum to the sample test tube. 3.0 ml of Bromocresol Green (BCG) reagent was added to the three test tubes. The tubes were mixed properly and incubated for 5 minutes at 20-25 ^{0}C. The absorbance of the sample (Asample) and that of the standard (Astandard) were read against the reagent blank at the wavelength of 630 nm. ALB was calculated thus;

$$\text{ALB Conc.}\left(\frac{\text{g}}{\text{dl}}\right) = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Cocentration of Standard}$$

Determination of Creatinine Concentration

Reagent blank, standard and sample test tubes were prepared separately. 50 µl of distilled water, standard solution and sample were added to reagent blank, standard and sample test tubes respectively. 500 µl of the working reagent were added into the test tubes and mixed. After 30 seconds, the absorbance A_1 of the sample and that of standard were read at the wavelength of 492 nm. After 2 minutes, the absorbance A_2 of standard and sample were read against the reagent blank at the same wavelength. Creatinine concentration was calculated from the formula;

$$\text{Creatinine conc.}\,(\text{mmol/L}) = \frac{\text{Change in Absorbance of Sample}}{\text{Change in Absorbance of Standard}} \times \text{Standard Conc.}$$

Determination of Urea Concentration

10 µl of distilled water were added to the sample, standard and blank test tubes separately. 100 µl of reagent 1 was added to all the test tubes, mixed and incubated at 37 0C for 10 minutes. Thereafter, 2.50 ml each of reagent 2 and reagent 3 were added to the three test tubes. The tubes were mixed immediately and incubated at 37 0C for 15 minutes. The absorbance of the sample (Asample) and that of standard (Astandard) were read against the blank at the wavelength of 546 nm. Urea concentration was calculated from the formula:

$$\text{Urea Conc.}\left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Standard Conc.}$$

Determination of Uric Acid Concentration

Test tubes were set up and labeled blank, standard and test samples. 1.0 ml of the working reagent was added into the test tube labeled blank. 1.0 ml of working reagent and 0.02 ml

standard solution were added into the standard test tube while, 1.0 ml of working reagent and 0.02 ml of the test sample were added into the sample test tube. The contents were mixed and incubated at room temperature for 5 minutes and the absorbance of the test samples and standard were read against the reagent blank at 520 nm. Uric acid content was calculated thus:

$$\text{Uric acid content } \left(\frac{\text{mg}}{\text{dl}}\right) = (\Delta \text{A520} - \text{b}) \div \text{a} \times \text{f},$$

Where,
a= slope of standard curve
b= intercept of standard curve
f= dilution multiple of tested samples

Assessment of amylase activity

Amylase activity was assayed by the method reported by Wroblewski and LaDue [16]. 0.5-50 µl of sample and 5 µl amylase positive control was added into the sample and positive control wells respectively. The volume was adjusted to 50 µl with distilled water. 0, 2, 4, 6, 8, and 10 µl of 2 M nitrophenol standard were mixed into the well plate and duplicated to generate 0, 4, 8, 12, 16, 20 nmol/ well nitrophenol standard. The total volumes were brought to 50 µl with distilled water. 100 µl of the reaction mixture was then added into each reaction and mixed. The optical density (OD) was measured immediately (T0) at 405 nm to get ODT0. The reaction was incubated at 25°C for various times (T1) and OD was measured again at 405 nm to get ODT_1. Amylase activity was calculated thus;

$$\text{Amylase Activity} = \frac{B}{T \times V} \times \textit{Sample Dilution Factor}$$

Where,
B= nitrophenol amount from standard curve (nmol).
T= time between T0 and T1 (min).
V= pre-treated sample volume added to the reaction well (ml).

