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BIOCHEMICAL CHANGES IN RATS AFTER TREATMENT WITH SOFT DRINK AND MENTHOL FLAVOURED CANDY

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KEY WORDS:
menthol candy, soft drink, transaminases, lipid profile

ABSTRACT

The current study looked at the biochemical variations that occurred in rats after they were given menthol flavoured candies and soft drinks. Seventy male Wistar albino rats in total were acquired, split up into ten treatments of seven rats each, and treated with the following regimen for forty-two days. Group 1 is the control. Groups 2, 3, and 4 received 8, 5, and 2.5 ml of La Casera/kg body weight. Groups 5, 6, and 7 received 0.34, 0.22, and 0.11 g of Tom-Tom/kg body weight. The dosages for groups 8, 9, and 10 were 8, 5, and 2.5 ml of La Casera/kg body weight, as well as 0.34, 0.22, and 0.11 g of Tom-Tom/kg body weight correspondingly. The results showed that the groups given Tom-Tom and La Casera had lower levels of total protein (TP) and albumin (Alb), and significantly ($p < 0.05$) higher serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol (T-Chol), triacylglycerol (TAG), and low-density lipoprotein cholesterol (LDL–C). Furthermore, there was a substantial ($p < 0.05$) increase in the serum levels of TP, Alb, and ALT between groups 7 and 5. Prolonged use of Tom-Tom mixed with La Casera should be avoided due to the detrimental alterations in biochemical parameters and subsequent health repercussions.

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БИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ У КРЫС ПОСЛЕ УПОТРЕБЛЕНИЯ ГАЗИРОВАННОГО НАПИТКА И МЕНТОЛОВЫХ КОНФЕТ

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КЛЮЧЕВЫЕ СЛОВА:
ментоловые конфеты, газированный напиток, трансаминазы, липидный профиль

АННОТАЦИЯ

В текущем исследовании изучались биохимические изменения, имевшие место у крыс после того, как им давали ментоловые конфеты и газированный напиток. Всего было получено семьдесят самцов крыс-альбиносов породы Вистар, разделенных на десять групп по семь крыс в каждой. Эксперимент проводился по описанной ниже схеме в течение сорока двух дней. Группа 1 — контрольная. Группы 2, 3 и 4 получали 8, 5 и 2,5 мл напитка «La Casera»/кг веса. Группы 5, 6 и 7 получали 0,34, 0,22 и 0,11 г конфет «Tom-Tom»/кг веса. Дозировки для групп 8, 9 и 10 составляли 8, 5 и 2,5 мл напитка

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«La Casera»/кг веса, а также 0,34, 0,22 и 0,11 г конфет «Tom-Tom»/кг веса, соответственно. Результаты показали, что в сыворотке крови у крыс, которым давали «Tom-Tom» и «La Casera», наблюдались более низкие уровни общего белка (TP) и альбумина (Alb), и значительно ($p < 0,05$) более высокие уровни аланинаминотрансферазы (ALT), аспартатаминотрансферазы (AST), щелочной фосфатазы (ALP), общего холестерина (T-Chol), триглицеридов (TAG) и холестерина липопротеинов низкой плотности (LDL–C). Кроме того, между группами 7 и 5 наблюдалось существенное ($p < 0,05$) увеличение сывороточных уровней TP, Alb и ALT. Вследствие пагубных изменений в биохимических параметрах и во избежание отрицательных последствий для здоровья следует избегать длительного употребления конфет «Tom-Tom» совместно с напитком «La Casera».

1. Introduction

A soft drink is a brew that typically consists of carbonated water, caffeine, phosphoric acid, sweetener (fruit juice, high-fructose corn syrup, sugar, or sugar substitutes), and other chemicals in the form of flavourings, colorants, and preservatives [1]. Soft drinks include Coca Cola, Pepsi, Fanta, Sprite, La Casera, Orange Drink, Chivita Orange Juice etc. Globally, the ingestion of soft drinks has increased within the last two to three decades [2]. Their impact on health is unknown, however epidemiological research has linked them to obesity, osteoporosis, liver and renal disorders [3]. Carbonated drinks have intentional caffeine additions that cause addiction in users because they are absorbed more quickly than other drinks [4].

In Spain, La Casera is a well-known brand of soft drinks. In Nigeria, it's one of the most popular soft drinks. This sweet carbonated drink is called gaseosa in Spanish. It can be served as a regular soda [5].

Cadbury produces the calming oval black and white sweet called Tom-Tom, which has a strong menthol flavour. A lozenge is a little tablet that is usually flavoured and sweetened, and it is designed to dissolve gradually in the mouth. Tom-Tom is widely available in Nigeria and provides fresh breath and instantaneous sweet comfort. Menthol is a monocyclic terpene alcohol. It is widely used in both food and medication and is generally regarded as being extremely safe. There have been incidents of menthol overconsumption leading to poisoning [6].

Tom-Tom contains the monocyclic terpene alcohol menthol. In general, it is regarded as quite safe and is extensively used in food and medicine. It has been revealed that codeine addicts use La Casera and Tom-Tom to form “gigabyte” solution (La Casera drink mixed with Tom-Tom candy) [7]. Case studies of menthol toxicity from excessive intake are documented in literature. But no case of lethal intoxication has ever been reported. The results of this investigation will help in ascertaining and establishing the possible biochemical adverse effects associated with combining Tom-Tom with La Casera [6].

