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Cannabis sativa in ethanol (Monkey Tail) and tramadol induced similar degrees of toxicity in adult female Wistar rats

Chinomso Friday Aaron[1], Emmanuel Iroham Akubugwo[1], Solomon Nnah Ijioma[2], Robert Ikechukwu Uroko[*3], Uche Okuu Arunsi[1], Kingsley Chijioke Ugwuanyi[2], Victor Chinwem Oguike[1]

[1]*Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria;*
[2]*Department of Zoology and Environmental Biology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria;*
[3]*Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria;*
***Email:** *ir.uroko@mouau.edu.ng*

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ABSTRACT

Background & Aim: This study was designed to evaluate the toxic effects of *Cannabis sativa* local gin formulation (CSLGF) and tramadol in adult female Wistar rats.

Experimental: *Cannabis sativa* local gin formulation (CSLGF) was prepared and was subjected to acute toxicity tests together with tramadol. In the main study, 25 rats were assigned into 5 groups (n = 5) in which the group 1 received no treatment, groups 2 and 3 received 25 and 50 mg/kg of tramadol, respectively while groups 4 and 5 received 25 and 50 mg/kg of CSLGF, respectively, for 21 days before the rats were sacrificed.

Results: Acute toxicity results indicated narrow margins of safety for CSLGF and tramadol with LD50 values of 123.0 and 133.0 mg/kg body weight, respectively. The rats treated with CSLGF lost weight significantly while that administered tramadol had lower weight gains when compared with the control ($P<0.05$). The red blood cell counts, packed cell volume and haemoglobin concentrations were significantly lowered by CSLGF ($P<0.05$). However, a significant increase in the number of platelets and white blood cells were observed in groups treated with both CSLGF and tramadol. Results of biochemical changes showed a significant increase in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, urea and creatinine with a concurrent decrease in total protein, albumin and globulin values following treatment with both agents ($P<0.05$), but serum electrolytes concentrations were not significantly altered ($P>0.05$). Treatment with CSLGF and tramadol also significantly lowered glutathione, superoxide dismutase and catalase activities but increased malondialdehyde concentrations when compared with control.

Recommended applications/industries: The findings show that although the toxic effects of CSLGF and tramadol in rats are similar, CSLGF appears to have higher toxicity potentials. Extending the current control on tramadol to CSLGF (monkey tail) is urgently needed in Nigeria to protect human lives.

1. Introduction

The upsurge in the number of people who engage in illicit drug use is currently an issue of globalconcern. *Cannabis sativa*, popularly called marijuana, is recognizedas the most widely used illicit substance

amongst youths of age 18 and above, who either smoke, cook with the substance or take it as a tincture in a solvent base. Recent reports have associated the current global increase in mental health problems with the use of *Cannabis sativa* (Degenhardt *et al*., 2010; Kumar *et al*., 2010). Other health challenges which have been linked to the substance are varying degrees of affecting the muscles and body extremities like fingers of the hands and toes of the feet. Tremors and intestinal dysfunctions have also been reported (Turner *et al*., 2020). Tramadol is a centrally acting synthetic analgesic agent,that has of late come under serious control by health agencies in many countries across the world due to its numerous scientifically established toxic effects which include but are not limited to carcinogenicity, mutagenicity, genotoxicity, cardiopulmonary disorders, seizures and deaths (Shadnia, 2008; Dhanjal *et al*., 2009; Belin, 2017; Jones *et al*., 2017).

Gender-based differences have been established in degrees of toxicity following intoxication by *Cannabis sativa* in males and females (Nicolson and Roberts, 2010). Although males are known to consume *Cannabis* more than females, the extent of use amongst females remains high, as a recent report has shown that about 15.4% of females 18 years and above engage in the use of cannabis globally (Haight *et al*., 2018). Females also experience more marijuana adverse effects than males due to hormonal differences and effects (Miller *et al*., 2001; Nicolson and Roberts, 2010). Sex hormones make females more sensitive to *Cannabis* toxicity than males and are the reason females experience higher degrees of toxicities affecting the liver, heart, brain and blood vessels following its use (Haight *et al*., 2018). The fact that androgens in males are known to offer some protection against cannabis toxicity on the heart appears to support the view of higher toxicity in females than males (Miller *et al*., 2001). Information on gender-based toxicity due to illicit use of tramadol remains very scanty, but an area of interest in the current study.

