RESEARCH ARTICLE

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Hepatic and Renal Biochemical Profile of Male Wistar Rats Following Ingestion of Lacatomtom Drink

C. F. Anyanwu[1*], U. O. Georgewill[1], N. U. Williams[2], O. T. Leslie[2], D. Nzei[2], G. C. Chioma[3]

[1] *Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, PMB 5323, Nigeria*
[2] *Department of Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt, Rivers State, PMB 5323, Nigeria*
[3] *Department of Paediatrics, University of Port Harcourt Teaching Hospital, Rivers state, PMB 5323, Nigeria*

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ABSTRACT: There is rising concern about the physical health risks of unconventional psychoactive drugs. A notable substance in this regard, "Lacatomtom" (LTT), has gained popularity among Nigerian youths. This study evaluated hepatic and renal biochemical profiles of male Wistar rats following ingestion of LTT drink. A total of 25 male wistar rats were randomly placed into 5 groups of 5 rats each. Group A (control): 0.5 ml of distilled water, group B (Vehicle) 0.5ml Lacasera©, and groups C, D, and E: 125, 500, and 1000 mg/kg LTT drink orally for 30 days. Plasma sera were used to analyse biochemical parameters like alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein, albumin, bilirubin, urea, creatinine, and blood electrolytes after treatments. Histopathological examination was carried out on liver and kidney sections. ALT levels were significantly higher in test group E (1000 mg/kg) than the control. LTT drink significantly (P>0.05) decreased ALP, total protein, and albumin levels in experimental group C compared to the control, but not in test groups D (500 mg/kg) and E (1000 mg/kg). Total and conjugated bilirubin, creatinine, urea, potassium (K+), sodium (Na+), and bicarbonate (HCO3-) levels in the blood did not differ significantly between the control and Lacatomtom-treated groups (C, D, and E). However, groups C and D had significantly lower chloride serum levels than the usual control. In conclusion, Lacatomtom may alter the homeostatic balance of the liver in a dose dependent manner. It is therefore recommended that public awareness campaigns be launched to educate adolescents on the potential health risk its consumption poses.

Keywords: Psychoactive drink, liver, alanine transaminase, alkaline phosphatase, kidney, toxicity.

INTRODUCTION

The persistent issue of substance abuse continues to pose a significant public health challenge, despite the diligent efforts of the Nigerian Drug Law enforcement agents to curb its prevalence (Khey et al., 2013). According to the World Health Organization (WHO, 2018), 'Adolescents' are 10-19 years old, 'Youth' are 15-24 years old, and 'Young People' are 10-24 years old (Abura-Meerdink and Albright, 2023). Generally, teenagers are recognized as valuable contributors to a country's growth and prosperity (Mohamad et al., 2018). However, drug addiction in adolescents and young people has been reported to result in serious implications (Mohamad et al., 2018).

According to a survey done on Nigerian secondary school students, 26% of them were reported to have tried alcohol at some point, and 13% of them were current users of alcohol (Fatoye et al., 2006; Asante and Kugbey, 2019). Adolescent illicit drug use is a severe problem that permeates our culture since kids are most vulnerable at this life-changing moment. Nigeria is currently among the countries in Africa with the highest rates of cannabis and amphetamine consumption, according to the United Nations Office on Drugs and Crime (UNODC, 2018). According to a recent survey, 4.4% of school-age teenagers in Nigeria were reported using amphetamines, a higher prevalence rate than that of other sub-Saharan African nations examined (Asante and Atorkey, 2023)

According to research, cannabis usage can impact the growing brain, which can lead to decreased academic achievement, attention problems, and dependency (Lawal et al., 2024). Over 15% of 15–29-year-old males and 6 % of women die from alcohol usage (Lawal et al., 2024). Youth alcohol usage is linked to stress, family violence, injury, suicide, sexuality, and other dangerous behaviours.

The surge in drug use and substance abuse, particularly among the youth and children, has witnessed a drastic increase due to societal advancements and medical progress. This trend has given rise to numerous adverse consequences within the community, including violence, crime, financial challenges, housing issues, and homelessness (Erhun et al., 2001). To address this escalating concern, the Nigerian government, through agencies like National Drug Law Enforcement Agency (NDLEA) and National Agency for Food & Drug Administration & Control (NAFDAC), has imposed bans on substances such as codeine, tramadol, and cannabis (Nwankwo et al., 2020). Despite these measures, youths, especially on university campuses, have devised

* chinwe.anyanwu@uniport.edu.ng

alternative concoctions like Lacatomtom (Lacasera© and Tom Tom) and Laca Maggi (Lacasera and Maggi) to serve as stimulants (Ijediogor et al., 2018).