Assessment of enzymes involved in glutamine metabolism

Determination of Glutamate Dehydrogenase (GDH) Activity

GDH activity was assayed according to the method reported by Lee and Lardy [17]. 100 µl of the reaction mixture containing 82 µl Assay Buffer, 8 µl GDH Developer and 10 µl Glutamate (2 M) was added to each well containing the test samples, positive controls and standards. They were mixed properly to ensure homogeneity. Samples and positive controls mixtures were incubated for 3 minutes at 37°C. The OD at 450 nm in a micro plate reader (A0) was measured, incubated for another 30 min to 2 hours at 37°C to measure OD at 450 nm again (A1). GDH activity was calculated thus;

GDH activity =

$$\frac{\text{B}}{TXV} X \textit{ Sample Dilution Factor}$$

Where
B= NADH amount from standard curve (nmol).
T= time incubated (min)
V= volume added into the reaction well (ml).

Determination of Glutaminase (GLS) Activity

Glutaminase activity was assayed by the method reported by Meister [18]. Four micro centrifuge tubes (Sample, Control, Standard and Blank) were arranged. 20 µl of sample and 200 µl of substrate were added to the sample tube. 20 µl of distilled water and 200 µl of substrate were added to the control tube. They were mixed and inserted in the oven at 37 °C for 1 hour. 300 µl of stop solution was added to both sample and control tubes, mixed and centrifuged at 8,000 rpm for 5 minutes. 130 µl of supernatant was added to both sample and control test tubes while 130 µl of standard was added to standard test tube. These were followed by addition of 130 µl of distilled water to the blank tube. Total of 50 µl of reaction buffer and 20 µl of the reagent were both added to all the test tubes. They were properly mixed again and the absorbances were read at 420 nm immediately. GLS activity was calculated using;

$$\text{GLS Activity} = \textit{Cstandard} \times \frac{(\textit{ODsample} - \textit{ODcontrol})}{(\text{ODstandard} - \text{ODblank})} \div \frac{\text{Cprotein}}{T \times 4}$$

Where
Cprotein= protein concentration,
Cstandard= concentration of standard,
T= reaction time;
4= factor.
OD= optical density

Assessment of brain monoamines

Dopamine Determination

\This was determined using the method reported by Kerma et al. [19]. 25 µL of the enzyme solution was used. The absorbance of the solution was read within 10 minutes, using a micro plate reader set to 450 nm. The

concentrations of the samples and the controls were read directly from the standard curve.

Serotonin Determination

This was determined using the method reported by Kerma et al. [19]. The concentrations of the samples and the controls were read directly from the standard curve. The calibration curve from which the concentrations of the samples were read was obtained by plotting the absorbance readings (mean absorbance) measured for the standards (y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

Statistical analysis

Data were analyzed with one-way ANOVA using the GraphPad Prism 8.0.2 (263). Post hoc Tukey test was used to compare between the groups. The data are presented as the mean ± standard error of means (SEM). Differences were considered significant when the $p<0.05$.

Results and discussion

Effects on brain monoamines

The results of brain monoamines in albino rats showed that serotonin and dopamine levels in the brain were significantly ($p<0.05$) lowered in tramadol administered rats in dose dependent manner when compared with the control as shown on fig. 1 and 2. The significant ($p<0.001$) decrease in 5-HT levels during tramadol exposure may be due to anabolic deficit caused by a decrease in amino acids in the brain.

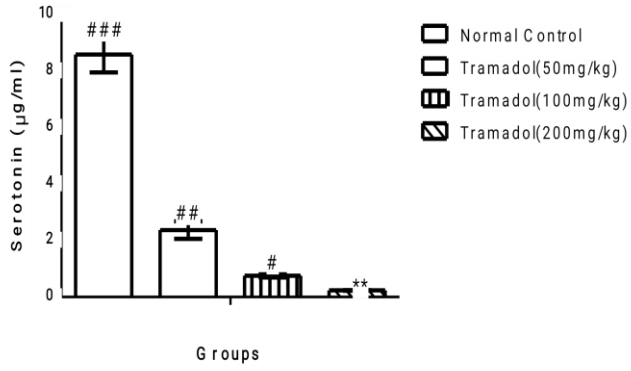

Fig. 1. Effect of Tramadol on serotonin level in brain of albino rats. Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$.

