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EFFECTS OF TRAMADOL ADMINISTRATION ON SOME BIOCHEMICAL PARAMETERS IN WISTER ALBINO RATS UNDER NON-INDUCTION OF PAIN.

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Abstract

Tramadol is one of the drugs that its abuse is of rapid increase in our society today, and there is little information on its associated side effects in non-pain induced condition. Hence, this study aimed at investigating the effect of tramadol under non-induction of pain on some biochemical parameters in albino rats. A total of twenty four (24) albino rats were used for this research. They were randomly divided into four groups of six (6) rats in each group; group 1 (normal control) received pellet and allowed free access to water; group 2, 3 and 4 (tramadol groups) 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, 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, nor-epinephrine, reduced glutathione (GSH), albumin and activities of catalase, super oxide dismutase (SOD), glutathione transferase (GST), glutathione peroxidase (GPx), glutaminase and lipase were significantly ($p<0.05$) lowered in rats administered tramadol only in dose dependent manner when compared to control. The results also revealed that administration of different doses of tramadol caused significant ($p<0.05$) increase in the activities of AST, ALT, ALP, α-amylase, glutamine dehydrogenase and levels of total bilirubin, uric acid, urea, creatinine and MDA in dose dependent manner when compared to control. The results generally revealed that intake of tramadol under non

induction of pain caused significant ($p<0.05$) alterations of some biochemical markers, which could lead to deleterious health consequences, hence its intake should be avoided in order to avoid further health consequences.

Keywords: Tramadol, Oxidative Stress, Non-pain induction, biochemical markers, Enzymes, Drug Abuse, liver functions.

Introduction

Drug abuse or drug dependence (as preferred by the world health organization) is the state of psychic or physical dependence, or both on a drug, following the intake of the drug on a periodic or continuous basis (Alhaji and Faruna, 2018). World health organization (WHO), found the term "abuse" ambiguous, it abandoned its use; instead the WHO glossary speaks of "harmful use" and "hazardous use. Harmful use is a pattern of psychoactive substance use that is causing damage to health. Hazardous use is a pattern of substance use that increases the risk of harmful consequences for the user. In contrast to harmful use, hazardous use refers to patterns of use that are of public health significance despite the absence of any current disorder in the individual user (Alhaji and Faruna, 2018). In the context of international drug control, drug abuse entails the use of any substance under international control outside therapeutic indications cum purposes, in excessive dose, or over an unjustified period of time (Haladu, 2003).

The economic effect of drug abuse deals with the loss of potential manpower, low productivity, and creation of an unfavorable environment for investors which of course will affect the Gross National income as well as the internally generated revenue (Haladu, 2003). Secondly, Government would be spending more funds on security created by insurgent as a result of drug abuse instead of using such funds to provide basic needs and infrastructural development. Drug abuse also tarnishes the image of the country by causing political unrest in the country (Haladu, 2003). Social effects of drug abuse in Nigeria comprises increasing criminal activities associated with drug abuse like robbery, burglary, rape, destructions of public properties, increasing rate of HIV/AIDS, the congestion of penitentiaries where the government spends more in the maintenance of prisoners, and of course the growing numbers of destitute which our social welfare administration system cannot carter for, thereby depleting government's budget (Alhaji and Faruna, 2018). It also increases number of political thuggery which resulted to killing of innocent citizen.

National Bureau of Statistics and Centre for Research and Information on Substance abuse revealed that prevalence of any drug use in Nigeria is estimated at 14.4 percent or 14.3 million people aged between 15 and 64 years (UNODC, 2018). The extent of drug use in Nigeria currently is

comparatively high when compared with the 2016 global annual prevalence of any drug use of 5.6 per cent among the adult population (UNODC, 2018; UNODC, 2016). UNODC (2018) reported that one in seven persons aged 15-64 years had used a drug (other than tobacco and alcohol) in the year 2017. Among every 4 drug users in Nigeria, one is a woman indicating that more men (annual prevalence of 21.8 per cent or 10.8 million men) than women (annual prevalence of 7.0 per cent or 3.4 million women) have been reported in the past for involvement in the abuse of drug use in Nigeria (UNODC, 2018). The highest levels of drug use were among those aged 25-39 years (UNODC, 2018). Cannabis and tramadol are the most commonly used drug (UNODC, 2018; UNODC, 2016). An estimated 15.5 per cent of the population or 15.2 million people had used cannabis and tramadol (non-medically) in the past year *UNODC, 2018*). Report have shown that non-medical use of these pharmaceutical opioids including tramadol in Nigeria is 7 times higher among men (18.8 per cent among men vs. 2.6 per cent of women) (UNODC, 2018). Specifically, an estimated 4.7 per cent of the population or 4.6 million people have used tramadol for non-medical purposes in the past year (UNODC, 2018).

Tramadol being one the most abused drug is a centrally acting 'atypical' opioid analgesic producing a synergistic analgesic effect provided by a μ-opioid receptor affinity coupled with inhibitions of synaptic reuptake of monoamine neurotransmitters such as 5-hydroxytryptamine (5-HT) and norepinephrine (Raffa, 2008). A product of tramadol's hepatic metabolism, o-desmethyl tramadol (M1), possesses 200 - 300 times the affinity for μ receptors than tramadol itself (Grond and Sablotzki, 2004). Tramadol is a potent analgesic medication prescribed worldwide for treatment of acute and chronic pains (Grond and Sablotzki, 2004; Gillman, 2005). Tramadol as a centrally acting analgesic with a multimode of action acts on serotonergic and noradrenergic nociception, while its metabolite O-desmethyltramadol acts on the μ-opioid receptor. Its analgesic potency is claimed to be about one tenth that of morphine (Miotto *et al.*, 2017). Tramadol immunotherapy does not usually provide adequate analgesia (Miotto, *et al.,* 2017). In chronic non-cancer pain, there is little evidence for the use of tramadol for more than three months.

The mechanisms of action of tramadol are mainly due to binding to the μ-opioid receptor and inhibition of the neuronal uptake of norepinephrine and serotonin (Grond and Sablotzki, 2004; Gillman, 2005). Cytochorome-P450 (CYP450) play a significant role *in the activation and* inactivation of many exogenous and endogenous compounds. *For* example, CYP2E1 metabolizes N-nitrosamines to genotoxic products that methylate DNA and other macromolecules (Sheweita, 2000). CYP2E1 is able to produce reactive oxygen species (ROS), leading to oxidative stress which

consequently induces different cytotoxic effects (Yu *et al*., 2010). CYP3A4 and CYP2D6 metabolize tramadol into more potent opioid analgesic metabolite M1 (Miotto *et al.,* 2017). In addition, CYP2D6 gene polymorphisms increase hepatotoxicity through the accumulation of tramadol bioactive metabolite (M1), and consequently induce oxidative stress (Arafa and Atteia, 2018). The opioid analgesic potency of tramadol is influenced by an individual's CYP genetics since poor metabolizers have experienced little conversion to the more active M1 opioid metabolite, whereas individuals with a high metabolic rates have experienced greatest analgesic effects. Detoxification of M1 opioid metabolite is mainly carried out through phase II reactions with glucuronic acid and/sulphate (Raffa, 2008). The toxic effects of tramadol could lead to the generation of more than one metabolites that are associated with neuro-hepatic and nephrotoxicity especially after long-term administration/therapy (Watson *et al.*, 2004).