Lacasera © soft drink is a non-alcoholic, sweetened, carbonated drink sold in plastic bottles in Nigerian markets, stores and fast-food outlets. It is also sold cold by street vendors to millions of travelers. It is the flagship product of Lacasera© Company Plc. founded in 2009 to advance national development (Van Eygen et al., 2005). The connection between Lacasera© and TomTom has been reported to be related to the controversial practice of mixing Lacasera© (an apple-flavored which contains Carbonated water, Sugar, Apple Flavor, Sodium Benzoate, Malic acid, Caramel) with TomTom (a menthol candy which contains sugar, Glucose syrup, Water, Menthol, Flavoring, Color (E153)) for the sake of euphoria (Emmanuel et al., 2022). According to Dumbili et al., (2020), the mixture is known to be used by young addicts to seek pleasure, pursue more intense highs, and maintain prolonged intoxication. Although studies have highlighted the toxic combination of methanol inside TomTom reacting with the carbon dioxide and water inside Lacasera©, along with other catalytic agents, leading to potential harm and previous reports identifying Lacatomtom as having psychotropic properties (Ijediogor et al., 2018), however, there is paucity of information on its specific impact on the biochemical and toxicological profile.

MATERIALS AND METHODS

Study Area

This research was carried out in Animal House of the pharmacology Department of the University of Port Harcourt, Rivers state, Nigeria.

Sample

The items were purchased at a local market located at the heart of Obio Akpor Local Government Area, Rivers State, Nigeria.

The sample Preparation (LTT Solution)

The samples were set to match Lacatomtom drinkers' dosage (i.e. 3 TomTom candies, dissolved in 35 ml of Lacasera© drink)

Sample A contained distilled water, while Sample B had just Lacasera©.

Samples C, D, and E included three tom-tom candies with a total weight of 11.45 g, dissolved in 350 mL of Lacasera© drink, yielding 32 mg per 1 mL.

Experimental Animal

Twenty-five (25) male Wistar rats weighing 190 - 200g (12 weeks old) were used in this study. The animals were procured from the animal house of the pharmacology department of the University of Port Harcourt, allowed to acclimatize to the standard laboratory conditions and fed standard commercial diet (Top Feed finisher) and water.

Ethical Approval

The study procedures were approved by the Research Ethics Committee of the Centre for Research Management and Development at the University of Port Harcourt (Reference Number: UPH/CEREMAD/REC/MM93/034). All experimental animals received humane treatment in accordance with the university's approved ethical guidelines and regulations for the use of research animals.

Experimental Design

Twenty-five male Wistar rats weighing 190g were randomly divided into five groups, with each group consisting of five rats. The rats were administered oral treatments daily via gavage as follows:

Group A (Normal control): 0.5 mL of distilled water.

Group B (Positive control): 0.5 mL of Lacasera Drink.

Group C: Lacatomtom Drink at a dose of 125 mg/kg.

Group D: Lacatomtom Drink at a dose of 250 mg/kg.

Group E: Lacatomtom Drink at a dose of 500 mg/kg.

Sacrifice and Collection of Samples

After the completion of the 30-day period, the rats were subsequently sacrificed. Blood samples were collected by cardiac puncture in plain tubes. Following centrifugation at 4000 RPM for 10 minutes, the serum was extracted and stored in Cryovials. Liver and kidney tissues were then removed and preserved in 10% formalin for histopathological examination using the method described by (Lillie, 1969) and stained with Hematoxylin and Eosin.

Laboratory Analysis

Heparinized bottles were punctured to measure plasma activity of Alanine transaminase (ALT), aspartate aminotransferase (AST), and Alkaline phosphatase (ALP), as well as total protein, albumin, total and conjugated bilirubin, creatinine, urea, sodium, potassium, chloride, and bicarbonate. After centrifuging blood at 4000rpm for 10 minutes, plasma was isolated from coagulated cells and placed in a separate vial. The vial was sealed in microcentrifuge tubes and kept at -20°C until analysis.

The plasma biochemistry was measured using commercial test kits. Diagnostic kits from Randox Laboratories, Northern Ireland, were used to assess ALT and AST activity according to Reitman and Frankel, (1957).

Serum ALP activity was measured using Teco Diagnostics kits (1268 N. Lakeview Ave, Anaheim, CA 92807) per Roy, (1970) using the thymolphthalein monophosphate technique (1-800-222-9880). The Jendrassik-Grof technique was used to measure plasma total and conjugated bilirubin using diagnostic kits from Randox Laboratories, Northern Ireland (Jendrassik & Grof, 1938).