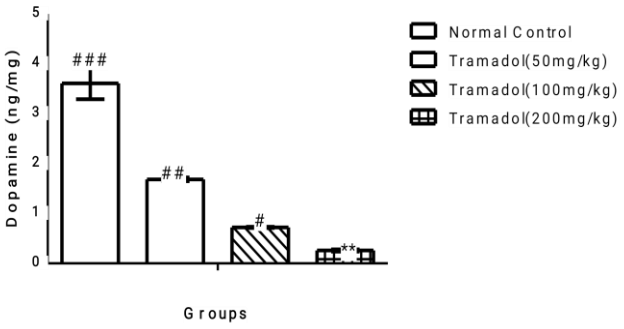

Fig. 2. Effect of Tramadol on Dopamine Level in brain of albino rats. Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$.

Effects on Oxidative Stress Indices

The results revealed that activities of catalase, SOD, GPx, GST and level of GSH were significantly ($p<0.05$) decreased while MDA level was significantly ($p<0.05$) elevated in tramadol administered albino rats in dose dependent manner when compared with the controls as seen in fig. 3 to 5. Results from this study suggest that free radicals generated by metabolism of tramadol causes significant reduction in protein concentration as well as the catalytic potential of enzymes such as GST in brain samples.

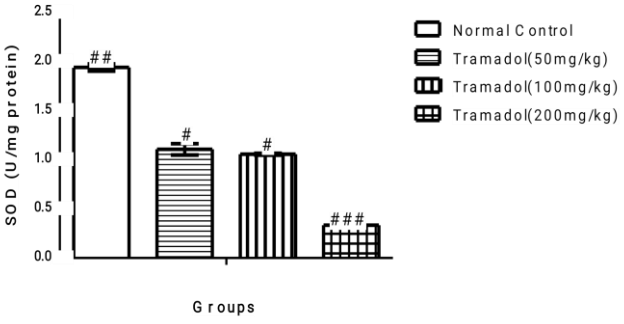

Fig. 3. Effect of Tramadol on SOD activity in Brain of Albino Rats. Data are shown as mean ±S.D (n=6). Mean values with the different signs are significantly different at $p < 0.05$.

Effects on liver function indices

The liver function indices were impaired in tramadol administered group as reflected by elevation of serum activities of ALT, AST, ALP and level of total bilirubin with decrease in the level of albumin when compared with the controls as shown in fig 6-7. Similar results were obtained by Atici et al. [20] who reported

significant ($p<0.05$) increase in the levels of serum ALT, bilirubin and AST in rats after long term usage of tramadol.

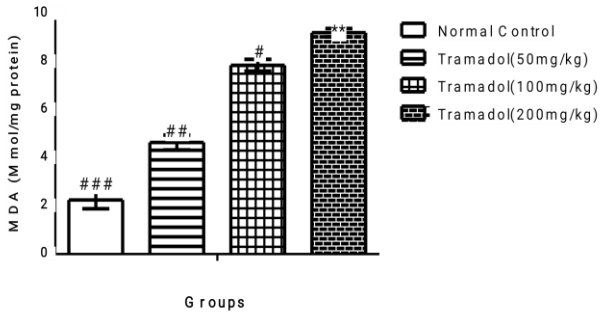

Fig. 4. Effect of Tramadol on MDA Level in Brain of Albino Rats. Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$.

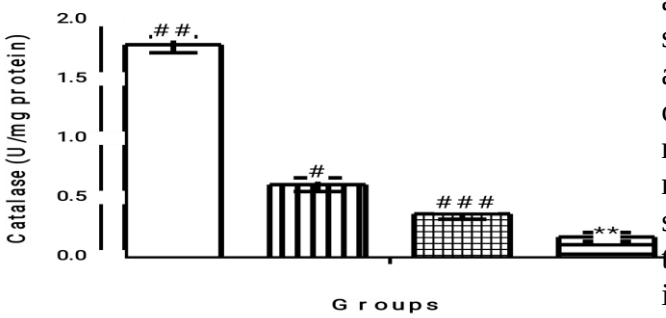

Fig. 5. Effect of Tramadol on Catalase Activity in brain of albino rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$.