Materials

Equipment and instruments used

The equipment and instruments used in this study were of good analytical quality/grade and they include the following; Stopper florescence flask (Pyrex, England), Kjeldalic flask (Pyrex, England), Electronic weighing balance (Pyrex, England), Crucible (USA), Desiccators (USA), Spectrophotometer (Sharwood Scientific Ltd, Cambridge UK), Electric oven (USA), Flame photometer (Sharwood Scientific Ltd, Cambridge UK), Glass column chromatography (Pyrex, England), Refrigerator (Haier Thermocool, England), Sohxlet apparatus (Bradford, England), Murfle furnace (Bradford, England), Calibrate precision pipettes (USA), Absorbent material (USA), Vortex mixer (USA), and Centrifuge (Gallenkamp, Germany).

Chemicals and Reagents

The chemicals and reagents used were of analytical grade. The chemicals were sourced from May and Baker, England; BDH, England and Merck, Darmstadt, Germany, while the reagents used were commercial kits and products of Randox, QCA, USA and Biosystem Reagents and Instruments, Spain.

Experimental animals

The Albino rats which were the only biological material used for this study, were purchased from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The rats 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.

Methods.

Total of 24 albino rats were randomly divided into four groups of 1-4 (N = 6); Group 1 (normal control): rats received pellet and allowed free access to water; groups 2, 3 and 4 (tramadol groups): the animals received 50, 100 and 200 mg/Kg body weight of tramadol, respectively (Abdel-Zaher *et al*., 2011 and Ghoneim *et al*., 2014). All the administrations were via oral intubation and were done once daily. After 30 days administrations, rats were anaesthetized, sacrificed and the samples were kept in the appropriate tubes for the subsequent analysis.

Determination of Biochemical MarkeDetermination of Brain Monoamines

Concentrations of brain monoamines (serotonin, dopamine and nor-epinephrine) were determined using the method reported by Kema et al., (1993).

Dopamine

Principle

Dopamine is extracted by using a cis-diol-specific affinity gel, acylated and then converted enzymatically. The competitive ELISA kit uses the micro titer plate format. The antigen is bound to the solid phase of the micro litre plate. The derivatised standards, controls and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

Procedure

About 25 µL of the enzyme solution (refer to Reagent Preparation) were added into the already prepared wells of the Dopamine Micro litre Strips. This was followed by addition of 25 µL of the extracted standards, controls and 50 µL of the extracted samples into the appropriate wells. Around 25 µL of Hydrochloric Acid was added to standards, controls and samples. The mixtures were incubated for 30 min at RT (20-25 °C) on a shaker (approx. 600 rpm). Then, 50 µL of the Dopamine Antiserum was added in all the wells and cover plate with Adhesive Foil. The mixtures were incubated again for 2 hours at RT (20-25 °C) on a shaker (approximately 600 rpm). The foils were removed followed by discarding or aspirating the content of the wells. The plates were washed 3x by adding 300 µL of Wash Buffer, then, the contents were discarded and blot dried each time by tapping the inverted plate on absorbent material. Around 100 µL of the Enzyme Conjugate were

added into the wells, followed by incubation for 30 minutes at RT (20-25°C) on a shaker (approximately 600 rpm). Then, the contents of the wells were discarded or aspirated again. The plates were washed again thrice by adding 300 µL of Wash Buffer, followed by discarding the content and blot drying each time by tapping the inverted plate on absorbent material. One hundred (100) µL of the substrate were added into the wells and incubated for 25 ± 5 minutes at RT (20-25 °C) on a shaker (approximately 600 rpm). One hundred µL of the Stock Solution was finally added to each well, followed by shaking the micro titer plate to ensure a homogeneous distribution of the solution. The absorbance of the solution in the wells was read within 10 minutes, using a micro plate reader set to 450 nm.

Levels of Malondialdehyde (MDA)

Malondialdehyde level was estimated by measuring thiobarbituric acid reactive substances (TBARS) by method reported by Ohkawa *et al.,* (1979).

Principle

The principle of this test was based on the fact that malondiadehyde (MDA) reacted with thiobarbituric acid to form a red or pink colored complex which in acid solution absorbed maximally at the wavelength of 532 nm.

Calculation

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

Serotonin

Principle

Serotonin is acylated and detected by the subsequent competitive ELISA kit, which uses the micro litre plate format. Antigens are bound to the solid phase of the microliter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using Tetramethylbenzidine (TMB) as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standard concentrations.

Procedure

Total of 100 µL of the acylated standards, controls and samples were added into the appropriate

wells of the serotonin/5-HIAA micro liter strips. Addition of 25 µL of the Serotonin Antiserum into the wells. They were cover plate with adhesive foil and incubated for 15-20 hours at 2–8 °C. 4. After that, the foil was removed and the contents of wells were discarded, washed 3 times by adding 300 µL of Wash Buffer. The contents were discarded again and blot dried each time by tapping the inverted plate on absorbent material. About 100 µL of the enzyme conjugate was added into the wells, then, incubated for 30 min at RT (20-25°C) on a shaker (approximately 600 rpm). After the incubation, the contents of the wells were discarded, followed by washing the plate 3x by adding 300 µL of Wash Buffer, discarding the content again together with blot drying each time by tapping the inverted plate on absorbent material. Around 100 µL of the Substrate were added into the wells and incubated for 20-30 minutes at RT (20-25°C) on a shaker (approx. 600 rpm). Finally, 100 µL of the Stop Solution were equally added to each well and the micro liter plate was shaking to ensure a homogeneous distribution of the solution. The absorbance of the solution in the wells were read within 10 minutes, using a micro plate reader set to 450 nm.

Calculation

Principle

Nor-adrenaline (nor-epinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then converted enzymatically. The competitive ELISA kit uses the micro liter plate format. The antigen is bound to the solid phase of the micro litre plate. The derivatised standards, controls and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

Procedure

About 25 µL of the enzyme solution (refer to Reagent Preparation) was added into the already prepared wells of the nor-epinephrine Micro litre Strips. This was followed by addition of 20 µL of the extracted standards, controls and samples into the appropriate wells. The mixtures were incubated for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm). About 50 µL of the nor-epinephrine Antiserum was added in all the wells and cover plate with adhesive foil. The foils were removed followed by discarding the content of the wells. The plates were washed 3x by adding 300

µL of Wash Buffer, then, the contents were discarded and blot dried each time by tapping the inverted plate on absorbent material. About 100 µL of the Enzyme Conjugate was added into the wells, followed by incubation for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm). Then, the contents of the wells were aspirated again. The plates were washed again 3 times by adding 300 µL of Wash Buffer, followed by discarding the content and blot drying each time by tapping the inverted plate on absorbent material. One hundred µL of the substrate was added into the wells and incubated for 25 ± 5 minutes at RT (20-25 °C) on a shaker (approximately 600 rpm). One hundred (100) µL of the Stop Solution was finally added to each well, followed by shaking the micro titer plate to ensure a homogeneous distribution of the solution. The absorbance of the solution in the wells was read within 10 minutes, using a micro plate reader set to 450 nm.

Calculation

The concentrations of the samples and the Controls were read directly from the standard curve. The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

Determination of Oxidative Stress Parameters

Catalase Activity

Activity of catalase (CAT) was determined according to method reported by Aebi (1983).

Principle

The principle was based on ultraviolet absorption of hydrogen peroxide which was measured at 240 nm. On the decomposition of hydrogen peroxide (H_2O_2) with catalase, the absorption decreased with time and from this reduction, catalase activity was measured.