Toxicity due to the use of medicinal plants has been reported,and has been worsened by the fact that in ethnomedicine, most of the herbal preparations are presented in an alcohol base, and may further increase toxicity due to possibly synergy between alcohol and the toxic components of the plant material used (Oshilonya *et al*., 2015). In southern Nigeria, the tincture popularly called "monkey tail" is usually prepared by soaking ground *Cannabis sativa* leaves into local gin for 2-3 days, after the resulting solution containing the extract is transferred into a fresh container and consumed by a large of youths for medicinal (analgesic) and lifestyle purposes. The toxic effects of alcohol have been widely reported (Teixeira *et al*., 2014; Zhou *et al*., 2017).

Bodyweight changes, haematological values, levels of liver and renal function parameters and antioxidant enzymes activities have remained major indicators of toxicity and good health and have over the years been used to measure the health status of both humans and animals (Oshilonya *et al*., 2015; Ijioma *et al*., 2019). For example, high values of liver function parameters like ALT, AST, ALP and bilirubin may correlate with liver damage even as above normal values of renal function parameters like urea and creatinine most often suggest possible kidney damage. Fall in glutathione, SOD and catalase activities coupled with a significant increase in MDA concentration also suggest an increased level of systemic toxicity due to oxidative effects of both endogenously and exogenously generated oxidants on body cells. It is therefore against this background that these parameters were carefully selected and employed in this study to evaluate the toxic effects of *Cannabis sativa* local gin formulation and tramadol in adult female Wistar rats.

2. Materials and Methods

2.1. Collection and identification of sample

The leaves of *Cannabis sativa* were obtained from IgbogeneEpie, Yenagoa Local Government Area of Bayelsa State, but Tramadol was purchased from a Patent Medicine Store in Umuahia, UmuahiaNorth Local Government Area of Abia State, both in Nigeria. The plant sample was taken to the Department of Forestry and Environmental Management, MichealOkpara University of Agriculture, Umudike for authentication. A sample specimen was deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike with voucher number (MOUAU/VPP/18/021). Tramadol was authenticated by a Pharmacist at the Federal Medical Center (FMC), Umuahia, Abia State, Nigeria.

2.2. Preparation of CSLGF and stock solution of tramadol

Following the indigenous protocol for the preparation of monkey tail, twenty grams of the dried and powdered *Cannabis sativa* was completely soaked in 100 mL of local gin (*Ogogoro*) and stirred intermittently within 48 hours. The resulting mixture was then filtered to obtain a filtrate and residue. The concentration of the filtrate (now monkey tail) was determined by drying the residue and reweighing it. With the weight of the residue being 14.70 g, the amount of the extract in 100 mL of the local gin was then 5.30 g, translating into 0.053 g/mL (53 mg/mL). The filtrate was then kept for use and is hereafter referred to as *Cannabis sativa* local gin formulation (CSLGF).The stock solution of tramadol was prepared by dissolving 100 mg of the drug in 100 mL of distilled water, representing a stock concentration of 1 mg/mL. This was preserved in a refrigerator for daily use.

2.3. Animals and experimental design

Seventy-five female albino rats were used for the study. Fifty of the rats were used for acute toxicity evaluation of the extract and tramadol while the remaining 25 were used for the sub-acute toxicity study. The rats which were obtained from the laboratory animal house of the College of Veterinary Medicine, Michael Okpara University, where the study was carried out, were kept under normal standard environmental conditions with access to feed (Vital Finisher mash) and water *ad libitum*. Experiments were carried out following the United States guidelines for care and use of experimental animals and as approved by the ethical committee of the host Department.

2.4. Acute toxicity (LD50) evaluation of the extract and tramadol

The method used by Ijioma *et al.* (2019) was adopted with little modification. Briefly, for each test substance, twenty-five rats were assigned to 5 groups (1-5) with each group administered a specific oral dose level of CSLGF or tramadol. The doses administered were group 1 (10 mg/kg), group 2 (50 mg/kg), group 3 (100 mg/kg), group 4 (120 mg/kg) and group 5 (200 mg/kg). After treatment, the animals were returned to their respective cages and were monitored for toxicity signs and mortalities within 24 hours and a further 7 days. Karber's formula stated below was used to calculate the acute toxicity value for each test agent.

$$LD_{50} = \frac{\text{LD100} \ - \sum (\text{Dd x Md})}{N}$$

Where:
LD_{100} = Dose that killed all animals in a given population
LD_{50} = Dose that killed 50% ina given populatio
$\sum$ (Dd x Md): Sum of all products of dose difference and mean death
N = Number of animals in each group.

