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Research Article

Investigation of the Hepatotoxicity of *Lacatomtom* Drink in Albino Rat

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ABSTRACT

"*Lacatomtom*" (LTT), commonly called 'gigabyte', is a dark-brown solution formed from the combination of *tomtom* candy with *lacasera* drink consumed by Nigerian youths. This study investigated the toxic effect of LTT at varying concentrations on albino rats' liver function enzyme profile. Thirty-six (36) male albino Wistar rats (*Rattus norvegicus*) with average body weights of 90-300g were placed in six (6) groups with (6) rats in each. Group A served as the control while the others were test groups. Group A was given access to only feed and water *ad libitum*. Group B, C and D were orally administered with 1 mL of LTT containing 37 mg, 18.5 mg and 9.25 mg respectively of *tomtom* in 1 mL of *lacasera* drink per kilogram body weight rat (0.1 mL per 100 g weight rat) once daily, while group E was given 1 mL containing 37 mg of *tomtom* in 1 mL of water per kilogram body weight rat. Group F was given 1 mL of *lacasera* drink per kg body weight rat. After the 7^{th} and 14^{th} day of oral administration, the rats were sacrificed. The sera collected were evaluated for enzymes activities. The results showed that liver enzymes, including aspartate transaminase, alanine transaminase and alkaline phosphatase levels increased significantly ($p < 0.05$) in the test groups compared to the control group. Also, there was a significant decrease ($p < 0.05$) in weight and liver-to-body weight ratio in rats administered with the test sample when compared with the control group. These findings suggest that the consumption of LTT drinks is toxic and can cause damage to liver with concomitant implications on human health.

Keywords: *Lacatomtom, Lacasera, Psychotic, Hepatotoxic, Liver enzyme*

INTRODUCTION

In recent times, youths, especially on campuses in Nigerian cities, have been seen clutching what seems to be a "bottle of water" of popular soft drinks. Upon closer look, one might discover that these are not water or conventional soft drinks therein. Most of them have concoctions, a mixture of different drugs and substances (stimulants) in them and are usually consumed by youths of within the age 12 to 30 years **(**Ijediogor *et al*., 2018**)**. Some examples of these stimulants include: caffeine, codeine, and tramadol (Gibson *et al.*, 2016). These Nigerian youths consume these stimulants for diverse reasons, but the common denominator is the desire to experiment (curiosity). Other factors include peer pressure, parental neglect, psychological disorders (depression, anxiety, grief and trauma), need for extra sexual strength, academic pressures, sleep induction, boredom, and enhancement of physical strength (Docherty, 2008; Ijediogor *et al*., 2018**).** There have been psychological and socioeconomic implications of the consumption of these stimulants. The physiological effects implicated include changes in eating and sleep patterns, neglect of personal care, hands and feet tremors, bloodshot eyes, unexplained need for money, excessive need for privacy, lack of coordination, and disorders of the kidney, lungs and liver (Pennings *et al.*, 2002; Shadnia *et al*., 2014; Wang *et al*., 2017; Youssef and Azza, 2016). Also, the socioeconomic effects of the consumption of these stimulants are seen in societal volatility, which includes increased armed robbery, kidnapping and rape cases (Adamu *et al*., 2018**;** Ijediogor *et al*., 2018; Jimoh and Bakare, 2014**).**

A typical case of the consumption of the psychotic drink called "*lacatomtom*" was reported by Mustapha (2018). *"Lacatomtom"* (LTT), also commonly called 'gigabyte', is a dark-brown solution formed from the complete dissolution

of *tomtom* candy with *lacasera* drink (Mustapha, 2018). It has a sharp, pungent and minty smell attributed mainly to the *tomtom* component. It also possesses a fizzy look which is a consequence of the reaction of *tomtom* candies with the *lacasera* drink, precisely because of the rapid nucleation of carbon dioxide gas as aided by menthol, carbonated water, sodium benzoate and aspartame (Kuntzleman *et al.*, 2017). Because LTT is a synthetic, consumer-made concoction, there are '2 gigabytes, 3 gigabytes and other gigabyte' concentrations depending on the quantity of the substance that satisfies the addict (Mustapha, 2018).