The following methods were used to measure plasma total protein (TP), albumin, urea, and creatinine: direct Biuret (Tietz, 1995), bromocresol green (Doumas et al., 1971), Urease-Berthelot (Fawcett and Scott, 1960), and modified Jaffe (Blass et al., 1974) using diagnostic kits from Randox Laboratories, Northern Ireland. Plasma potassium (K+), sodium (Na+), bicarbonate (HCO-3) and chloride (Cl-) were measured using colorimetric methods (Wheeler, 1998).

Sacrifice and Collection of Samples

The data obtained was collated, tabulated and statistically analysed. Results are given as Mean ± SEM for each group, statistical evaluation was done by one way ANOVA, followed by Tukey's post-hoc test.

RESULTS

The impact of LTT drink on hepatic and renal biochemical profile of male Wistar rats are summarized in table 1 and 2. Ingestion of LTT drink by wistar rats at doses of 125, 500 and 1000 mg/kg for 30 days had a significant ($P<0.05$) decrease in the AST, ALT and ALP levels of experimental test groups C, D, and E respectively compared to the Vehicle Control B. Although a significant increase in ALT levels was observed in test group E (1000 mg/kg) compared to the normal control A, there was no significant variation in ALT levels of test groups C (125 mg/kg) and D (500 mg/kg) compared to Normal control. Although there was a significant decrease in serum concentration levels of ALP in experimental groups C, D, E, compared to the vehicle control, there was a significant decrease in ALP levels of experimental group C when compared to the normal control.

Although the serum concentration levels of total protein and albumin of rats ingested with 125 mg/kg (group C) of lacatomtom drink decreased significantly ($P>0.05$) relative to the normal control, serum concentration levels of total protein and albumin in test groups D (500 mg/kg) and E (1000 mg/kg) did not differ significantly ($P>0.05$) from the control (Table 1).

The mean concentration serum bilirubin (total and conjugated) of rats ingested with lacatomtom drink (C, D and E) did not differ significantly from the normal control (Table 1).

Table 1. *Effect of Lacatomtom Drink on Hepatic Biochemical Parameters following ingestion for 30 days*

Groups	Parameters						
	AST (u/L)	**ALT (u/L)**	**ALP (u/L)**	**Total Protein (g/L)**	**Albumin (g/L)**	**Total Bilirubin (umol/L)**	**Conjugted Bilirubin (umol/L)**
A	23.20±1.28[a]	10.00±1.64[a]	44.20±2.75[a]	76.40±1.36[a]	47.40±1.29[a]	4.94±0.17[a]	3.44±0.22[a]
B	44.60±2.98[b]	33.80±4.51[b]	70.80±2.63[b]	58.60±2.21[b]	37.20±1.66[b]	9.16±0.57[b]	6.54±0.32[b]
C	30.80±2.67[a]	14.80±2.08[a]	28.00±2.21[a b]	63.40±2.29[b]	40.40±1.78[b]	6.86±0.53	4.00±0.34[a]
D	24.80±2.18[a]	15.60±1.86[a]	34.80±3.18[a]	69.00±2.10[a]	43.00±1.55	5.40±0.40[a]	2.90±0.10[a]
E	23.40±2.29[a]	21.00±1.30[ab]	55.20±2.73[a]	72.00±1.84[a]	46.40±1.08[a]	5.56±0.84[a]	3.08±0.36[a]

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with group A (Normal Control) and group B (vehicle). bp<0.05 was considered as significant versus the Normal control (Group A); ap < 0.05 was considered significant versus the vehicle (Group B).

There is no significant difference in the serum concentration levels of creatinine, urea and potassium (k+), sodium (Na+) and bicarbonate (HCO3-) of rats ingested with lacatomtom drink (C, D and E) when compared with the normal control as shown in table 2. However, there is a significant decrease in serum chloride levels of test group C and D rats ingested with lacatomtom drink compared with the normal control (Table 2).