Procedure

Exactly 2.5 ml of phosphate buffer and 2 ml of hydrogen peroxide (H2O2) were added to the test tube. After that, 0.5 ml of the sample was also added to the test tube. 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.

Calculation

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 can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

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

Activity of SuperoxideDismutase

Superoxide dismutase (SOD) was assayed by the method reported by Marklund and Marklund (1974).

Principle

The superoxide dismutase ability to block the autoxidation of adrenaline was the basis of the SOD assay. Superoxide generated by xanthine oxidase is known to cause the oxidation of adrenaline to adenochrome. The production of the adenochrome formed per superoxide added increased with elevation of pH and also with increased adrenaline.

Procedure

Exactly 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.

Calculation

Super Oxide Dismutase (SOD) activity was measured by determining the inhibition of auto oxidant of adrenalins as shown below.

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

Glutathione-S-transferase (GST) activity of tissues was measured spectrophotometric method by the method reported by Habig *et al.*, (1974).

Principle

Glutathione-S-Transferase Assay Kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.

Procedure

Three wells were prepared and designated as non-enzymatic well, sample well and control well. Exactly 170 µL of Assay Buffer and 20 µL of glutathione were added to non- enzymatic wells. Exactly 150 µL of Assay Buffer, 20 µL of glutathione and 20 µL of the reconstituted GST-control were added to control wells. Around 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) (working reagent) were added to all the wells. The well plate were carefully shook for a few seconds to mix properly and the absorbance were read once every minute at 340 nm using a plate reader to obtain at least 5 time points.

Calculation

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

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

Δ A340/min = Change in absorbance at 340 nm per minute

Activity of GlutathionePeroxidase

Glutathione peroxidase (GPx) activity was estimated using the method reported by Flohe and Gunzler (1984).

Principle

Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) and oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) to form NADP+ and recycling the GSH. This is measured at 420 nm.

Reaction Principle:

Principle

Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) and oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) to form NADP+ and recycling the GSH. This is measured at 420 nm.

Reaction Principle*:*

GPx

2GSH+ ROOH ⟶ ROH + GSSG + H2O GR

GSSG + NADPH + H^+ ⟶ $NADP^+$+ 2GSH

Procedure

The reaction mixtures were prepared by putting in a test tube 14.0 ml of distilled water, 2.0 ml 5% pyrogallol solution, 1.0 ml of 0.147M H2O2 solution and 2.0 ml of 0.1M phosphate buffer (pH 6.0). The mixture was then equilibrated at 20 ^oC for about 5 minutes, after which there was the addition of 1.0 ml of the sample solution with mixing of the resulting solution. This was folowed by the addition of 1.0 ml of 2.0 N 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 (prepared like the test except that no sample is added to it and 15 ml of distilled water is used where 14 is used in the test solution).

Activity of Glutathione-s-transferase

Calculation

The activity of peroxidase can be 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 420nm corresponding to 1mg% purpurogallin in ether. df = dilution factor (if used during the study)

Level of Reduced glutathione

Reduced glutathione level was determined by the method reported by Jollow *et al.*, (1974).

Principle

This was based on the formation of relative stable yellow colour when Ellman's reagent is added to a sulforhydryl compound, 2-nitro-5 thiobenzoic acid. Ellman's reagent produce chromophoric product as a result of the reaction with reduced gluthathione absorbing at 412 nm. The absorbance at 412 nm is proportional to the gluthathione content.

Procedure

One mililitre of the sample was added 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 were 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.

Calculation

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

Some liver and Kidney Markers Assay

The activities of ALT and AST were assayed according to the method reported by Reitman and Frankel (1957). ALP activity was assayed by colorimetric method as reported by Klein et al., (1960).

Alanine Aminotransferase (ALT) Activity

Principle

α-oxoglutanate react with L-alanine to yield L-glutamate and pyrurate and ALT activity is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4- dinitrophenylhydrazine.

α-oxoglutarate + L-alanine ⟶ GGT L-glutamate + pyruvate

Procedure

Two test tubes were prepared, reagent blank and sample test tubes. Exactly 0.1 ml of the sample was added to the sample test tube. Then 0.5 ml of solution RI were added to both blank and sample test tubes followed by addition of 0.1 ml of distilled water to the reagent blank only. The tubes were mixed and incubated for exactly 30 minutes at the temperature of 37^0C. After that, 0.5 ml of solution R2 was added to the two test tubes. The test tubes were mixed and allowed to stand for exactly 20 minutes at 20-25 ^{0}C. Exactly 0.5 ml of 0.4N 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.

Calculation

The activity of ALT in the serum was obtained from the standard table.

Aspartate Aminotransferase (AST) Activity

Principle

This was based on the reaction between α-oxoglutarate and L-aspartate to yield L- glutamate and oxaloacetate catalyzed by GOT. AST was then measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

α-oxoglutanate + L-aspartate ⟶ GOT L-glutanate + oxaloacetate

Procedure

Two tests tubes were arranged, the sample and reagent blank test tubes. To the sample test tube, exactly 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, 0.1ml 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 exactly 20 minutes at 20-25^0C. Finally, 5.0 ml of 0.4N sodium hydroxide was added to each of the test tubes, mixed and after 5 minutes, the absorbance of sample (A sample) was read against the reagent blank at the wavelength of 546 nm.

Calculation

AST activity was obtained from the standard table.

Alkaline Phosphatase (ALP)Activity

Principle

The principle of this test was based on the hydrolysis of p-nitrophenylphosphate to yield phosphate and p-nirophenol catalyzed by ALP.

p-nitrophenylphosphate + H_2O ⟶ ALP Phosphate + p-nitrophenol.

Procedure

Exactly 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 timer start simultaneously. The absorbance was read again after 1, 2 and 3 minutes to get the changes in absorbance.

Calculation

ALP (U/L) = 2760 x ΔA nm/minutes

Determination of Total Bilirubin Concentration.

Principle

The principle was based on colorimetric method whereby total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure

Two cuvettes were prepared and arranged, the sample blank and sample cuvette. Exactly 4 drops (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^{0}C. Finally, 1000 µl of reagent four was added to both sample blank and sample cuvette, mixed and incubated for a further 5–30 minutes at 25 ^{0}C and the absorbance of the sample against sample blank was read at the wavelength of 578 nm.

Determination of Albumin Level (ALB)

Principle

The principle was based on the quantitative binding of albumin to the indicator 3, 3', 5, 5'-tetrabromo-m cresol sulphonephthalein (bromcresol green BCG). The albumin-BCG- complex absorbed maximally at 578 nm and the absorbance was directly proportional to the concentration of albumin in the sample.

Procedure

Test tubes were prepared and designated as reagent blank, standard and sample test tubes. Exactly 0.01 ml of distilled H_2O was added to the reagent blank. Then 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. Finally, 3.0 ml of 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 (A sample) and that of the standard (A standard) were read against the reagent blank at the wavelength of 630 nm.