Drug abuse is a serious problem that have been reported among Nigerian youth in which La Casera mix with Tom-Tom is one of those drugs that is recently abused. Following the ban by the Federal Government of Nigeria on the import and sale of codeine syrup, users reportedly turned to beverages, mainly La Casera drinks to mix with Tom-Tom sweets to create a substance known as "gigabyte" as an alternative [8]. This mixture may have higher effect of intoxication than real hard drugs or alcohol, and could have effect on various biochemical parameters. However, no study has been reportedly done on the effect(s) of this mixture. This study looked ~~into~~ at the biochemical alterations that occurred in rats treated with soft drinks and candies flavoured with menthol. Histological analysis and test on the extent of oxidative damage in the tissue were also performed [9].

Pure menthol consumption is risky, and excessive use of menthol lozenges puts users at risk for overdosing on the substance [10]. There have been reports of gastrointestinal, neurological, and cutaneous symptoms linked to long-term menthol consumption [11]. Excessive menthol consumption has also been linked to coma, convulsions, ataxia, agitation, and dizziness [12]. Codeine addicts now embark on the use of La Casera and Tom-Tom candy to form “gigabyte” (mixture made with La Casera drink and Tom-Tom candies). Moreover, authors in [13] noted that the amount of drugs an addict needs to feel fulfilled varies from 2 to 10 gigabytes, depending on the amount utilized.

The liver which is the primary site for the metabolism of drugs gets overwhelmed in the case of an overdose [14]. This can cause toxicity and oxidative stress [15]. An adverse drug effect refers to any injury occurring at the time a drug is used [16], whether or not it is identified as a cause of the injury [17]. An undesired or dangerous reaction that occurs after a drug or combination of drugs is administered under normal use circumstances and is thought to be related to the drug is known as an adverse drug reaction (ADR) [18]. Usually, an ADR calls for stopping the medication or lowering the dosage [19]. ADRs can happen after taking a medication for an extended period of time [20], after a single dose, or as a result of taking two or more medications together [21].

Moreover, oxidative stress induced liver dysfunctions demonstrated by considerable increase in alkaline phosphatase (ALP) [22], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities [23]. Recent study on alternations in the oxidative stress levels of Wistar rats who received menthol candy and soft drink have been reported [10]. Also, observed alteration in triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL–C), high-density lipoprotein cholesterol (HDL–C) and total cholesterol (TC) have been stated in oxidative stress induced liver damage [24].

This study was centred on the evaluation of serum transaminases and lipid profile of rats administered La Casera mixed with Tom-Tom candy.

2. Materials and methods

2.1. Reagents and chemicals

All the chemicals and reagents utilized in this investigation were of analytical quality. Tom-Tom (TT) candies and La Casera (LC) drinks were bought at the Abraka Main Market in Delta State, Nigeria.

2.2. Investigational processes

Seventy male albino rats (Wistar strain) with body weight ranging from 125 to 175 g were obtained and used for the entire duration of this study. They were fed with water and growers mash (laboratory diet) randomly. The rodents were randomly divided into 10 treatments or groups with each treatment containing seven rats as follows:

Group 1 is the control group, which was given a typical laboratory meal (growers mash) and unlimited water.

Group 2: 8 ml of LC per kilogram of body weight;
Group 3: 5 ml of LC per kilogram of body weight;
Group 4: 2.5 ml of LC per kilogram of body weight;
Group 5: 0.34 g of TT per kilogram of body weight;
Group 6: 0.22 g of TT per kilogram of body weight;
Group 7: 0.11 g of TT per kilogram of body weight;
Group 8: 0.34 g of TT in 8 ml of LC per kilogram of body weight;
Group 9: 0.22 g of TT in 5 ml of LC per kilogram of body weight;
Group 10: 0.11 g of TT in 2.5 ml of LC per kilogram of body weight;

La Casera and Tom-Tom were administered orally to rats in groups 2 through 10 for 42 days (either in combination or singly); when the study was completed (after 41 days), all the rats used were exterminated in a chloroform-filled airtight glass chamber; and after being surgically opened, blood samples and liver were obtained for use in biochemical analyses.

2.3. Biochemical analyses

2.3.1. Estimation of liver function markers

On a weekly basis, the glucose level was recorded, as well as the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). Using commercially available diagnostic kits (Randox Laboratories Limited, England), colorimetric measurements **(**USB4000, Ocean Optics, Dunedin, FL, USA**)** of the total proteins and the albumin in the kidney and serum were taken in accordance with normal methods [25].

2.3.2. Blood glucose

The fasting blood glucose level in the experimental rats was examined periodically during the treatment via prick method using advanced Accu-Chek glucometer (Accu-Chek Inform II, 2003) (Roche Germany). Glucose test strip was inserted in the glucometer, and then a blood drop placed onto the green field of the test strip. The blood glucose result was read in mg/dL [26].

2.3.3. Determination of alkaline phosphatase

The approach by Okpoghono et al. [27] was used to measure the alkaline phosphatase activity. Alkaline phosphatase substrate of 0.05 ml was added to tubes with labels, and it was allowed to equilibrate at 37 °C for three minutes. The tubes containing the control and sample were introduced and mixed at intervals of 0.05 ml of standard. At 37 °C, this was incubated for exactly 10 minutes. Alkaline phosphatase colour developer was then added

in 2.5 ml increments and thoroughly mixed. At 580 nm, spectrophotometry (USB4000, Ocean Optics, Dunedin, FL, USA) was used to measure absorbance against a reagent blank comprising distilled water.

