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ACUTE AND CHRONİC DEGREE OF TOXICITY IN TRAMADOL CONSUMPTİON ON PLASMA PROTEIN, *ASPARTATE AMİNOTRANSFERASE (AST), ALANİNE AMİNOTRANSFERASE (ALT) AND ALKALİNE PHOSPHATASE (ALP)* İN ADULT WISTAR RATS

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Abstract – Although the mechanism of hepatotoxicity from tramadol overdoses is unknown, it is most likely caused by direct hepatocellular injury, either as a result of ischemia or mitochondrial toxicity. Overdosing on tramadol has been reported to cause acute liver failure. Tramadol-related minor enzyme increases are often asymptomatic, self-limited, and resolve even when medication is continued. This investigation examines the short- and long-term effects of tramadol use on liver enzymes in an animal model. The study involved sixty (60) mature Wistar rats of both sexes. Tramadol (300g) were administered to the animals in the experimental and control groups in the following ways: Before being sacrificed, Group A (n = 5 Males and 5 Females) received no treatment within the study's time frame. Group B (n = 5 Males and 5 Females) received tramadol 30 mg/kg body weight for 7 days and were sacrificed; Group C (n = 5 Males and 5 Females) received tramadol 30 mg/kg body weight for 14 days and were sacrifice; Group D (n = 5 Males and 5 Females) received tramadol 30 mg/kg body weight for 21 days and were sacrifice, Group E (n = 5 Male and 5 Female) received tramadol 30 mg/kg body weight for 42 days and were sacrificed, while Group F (n = 5 Male and 5 Female) withdrew for 3 weeks after receiving tramadol 30 mg/kg for three weeks before sacrificing. Liver was removed from the animals for biochemical examination. The findings of the SPSS analysis on the generated data were expressed as mean SEM. After three and six weeks of tramadol administration, the results obtained demonstrated a progressive increase in weight and an increase in the activities of ALT, ALP, and AST in the plasma while decreasing the level of total protein, albumin, direct bilirubin as well as indirect bilirubin as compared to the control rats. Therefore, this study comes to the conclusion that tramadol has harmful effects, both acute and chronic, on the structure and operation of hepatic tissue in wistar rats. As a result, tramadol use needs to be monitored.

Keywords – Tramadol, Liver Enzymes, Wistar Rtas

1. INTRODUCTION

An opioid analgesic called tramadol is used to treat mild to moderate pain (Ryan, 2019; Ojieh et al., 2022). It is a synthetic codeine analogue that is effective against mild-to-moderate pain, but is not as effective as standard opioids and is not advised for severe pain (Leppert, 2009; Leppert, 2011; Ryan, 2019). It also mildly inhibits serotonin and norepinephrine reuptake. Serum aminotransferase levels can be raised in a small percentage of tramadol-taking patients, especially with high dosages, according to Chalasani et al. (2008). Tramadol overdoses, whether intentional or unintentional, have been known to result in fatal cases of severe liver failure and respiratory arrest (Chalasani et al., 2008). However, in these situations, the liver damage can have been brought on by the respiratory arrest-related shock, hypoxia,

or ischemia. Tramadol overdose-related liver damage has also been linked to hyperammonemia, lactic acidosis, and hepatic steatosis, all of which are indicative of a direct injury to the mitochondria (Reuben et al., 2010). Acetaminophen taken alone or in conjunction with tramadol has been linked in certain cases to acute liver failure following tramadol overdose (Reuben et al., 2010). If it happens at all, clinically obvious idiosyncratic liver impairment caused by tramadol must be quite uncommon. However, clinically obvious drug-induced liver damage has not been linked to the pharmacologic use of tramadol.

Although the exact mechanism of hepatotoxicity caused by tramadol overdoses is unknown, direct hepatocellular injury—possibly as a result of ischemia or mitochondrial toxicity—is most likely to be the cause (Björnsson et al., 2013). Tramadol is metabolized by the liver, primarily by CYP2D6 and 3A4 to its active form, which can lead to problematic drug-drug interactions (Björnsson et al., 2013). CYP2D6 is an enzyme that contributes to the metabolism of about 25% of all medications (Miotto et al., 2017). Therefore, medications with the ability to inhibit or induce these enzymes may interact with tramadol. Tramadol use is associated with modest enzyme increases, which are often low, asymptomatic, and self-limited and resolve even with continued therapy (Loughrey et al., 2003).