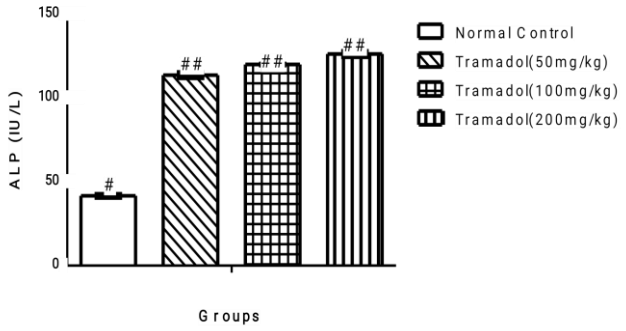

Fig. 6. Effect of Tramadol on ALP Activity in Albino Rats. Data are shown as mean ±S.D (n=6). Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$

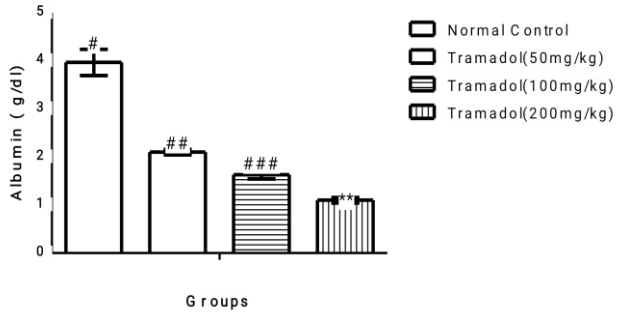

Fig. 7. Effect of Tramadol on Albumin Level in Albino Rats. Data are shown as mean ±S.D (n=6). Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $p < 0.05$.

Effects on kidney function

Impairment of the renal functions in tramadol administered albino rats was indicated by a significant ($p<0.05$) increase in serum urea, uric acid and creatinine when compared with the control groups, as shown on fig. 8-9. Similar results were reported by Atici et al [20] who reported a significant ($p < 0.05$) increase in serum urea, uric acid and creatinine levels in tramadol-administered rats. The significant increase in creatinine and urea levels compared with the control suggested that the filtration activity of the kidney was compromised by tramadol administration.

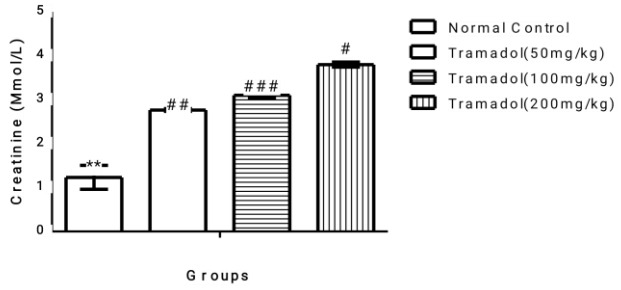

Fig. 8. Effects of Tramadol on Creatinine Level in Albino Rats. Data are shown as mean ±S.D (n=6). Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at $P < 0.05$.

Effects on alpha amylase activities

The result revealed that activities of alpha amylase were significantly ($p<0.05$) elevated in tramadol administered albino rats when compared with control as seen in fig. 10. The rise in amylase activity in this study may be due to a dyslipidemia.

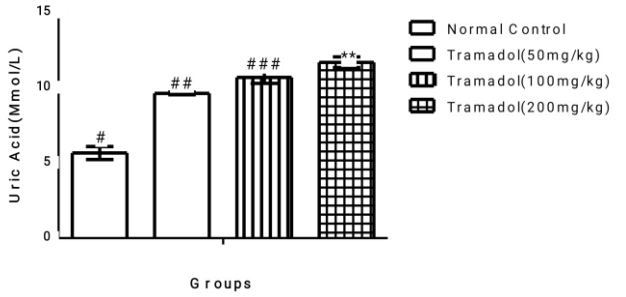

Fig. 9. Effects of Tramadol on Uric Acid Level in Albino Rats. Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at P < 0.05.