2.5. Sub-acute toxicity evaluation of CSLGF and Tramadol

Twenty-five female albino rats assigned to 5 groups of 5 rats each were treated as shown below via the oral route for 21 days:

Group 1: 0.5 mL of water (control)
Group 2: 25 mg/kg of tramadol
Group 3: 50 mg/kg of tramadol
Group 4: 25 mg/kg of CSLGF
Group 5: 50 mg/kg of CSLGF

At the end of treatment, each animal was sacrificed by cervical dislocation for blood collection by cardiac puncture into EDTA and plain bottles for haematological and all serum biochemical studies including liver and renal functions tests and antioxidant enzyme assays. The body weights of the rats were measured on day 1 and day 21 of treatment.

2.6. Determination of haematological, biochemical and antioxidant values

Haematological values including red blood cells count (RBCC), packed cell volume (PCV), haemoglobin (Hb), white blood cells count (WBCC), platelets count (PLTC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined at once for each blood sample in an automated haematologyanalyser (BC-2300, Mindray Company, China). Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, bilirubin, urea, creatinine, sodium, potassium, chloride and bicarbonate were determined using commercial test kits with strict adherence to procedures prescribed by the producer, Randox

Laboratories, UK. Antioxidant parameters including superoxide dismutase (SOD), catalase, reduced glutathione and Malondialdehyde (MDA) were determined in serum according to the protocols adopted by Sun *et al*. (1988) and Kanu *et al*. (2016).

2.7. Statistical analysis

Results were presented as mean values ± standard deviations (mean ± SD). The replicates in each treatment were subjected to one-way analysis of variance (ANOVA) and the difference between the samples mean were tested by Tukeyposthoctest using R-statistics software version 3.03. P-values ≤ 0.05 were considered statistically significant.

3. Results and discussion

3.1. Acute toxicity values of CSLGF and Tramadol

While no mortality was recorded in groups treated with up to 50 mg/kg body weight of CSLGF, 20%, 60% and 100% mortalities were observed in the 100, 200 and 200 mg/kg treatment groups respectively giving rise to an acute toxicity value of 123.0 mg/kg body weight in the female rats (Table 1). In the case of tramadol, 20%, 40% and 100% mortalities were observed in groups treated with 100, 120 and 200 mg/kg body weight of the substance respectively and yielded an acute toxicity value of 133.0 mg/kg body weight (Table 2). The degree of toxicity caused by CSLGF and tramadol was first made evident by their LD_{50} values of 123 mg/kg and 133 mg/kg body weight respectively. These acute toxicity values suggest narrow margins of safety for both agents with CSLGF appearing to be more toxic than tramadol having as the former had a slightly lower LD_{50} value than the latter. The animals that died across the groups in the course of the acute toxicity test may have lethal doses of both substances. The consumption of toxic doses of *Cannabis sativa* has been linked with toxicity signs like dizziness, drowsiness, nervous problems and liver damageand may have played various roles leading to the death of the animals (Turner *et al*., 2020). Not to be forgotten is the fact that *Cannabis sativa* as used is contained in an alcohol base which is another major cause of systemic toxicity (Teixeira *et al*., 2014). This is in addition to the toxic effect of the major component of *Cannabis sativa*, tetrahydrocannabinol, a component that has been reported to be toxic to rats (Turner *et al*., 2020). Systemic toxicity following tramadol use is also well established (Youssef and Zidan, 2015).

Table 1. Result of acute toxicity evaluation of CSLGF.

Dose (mg/kg)	Number of deaths	Percentage mortality	Dose difference (DD)	Mean death (MD)	DD × MD
10	0	0	40	0	0
50	0	0	50	0.5	25
100	1	20	20	2.0	40
120	3	60	80	4.0	320
200	5	100	-	-	-

LD_{50} = 123.0 mg/kg body weight

Table 2. Result of acute toxicity evaluation of tramadol**.**

Dose (mg/kg)	Number of deaths	Percentage mortality	Dose difference (DD)	Mean death (MD)	DD × MD
10	0	0	40	0	0
50	0	0	50	0.5	25
100	1	20	20	1.5	30
120	2	40	80	3.5	280
200	5	100	-	-	-

LD_{50} = 133.0 mg/kg body weight

3.2. Effect of CSLGF and tramadol on body weight of female rats

Both CSLGF and tramadol negatively affected the body weights of treated female rats when compared with control (P<0.05). While body weight gains in the groups treated with tramadol were only slight and significantly lower than that of the control rats, those treated with CSLGF had weight loss to the tune of 2.11±0.36% and 3.03±0.40% in groups treated with 25 mg/kg and 50 mg/kg, respectively (Table 3). The loss in body weight observed especially in the CSLGF treated groups is another pointer to the toxic effects of both substances. Increased metabolism of body fat,

decreased intestinal food transit and low appetite are key players in weight loss and may have been enhanced by both CSLGF and tramadol (Cummings and Overduin, 2007). Females have higher body fat than males and as such are expected to have a higher degree of weight loss in the case where fat metabolism prevails.