Table 2. *Effect of Lacatomtom Drink on Hepatic Biochemical Parameters following ingestion for 30 days*

Groups	Parameters					
	Urea (mmol/L)	**Creatinine (µmol/L)**	**K (mmol/L)**	**Na (mmol/L)**	**Cl (mmol/L)**	**HCO_3 (mmol/L)**
A	3.34±0.19 [a]	72.20±2.96 [a]	4.18±0.23	143.80±2.54	80.60±2.98 β	23.60±0.93
B	5.16±0.23[b]	105.00±4.29[b]	4.22±0.23	142.40±2.89	67.80±1.39[b]	27.60±0.68
C	3.68±0.12[a]	76.00±2.43 [a]	3.90±0.20	138.80±4.52	62.60±1.60[b]	24.40±1.08
D	3.80±0.07 [a]	79.00±1.30 [a]	4.66±0.18	147.00±3.42	65.20±1.07[b]	25.00±1.00
E	2.92±0.12 [a]	62.40±1.91 [a]	4.38±0.21	137.40±4.50	79.20±2.89 [a]	25.40±1.44

Results are given as Mean ± SEM for each group. Statistical evaluation was done by one-way ANOVA, followed by Tukey's post-hoc test. Experimental groups are compared with group A (Normal Control) and group B (vehicle). bp<0.05 was considered as significant versus the Normal control (Group A); ap < 0.05 was considered significant versus the vehicle (Group B).

The photomicrographs of liver and kidney sections of rats ingested with lacatomtom drink did not show any abnormality in the histoarchitecture in comparison with the control (Figure 1 and Figure 2).

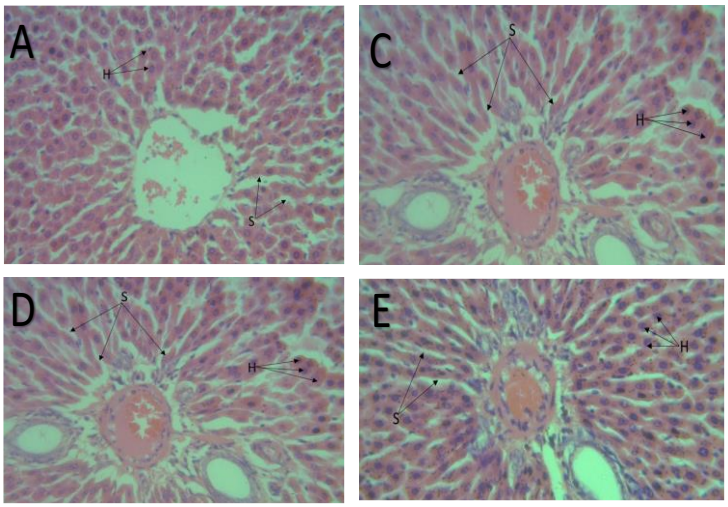

Figure 1. *Photomicrographs of liver sections of rats from groups A (Control), C, D and E (LTT doses of 125, 500 and 1000mg/kg respectively) treated for 30days; stained with H&E (×400). No obvious histological change in the liver of rats ingested with LTT drink relative to the control. No obvious change in the histoarchitecture of the liver sections of LTT ingested rats when compared with the control. The sinusoids (S) are seen radiating with very prominent hepatocytes(H)*

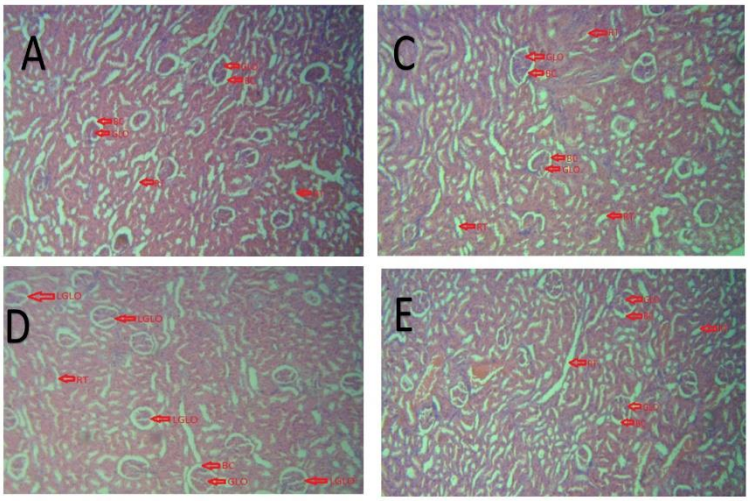

Figure 2. *Photomicrographs of kidney sections of rats from groups A (Control), C, D, and E (LTT drink doses of 125, 500 and 1000 mg/kg respectively) treated for 30 days stained with H&E (×400). No obvious histological change in the kidney of LTT treated rats relative to the control. The photomicrograph shows the glomeruli (G) with normal tuffs, surrounded by patent Bowman's capsules (BC)*