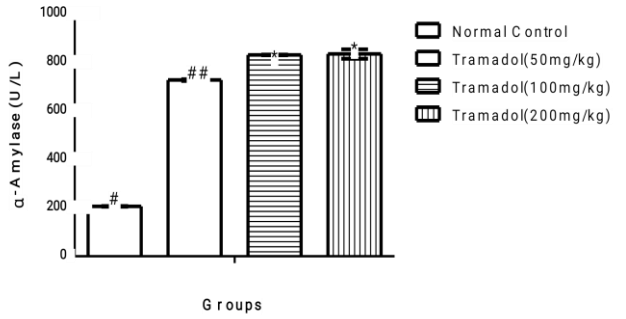

Fig. 10. Effects of Tramadol on α-Amylase Activity in Albino Rats. Data are shown as mean ±S.D (n=6). Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at p < 0.05.

Effects on enzymes involved in glutamate metabolism

The results revealed a significant ($p<0.05$) increase in glutamine dehydrogenase (GDH) activity and significant ($p<0.05$) decrease in glutaminase activity in the brain following the administration of tramadol in relation to normal control as seen in fig. 11-12. Glutamatergic transmission may have a role in learning and memory, central pain transduction, and the pathophysiology of neuronal death after brain injury. Alterations in the activities of key enzymes of glutamate metabolism signify modulation in the turnover rates of glutamate. Glutamate can be utilized in the brain predominantly either as a metabolite in TCA cycle or for the synthesis of glutamine. This invariably suggested that the enzymes involved in glutamate metabolism in the brain were compromised by the tramadol toxicity.

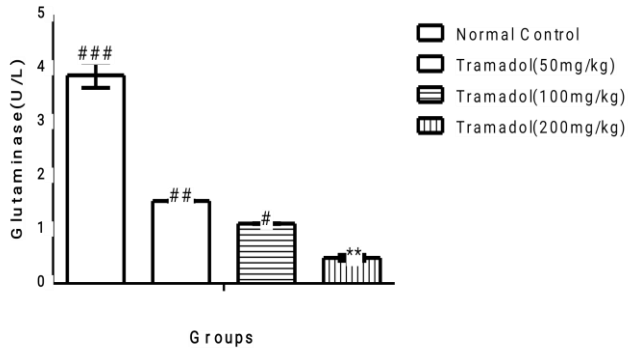

Fig. 11. Effects of Tramadol on Glutaminase Activity in Albino Rats. Data are shown as mean ±S.D (n=6). Mean values with different signs are significantly different at p < 0.05.

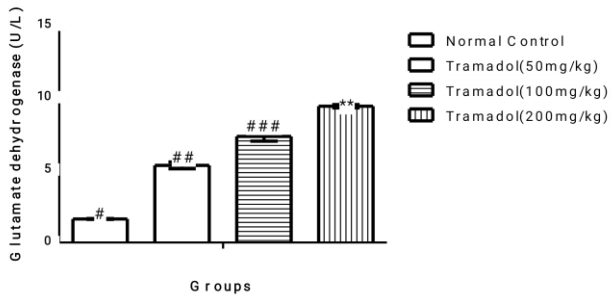

Fig. 12. Effects of Tramadol on Glutamate Dehydrogenase Activity in Albino Rats. Data are shown as mean ±S.D (n=6). Mean values with the different signs are significantly different at p < 0.05.

Conclusions

The results from this study generally revealed that intake of tramadol under non-induction of pain caused significant alterations in the brain, liver, kidney and pancreatic function markers of albino rats as seen in the elevated increase in AST, ALT, ALP, MDA, total bilirubin, uric acid, urea, creatinine, α-amylase, and glutamate dehydrogenase in a dosage dependent manner in the albino rats. Based on this, we recommend that other tissues should be assessed to ascertain the degree of toxicity of this drug. More so, we recommend that disease conditions associated with pains may also be induced on experimental model and treated with this drug to obtain its level of toxicity under pain induction.

Conflict of interest

The authors of this work declare no conflict of interest

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