2.3.4. Determination of aspartate aminotransferase

AST activities were measured using the technique by Ozer et al. [28]. 250 µl of reagent 1 were dispensed into test tubes marked as sample and blank. 50 µl of samples were then added and combined. After incubating the tubes for 1 hour at 37 °C, 250 µl of the second reagent were added. Working NaOH reagent in amounts of 2.5 ml was added, and it was let to stand for 10 minutes. At 540 nm, the absorbance (USB4000, Ocean Optics, Dunedin, FL, USA) was measured against the reagent blank.

2.3.5. Determination of alanine aminotransferase

The approach by Hassan et al. [29] was used to determine the activity of alanine aminotransferases. 250 µl of reagent 1 were dispensed into the sample and blank test tubes. 50 µl of sample and 50 µl of distilled water were put to test tubes for the sample, which were then incubated at 37 °C for 60 minutes. Reagent 2 was added, stirred, and incubated for 20 minutes at 20 to 25 °C with 250 µl of the solution. 2.5 ml of the active NaOH reagent was added to each test tube, thoroughly mixed, and then left to stand for 10 minutes. Absorbance (USB4000, Ocean Optics, Dunedin, FL, USA) was measured at a wavelength of 540 nm against the reagent blank.

2.3.6. Determination of albumin

To estimate albumin level, the approach by Charmier et al. [30] was applied. In a test tube with the labels of blank, standard, and sample, 3 ml of bromocresol green (BCG) regent were dispensed. Samples containing 10 µl were transferred to tubes and left for five minutes. The absorbance was measured at 580 nm by spectrophotometry (DT-Mini-2, Ocean Optics, Dunedin, FL, USA).

2.3.7. Estimation of total protein

Using the approach by Charmier et al. [30], the total protein was measured. 1 ml of protein regent was poured into each of the sample, standard, and blank test tubes, followed by the addition of 20 µl of sample. After 30 minutes, the absorbance against the reagent blank was measured at 546 nm (DT-Mini-2, Ocean Optics, Dunedin, FL, USA).

2.3.8. Estimation of lipid profile

Commercial diagnostic kits were bought from Randox Laboratories Limited, England. Standard approach was used to determine the HDL cholesterol, total cholesterol and triacylglycerol levels. Low density lipoprotein (LDL)-cholesterol was estimated thus: LDL – C (mg/dl) = (TC – HDL) – TAG/5 [29].

2.3.9. Determination of total cholesterol

The method by Nagababu et al. [31] was taken for total cholesterol determination. Cholesterol regent (1 ml) was taken in a labeled test tube, 10 µl of the sample was added into the tube swirled. The tubes that contained the specimen and the reagent were allowed to stand for 5 minutes. The colour of the solution changed to pinkish red after which the absorbance was measured at 530 nm (USB4000, Ocean Optics, Dunedin, FL, USA).

2.3.9. Determination of triacylglycerol

This assay was carried using the method by Nagababu et al. [31]. Triacylglycerol regent (1 ml) was put in tubes and labelled after which the standard reagent and 10 µl of sample (distilled water) were put in their respective tubes and whisked. The specimen and reagent containing tubes were allowed to stand for 5 minutes at ambient temperature. Following blanking, the absorbance was measured at 540 nm (USB4000, Ocean Optics, Dunedin, FL, USA).

2.3.10. Determination of high-density lipoprotein cholesterol

The technique by Ozer et al. [28] was used to calculate HDL–C. To the test tube marked as "CHOLESTROGEN REGENT," 1 ml was inserted. Thereafter the standard reagent and 25 µl of the sample (distilled water) were then put into their individual tubes. The contents of each tube were thoroughly mixed for five minutes. Values were recorded at 500 nm (USB4000, Ocean Optics, Dunedin, FL, USA).

2.4. Histopathology analysis

The liver histology was performed using the methodology by Perry et al. [32]. For 48 hours, the tissues were left to repair in 10% formyl saline. In a tissue cassette with prelabels, the tissues were sliced into 3 mm thick pieces. 70% alcohol was used to obtain dehydrated pieces that were passed through 90% alcohol and chloroform for different time lengths. Afterwards, they were moved into changes of molten paraffin wax for twenty minutes all in a temperature 57 °C oven. Successive 5 µm thick sections of a hard block tissue were obtained and stained with eosin and haematoxylin stains. Following the staining, the tissue was passed through a blend of equivalent concentrations of alcohol and xylene. Clearance was done after which the tissue was dried in the oven coloured video digital camera-aided photomicrographs were taken. The video camera was placed on an Olympus light microscope (Olympus UK Limited, United Kingdom)

2.5. Statistical analysis

The data obtained were expressed as mean ±SD and analysis of the group mean and variance (ANOVA) was compared by least significant difference (LSD). The SPSS-PC programme package (version 22.0) will be used for statistical analysis.

2.6. Ethical approval

It was approved ethically to conduct the experiment. The protocol for managing lab humans/animals during the study was adhered to. Additionally, authors collaborated with the technicians in the lab and humans/animal house.

The protocol for managing laboratory animals during the study was adhered to. Ethical approval was obtained from the Faculty Research Ethics Committee, Faculty of Science, Delta State University of Science and Technology, Ozoro (Ethical approval number: FOS/DSUST/23/6543). The research was carried out according to the guidelines of the ethics committee and the protocol was approved by FOS/DSUST/23/6543.