Table 3: Results on the effects of CSLGF on body weight changes in female rats.

Treatment Groups	Body weight on day 1 (g)	Body weight on day 21 (g)	Weight gain (g)	% weight gain/loss
Control	224.28±8.23^{b}	262.00±14.15^{c}	28.82±4.46^{d}	12.81±2.25^{d}
Tramadol (25 mg/kg)	248.16±10.20^{c}	260.30±15.33^{b}	12.14±3.30^{c}	7.27±1.98^{c}
Tramadol (50 mg/kg)	184.16±4.55^{a}	184.24±10.87^{a}	0.08±0.01^{a}	0.04±0.09^{a}
CSLGE (25 mg/kg)	229.46±6.43^{b}	224.62±12.48^{b}	-4.84±0.37^{b}	-2.11±0.36^{b}
CSLGE (50 mg/kg)	191.62±3.69^{a}	185.82±10.01^{a}	-5.80±0.75^{b}	-3.03±0.40^{b}

Values are presented as mean ± standard deviation (n = 5) and values with different letter superscripts are significant at P<0.05 from any paired mean within the column. Also, values with (-) = loss while those without (-) indicate gain.

3.3. Effects of CSLGF and Tramadol on the haematological values of female rats

While tramadol did not significantly alter the values of RBC, PCV and Hb in the female rats, treatment with CSLGF lowered the values of these parameters significantly in the rats when compared with control (P<0.05). MCV, MCH and MCHC values were also higher in these CSLGF treated groups than in control (P<0.05). Platelets count remained unaltered in the tramadol treated female rats but increased significantly in groups treated with CSLGF (P<0.05). Significant elevations were also observed in WBC values following treatment with both CSLGF and tramadol with severe occurrence in the CSLGF groups (Table 4). The fall in RBC parameters in the CSLGF treated female rats may be linked to the alcohol content of CSLGF. Alcohol reportedly causes anaemia due to itshaemolytic effect on the RBC, ability to disrupt erythropoietic processes in the bone marrow and the role it plays in the lowering of folic acid absorption in the gastrointestinal (Zhou *et al*., 2017). Although the information on the haematoxic effects of *Cannabis sativa* is scanty, its major toxic component, tetrahydrocannabinol, may cause anaemia due to its reported suppressive effect on bone functions in rats (Turner *et al*., 2020). On the other hand, tramadol administration did not alter RBC parameters in the rats, but like CSLGF, elevated WBC values in the rats, a condition which in this study may be associated with liver inflammation due to the impact of these substances (Chung *et al*., 2016). The increase in platelets number following treatment with both tramadol and CSLGF is consistent with results of similar studies (Gautheir, 2005; Obembe *et al*., 2015), and further suggests that the use of these agents may contribute to increasing cases of thrombosis, cardiovascular shock and myocardial infarction usually seen in thrombocytopenia and also associated with alcohol and narcotics addiction (Randal, 2004; Ijioma, 2015).

Table 4. Effects of CSLGF and Tramadol on the haematological values of female rats.

Parameters	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGE (20mg/kg)	CSLGE (40mg/kg)
RBC(10^{12}/L)	7.56±0.10^{c}	7.48±0.09^{c}	7.46±0.10^{c}	5.56±0.25^{b}	5.28±0.19^{a}
PCV (%)	47.76±0.57^{d}	46.28±0.43^{c}	46.04±0.56^{c}	37.62±0.86^{b}	33.39±0.97^{a}
Hb (g/dL)	13.24±0.41^{e}	12.68±0.22^{b}	11.66±0.82^{c}	11.24±0.50^{b}	10.68±0.22^{a}
WBC(x10^{9}/L)	10.56±0.62^{a}	12.70±0.26^{b}	12.94±0.23^{c}	14.26±0.39^{d}	15.18±0.10^{e}
PLT(x10^{9}/L)	722.40±22.08^{a}	758.40±13.80^{d}	753.80±14.13^{c}	790.80±5.89^{b}	870.40±51.51^{a}
MCV(fl)	62.20±0.29^{a,b}	61.84±0.22^{a}	61.74±1.24^{c}	67.74±2.02^{c}	63.32±3.13^{b}
MCH (pg)	17.54±0.40^{c}	16.94±0.27^{b}	15.62±1.20^{a}	20.24±0.27^{d}	20.24±0.42^{d}
MCHC (g/L)	277.24±5.61^{c}	274.00±4.06^{b}	253.36±19.28^{a}	298.74±9.20^{d}	320.12±13.07^{e}

