Tropical Journal of Drug Research May 2025; 2 (5): 117 - 129
ISSN: 2476-8065 (Electronic)
Available online at http://www.tjdr.org
https://doi.org/10.26538/tjdr/v2i5.1

Original Research Article

In vivo analysis of Neuromodulatory and Biochemical Effects of Lacatomtom Drinks on Wistar Rat Brain

Friday T Emmanuel[1]*, Gideon A Gyebi[1], Samson A Oka[1], Adams A Omada[1], Yahaya Junaidu[1], Ayeni Gideon[3], Gideon Obaje[1], Idowu O Owemidu[4], Emmanuel O Jimoh[5], Joel M Omale[6], Musa O Iliyasu[7], Negedu N Muhammed[4], Godwin A Ewu[5] and Olajumoke R Oloniyo[8]

[1]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba, Nigeria.*
[2]*Department, Faculty of Basic Medical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba. Nigeria*
[3]*Department of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, Anyigba, Nigeria.*
[4]*Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba. Nigeria*
[5]*Department of Community Medicine, Faculty of Basic Clinical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba. Nigeria.*
[6]*Pharmacy Unit, Health Services, Prince Abubakar Audu University, Anyigba. Nigeria*
[7]*Department of Anatomy. Faculty of Basic Medical Sciences, College of Health Sciences, Prince Abubakar Audu University, Anyigba. Nigeria*
[8]*Department of Food, Nutrition and Home Sciences, Faculty of Agricultural Science, Prince Abubakar Audu University, Anyigba, Nigeria*

*For correspondence: Email: friday.et@ksu.edu.ng, emmfriday@gmail.com, +2348069608367, +2348068956979

Sent for review: 13April 2025 Revised accepted: 12 May 2025

Abstract

Purpose: This study investigated the neuromodulatory and biochemical effects of Lacatomtom, a mixture of a soft drink and candy, on Wistar rat brains.
Methods: This research examined the activities of ATPases (Mg2+, Na+/K+, Ca2+/Mg2+), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and levels of dopamine, serotonin, tumor necrosis factor-alpha (TNF-α), sodium ions (Na+), glutathione peroxidase (GPx), bicarbonate (HCO3-), and creatinine kinase (CK). Wistar rats were divided into four groups: control (water), Lacatomtom (LTT), Lacasera (LC) alone, and Tomtom candy dissolved in water (TTW).
Results: Results revealed significant increases in AChE and BChE activities, particularly in the LTT group, suggesting potential cholinergic dysfunction. Elevated ATPase activity was observed in the LTT and LC groups, indicating altered energy metabolism. Furthermore, increased levels of nitrite and protein suggested oxidative stress.
Conclusion: These findings indicate that LTT induces significant neurochemical alterations, potentially leading to neurotoxicity.

Keywords: Lacatomtom, Neurotoxicity, ATPase, Acetylcholinesterase, butyrylcholinesterase, lacasera, psychoactive.

Tropical Journal of Drug Research is indexed by Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals
(DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The consumption of unconventional mixtures, such as Lacatomtom (LTT) (a combination of a specific Lacasera LC and candy), has raised concerns regarding potential neurotoxic effects. These mixtures often contain high concentrations of sugars, artificial sweeteners, and other chemical compounds, raising concerns about their potential to disrupt normal brain function. The practice of combining seemingly disparate food and beverage items into novel concoctions, while sometimes driven by act of use it as stimulant or cultural trends and economic necessity, presents a unique challenge to our understanding of food-drug interactions and their impact on human health. In regions like Nigeria, the consumption of these mixtures, particularly among younger populations, has become a growing trend, often with limited awareness of the potential health risks involved.

The appeal of such mixtures often lies in the perceived synergistic effects of their components. For instance, the high sugar content of soft drinks may enhance the palatability and immediate energy boost, while the presence of stimulants in candy could potentiate the effects of caffeine or other psychoactive substances that may be present, either as intended ingredients or unintended contaminants. This creates a complex interplay of chemicals, where the effects of individual components are modified and potentially amplified, leading to unpredictable and potentially harmful consequences, particularly concerning the delicate balance of neurotransmitters and enzymatic activity in the brain.