Acute liver failure brought on by tramadol overdose can be fatal and requires intensive medical care. Acute overdoses may interact with other substances that can harm the liver, including acetaminophen (Loughrey et al., 2003).

Patients and medical professionals face a severe issue as a result of the negative consequences of tramadol used in clinics (Asis and Navarro, 2009). According to estimates by Shin et al. (2017), around 10% of medications have serious, unfavourable side effects. Hussaini and colleagues, 2013). Given that medication-induced side effects are challenging to identify due to pre-existing medical disorders, multiple drug usage, and a lack of diagnostic criteria, this figure is likely underestimated (Asis and Navarro, 2009). Therefore, this study assesses the level of toxicity of tramadol on plasma protein, direct and indirect bilirubin levels, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels in adult wistar rats.

2.0 MATERIALS AND METHODS

Chemicals and drugs

The Demerk pharmacy in Obiaruku, Delta State, Nigeria sold tramadol. All other substances, including medications, were of analytical grade.

Experimental animal

At the Faculty of Basic Medical Sciences Animal House at Delta State University in Abraka, Nigeria, sixty (60) mature Wistar rats—30 males and 30 females—were purchased for this study and housed in metabolic cages. They were fed a daily mash diet made for animal feed farmers by Top Feed, a company located in Sapele, Delta State. 17.0% protein, 4.5% minimum fat, 0.96% minimum calcium, 3.92% useable minimum phosphorus, and 2450 kcal energy are all included in the feed, in addition to unlimited amounts of water.

Drugs preparation and administration

To create a stock solution containing 6 mg/ml of tramadol, 300 mg of the drug were dissolved in 50 ml of water. Oral administration was the method used.

Experimental Design:

Group A (5 males and 5 females): Untreated control

Rats in Group B (n = 5 male and 5 female) received 30 mg/kg of tramadol daily for 7 days before being sacrificed.

Rats in Group C (n = 5 male and 5 female) received tramadol at a dose of 30 mg/kg body weight daily for 14 days before being sacrificed.

Rats in Group D (n = 5 male and 5 female) received tramadol at a dose of 30 mg/kg body weight every day for 21 days before being sacrificed.

Rats in Group E (n = 5 male and 5 female) received tramadol 30 mg/kg body weight for 42 days before being sacrificed.

Rats in Group F (n = 5 male and 5 female) were given tramadol 30 mg/kg for three weeks before being sacrificed.

Sample Collection

Rats were dislocated at the cervical spine to cause death, then each were placed on its dorsal surface. A laparotomy were then performed to expose the internal organs, and blood were drawn through heart puncture and deposited in blood sample containers for biochemical analysis.

Determination of Body Weight and Organ Weight

$$\text{Percentage weight change (\%)} = \frac{\boldsymbol{final - intialbodyweight\ (g)}}{\boldsymbol{intialbodyweight\ (g)}} \boldsymbol{X} \frac{\mathbf{100}}{\mathbf{1}}$$

Body weight of experimental animals was checked at week 0 before tramadol administration and last day of experiment before sacrifice. Percentage weight change was later calculated as follows.

Biochemical Analyses

Aspartate Amino Transferase (AST) Determination:

This was done using a pair reaction of malate dehydrogenase (MDH) and NADH, which was the first kinetic test of AST for diagnostic purposes disclosed by (Karmen, et al., 1955).

Alanine Amino Transferase (ALT) Estimation

Reitman and Frankel (1957) used the same enzymatic reaction sequence for their ALT test. 2-oxoglutarate with L-alanine. By keeping an eye on the quantity of pyruvate hydrazone that 2,4 dimitrophanylhydrazine forms, alanine aminotransferase activity was monitored.

Alkaline phosphatase (ALP) Assay

Alkaline phosphatase (ALP) is a class of cytomembrane-related enzymes that acts on various phosphate substrates through hydrolysis and transfer activity. ALP enzyme determination was done using the technique used by Ossai et al. in 2021.