Calculation

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

Results

Effects of Tramadol on Brain Monoamines in Albino rats

The results of brain monoamines in albino rats showed that serotonin, dopamine and nor-epinephrine levels in brain were significantly lowered at ($p<0.05$) in tramadol administered rats in dose dependent manner when compared to control as shown in figure 1

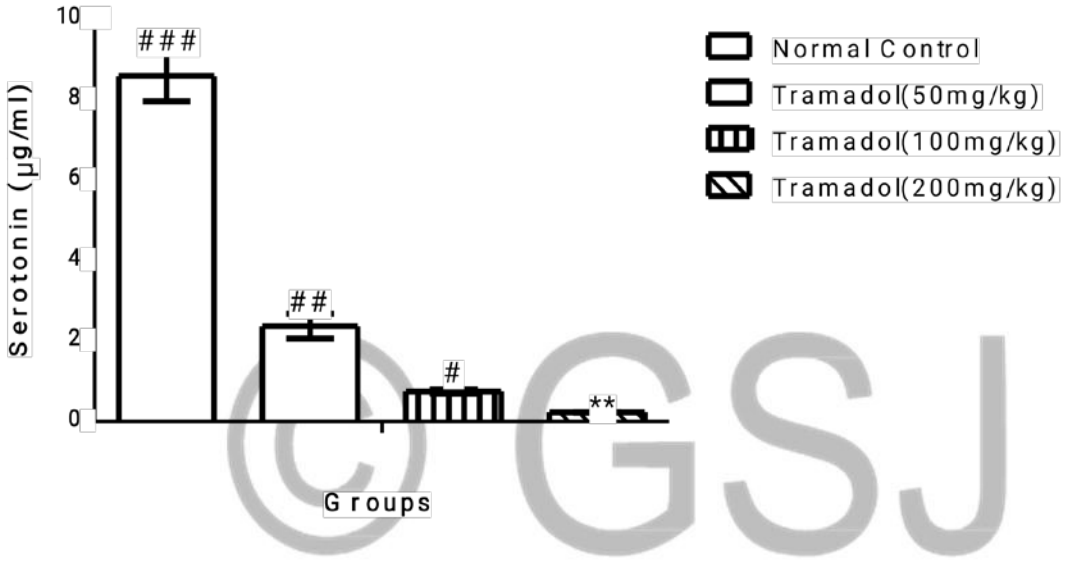


Figure 1: Effect of Tramadol on Serotonin Level in brain of albino rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at ($p<0.05$).

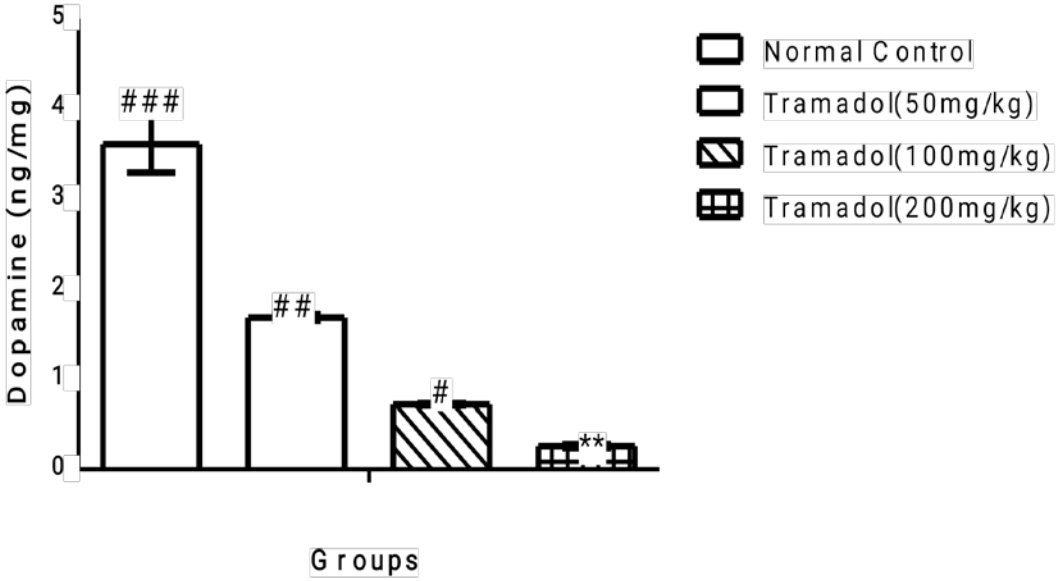
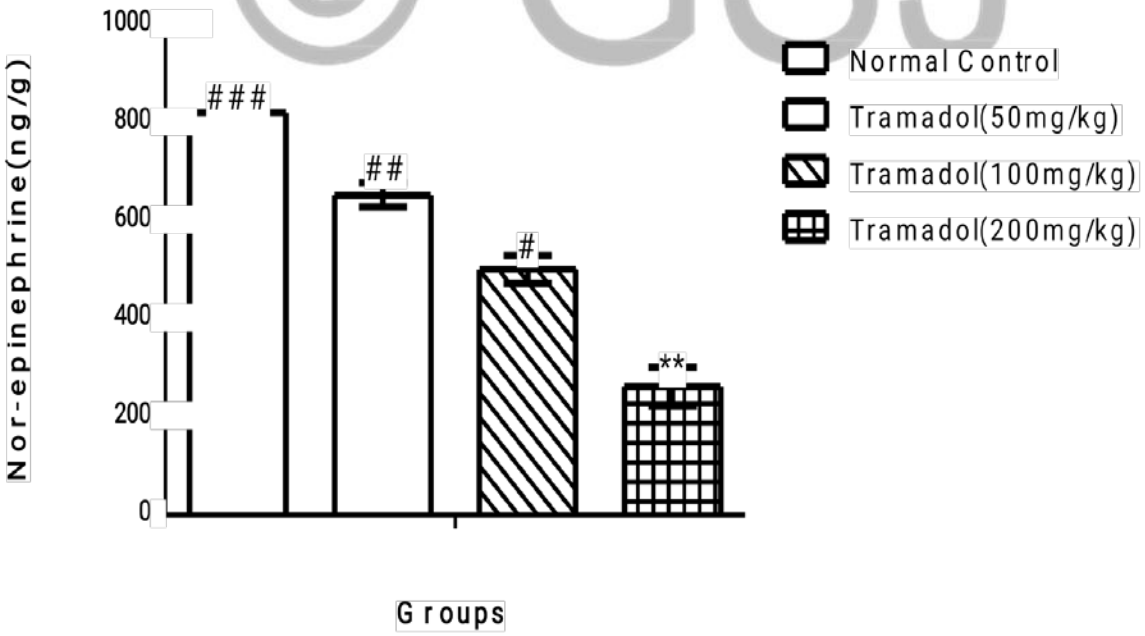


Figure2: Effect of Tramadol on Dopamine Level inbrainofalbinorats.Dataare shown as mean ± S.D (n = 6). Mean values with different signs are



significa

ntly different at ($p<0.05$)

Figure 3: Effect of Tramadol on Nor epinephrine 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 of Tramadol Exposure on Oxidative Stress Indices in Brain of Albino Rats

The results of oxidative stress indices of brain of albino rats showed that the activities of catalase, super oxide dismutase (SOD), glutathione transferase (GST), glutathione peroxidase (GPx) and level of reduced glutathione (GSH) were significantly ($p<0.05$) lowered in tramadol administered rats in dose dependent manner when compared to control while malondialdehyde (MDA) level was significantly elevated in the same manner when compared to control as shown in Figures 4 - 8