The consumption of LTT is possibly necessitated by the ban on the importation and sale of codeine by the Federal government of Nigeria resulting in a skyrocketed price of the product. Therefore, the quest for cheaper alternatives has prompted the Nigerian youths to utilize new concoctions such as LTT (Ijediogor *et al*., 2018**;** Mustapha, 2018). Similarly**,** Ijediogor *et al*. (2018) and Mustapha (2018) reported other "new wave" techniques (as they are called) which took the form of the mixture of methylated spirit and Cola drink, *lacasera* and magi seasoning cubes *("lacamaggi"*), cobwebs and water, codeine, refnol, tramadol, cannabis and water (omi gutter), tramadol, codeine, benzodiazepine and Hollandia yogurt (jiko).

Because the liver is the center of detoxification, exposure to hepatotoxicants alters the homeostatic balance of various liver enzymes indicative of hepatic injuries such as cellular degeneration, necrosis, cirrhosis, cholestasis or vascular injury. Alanine transaminase (ALT) is a cytoplasmic/mitochondrial enzyme predominantly found in the liver and to a lesser extent in the kidney, heart, and skeletal muscles (Satyanarayana and Chakrapani, 2007). Being a cytoplasmic/mitochondrial enzyme, ALT leaks into the serum upon hepatocellular injury (Ozer *et al*., 2008; Shimizu, 2008). Aspartate transaminase (AST) is another hepatic enzyme responsible for aspartate metabolism. It is a cytosolic and mitochondrial enzyme also found in the kidney, pancreas, erythrocyte, brain, and skeletal muscles (Satyanarayana and Chakrapani, 2007). Although the sensitivity of AST serological test is lower than that of ALT, the levels of AST, when combined with ALT and ALP, can be used to monitor hepatic disorders (McPherson and Pincus, 2017). On the other hand, ALP is a membrane-bound enzyme that is usually found in the liver, bone, placenta, and intestine. The bile duct is blocked for a disordered liver, and ALP accumulates in the serum (Beaussier *et al*., 2007).

Therefore, the serological quantification of AST, ALT, and ALP, which are valuable markers of liver disease, is a reliable approach to comprehending the spectrum of liver injuries. This study, therefore, investigated the acute and chronic toxicity of *"lacatomtom"* drink on liver function enzymes, specifically AST, ALT, and ALP.

MATERIALS AND METHOD

Sample

Lacasera drinks (The *Lacasera* Company Plc, Nigeria) and *tomtom* candies (Cadbury Nigeria Plc, Nigeria) were purchased from Anyigba market, Dekina Local Government Area, Kogi State in Nigeria.

Experimental Animal

Thirty-six (36) male Albino Wistar rats (*Rattus norvegicus*) with average body weights of 90-300g were obtained from the Animal House of Kogi State University, College of Health Sciences, Kogi State, Nigeria. Animals were then transferred to the animal house of the Biochemistry Department, Kogi State University and acclimatized for two weeks before the experiment. The animals were kept under standard conditions; a well-ventilated animal room with at normal room temperature under 12 hours' daylight photoperiod regimen and access to rat feed pellets and water *ad libitum*. The experimental animals used in this research were handled in line with the guidelines of International Laboratory Animal use and Care (National Research Council, 2011).

Chemicals and Reagents

The chemicals and reagents were of analytical grade, and key ones used include Aspartate transaminase (AST) kit (Randox Laboratories Ltd, UK), Alanine transaminase (ALT) kit (Randox Laboratories Ltd, UK), Alkaline phosphatase (ALP) kit (Teco Diagnostics, USA), Dettol antiseptic (Reckitt Benckiser Ltd, Nigeria) and Detergent (Natural Prime Resources Nigeria Ltd, Nigeria).

Equipment and Apparatus

Prominent ones include UV/VIS Spectrophotometer with cuvette (UV752, Shanghai Youke Instruments Co Ltd, China), centrifuge (SM 800B, Surgifriend Medicals, England), water bath (HH-W21-Cr42II, IndiaMart, India), dissecting kits (Real star, Germany), stopwatch (E0700, Tecno T340, China), cryovials (CJS Smart, Nigeria) and micropipette with tips (CE-IVD, Labmat, India).