Assay for Total Protein

Principle:

Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Sample: Serum, heparinized plasma or EDTA plasma.

Materials: Biuret Reagent, Blank Reagent, Standard.

Reagent:

i. For the biuret reagent (stock solution), 400 ml of 0.2N (0.8%) NaOH was used to dissolve 45 g of sodium potassium tartrate. Next, 15 g of copper sulphate and 5.0 g of potassium iodide were added, and the volume was then increased to 1 litre using 0.2N NaOH.

ii. To make the biuret working solution, 200 ml of the stock were diluted to a volume of 1 litre using 0.2 N NaOH that included 5 g/l potassium iodide.

iii. Tartarate iodide solution: In 1 litre of 0.2N NaOH with 5 g/l potassium iodide, 9g of sodium potassium tartarate was dissolved.

iv. Standard protein: Rats used in experiments provided pooled sera devoid of hemolysis. Kjedahl's method, in which proteins are digested by boiling in the presence of sulphuric acid and a catalyst, was used to determine the protein concentration. Store between 2 and 8 oC.

Procedure

There were four test tubes with the labels "Test," "Test Blank," "Standard," and "Blank," each containing 5.0 millilitres of working biuret reagent and 0.05 millilitres of distilled water. "Test" stood for "test," which contained 5.0 millilitres of working biuret reagent and 0.05 millilitres of serum.All test tubes were combined and heated to 20 to 25 oC for 10 minutes.By zeroing the spectrophotometer with the reagent blank, the absorbance of the sample (A

sample) and the standard (A standard) at 540 nm (green filter) were measured.

Calculation

When measurements were taken at 540nm, total protein concentration was calculated as follows:

Total Protein conc. (g/l) =190 multiplied by A sample

Total Protein conc. (g/l) =19 multiplied by A sample

When using standard

Total protein conc. = A sample X standard concentration

A standard

Determination of Bilirubin Level

Direct and indirect bilirubin levels were measured to determine the in vitro quantity of serum bilirubin.

Principle of Test

High levels of conjugated or direct bilirubin show that bile is not being effectively expelled, therefore a bile duct or gall bladder obstruction may exist. By deducting the direct bilirubin level from the total bilirubin value, the unconjugated or indirect bilirubin level can also be calculated. Unconjugated bilirubin levels above normal suggest either that the liver is not actively repairing the hemoglobin it is receiving or that excessive amounts of hemoglobin are being degraded.

The Jendrassik and Grof (1938) colorimetric method can be used to assess bilirubin levels. In an alkaline media, direct bilirubin and diazotized suphanilic acid combine to generate a blue-colored complex. By reacting with diazotized sulphanilic acid in the presence of caffeine, which releases albumin-bound bilirubin, total bilirubin can be measured. A spectrophotometer can be used to detect the amount of absorbance and calculate the level of bilirubin to determine the reaction's outcome and the intensity of the complex that is produced.

Procedure

For total bilirubin test:

The reagents R1, R2, and R3 from the bilirubin kit were combined with the sample and let to stand for 10 minutes at 20 to 25 degrees Celsius. The sample was mixed with billirubin reagent (R4) and let to stand for 5–30 minutes at 20–25 oC. The above process was used to prepare the sample blank but without the sample. The sample's absorbance was then measured at 578 nm in comparison to the sample blank.

For direct bilirubin:

R1, R2, and 0.9% NaCl from the bilirubin kit were combined with the sample and let to stand for 10 minutes at 20–25 oC. The above process was used to prepare the sample blank but without the sample. The sample's absorbance was then measured at 578 nm in comparison to the sample blank.

Calculation

Total Bilirubin (µmol/l) = 185 X ATB (578nm)

Total bilirubin (mg/dl) = 10.8 X ATB (578nm)

Direct Bilirubin (µmol/l) = 246 X ADB (546nm)

Direct bilirubin (mg/dl) = 14.4 X ADB (546nm)

Where A = Absorbance

Statistical analysis

Results were expressed as mean values standard error of mean (Mean SEM) after data were processed by comparing values for various treatment groups with values for individual controls. Using SPSS version 23 software, significant differences between the control and experimental groups were examined using the student's t-test and analysis of variance ANOVA. The results were deemed significant at P-values of less than 0.05 (P0.05). Microsoft Excel (2007) was used to create the graphic representations.