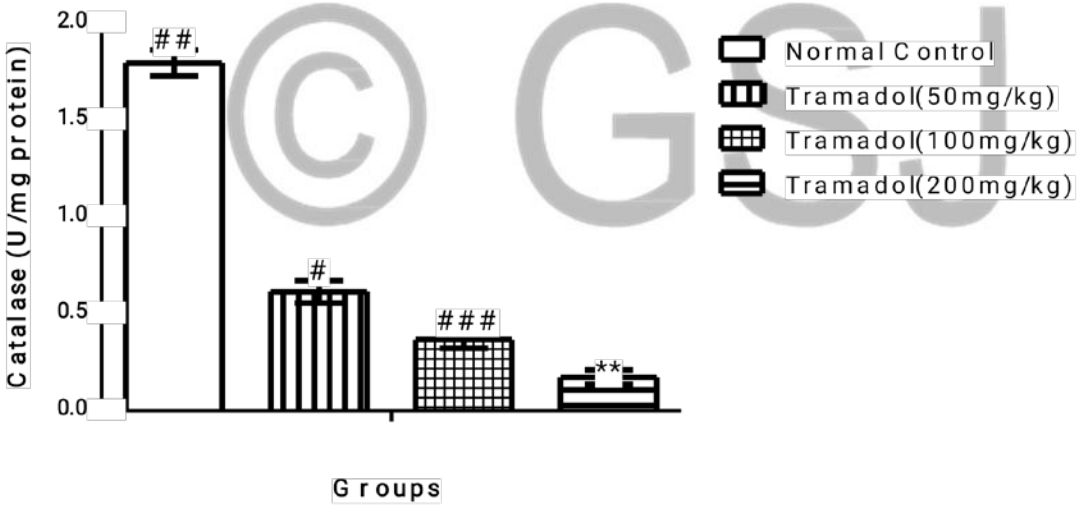


Figure 4: Effect of Tramadol on Catalase Activity in brain of albino rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at ($p<0.05$).

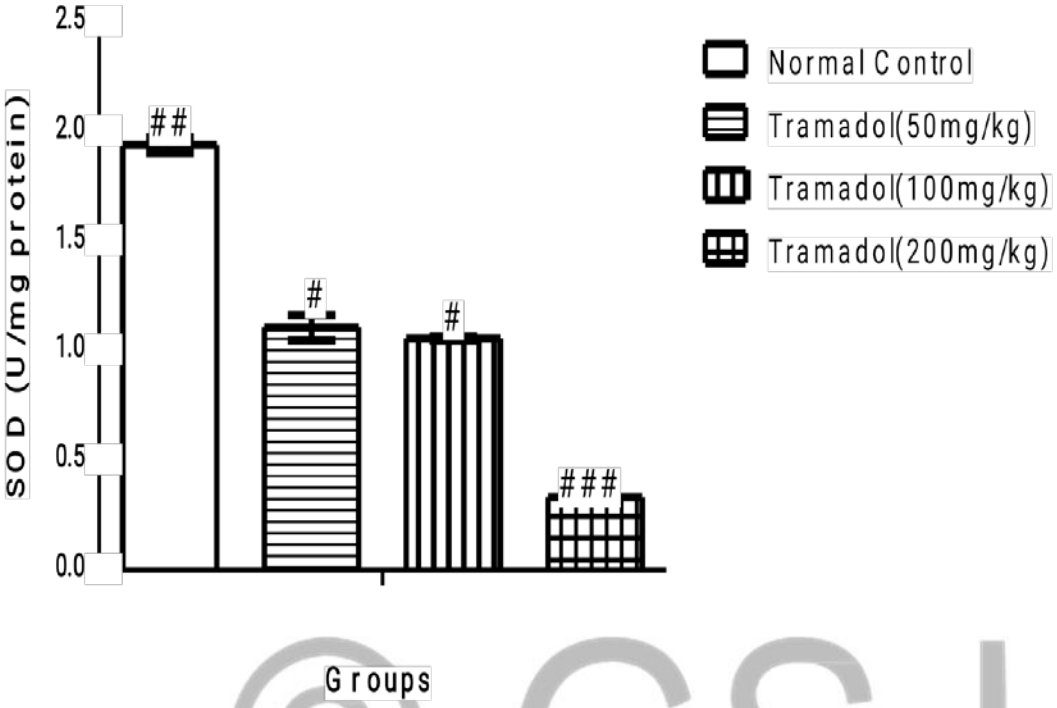


Figure 5: Effect of Tramadol on SOD Activity in Brain ofAlbino Rats. Data are shown as mean ± S.D (n=6). Mean values with the different signs are significantly different at ($p<0.05$).

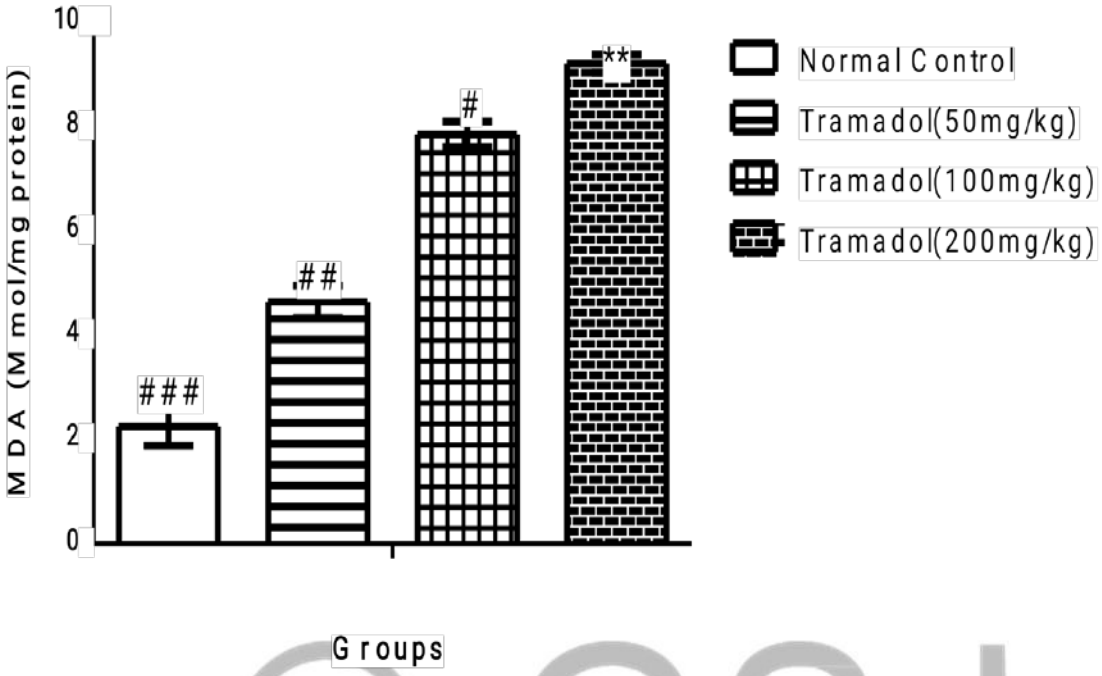


Figure 5: Effect of Tramadol on MDA Level in Brain of Albino Rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at $p<0.05$.