3. Results

3.1. Blood glucose level of rats administered TT, LC, and TT mixed with LC

The blood glucose level results of rats administered TT, LC and TT mixed with LC for a period of six weeks are shown in Figure 1. In the first week, groups 2, 5, 6, and 7 showed no discernible changes in their glucose levels compared to the control, while groups 3, 4, 8, 9 and 10 showed a substantial increase ($p < 0.05$) in their blood glucose levels. Moreover, group 3 did not exhibit a significant difference from group 4, although group 2 did exhibit a considerable drop ($p < 0.05$) compared to group 4. Additionally, there was no discernible variation in group 10's blood glucose level when compared to groups 8 and 9. Furthermore, group 7 did not exhibit a statistically significant difference compared to groups 5 and 6. There was no significant difference between groups 2 and 5, while group 8's blood glucose level rose considerably ($p < 0.05$) in comparison to group2. Groups 7, 8, 9, and 10 did not significantly differ in their blood glucose levels from the control on weeks 4 and 5, however groups 2, 3, 4, and 5 did significantly rise ($p < 0.05$) in comparison to the control. That being said, between groups 4 and 3, there was no obvious difference. Furthermore, no discernible variation was observed between groups 8 and 9 and group 10. On the other hand, group 7 showed a substantial rise ($p < 0.05$) in comparison to group 5. Additionally, group 2 showed a substantial decrease ($p < 0.05$) in comparison to groups 5 and 8. During week six, there was no significant difference observed between groups 6 and 7, while groups 2, 3, 4, 5, 8, 9 and 10 showed a significant ($p < 0.05$) increase in blood glucose compared to the control. Additionally, there was no discernible change in group 4's blood glucose levels when compared to groups 2 and 3. Furthermore, group 10 did not exhibit a statistically significant difference from groups 8 and 9. Conversely, group 5 exhibited a substantial rise ($p < 0.05$)

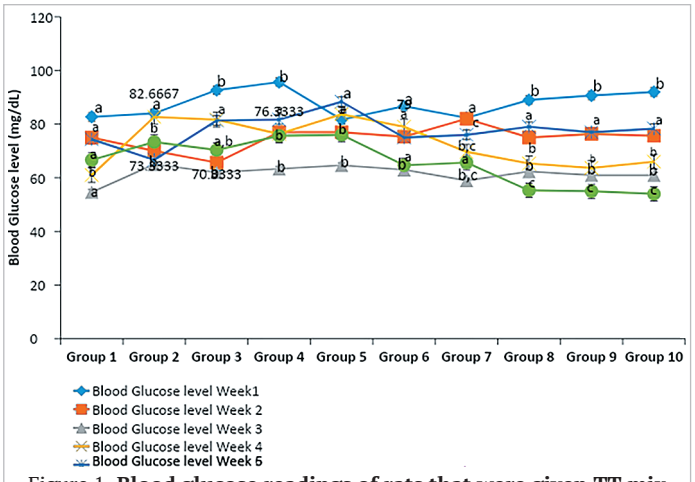


Figure 1. **Blood glucose readings of rats that were given TT mix with LC**

Mean values (n5) in the same line with different superscript letter differ significantly at $p < 0.05$.

Рисунок 1. **Уровни глюкозы в крови у крыс, которым давали смесь TT и LC**

in comparison to group 7, whereas group 6 showed no significant difference in comparison to group 7. Furthermore, no statistically significant difference was seen between group 2 and group 5, although a substantial rise ($p < 0.05$) was noted between group 2 and group 8.

3.2. *AST, ALT, ALP, albumin and total protein of rats given TT, LC and TT mixed with LC*

The effect of TT, LC and TT mixed with LC on liver function markers of rats is displayed in Table 1 and Table 2. There was a significant increase ($p < 0.05$) observed in serum AST, ALT, ALP activity and decrease in albumin and total protein of rat in groups 2, 3, 5, 6, 8, 9 and 10 compared to the control. Also, there was a significant decrease recorded in group 4 compared to groups 2 and 3. However, there was no significant difference observed in group 10 compared to groups 8 and 9. Furthermore, group 7 had an obvious decrease ($p < 0.05$) compared to groups 5 and 6. In addition, there was an important decrease in group 2 compared to group 8.

Table 1. **Serum AST, ALT and ALP activities of rats administered TT, LC and TT mixed with LC**

Таблица 1. **Активность АСТ, АЛТ и ЩФ в сыворотке крови у крыс, которым давали ТТ, LC, а также смесь ТТ и LC**

	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)
Group 1	151.40 ± 32.56^{a}	56.00 ± 13.78^{a}	120.80 ± 57.66^{a}
Group 2	180.60 ± 31.86^{b}	77.00 ± 22.69^{b}	153.60 ± 48.40^{b}
Group 3	175.40 ± 48.60^{b}	68.40 ± 21.46^{c}	140.20 ± 52.16^{c}
Group 4	150.60 ± 37.87^{a}	61.40 ± 23.94^{d}	120.40 ± 50.30^{a}
Group 5	$170.80 \pm 45.63^{b, c}$	$65.00 \pm 8.66^{c, d}$	135.20 ± 53.13^{c}
Group 6	176.00 ± 42.19^{b}	60.80 ± 15.73^{d}	$130.60 \pm 39.01^{c, d}$
Group 7	149.00 ± 15.95^{a}	54.40 ± 11.86^{a}	110.60 ± 25.38^{e}
Group 8	202.60 ± 25.07^{d}	83.20 ± 20.26^{b}	165.40 ± 36.48^{f}
Group 9	205.80 ± 54.30^{d}	81.40 ± 31.26^{b}	166.00 ± 33.08^{f}
Group 10	202.80 ± 38.64^{d}	80.00 ± 21.94^{b}	164.40 ± 52.29^{f}

The values are expressed as mean ± SD. Mean values ($n = 5$) in the same column with changed letter differ at $p < 0.05$.