Values are presented as mean ± standard deviation (n = 5) and values with different letter superscripts are significantly (P<0.05) different from any paired mean across the row.

3.4. Effect of CSLGF and tramadol on liver and renal function parameters in rats

Total protein and albumin concentrations were significantly lower in the CSLGF and tramadol treated female rats than in the control (P<0.05) with a higher degree of fall observed in the CSLGF treated groups. The activities of liver enzymes including AST, ALT and ALP were also found to be higher in the CSLGF and tramadol treated rats than in control (P<0.05). Total bilirubin concentration in the test animals also followed the same trend as that of the liver enzymes, being higher in the treated female rats and of a higher degree in the CSLGF treated groups (Table 5). Renal function parameters were not so much affected following treatment with both CSLGF and tramadol, although urea and creatinine values in groups treated with CSLGF were significantly higher than control values (P<0.05). The concentrations of serum electrolytes like Na^+, K^+, Cl^- and HCO^{-3} did not also change significantly (P>0.05). Potassium concentration was found to be lower than the control value in groups administered higher and lower doses of tramadol and CSLGF respectively (Table 6).

Since liver function affects most body organs, measurement of changes in the concentration of liver biomarkers may therefore be a means of evaluating the toxic effects of substances (Akomas *et al*., 2007). The decline observed in serum total protein concentration suggests possible liver dysfunction (Thepa and Walla, 2007). The damage done to the liver cells by both CSLGF and tramadol also corroborates with observed increase in ALT, AST and total bilirubin concentrations (Oshilonya *et al*., 2015), even as elevated ALP value may be due to possible biliary tract obstruction and further liver damagecaused by metabolites generated from the repeatedly administered CSLGF and tramadol administrations (Shahjahan *et al*., 2004). Goeringer *et al*. (1997) had reported that metabolites from ingested toxic substances may cause greater toxic effects to liver cells than their parent substances and may in the process also affect other organs like the kidneys, hence the observed increase in the levels of serum urea and creatinine. A similar result was obtained in a study carried out to determine the impact of tramadol on the renal function of rats (Atici *et al*., 2005).

Table 5. Effect of CSLGF and Tramadol on liver function parameters in female rats.

Parameters	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
TP (g/dl)	8.10±0.16^{d}	7.74±0.18^{c}	7.84±0.21^{c,d}	6.40±0.16^{a}	5.74±0.40^{a}
Alb (g/dl)	4.82±0.31^{c}	3.12±0.15^{b}	3.19±0.20^{b}	2.45±0.13^{c}	2.50±0.14^{c}
Glb (g/dl)	3.28±0.24^{a}	4.58±0.13^{c}	4.64±0.11^{c}	3.75±0.50^{b}	3.27±0.44^{a}
AST (u/l)	36.60±0.89	100.80±4.44^{b}	140.00±3.16^{d}	154.00±4.69^{d}	162.40±6.50^{e}
ALT (u/l)	29.80±1.48^{a}	69.40±1.67^{b}	110.00±8.12^{c}	111.20±2.59^{c}	118.80±6.14^{d}
ALP (u/l)	97.60±1.67^{a}	153.80±7.09^{b}	187.20±6.69^{b}	209.80±13.14^{b}	238.40±7.64^{c}
TB (mg/dl)	0.39±0.04^{a}	0.50±0.02^{b}	0.52±0.05^{b}	0.96±0.07^{c}	1.23±0.11^{d}

Values are presented as mean ± standard deviation (n = 5) and values with different letter superscripts are significantly (P<0.05) different from any paired mean across the row.

Table 6. Effect of CSLGF and Tramadol on renal function parameters in female rats.