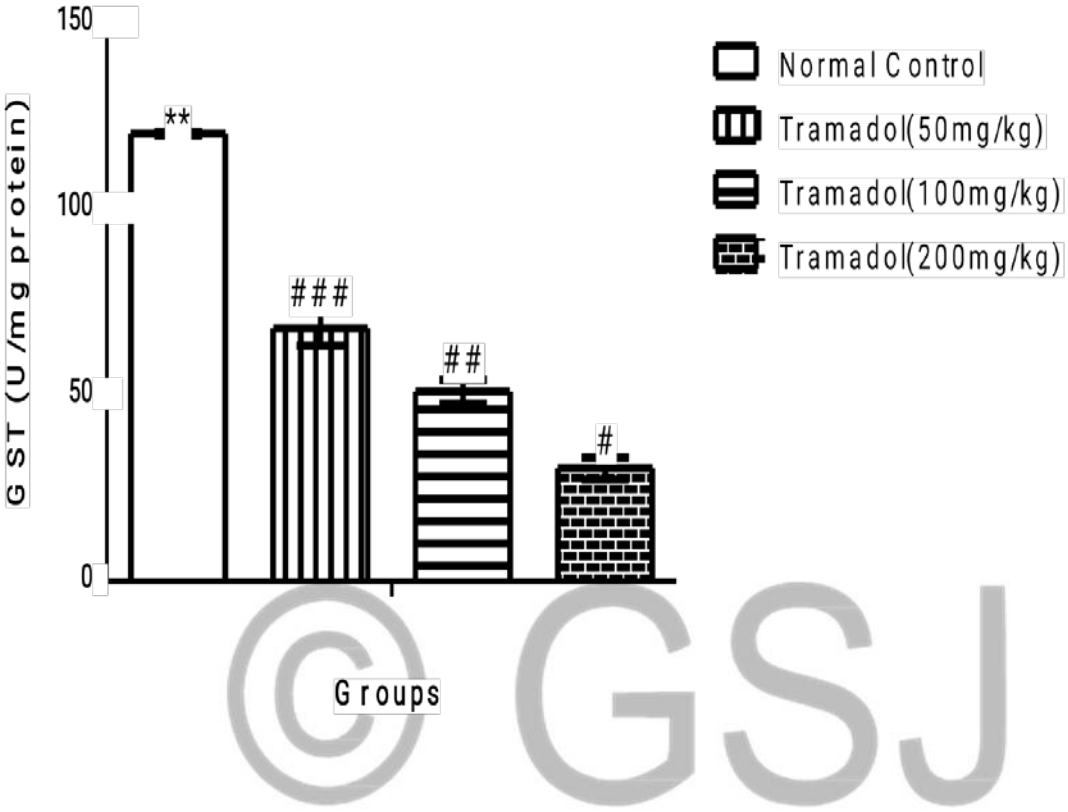


Figure 6: Effect of Tramadol on GST Activity in Brain of Albino Rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at P<0.05.

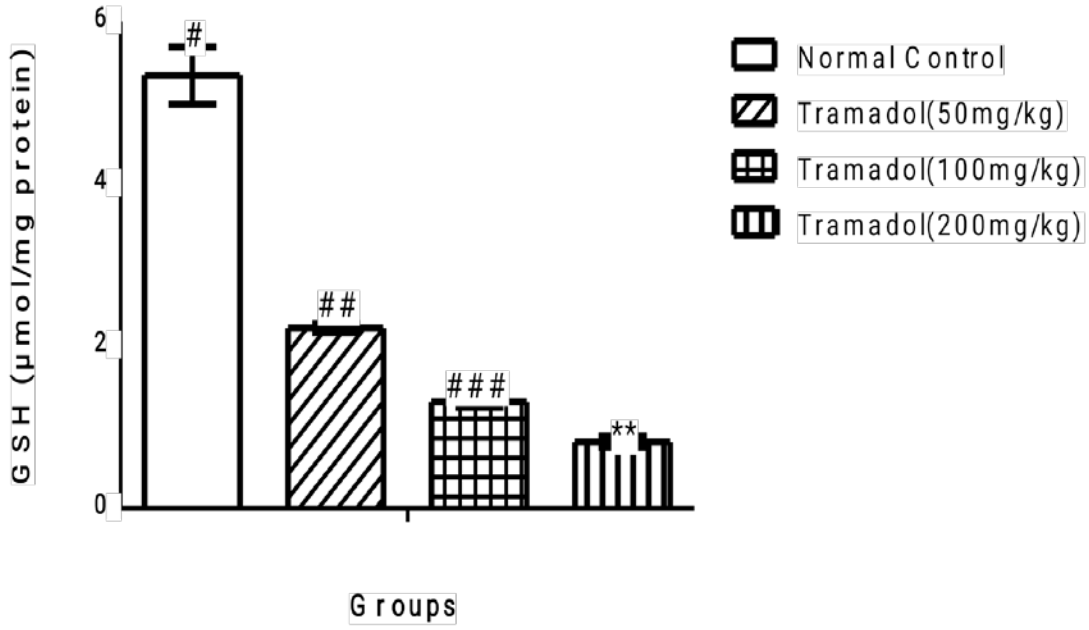


Figure 7: Effect of Tramadol on GSH Level in Brain of Albino Rats. Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at $p<0.05$.

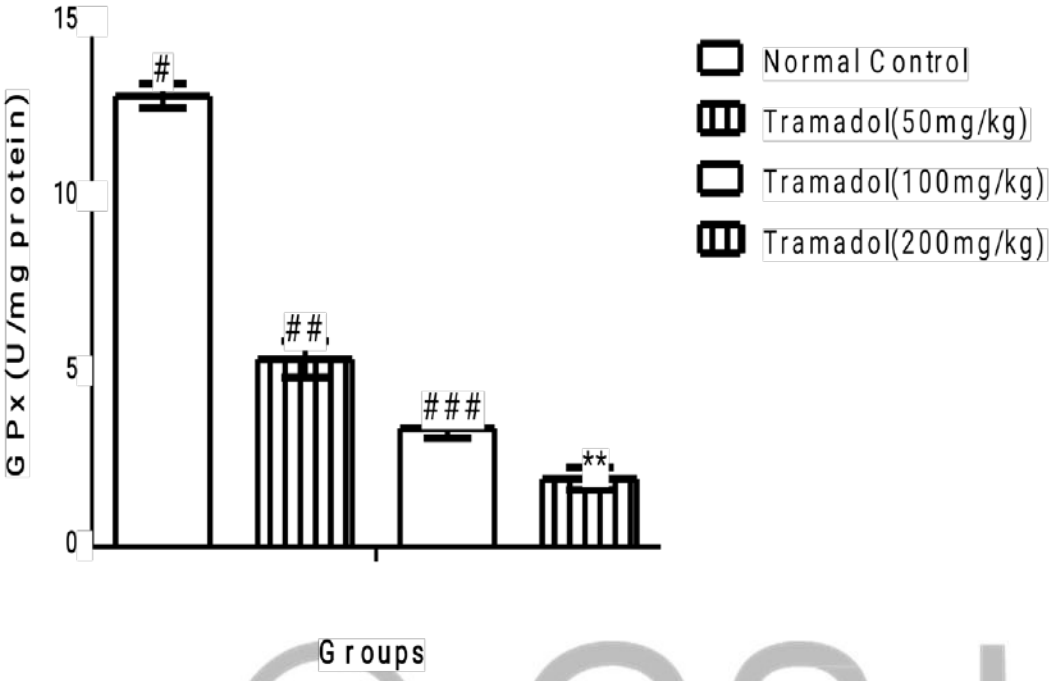


Figure 8: Effect of Tramadol on GPx Activity in Brain of Albino Rats. Data are shown as mean ± S.D (n=6). Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at $p<0.05$.

Effects of Tramadol Exposure on Liver Function Indices in Albino Rats

The results showed that the activities of AST, ALT, ALP and level of total bilirubin were significantly ($p<0.05$) elevated in tramadol exposed rats in a dose dependent manner when compared to control while albumin level was significantly lowered in the same manner when compared to control as shown in Figures 9-18.