Table 2. **Effect of TT, LC and TT mixed with LC on serum albumin and total protein level of rats**

Таблица 2. **Влияние ТТ, LC, а также смеси ТТ и LC на сывороточные уровни альбумина и общего белка у крыс**

	Albumin (g/dL)	Total protein (g/dL)
Group 1	5.44 ± 0.05^{a}	8.22 ± 0.5^{a}
Group 2	3.82 ± 0.21^{b}	7.44 ± 0.70^{a}
Group 3	3.34 ± 0.25^{b}	7.46 ± 0.48^{a}
Group 4	5.40 ± 0.14^{a}	8.66 ± 1.04^{a}
Group 5	$4.72 \pm 1.69^{a, b}$	$6.92 \pm 0.42^{a, b}$
Group 6	$4.62 \pm 1.69^{a, b}$	7.48 ± 0.33^{a}
Group 7	6.36 ± 0.18^{c}	8.10 ± 0.68^{a}
Group 8	2.78 ± 0.13^{b}	5.92 ± 1.40^{b}
Group 9	2.40 ± 0.15^{b}	5.54 ± 0.24^{b}
Group 10	2.70 ± 0.29^{b}	5.40 ± 0.55^{b}

The values are expressed as mean ± SD. Mean values ($n = 5$) in the same column with changed letter differ at $p < 0.05$.

3.3. *Lipid profile of rats that were given TT, LC and TT mixed with LC*

The blood lipid profile of rats given TT, LC, and TT combined with LC is displayed in Table 3. Rats in groups 2, 5, 6, 8, 9, and 10 had significantly higher serum levels of Total-Chol, TAG, and LDL–Chol ($p < 0.05$) when compared to the control. Additionally, group 4's serum Total-Chol, TAG, and LDL–Chol were significantly lower than those of groups 2 and 3. On the other hand, group 10's Total-Chol, TAG, and LDL–Chol did not significantly differ from groups 8 and 9. Moreover, compared to groups 5 and 6, group 7 experienced a substantial drop ($p < 0.05$). Furthermore, in group 2, serum LDL–Chol did not differ significantly from groups 5 and 8. Rats in groups 2, 3, 5, 6, 8, 9, and 10 had significantly lower serum HDL–Chol levels ($p < 0.05$) than the control group, whereas groups 4 and 7 did not significantly vary from the control group. Additionally, group 2's results were significantly lower than those of groups 4 and 3. However, when group 10 was compared to groups 8 and 9, no discernible differences were seen. Additionally, when compared to groups 5 and 6, group 7 showed a substantial increase ($p < 0.05$). Furthermore, group 2's serum HDL–Chol considerably decreased ($p < 0.05$) when compared to group 5, but group 2's serum HDL–Chol did not significantly differ from group 8's values.

Table 3. **Changes in serum lipid profile of rats given TT, LC and TT mixed with LC**

Таблица 3. **Изменения в липидном профиле сыворотки крови у крыс, которым давали ТТ, LC, а также смесь ТТ и LC**

	Total-Chol (mg/dL)	TAG (mg/dL)	HDL–Chol (mg/dL)	LDL–Chol (mg/dL)
Group 1	42.00 ± 14.38^{a}	60.60 ± 17.14^{a}	30.80 ± 1.64^{a}	13.60 ± 6.54^{a}
Group 2	55.20 ± 15.65^{b}	80.20 ± 11.60^{b}	16.40 ± 2.88^{b}	21.20 ± 10.23^{b}
Group 3	58.00 ± 14.73^{b}	56.00 ± 25.60^{a}	20.80 ± 2.77^{b}	18.40 ± 12.34^{b}
Group 4	40.00 ± 13.63^{a}	42.60 ± 8.14^{d}	31.80 ± 2.16^{a}	10.00 ± 5.04^{a}
Group 5	$50.00 \pm 15.09^{b, c}$	$70.00 \pm 22.07^{a, e}$	$23.00 \pm 2.23^{b, c}$	18.20 ± 9.23^{b}
Group 6	$47.00 \pm 6.44^{b, c}$	$64.20 \pm 11.90^{a, e}$	$26.60 \pm 5.59^{b, c}$	$15.00 \pm 3.80^{a, b}$
Group 7	36.40 ± 9.60^{d}	55.60 ± 15.43^{a}	32.60 ± 3.04^{a}	11.60 ± 9.34^{a}
Group 8	60.40 ± 19.15^{c}	92.00 ± 11.33^{f}	15.40 ± 2.79^{b}	22.40 ± 16.92^{b}
Group 9	61.60 ± 6.14^{c}	91.60 ± 25.03^{f}	16.80 ± 5.93^{b}	20.60 ± 1.51^{b}
Group 10	61.20 ± 24.80^{c}	87.00 ± 24.70^{f}	15.60 ± 5.85^{b}	22.00 ± 17.27^{b}

The values are expressed as mean ± SD. Mean values ($n = 5$) in the same column with different letter differ at $p < 0.05$.