Sample Preparation (LTT solution)

The samples were prepared in accordance with the dosage used by the lacatomtom drink users as follows;

Sample A: Distilled water was used; *Sample B:* Three (3) tomtom candies weigh altogether 13.16 g were dissolved in 350 mL *lacasera* drink to obtain 37 mg of *tomtom*in 1 mL of lacasera drink; *Sample C:* 18.5 mg of *tomtom* in 1mL of lacasera drink was prepared by adding equal volumes of

distilled water to equal volumes of solution taken from sample A; *Sample D:* 9.25 mg of *tomtom* in 1 mL of *lacasera* drink was prepared by adding equal volumes of distilled water to equal volumes of solution taken from sample B; *Sample E:* 37 mg/mL of *tomtom* solution was prepared by dissolving 13.16 g (3 candies) of *tomtom* in 350 mL of distilled water; *Sample F:* lacasera drink was used.

Experimental Design

Groupings

The rats were randomly placed into six groups: A, B, C, D, E and F, with each group having six rats.

Administration of Samples

Sample solution was administered orally to each rat in each group accordingly. *Group A* was given 1 mL of water as control (1 mL of water only); *Group B* was given 1 mL of LTT drink per kg body weight rat of sample B containing of 37 mg of tomtom in 1 mL of lacasera once daily; *Group C* was given 1 mL of LTT drink per kg body weight rat of sample C containing 18.5 mg of *tomtom* in 1 mL of *lacasera* once daily; *Group D* was given 1 mL of LTT drink per kg body weight rat once daily of sample D containing 9.25 mg of *tomtom* in 1 mL of *lacasera* drink once daily; *Group E* was given 1 mL of LTT drink per kg body weight rat once daily of sample E containing 37 mg/mL of *tomtom* solution once daily; *Group F* was given 1 mL of LTT drink per kg body weight rat sample F containing lacasera drink only.

In addition, all the rats were fed with rat feed pellets and water normally with adequate care every day.

Animal Sacrifice and Blood Collection

At the end of the 7th and 14th days, the rats were fasted overnight and three (3) animals each were then sacrificed twenty-four (24) hours after the last administration at days 7 and 14, respectively. The blood was collected through the jugular vein into the plain tube. The sera were then collected into cryovial after centrifugation at 4000 RPM for 10 minutes. The collected sera were assayed for liver enzymes within 24 hours of sample collection, but the remaining sera were preserved in the freezer at -2 $^{\circ}$C for future reference. The liver organs were also collected and weighed for further studies.

Serum Assay for Liver Function Enzymes

Serum AST and ALT activity were assayed according to the method of Reitman and Frankel (1957) following the specification of the Randox kit manufacturer. The Serum ALP was analyzed using the method described by Demetrious (1974) following the description of the Teco kit manufacturer.

Data Analysis

The data obtained from the results were collated, tabulated and statistically analyzed. The analyzed data were presented as mean $\pm$ standard deviation (SD) of triplicate determinations for day 7 and day 14 each. Significant difference was established at 5% level ($p< 0.05$) using ANOVA (GraphPadInstat).

RESULTS

Table 1 below shows the Percentage (%) weight gain and liver-to-body weight ratios of rats administered with LTT drink at days 7 and 14 in each group. The results from Table 1 showed a general increase in the body weight of rats in each group after oral administration with LTT drink on days 7 and 14 with few exceptions. Unexplainable weight losses were observed in this order: 0.9 % weight loss at day 14 for group A, 0.1 9% weight loss at day 14 for group B, 1.33 % weight loss at day 7 for group C, 3.19 % weight loss at day 14 for group D. However, a general increase was observed in the body weights of rats in the various groups when compared to the control group (group A). Even when a 0.9 % weight loss was observed in the control group at day 14, the weight losses in the other groups (except for group B at day 14) were seen to be lower when compared to the control group (group A) indicative of increased body weight relative to the control. Also, the result from Table 1 showed an increased liver weight-to-body weight ratio on the general view when compared with the control, indicative of a reduced liver weight proportion. Exceptions were observed with groups C and F that had reduced liver weight-to-body weight ratios when compared with that of the control.