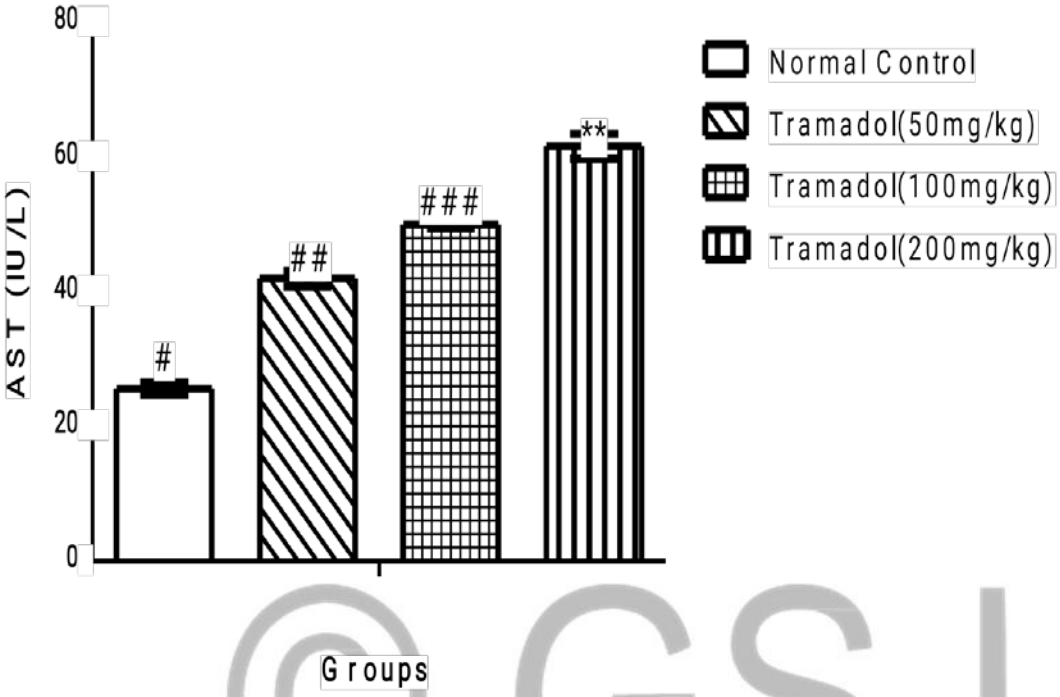


Figure 9: Effect of Tramadol on AST Activity in Albino Rats. Data are shown as mean

± S.D (n=6). Data are shown as mean ± S.D (n=6). Mean values with the different signs are significantly different at $p<0.05$.

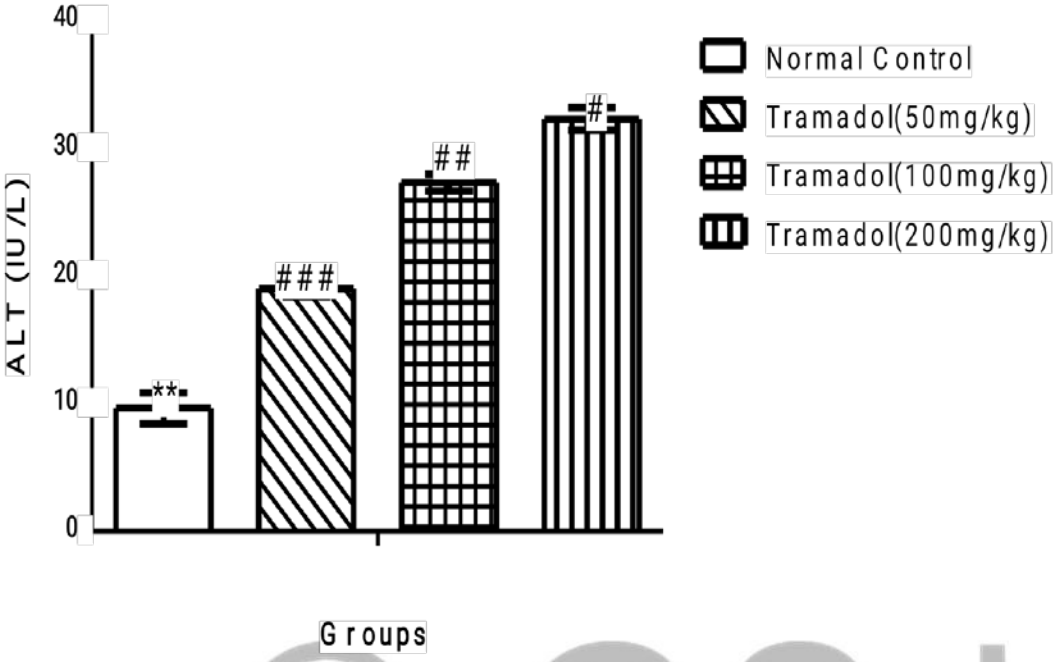


Figure 10: Effect of Tramadol on ALT Activity in Albino Rats. Data are shown as mean ± *S.D (n=6*). Data are shown as mean± S.D (n=6). Mean values with the different signs are significantly different at $p<0.05$.

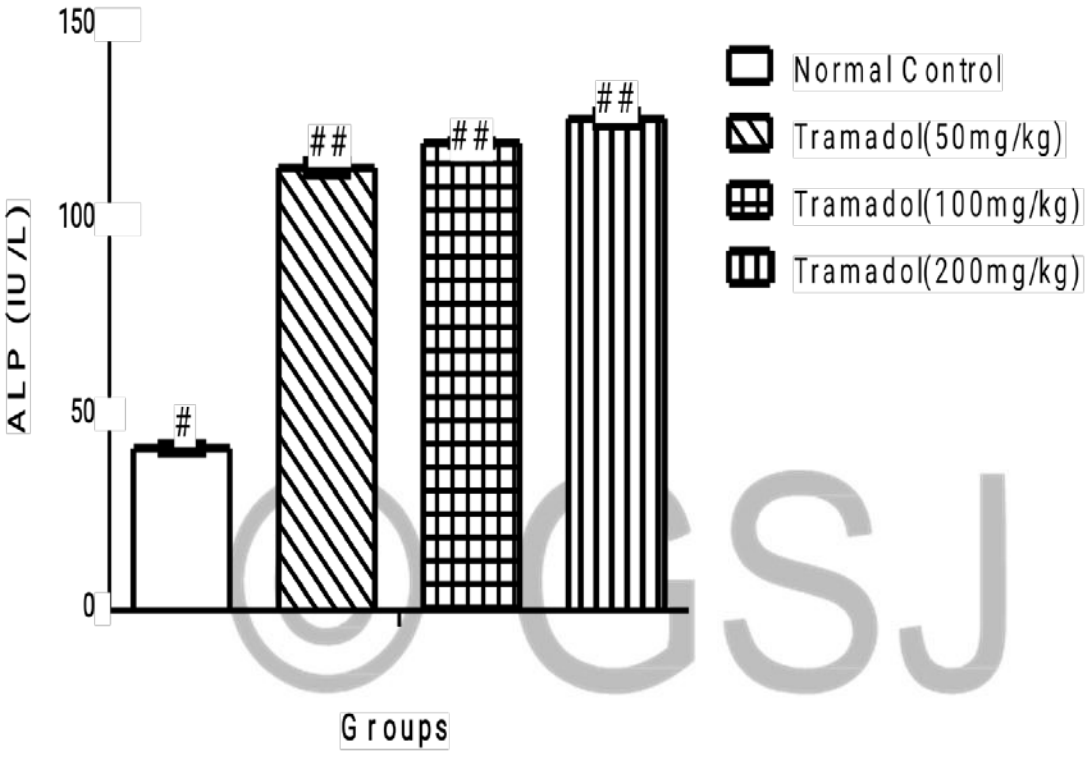


Figure 11: Effect of Tramadol on ALP Activity in Albino Rats. Data are shown as mean

± S.D (n=6). Data are shown as mean ± S.D (n=6). Mean values with different signs are significantly different at $p<0.05$.