3.4. *Liver histology results of the rats that received TT, LC and TT mixed with LC*

The liver histology results of rats administered TT, LC and TT mixed with LC are illustrated in Figure 2 (group 1 to 10). The liver histology of Group 1 (control) had central vein (CV) and normal hepatic cell (HC). Group 2 had hepatic cell (HC) and inflammation (I) of portal vein (PV). Group 3 showed mild inflammation (I) of PV. Group 4 had no damage to hepatic cell. Groups 5 and 6 indicated congestion (C) of PV. Group 7 had normal HC and CV. Groups 8, 9 and 10 indicated severe inflammation (I) and necrosis (N).

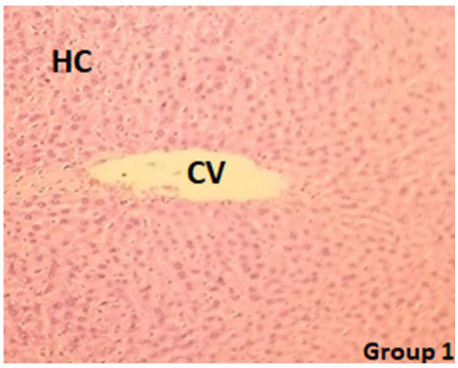
4. Discussion

Tom-Tom is an oval black and white sweet with a strong menthol flavour that is calming. There have been cases reported of menthol poisoning brought on by excessive use [6]. Nigerians are familiar with La Casera, a typical Spanish soft drink. It is suitable for serving as a typical soda [12]. Individual and societal chronic soft drink use is concerningly rising [12]. The goal of the current study was to look at the biochemical characteristics of rats given LC, TT, and LC combined with TT. The blood glucose levels of the rats given TT mixed with LC was measured, and the results showed that, from the first week to the fifth week, no discernible disparity between the glucose levels of groups 6, 7, 8, 9, and 10 and the control was observed. On the other hand, the consumption of LC may have contributed to the significant ($p < 0.05$) rise in groups 2, 3, and 4 during week 4 as compared to the control. This aligns with the research conducted by Alsunni et al. [33], who suggested that soft drink caffeine content could be the cause of an increase in blood glucose levels. Adrenaline is released by caffeine, which raises blood sugar levels [34]. Research has also indicated that the use of certain soft drinks may cause an increase in blood glucose levels because of their high glycaemic index and other ingredients including caffeine, high fructose, aspartame, and caramel [35].

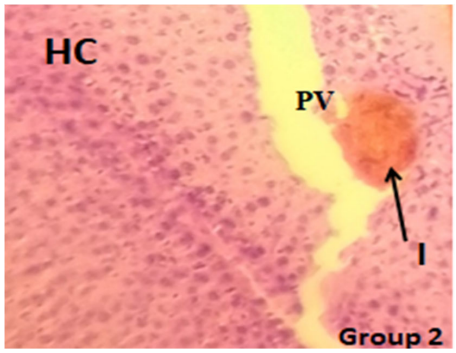
The rats that were given TT and LC in amounts of 0.11 g in 2.5 ml, 0.22 g in 5 ml, and 0.34 g in 8 ml per kg body weight on week 6 showed lower blood glucose levels than those on weeks 1, 2, 3, and 5, which may indicate that menthol's anti-hyperglycaemic action is attenuated. Research has shown menthol, a naturally occurring monoterpene, to be great at reducing high blood glucose by inducing the secretion of insulin and improving the activity of glucose metabolic enzymes in experimental animals [36]. Glucokinase is the enzyme that sensors glucose in pancreatic beta cells and liver cells. The enzyme also fundamentally determines flux via glycolysis in both cell types besides been critically implicated in the regulation of glucose. The high glucose level in rats observed in this study might be due to a deficiency of direct stimulation of glycolysis by insulin in tissues. It was reported that a deficiency is the cause of the decrease in the activity of glucokinase in rats' liver at high glucose levels [37].

Interestingly, menthol was seen to improve the secretion of insulin which consequently improved glucokinase activity. Increase in the activity of glucokinase leads to greater utilization of glucose which in turn leads to its decrease in the blood [38].

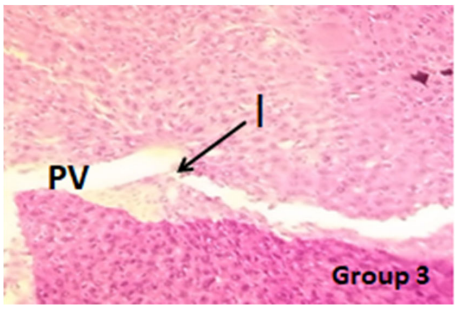
Rats given TT and LC in amounts of 0.34 g in 8 ml, 0.22 g in 5 ml, and 0.11 g in 2.5 ml per kg body weight showed a significant ($p < 0.05$) increase in serum AST, ALT, and ALP compared to the control group (Table 1). This increase is likely the result of liver damage caused by the high dose of TT mixed with LC.