Table 2 below shows the liver enzymes activities' results of the rats on the 7th and 14th days. The results from Table 2 above indicate that ALT and ALP activities increased in all the groups from day 7 to 14. However, AST levels decreased from day 7 to day 14 in all the groups. ALT and ALP activities were increased in all the groups from day 7 to 14. In contrast, AST levels in all the groups were decreased from day 7 to 14. But when compared to the control group (group A), there were observed elevated activities of AST, ALT, and ALP for days 7 and 14 respectively.

DISCUSSION

Hepatotoxicants have been shown to cause hepatic injury (cellular degeneration, necrosis, cirrhosis, cholestasis, or vascular injury) by compromising the hepatocellular membrane, which results in the alteration of the homeostatic balance of liver enzymes and loss of functional mass of the liver (Arika *et al.*, 2016). This present study, therefore, evaluated the hepatotoxicity profile of LTT by measuring

the effect of oral administration of LTT on AST, ALT, and ALP level, and the liver-to-body weight ratio.

As shown in Table 1, this study indicated an overall significant increase in the body weights of the rats orally administered with LTT drink compared with rats given the control (group A), but an exception with group B at the 14th day. Although there is no rationale that account for this exception, these results agree with the findings of Umoh and Jimmy (2017). They worked on caffeinated drinks and reported that they stimulate elevated cortisol production, that promotes weight gain via visceral storage and mobilization

Table 1. Percentage (%) Weight Gain and Liver-to-body Weight Ratios of Rats Administered with "Lacatomtom" Drink at Days 7 and 14 in Each Group.

Groups	Body weight at day 1 (g)	Body weight at day 7 (g)	% body weight gain	Weight of liver (g)	Body weight at day 1 (g)	Body weight at day 14 (g)	% body weight gain	Weight of liver (g)	Ratio of liver weight: to body weight
A	194.00±4.24	194.50±6.36	0.26	6.38±0.23	223.50±23.33	221.50±14.85	0.90*	7.27±0.10	1:30
B	165.00±39.60	175.00±45.25	6.06	5.38±1.09	258.50±89.80	258.00±86.27	0.19*	7.67±2.23	1:34
C	150.00±4.24	148.00±4.24	1.33*	4.82±0.34	149.00±12.73	169.50±6.36	13.76	5.96±0.31	1:28
D	172.50±30.41	179.00±32.53	3.77	6.54±1.76	298.00±94.65	288.50±79.90	3.19*	8.24±1.56	1:35
E	181.50±21.92	190.50±20.51	4.96	6.74±1.02	159.00±8.49	175.00±7.07	10.06	5.73±0.86	1:31
F	156.00±1.41	169.50±4.95	8.65	6.09±1.32	180.50±34.65	191.00±56.57	5.65	7.55±0.10	1:25

Measured values are expressed as mean + standard deviation (SD) of triplicate determination. Values with * indicate % Body weight loss

Table 2. Results of Liver Enzymes Activities of Rats Administered *"Lacatomtom"* Drink