Parameters	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
Creatinine (mg/dl)	0.41±0.07^{a}	0.36±0.03^{a}	0.37±0.03^{a}	0.61±0.03^{b}	0.69±0.01^{b}
Urea (mg/dl)	16.79±0.75^{a}	18.70±1.69^{b}	21.29±1.21^{c}	22.88±0.81^{d}	24.08±1.66^{d}
Sodium (mEq/L)	148.08±1.57^{b}	143.24±2.62^{a}	144.48±1.19^{a}	147.22±1.58^{a,b}	150.10±3.09^{c}
Potassium (mEq/L)	5.25±0.10^{a}	5.28±0.13^{a}	4.96±0.10^{a}	4.97±0.22^{a}	5.22±0.13^{a}
Chloride (mEq/L)	89.84±4.16^{a,b}	92.27±0.95^{a,b}	98.27±3.68^{c}	88.35±4.69^{a}	94.76±1.33^{b}
Bicarbonate (mmol/L)	22.22±2.08^{a}	23.28±1.66^{a,b}	23.16±1.14^{a,b}	24.78±0.62^{a,b}	23.84±0.86^{b}

Values are presented as mean ± standard deviation (n = 5) and values with different letter superscripts are significantly (P<0.05) different from any paired mean across the row.

3.5. Effect of CSLGF and tramadol on antioxidant parameters in female rats

The higher dose of tramadol and all doses of CSLGF significantly lowered serum glutathione and superoxide dismutase concentrations in the treated female rats (P<0.05) while the concentration of catalase was only significantly than control in a group-administered higher dose of CSLGF. Malondialdehyde concentration in all rats administered CSLGF and tramadol was significantly higher than the control value, but the level of increase was higher in the CSLGF treated rats (Table 7). Measurement of lipid peroxidation may help to

identify the degree of oxidative stress on body cells and to a large extent explains the presence of toxicity (Akomas *et al*., 2007). Therefore high MDA values with concurrent falls in the activities of antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase may corroborate with increasing toxicity due to fall in the body's antioxidant defence line usually strengthened by these antioxidant enzymes (Kanu *et al*., 2016). These antioxidant agents together with those from food (vitamins C and E) counter oxidative stress and toxicity by scavenging free radicals in biological systems (Ijioma *et al*., 2019). A condition where generated free radicals are higher than the levels of these antioxidant agents still amounts to increasing toxicity and may have played out in this study following sustained daily administrations of CSLGF and tramadol to the experimental rats and also accounts for the increased MDA values in the test female rats. While tramadol toxicity via this pathway has been reported, that of CSLGF may also be by the same pathway due to the oxidative effects of tetrahydrocannabinol in *Cannabis sativa* coupled with that of its alcohol base (Ismail *et al*., 2015).

Table 7. Effect of CSLGF and Tramadol on antioxidant parameters in female rats.

Parameters	Control	Tramadol (25mg/kg)	Tramadol (50mg/kg)	CSLGF (25mg/kg)	CSLGF (50mg/kg)
GSH (iu/l)	44.88 ± 0.88^{c}	45.42 ± 1.53^{c}	42.34 ± 1.87^{b}	41.05 ± 1.29^{b}	34.72 ± 1.06^{a}
SOD (iu/l)	27.97 ± 1.16^{b}	27.65 ± 0.74^{b}	$25.55\pm0.52^{a,b}$	25.62 ± 0.56^{b}	21.23 ± 0.38^{a}
CAT (iu/l)	22.08 ± 0.78^{c}	22.31 ± 0.55^{c}	$21.21\pm1.70^{b,c}$	21.42 ± 0.81^{b}	15.99 ± 1.33^{a}
MDA (mmol/l)	0.55 ± 0.13^{a}	0.62 ± 0.17^{b}	0.74 ± 0.83^{c}	1.15 ± 0.11^{d}	2.87 ± 0.29^{e}

Values represent the mean ± SD for N =5. Values in the same row bearing the same letter of the alphabet are not significantly different from each other ($p>0.05$). GSH, reduced Glutathione; SOD, Superoxide Dismutase; CAT, Catalase; and MDA, Malondialdehyde.

4. Conclusion

In adult female Wistar rats, *Cannabis sativa* local gin formulation (CSLGF) and tramadol have shown similar oxidative, hepatotoxic and nephrotoxic effects with severe haematotoxicity observed only in animals administered CSLGF. Acute toxicity values for substances also indicate similar toxicity potentials with narrow safety margins. It may therefore important to extend the current global control on tramadol to CSLGF (monkey tail) for the safety of humanity.

5. References

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