3. RESULTS

Table 3.1. Acute and chronic effects of Tramadol consumption on body weight and percentage change in body weight of male Wistar rat

GROUP	INITIAL WEIGHT (g)	FINAL WEIGHT	PECENTAGE WEIGHT CHANGE
A	161.40±16.42	194.60±19.44	20.79±4.24[a]
B	139.80±2.60	140.12±6.29	0.20±3.87[b]
C	137.00±3.23	153.60±5.24	12.05±1.91[a]
D	138.20±5.35	142.80±14.26	2.89±7.89[b]
E	152.80±13.17	171.40±15.46	15.24±13.06[a]
F	172.20±21.29	164.20±7.05	2.33±14.23[b]

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. aP<0.05 indicate significant increase and bP>0.05 indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.2: Acute and chronic effects of Tramadol consumption on organ weight of male Wistar rat

GROUP	Liver weight (gm)	Relative liver weight (%)
A	5.97±0.54	3.10±0.16
B	4.49±0.20	3.20±0.06
C	4.74±0.35	3.10±0.23
D	3.67±0.46	2.55±0.08
E	5.49±0.36	3.23±0.11
F	4.80±0.14	2.93±0.07

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. [a]P<0.05 indicate significant increase and [b]P>0.05 indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks

Table 3.3: Acute and chronic effects of Tramadol consumption on body weight and percentage change in body weight of female Wistar rat

GROUP	INITIAL WEIGHT (g)	FINAL WEIGHT	PECENTAGE WEIGHT CHANGE
A	212.00±7.14	214.97±9.90	1.45±3.82[b]
B	193.00±6.68	184.93±7.10	-4.22±0.43[c]
C	195.00±14.28	196.25±11.62	2.83±11.43[b]
D	181.75±16.36	193.00±7.31	8.52±9.79[a]
E	202.75±14.41	183.50±8.41	-9.00±2.31[c]
F	180.75±14.87	192.00±15.64	6.25±0.71[b]

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. [a]$P<0.05$ indicate significant increase, [b]$P>0.05$ indicate no significant difference and [c]$P<0.05$ indicate significant decrease

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.4: Acute and chronic effects of Tramadol consumption on organ weight of female Wistar rat

GROUP	Liver weight (gm)	Relative liver weight (%)
A	6.67±0.43	3.14±0.31
B	5.23±0.12	2.83±0.05
C	4.60±0.54	2.34±0.25
D	5.49±0.15	2.86±0.15
E	6.62±0.48	3.60±0.16
F	7.18±0.86	3.71±0.17

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. [a]$P<0.05$ indicate significant increase and [b]$P>0.05$ indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.5: Acute and chronic effects of Tramadol consumption on liver biomarkers of male Wistar rat

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)
A	49.17±4.01	52.71±2.15	99.23±9.61
B	54.51±9.50	63.48±8.04	172.22±32.14
C	47.10±1.53	66.85±9.14	129.85±13.48
D	44.64±1.35	54.69±5.69	165.70±11.62
E	61.41±8.86	62.46±2.86	124.55±1.92
F	50.02±4.40	59.14±6.96	109.69±5.74

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. $^{a}P<0.05$ indicate significant increase and $^{b}P>0.05$ indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.6: Acute and chronic effects of Tramadol consumption on liver biomarkers of female Wistar rat

GROUP	ALT (U/L)	AST (U/L)	ALP (U/L)
A	33.30 ± 2.02^{a}	47.13 ± 6.59^{a}	110.81 ± 15.11^{a}
B	45.22 ± 3.08^{b}	57.29 ± 96.29^{c}	160.45 ± 11.16^{c}
C	80.44 ± 24.38^{c}	63.55 ± 5.95^{c}	159.02 ± 7.32^{c}
D	105.64 ± 6.86^{c}	54.22 ± 2.52^{c}	154.24 ± 11.62^{c}
E	152.29 ± 67.29^{c}	65.80 ± 4.70^{c}	134.96 ± 4.94^{c}
F	65.77 ± 86.19^{c}	47.70 ± 2.04^{b}	115.09 ± 11.25^{b}