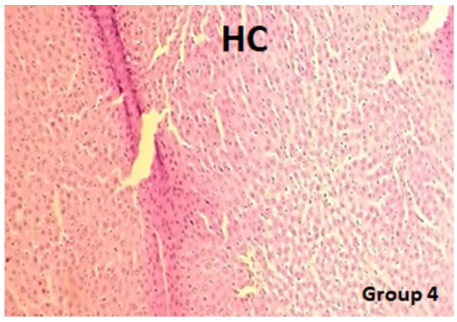
A) Group 1: control, showing normal hepatic cell (HC) and central vein (CV). (H&E stain, 400×)



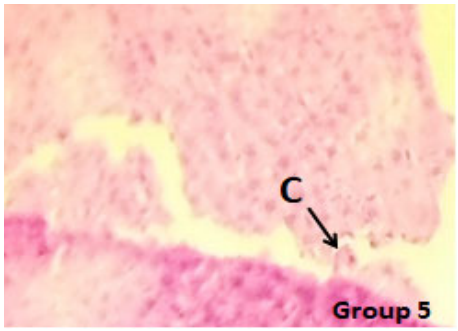
B) Group 2: 8 ml of LC/kg body weight, showing hepatic cell (HC) and inflammation (I) of portal vein (PV). (H&E stain, 400×)



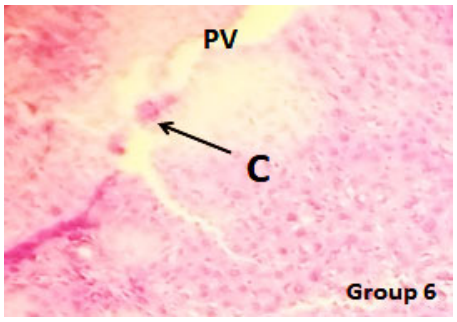
C) Group 3: 5 ml of LC/kg body weight, having slight inflammation (I) of portal vein (PV). (H&E stain, 400×)



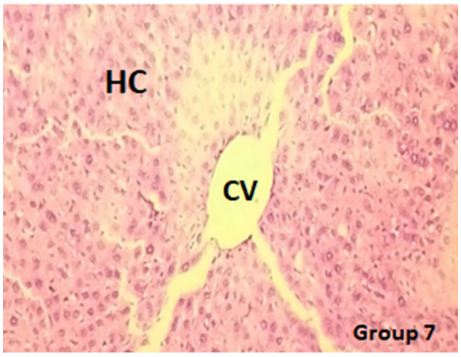
D) Group 4: 2.5 ml of LC/kg body weight, with no damage to hepatic cell (HC). (H&E stain, 400×)



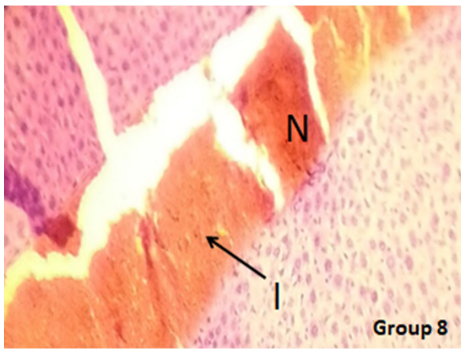
E) Group 5: 0.34 g of TT/kg body weight, showing congestion (C) of portal vein (PV). (H&E stain, 400×)



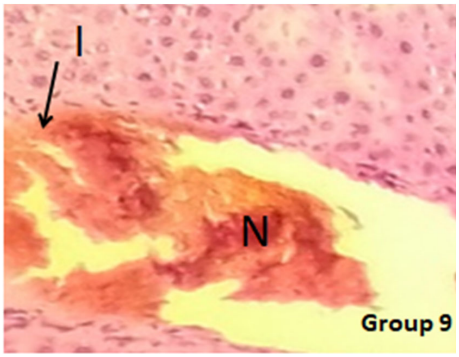
Group 6: 0.22 g of TT/kg body weight, demonstrating congestion (C) of portal vein (PV). (H&E stain, 400×)



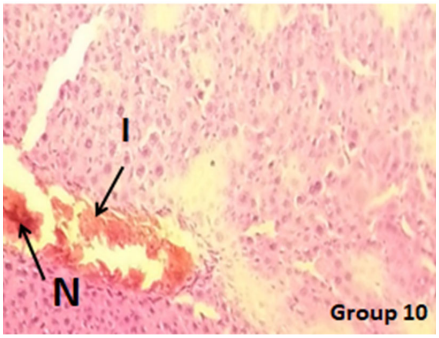
Group 7: 0.11 g of TT/kg body weight, showing normal hepatic cell (HC) and central vein (CV). (H&E stain, 400×)



Group 8: 0.34 g of TT in 8 ml of LC/kg body weight, showing severe inflammation (I) and necrosis (N). (H&E stain, 400×)



Group 9: 0.22 g of TT in 5 ml of LC/kg body weight, showing severe inflammation (I) and necrosis (N). (H&E stain, 400×)



Group 10: 0.11 g of TT in 2.5 ml of LC/kg body weight, showing inflammation (I) and necrosis (N). (H&E stain, 400×)

Figure 2. **Liver histology of rats administered TT, LC and TT mixed with LC**
Рисунок 2. **Гистология печени у крыс, которым давали TT, LC, а также смесь TT и LC**

The results are identical with those by Dubey et al. [39], who found that rats given soft drinks had higher serum levels of AST, ALT, and ALP than rats in the control group. According to Hasan et al. [40], biochemical indicators indicating liver injury include elevated serum levels of the activity of AST, ALT, and ALP. Rats' serum ALT levels increased, which suggests that liver function may be compromised [41]. However, significant decrease was recorded in AST, ALT and ALP of rats given 2.5 ml of LC/kg (group 4) in comparison with 8 ml of LC/kg (group 2) and 5 ml of LC/kg (group 3). Interestingly, at low dose of 2.5 ml of LC/kg and 0.11 g of TT/kg, no significant difference was noted compared to the control.