	AST (IU/L)		ALT(IU/L)		ALP (IU/L)	
	Day 7	**Day 14**	**Day 7**	**Day 14**	**Day 7**	**Day 14**
A	43.75 ± 5.94^{ade}	33.25 ± 2.48^{ae}	6.40 ± 1.81^{a}	8.40 ± 1.92^{ade}	32.36 ± 0.97^{a}	38.12 ± 3.49^{a}
B	121.10 ± 22.77^{b}	96.78 ± 14.11^{b}	37.36 ± 2.60^{b}	42.64 ± 10.30^{b}	59.24 ± 0.76^{b}	106.67 ± 1.85^{b}
C	102.73 ± 0.74^{bc}	95.55 ± 3.47^{bc}	35.84 ± 4.53^{bc}	40.16 ± 5.66^{bc}	52.17 ± 0.82^{c}	82.32 ± 1.44^{c}
D	74.55 ± 3.96^{cd}	71.75 ± 2.48^{bcd}	26.96 ± 0.80^{bcd}	28.80 ± 2.49^{bcd}	47.47 ± 0.52^{d}	77.25 ± 2.05^{cd}
E	64.40 ± 5.45^{cde}	51.63 ± 8.17^{de}	21.20 ± 5.77^{de}	22.96 ± 5.09^{bcde}	56.67 ± 0.82^{be}	90.58 ± 0.62^{e}
F	66.85 ± 6.44^{acdef}	53.55 ± 12.37^{adef}	9.44 ± 1.13^{aef}	10.16 ± 4.64^{adef}	53.44 ± 1.90^{cef}	83.92 ± 1.03^{cdef}

Measured values are expressed as mean + standard deviation (n = 3) and values with different superscripts down the column are significantly different ($p< 0.05$).

of triacylglycerol (Umoh and Jimmy, 2017). Although the weight gain is similar to that described by Umoh and Jimmy (2017), the pathway describing such weight gain in LTT is not yet known. Also, the observed increase in the liver weight-to-body weight ratio of rats given LTT drink compared with the control (which is lower than the standard liver weight-to-body weight ratio; 1:40), indicates a decreased liver weight relative to the body weight of rats administered with LTT drink. However, this finding contradicts the result of Spindler and Madsen (1992) on menthol, which showed increased liver weight proportion. But the reduced liver weight proportion observed in this study may result from functional mass loss associated with atrophy or significant hepatocellular injury.

In addition, the results from Table 2 below indicate that AST, ALT, and ALP activities increased in all the groups administered with LTT compared to the control at the 7th and 14th days. Day 14 had elevated levels of ALT and ALP compared to day 7 in all the groups, while day 14 had lowered AST levels when compared to day 7. Therefore, an overall increase in the liver enzymes (AST, ALT, and ALP) activities observed in the rats administered with LTT drink shows that LTT drink is a hepatotoxicant since it alters the homeostatic balance of the liver enzymes. As Arika *et al.* (2016) have demonstrated, hepatic injury permits the leakage of the liver enzymes, which is an indication of a compromised hepatocellular membrane. Similarly, the results of this study are in line with works done on compounds found in LTT. Agomuo *et al.* (2017) and Manne and Saab (2015) demonstrated increased levels of AST and ALT in a study with caffeine, but the ALP levels were decreased. Elevated AST and ALT levels, with no evaluation of ALP levels, were also reported by Oyewole *et al*. (2012) who showed hepatorenal toxicity of sodium benzoate in albino Wistar rats. Although Vo *et al*. (2003) showed no change in AST and ALT levels upon oral administration of rats with menthol, an elevated ALP level was reported. Other studies using aspartame also showed significantly elevated levels of AST, ALT, and ALP in rat models (Ashok *et al*., 2014; Prokić *et al*., 2015). The hepatotoxicity outcome of LTT compared with the above studies suggests that the hepatotoxicity of LTT may be attributed to the various constituents of "lacatomtom" drink such as menthol, aspartame, sodium benzoate, and caffeine.

CONCLUSION

In conclusion, since LTT drink reduced the liver-body weight ratio, increased the activities of AST, ALT and ALP, which are indications of liver dysfunctions, LTT drink may be considered hepatotoxic for human consumption. Further pre-clinical studies using animal models and clinical studies should be conducted to support the findings from this research through studies that would investigate its neurotoxicity and genotoxicity. More so, the active components responsible for the psychotic effect should be identified and characterized.

AUTHORS' CONTRIBUTIONS

FTE designed the research work and analyzed the biochemical parameters. He was also involved in writing the manuscript. SEA carried out the preparation of *lacatotom*, care, and administration of the drink to the animals with instructions from the first author. He was involved in writing the manuscript. SM reviewed the concept, design and contributed to the draft of the Manuscript. SEA took part in the drink administration, analysis, and draft of the manuscript together with IOO.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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