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. $^{a}P<0.05$ indicate significant increase, $^{b}P>0.05$ indicate no significant difference and $^{c}P>0.05$ indicate significant difference with control

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.7: The short and long term effects of Tramadol consumption on serum proteins level of male Wistar rat

GROUP	TP	ALB	Dir bil	Indir bil
A	10.59±1.36[a]	4.10±0.38[b]	8.19±1.33[b]	12.92±1.80[a]
B	23.63±6.57[c]	5.93±0.44[b]	10.11±0.51[b]	16.24±1.34[c]
C	19.33±2.16[c]	5.91±0.73[b]	8.23±0.95[b]	15.57±0.82[b]
D	54.53±4.41[c]	7.49±0.22[b]	10.62±0.48[b]	15.76±1.05[b]
E	38.93±4.25[c]	4.80±0.54[b]	7.55±0.68[b]	16.8627±0.88[c]
F	47.42±4.69[c]	5.44±0.33[b]	7.68±0.43[b]	13.4442±1.75[b]

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. [a]P<0.05 indicate significant increase and [b]P>0.05 indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

Table 3.8: The short and long term effects of Tramadol consumption on serum proteins level of male Wistar rat

GROUP	TP	ALB	Dir bil	Indir bil
A	15.38±0.88	6.88±0.76	7.13±0.77	10.76±0.86[a]
B	19.06±0.22	9.06±0.22	9.06±0.22	17.06±1.42[c]
C	21.77±4.29	8.63±1.09	6.44±0.30	14.59±2.17[b]
D	28.43±8.75	8.23±1.50	10.08±0.21	15.69±1.18[b]
E	30.13±0.18	10.05±0.15	7.74±1.38	14.32±1.88[b]
F	36.79±2.39	9.21±1.53	9.30±1.09	17.45±1.44[c]

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. [a]P<0.05 indicate significant increase and [b]P>0.05 indicate no significant difference

KEY: GROUP A = Normal Untreated rats, GROUP B = one week treatment with tranadol 30mg/kg. GROUP C = Received tramadol 30 mg/kg for two weeks, GROUP D = Received tramadol 30 mg/kg for three weeks, GROUP E = Received tramadol 30 mg/kg for six weeks and GROUP F = withdraw from receiving tramadol 30 mg/kg after three weeks.

4.0 DISCUSSION

The most widely used over-the-counter medications worldwide are analgesics (Ossai *et al.,* 2021; Ojieh *et al.,* 2022; Ossai *et al.,* 2023). Tramadol hydrochloride, a synthetic counterpart of codeine, is a centrally acting analgesic medication with minimal affinity for opioid receptors (Desmeules *et al.,* 1996). As compared to the parent medication, tramadol's M1 metabolite exhibits a greater affinity for opioid receptors (Desmeules *et al.,* 1996). Tramadol has attracted a lot of attention since it was first introduced in the 1970s since it is used to treat moderate to severe pain (De Decker *et al.,* 2008). Tramadol misuse is on the rise among young adults today, with male abuse being more prevalent in most nations around the world and especially among Africans (Ossai *et al.,* 2023). With significant morbidity and mortality (Jones and Story, 2005; Chandrasekaran *et al.,* 2007), tramadol overdose and chronic use have been two of the most common causes of drug poisoning in recent years, particularly in young adults with a history of substance abuse and mental disorders (Shadina *et al.,* 2008).

Having reported the LD50 of tramadol in mice and rats to be 350 mg/Kg and 300 mg/Kg body weights respectively (Matthiessen *et al.,* 1998), Ossai *et al.* (2023) had recommended a daily dose of 50-100 mg of tramadol between four to six hour intervals due to the increased risk of side effects with higher doses.

Tramadol misuse, especially among opiate addicts, has previously been linked to increased body weight in users (Mohammed and Mahmoud, 2019). Tables 3.1 and 3.3 provide information on the body weights of both male and female tramadol-induced Wistar rats. When comparing the final weight gain of the rats to their baseline body weight, a progressive weight growth was seen after three and six weeks of the treatment of tramadol (30mg/kg). Additionally, the growth with groups C and E was comparable to that of the control group A.