DISCUSSION

The liver and kidney are important organs in animals, responsible for metabolism and detoxification. Assessing liver and kidney functions is crucial for assessing medications and psychostimulant toxicity, as they play a crucial role in animal survival. Plasma serum biochemistry tests, often known as liver and kidney function tests, offer information on organ function. The liver function test assesses liver health by assessing protein levels, liver enzymes, and bilirubin levels (total and conjugate) in the blood (Obinna et al., 2018). Aspartate Transaminase (AST) enzyme is found in several bodily organs, including the kidney, liver, heart, brain, and muscle while Alanine Transaminase (ALT) enzyme is mostly present in the liver. Alkaline Phosphatase (ALP) enzyme on the other hand, is located in bone, liver, and bile ducts. Therefore, damage to or inflammation in the organs containing these enzymes might result in elevated blood levels. Increased levels of these in the bloodstream may indicate organ damage or illness (Finelli, 2023). Although increased serum concentration levels of AST , ALT, and ALP are indicators of liver damage or illness, ALT is more specific to the liver since AST and ALP may also be raised in conditions affecting other organs such the heart or muscles.

This study found that LTT drink ingested by wistar rats for 30 days significantly ($p>0.05$) increased the serum concentration levels of ALT in a dose dependent manner while increasing ALP levels in the same manner with a significant ($p<0.05$) reduction seen in test group C (125 mg/kg LTT drink). This finding contrasts with findings by Emmanuel et al., (2022) who reported elevated ALT, AST and ALP levels in albino rats administered lacatomtom drink for 14days. However, ALP which did not significantly increase in our study, was increased in the latter. This disparity may be as a result of the disparity in doses used as well as the duration of treatment. Surprisingly, there was a significant increase in all the hepatic biochemical parameters in the vehicle group when compared to the normal control. This could be as a result of the presence of the preservative sodium benzoate which is found to be present in the vehicle. Studies have demonstrated the possible hepatotoxic effect of sodium benzoate in a dose dependent manner (Khan et al., 2022). It is therefore plausible that the hepatotoxic impact of the vehicle could be as a result of the presence of its preservative, sodium benzoate. Therefore, it is safe to say that lacatomtom drink might interfere with the homeostatic function of the liver and as such may be said to be potentially hepatotoxic in a dose dependent manner as evidenced by the significant increase in ALT enzyme which is said to be specific for liver damage or illness.

The result of this study shows that lacatomtom drink did not have any observable renal effects after administration for 30 days. This study is in tandem with that reported by Okorie et al., (2022), who reported a possible time dependent nephrotoxic impact of LTT drink evidenced by elevated renal biochemical parameters following chronic administration of LTT drink at a dose of 0.01mg/g twice daily for 42days . Although our study administered LTT drink daily, that of the latter was done twice daily. However, at acute administration in the latter study, the renal biochemical parameters where not significantly elevated. The non-significant difference in serum concentration levels of urea, creatinine, potassium and sodium as seen in our study, may be as a result of the disparity in doses, frequency as well as the duration of administration of LTT drink used, and plausible variations in the preparation or formulation of the LTT drink between studies.

However, in this study, there was a significant rise in serum urea and creatinine levels in the vehicle control group, relative to the control. Increase in serum urea and creatinine levels typically occurs when kidney function, specifically its ability to filter

fluid in the body, is reduced. This could be as a result of the presence of sodium benzoate, which has been hypothesized to have either impacted creatinine metabolism, potentially leading to increased synthesis, or compromised the functional capacity of kidney tissues responsible for tubular excretion (Manal Said and Nawal, 2012).

CONCLUSION

This study suggests that lacatomtom drink may affect the liver's homeostatic balance and be dose- and time-dependently hepatotoxic and nephrotoxic respectively. Thus, social media, public service announcements and health education programmes should be used to inform consumers of Lacatomtom's health risks.

ACKNOWLEDGEMENTS

The authors are grateful to 'Lively Stone Diagnostic Laboratory', for providing the facility for the hepatic and renal biochemical parameter assay.

CONFLICT OF INTEREST

The products employed in this study are extensively widespread and consumed in our research region and nation. The authors would like to emphasise that there are no conflicts of interest between them and the manufacturers of these products. We use these items only for academic and research reasons, with the goal of furthering knowledge. It should be emphasised that this research received no financial assistance or funding from any manufacturing business. Instead, the research was carried out alone by the authors using their own personal labour and resources. The presence of particular items in this research should not be interpreted as a basis for legal action or any type of dispute. Our main goal is to seek information and make intellectual progress.

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