Discussion

Exposure of tramadol to albino rats caused significant ($p<0.05$) decrease in the levels of brain monoamine neurotransmitters (serotonin, dopamine and nor-epinephrine) relative to the normal control (Figure 1-4). This result is in agreement with the study of Essam et al., (2014) who reported that oral administration of tramadol once daily for 28 days significantly ($p<0.05$) decreased the monoamine neurotransmitter (nor-epinephrine (NE), serotonin (5-HT) and dopamine (DA)) levels in streptozotocin-induced diabetes in sprague-dawley rats during painful diabetic neuropathy. It also correlate with the work of El-Baky and Hafez (2017) who reported a significant ($p<0.001$) increase in plasma monoamine oxidase (MAO) leading to serotonin (5-HT) and dopamine decrease in brain tissues proteins ($p<0.001$) in tramadol administered albino rats.

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, with the consequent decrease in 5-HT synthesis (Manjarrez et al., 1998). The decreases in neurotransmitters induced by tramadol may also be due to its ability to block their uptake (Halfpenny et al., 1999) and inhibit its reuptake (Zacny, 2005) as well as the interaction between the tramadol metabolite O-desmethyltramadol and the monoamine neurotransmitter receptors (Frink *et al.*,1996).

Oxidative Stress

This research investigations 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 when compared to controls. This finding is in agreement with the following findings: Hanaa and Ayman (2019) who reported that administration of tramadol to albino rats significantly ($p<0.05$) increased cerebral lipid peroxidation and decreased GSH content and expression of the antioxidant enzymes (catalase, superoxide dismutase, peroxidase, glutathione reductase, glutathione-S- transferase, glutathione peroxidase, quinone reductase). El-Baky and Hafez (2017) who reported that tramadol in both low and high dose ingestion lead to expression of plasma peroxidation value (MDA) but reduced glutathione (GSH) level and superoxide dismutase (SOD) activity in plasma were significantly ($p<0.05$) decreased.

The study results have shown that tramadol intoxication lead to significant reduction in enzymes such as SOD, POD and CAT in brain tissue. It also suggested 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. Reactive oxygen species formed by tramadol

intoxication can covalently bind to macromolecules and in cellular membranes, hence promoting lipid peroxidation (Khan and Younus, 2011; Saeed *et al*., 2012). Peroxidation of polyunsaturated fatty acids generates malonaldehydes (Khan and Younus, 2011; Saeed *et al*., 2012). Increase in MDA levels indicated that tramadol induced oxidative damages in brain tissues (Clemedson *et al*., 1990; Krasteva *et al*., 2007 and Park *et al.*, 2008).

Reduced glutathione is an important thiol protein, which participates in different defense processes against oxidative stress. It eliminates superoxide anion and H_2O_2 from the body. Low level of GSH as a result of tramadol toxicity could be responsible for tissue injury and various anomalies related to oxidative stress (Limon Pacheco *et al.*, 2007). GSH is a well-known endogenous antioxidant (Drechsel and Patel, 2008). From the present results and previous studies, it has been shown that tramadol caused reduction in GSH levels. GSH normally acts through a combination of various reduction and conjugation reactions to protect cells against both exogenous toxicants and the reaction of endogenous compounds (Krzywanski, *et al.*, 2004). One possible explanation for the decreased level of GSH is the defective synthesis, utilization, and degradation of GSH as well as changes in the activity of glutathione peroxidase and glutathione reductase as presented in this report. SOD eliminates peroxide anion radicals and inhibits the propagation of fatty degeneration (Escobar et al., 1996). Decreased SOD and CAT activities in tramadol administered albino rats are linked up to exhaustion of the enzymes as a result of increased generation of reactive oxygen species due to tramadol exposure.

In the present study, the liver functions 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. Similar results were obtained by Wu et al., (2001) and Atici et al., (2005) who reported significant ($p<0.05$) increase in the levels of serum ALT, bilirubin and AST in rats after long term usage of tramadol. Abdelraouf et al., (2015) also reported that serum activities of ALT, ALP, AST and LDH and levels of direct and total bilirubin were significantly ($p<0.05$) higher in tramadol abuse group compared to the control group. Similarly, Elkhateeb et al., (2015) reported a significant ($p<0.05$) elevation in liver enzymes such as ALT, AST, GGT and bilirubin level in tramadol administered albino rats. This findings equally concur with the work of Essam et al., (2014) who reported that administration of 100 mg/Kg therapeutic dose of tramadol to diabetic animals resulted in a significant ($p<0.05$) increase in the activities of AST and ALT when compared with the control group during painful diabetic neuropathy and Abiodun et al., (2019) who also

reported that tramadol intake at different doses significantly ($p<0.05$) increased plasma levels of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) with reduction in total protein levels.

The role of the liver in tramadol metabolism predisposes it to toxic injury associated with tramadol (Matthiessen *et al.,* 1998). The hepatic function in drug metabolism involves converting drugs and other compounds into products (metabolites) that are more easily excreted (Coughtrie et al.,1989; Milne et al.,1997; Tolman, 1998). However, metabolites may have a higher activity and/or a greater toxicity than the original drug. Serum ALT is relatively specific, affected early by hepatotoxicity and is considered an excellent marker of cellular necrosis, as it is a cytoplasmic enzyme (Moss and Handerson, 1999). Thus, a rise of ALT activity in diseases is associated with injury of hepatocytes like in viral hepatitis. On the other hand, AST is mainly a mitochondrial enzyme, its elevated level in the serum is not specific of the hepatic disorder, as it is used mainly to diagnose and to verify persistent cellular injury with other enzymes like ALT (Vozarova et al., 2002). The serum ALP is related to the function of hepatic cell and its increase in serum may be due to increased synthesis of the enzymes in presence of increasing biliary pressure (Tripathi, 2013). General elevation in hepatic markers (ALT, AST and ALP) in the swerum could be a secondary event following tramadol-induced lipid peroxidation of hepatocyte with the subsequent increase in the leakage of these biomarkers from the liver (Nehru and Anand, 2005). Lipid peroxidation of cell membranes leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability, all of which lead to leakage of the enzymes from the liver cells. Generally, an increase in this enzyme indicates injury or toxicity to the organ. Hence, long-term use of tramadol intake is more harmful to liver and can cause a serious cellular toxicity and a liver failure (Rukhshanda et al., 2014).

Conclusion

The results 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.

Recommendations

Based on the results of the study, we recommend that the following should be done;

Other tissues should be assed to ascertain the degree of toxicity of this drug.

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.

Concomitant intake of tramadol and any supplement should be encouraged to reduce associated toxicity of tramadol.

The government should create an awareness to enlighten and educate the general public on the toxicity associated with tramadol intake under non- induction of pain.

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