This is comparable to a study by Shuey *et al.* (2008) which found that wistar rats given with tramadol at doses of 40 mg/kg and 80 mg/kg for 8 weeks experienced a significant increase in body weight. A significant difference in body weight between control and tramadol-induced rats was also found in Mohammed and Mahmoud's (2019) research after giving the rats 30 mg/kg and 60 mg/kg of tramadol for 8 weeks, respectively. As with antidepressant medications like venlafaxine (Effexor), tramadol increases levels of the neurotransmitters serotonin and norepinephrine in the brain, which is thought to be part of the mechanism behind the drug's ability to reduce depressive and obsessive-compulsive symptoms as well as why it causes an increase in body weight in those who take it (Raffa *et al.,* 2012).

When compared to the starting weight before tramadol administration, the organ (liver) weight similarly exhibits a relative weight change with the animal's bodyweight following tramadol (30mg/kg) treatment (Tables 3.2 and 3.4). These results corroborated those of Ossai et al. (2021) and Ojieh et al. (2022). The neurotoxic effect of prolonged tramadol usage is mediated by oxidative stress, inflammation, and apoptosis and promotes body and organ weight gain in an animal model, according to a paper by Mohammed and Mahmoud (2019).

The liver's role in the metabolism and excretion of tramadol may help to explain these findings (Shah *et al.,* 2013). The liver primarily uses N- and O-demethylation to break down tramadol hydrochloride before conjugating it with glucuronic acid and sulphate. O-desmethyl tramadol, the active metabolite, has double the analgesic effectiveness of the parent drug and has greater affinity for mu-opioid receptors (Lee *et al.,* 2011).

Due to the liver's crucial function in drug metabolism, the majority of medications have been linked to hepatotoxicity (Wu *et al.,* 2001). The primary process by which medicines and other chemicals are transformed into products that can be eliminated more readily and typically have lower pharmacologic action than the powerful molecule is hepatic function (Loughrey *et al.,* 2003; Rafati *et al.,* 2012). The activity and/or toxicity of metabolites may be higher than those of the parent medication. As a result, the liver is vulnerable to toxic damage due to its central involvement in drug metabolism (Matthiessen *et al.,* 1998; Ojieh *et al.,* 2022). Due to the liver's crucial function, almost every medicine has been linked to hepatotoxicity (Tolman, 1998). Drug metabolites from the liver are discharged in the kidneys, where some of them may harm cells and result in malfunction of the liver (Singhal *et al.,* 1998).

As evidenced by an increase in the plasma activities of ALT, ALP, and AST when compared to the control rats, the liver functions of the male and female rats treated with tramadol in the current investigation were both compromised. This result is comparable to earlier studies that showed a considerable rise in serum ALT, AST, and ALP levels in rats after chronic tramadol use (Wu *et al.,* 2001; Atici *et al.,* 2005). The elevated ALT, AST, and ALP plasma activities in this study are symptomatic of liver toxicity since the liver is an organ that detoxifies hazardous substances and chemical medications in the body (Vozarova *et al.,* 2002; Ossai *et al.,* 2021). The increased plasma level of these enzymes in tramadol-treated rats may therefore be the result of necrosis or damage to the liver cell membrane that allows the enzymes to leak into the blood circulation (Loughrey *et al.,* 2003). The increased secretion of these liver enzymes may be accompanied by acute cell necrosis. Similar findings were supported by experimental tests of acute and chronic morphine therapy in mature albino rats, which showed that morphine can cause necrosis in the liver during its metabolism (Atici *et al.,* 2005). In rats receiving morphine treatment, hemorrhage and cytolysis were also seen (Atici *et*

al., 2005). In the hepatic tissue, however, they only observed perivenular hydropic degeneration (Atici *et al.,* 2005). Another study showed that the morphine and tramadol group's postoperative effects on the histopathology of the liver in rabbits underwent isoflurane anesthesia, hepatocyte degeneration, central vein dilatation, and mononuclear cellular infiltration were more severe than those of the control group. Additionally, the tramadol group had higher sinusoidal dilatation and cell membrane degradation of the hepatocytes than the morphine group did (ZuhtuUtku *et al.,* 2006). These findings imply that the liver tissue may experience certain modifications as a result of morphine and tramadol use.