This could be because excessive carbonated drink consumption can cause liver damage and raise liver enzyme activity, even at lower dosages when it may not be hazardous [41]. However, the rats that received TT and LC in amounts of 0.34 g in 8 ml, 0.22 g in 5 ml, and 0.11 g in 2.5 ml per kg body weight did not exhibit significantly different blood AST, ALT, or ALP activity. This may be the result of TT combined with LC having the same dose concentrations. Additionally, blood levels of AST, ALT, and ALP were significantly lower ($p < 0.05$) in rats given a low dose of 0.11 g of TT/kg than in rats given a high dose of 0.34 g and 0.22 g of TT/kg. Furthermore, in comparison to rats given 8 ml of LC/kg, rats given 0.34 g of TT and 0.34 g of TT in 8 ml of LC/kg showed a substantial increase in ALT and ALP, according to the study. The effect could be as a result of the menthol constituents Tom-Tom candy. Study have shown that intake of menthol at high dose could be toxic to the liver [42].

In this study, the liver histology of control (group 1), groups 4 and 7 demonstrated average hepatic cell and central vein; no damage was indicated (Figure 2A, Figure 2D, Figure 2G). On the other hand, groups 2, 3, and 5 displayed portal vein inflammation and congestion. Rats administered TT and LC in amounts of 0.34 g in 8 ml, 0.22 g in 5 ml, and 0.11 g in 2.5 ml per kg body weight similarly showed signs of severe inflammation and necrosis (groups 8, 9 and 10). The increased AST, ALT, and ALP levels in the livers of the rats in these groups may be supported by this damage.

When compared to the control group (Table 2), rats in groups 2, 3, 7, 8, 9 and 10 had significantly lower serum levels of albumin and total protein ($p < 0.05$). This could be the result of tissue damage caused by high doses of TT, LC, and TT mixed with LC. A steady increase in the consumption of soft drinks may be linked to a significant drop in albumin and total protein [43]. There has also been evidence of a link between albuminuria — a sign of early kidney damage — and sugar-sweetened soda drinks [44]. According to Elbendary et al. [45], a prolonged consumption of soft drinks has been linked to damage to the kidneys and liver, which is the primary source of albumin.

Prolonged hepatic cell death lowers serum levels of albumin and total protein and increases hepatic releases, which worsen hepatic dysfunction [46]. However, when comparing the rats administered 8 ml of LC/kg to the group given 0.34 g of TT/kg and 0.34 g of TT in 8 ml LC/kg body weight, respectively, no discernible differences were shown in the levels of albumin and total protein. When comparing a little dose of 2.5 ml of LC/kg body weight (group 4) to the high dose of 5 and 8 ml of LC/kg (groups 2 and 3), a substantial increase ($p < 0.05$) was seen in total protein and albumin. This may indicate that there may be no correlation between reduced use of carbonated drinks and protein degradation [47].

In contrast, rats fed 0.22 g of TT in 5 ml of LC and 0.11 g of TT in 2.5 ml of LC/kg body weight (groups 9 and 10) demonstrated no significant difference in total protein and albumin compared to 0.34 g of TT in 8 ml of LC/kg (group 8). Furthermore, serum albumin and total protein levels significantly increased ($p < 0.05$) in rats given a modest dose of 0.11 g of TT/kg body weight (group 7) in comparison to 0.22 and 0.34 g of TT/kg.

Low dose of TT intake may provide low menthol which might have stimulated protein synthesis. Paraskeuas et al. [48] reported that inclusion of menthol at the low rate of 100 to 150 mg/kg in feed improved the nutrient digestibility, thus enhancing the growth measures and protein level in broiler chickens. In this investigation, rats given LC in amounts of 8 and 5 ml/kg, TT in amounts of 0.34 g/kg, and TT mix with LC in amounts of 0.34 g in 8 ml, 22 g in 5 ml, and 0.11 g in 2.5 ml/kg demonstrated a considerable increase ($p < 0.05$) in serum Total-Chol, TAG, and LDL–Chol and a decrease in HDL–Chol when compared to the control group (Table 3). Intake of TT and LC at high dose may lead to alteration of lipids. Gugliucci et al. [49] stated that excess of carbonated drinks is considerably linked with the elevation of triglycerides, which may add to cardiovascular disease and is also a variable marker for diabetes mellitus. Increased levels of triglycerides in blood thus indicated to be predisposing for cardiovascular diseases [50]. Additionally, considerable decrease was seen in serum Total-Chol, TAG and LDL–Chol in the rats that were administered 2.5 ml of LC/kg in comparison to 8 ml and 5 ml of LC/kg. This should mean that intake of LC lower Total-Chol, TAG, LDL–Chol and increase HDL–Chol. Moreover, a considerable reduction ($p < 0.05$) was observed in serum LDL–Chol of the rats that took 2.5 of LC/kg (group 4) in comparison to those given 8 and 5 of LC/kg. Furthermore, little dose of TT of 0.11 g/kg considerably reduced serum LDL–Chol ($p < 0.05$) compared to elevated doses of 0.22 g and 0.34 g/kg. This clearly illustrates that low LC and TT doses may not have effect on the alteration of the lipids.

5. Conclusion

Conclusively, a continually consuming of La Casera, Tom-Tom or La Casera mixed with Tom-Tom triggered alterations in serum lipid profile and liver function markers. The liver histopathological examination of the rats that administered TT mix with LC, LC only and TT only at high dose had severe alterations in comparisons to that of rats given low dose. Taken together, these confirmed that TT and LC at high dose or TT mix with LC triggered biochemical alterations. It is therefore recommended that intake of TT mix with LC or constant consumption of TT and LC should be discouraged because of the noticeable side effects associated with their consumption, especially at over a long period of time.

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