Plasma protein synthesis is primarily done in the liver (Wu *et al.,* 2001). Rough endoplasmic reticulum performs the synthesis, which is then released into the hepatic sinusoid (Osadolor and Omo-Erhabor, 2017). The availability of amino acids, catabolic situations, the activities of cytokines and hormones, and/or congenital deficient states have all been shown to alter protein synthesis in addition to decreased hepatic function (Robert, 2012).

The concentration of substances produced by the liver is typically determined by one of the following methods: measurement of the serum content of substances known to change in concentration as a result of hepatic damage, measurement of substance concentration from injured liver cells, evaluation of the liver's capacity to perform a metabolic task, such as conjugation or detoxification, and/or evaluation of the enzyme activity and substrate content of organelles and cells.

Tramadol's effects on hepatic integrity, test for cholestasis, and synthetic function were identified. Despite not being due to a decrease in albumin, as indicated in tables 3.7 and 3.8, there was a significant fall in serum total protein in this study. According to Osadolor and Omo-Erhabor (2017), albumin is a typical indicator of hepatocyte capacity to perform synthetic activity. According to Sembulingam and Sembulingam (2010), albumin and globulin fractions make up the majority of blood total protein. Any component that makes up the total protein levels in serum can decrease without affecting the total protein levels in the blood. Tramadol's impact on any of the globulin fractions may have caused the drop, suggesting that long-term use of the drug could be harmful to immunological responses. The serum globulin level was not assessed in this investigation, though. Tramadol may have an immune-enhancing effect, as suggested by Zhihen and colleagues (2006), while Sacerdote and colleagues (1997) suggest it may be helpful in treating patients for whom immunosuppression may not be appropriate. Since the liver is known to have a reserve capacity, a reduction in protein content can only be noticed in cases of severe liver damage. The findings of this study may possibly be explained by the comparatively long half-lives of liver proteins, which for albumin range from 19 to 21 days (Osadolor and Omo-Erhabor, 2017). Acute hepatic impairment has been associated with minimal increases in plasma protein concentrations (Robert, 2012). In a study on "hepatic DNA damage and abnormality in serum protein pattern due to long-term tramadol use in rats," Laila (2012) also noted changes in serum protein levels.

The orange-yellow pigment known as bilirubin is derived from heme and is mostly produced by the red blood cell turnover process (Sembulingam and Sembulingam, 2010). In the liver, it is extracted, bio-transformed, and eliminated through the urine and bile (Osadolor and Omo-Erhabor, 2017). Its estimation provides details regarding the liver's conjugative activity (Guyton and Hall, 2006). When treated groups were compared to control groups, the study's findings in tables 3.7 and 3.8, respectively, demonstrated a substantial decrease in total and direct bilirubin readings. Showing that tramadol has no effect on the liver's ability to conjugate.

Abdelraouf *et al.* (2015) provided evidence that tramadol did not significantly differ between abusers and control groups, with the exception of abusers who had been abusing the drug for longer than five years.

5.0 SUMMARY OF FINDINGS

1. Taking tramadol increases body weight and organ weight regardless of time, although compared to acute usage, a considerable rise was seen with chronic use.
2. The liver enzymes ALT, ALP, and AST significantly rise with the administration of tramadol for both acute and chronic disorders.

3. Consuming tramadol both acutely and chronically lowers total protein levels while having little effect on albumin levels

4. Consuming tramadol lowers levels of both total and direct bilirubin in both acute and chronic conditions

6.0 CONCLUSION

According to the results of the current study, tramadol use has harmful effects on the structure and function of hepatic tissue in wistar rats in both acute and chronic situations. As a result, its use must be under strict control.

Recommendations

Before starting therapy, patients who will be receiving long-term tramadol treatment should be informed of the potential liver consequences of this medication. It is advised to monitor liver function tests. Tramadol hydrochloride should be tapered and discontinued if there are irregularities, if this is clinically appropriate.

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