Soft drinks, a major component of these mixtures, are typically characterized by their high sugar content, acidic pH, and the presence of artificial flavors and colors. The excessive consumption of sugar, particularly in the form of fructose and glucose, has been consistently linked to a range of metabolic disorders, including insulin resistance, type 2 diabetes, and obesity.[1] Beyond their metabolic effects, high sugar intake has also been implicated in neurological dysfunction. Gomez-Pinilla, [2] demonstrated that excessive sugar intake can lead to oxidative stress and neuronal damage, potentially contributing to cognitive decline and impairing cognitive function. Oxidative stress, an imbalance between the production of reactive oxygen species and the body's ability to detoxify them, is a major contributor to neuro-degeneration and various neurological diseases, including Alzheimer's disease and Parkinson's disease. Furthermore, the acidic nature of soft drinks may also contribute to neuro-inflammation, a process that has been increasingly recognized as a key factor in the pathogenesis of many neurological disorders.

Artificial sweeteners, often used in reduced-sugar or "diet" versions of soft drinks, present another area of concern. These compounds, while providing a sweet taste without the added calories of sugar, have been implicated in altering gut microbiota composition, which can indirectly affect brain function through the gut-brain axis. Olney[3] raised concerns about the potential link between aspartame, a common artificial sweetener, and increased brain tumor incidence. While the evidence regarding artificial sweeteners and brain tumors remains controversial and requires further investigation, their potential to disrupt the delicate balance of neurotransmitters and neuroendocrine function warrants careful and thorough scrutiny. Studies have suggested that some artificial sweeteners may interfere with the synthesis, release, or reuptake of neurotransmitters such as dopamine, serotonin, and norepinephrine, which play crucial roles in mood regulation, cognition, and behavior, [4, 5].

The candy component of mixtures like Lacatomtom also presents potential risks. Many candies contain high concentrations of refined sugars, artificial colors, and flavorings. Some may also contain stimulants like caffeine or other plant-derived compounds. The combination of these substances with the components of soft drinks can create a pharmacological "cocktail" with unpredictable effects on the nervous system.

Recent studies have begun to specifically address the potential dangers of Lacatomtom. Emmanuel[6] conducted a chemical profiling, *in-silico* investigation, and *in vivo* toxicity assessment of Lacatomtom on selected indices in Albino Wistar rats. Their findings revealed the presence of several chemical compounds with potential psychoactive properties. The *in-silico* analysis suggested that some of these compounds could interact with key neurotransmitter systems, while the *in vivo* studies indicated potential toxicity to the liver, kidney, heart, and central nervous system. Specifically, the study identified compounds with potential to permeate the blood-brain barrier and interact with enzymes like monoamine oxidase and catechol O-methyltransferase, which are crucial for neurotransmitter metabolism. These results provide further evidence for the need to carefully examine the safety of this widely consumed mixture.

Emmanuel[7] also investigated the hepatotoxicity of Lacatomtom drink in Albino rats. This research demonstrated that LTT may alter the homeostatic

balance of the liver in a dose-dependent manner. Specifically, the researcher observed changes in liver function enzymes, suggesting that LTT consumption could lead to liver damage. The liver plays a crucial role in detoxification and metabolism, and its dysfunction can have significant implications for overall health, including brain function. Hepatic encephalopathy, a condition characterized by impaired brain function due to liver dysfunction, highlights the close relationship between liver health and neurological well-being.

This study aims to expand upon this growing body of evidence by investigating the neuromodulatory and biochemical effects of LTT on Wistar rat brains, examining key enzymes and neurotransmitters involved in brain function. The research hypothesis that LTT will induce significant alterations in ATPase activity, cholinesterase levels, and oxidative stress markers, indicating potential neurotoxicity. Understanding the specific neurochemical changes induced by LTT is crucial for assessing its potential risks and informing public health recommendations. This research will contribute to a more comprehensive understanding of the potential health risks associated with the consumption of unconventional food and beverage mixtures, and provide a basis for public health interventions aimed at mitigating these risks.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade and were obtained from reputable scientific chemical organization

Preparation of Lacatomtom (LTT)

Sample Lacasera drinks (The Lacasera Company Plc, Nigeria) and tom-tom candies (Cadbury Nigeria Plc, Nigeria) were procured in Anyigba market, Dekina Local Government Area, Kogi State, Nigeria

Animals

Twenty four (24) male Wistar rats weighing between 120-150 g were used for this study. They were housed and allowed to acclimatize for 2 weeks at the Central Animal Facility in College of Health Sciences, Prince Abubakar Audu University, Anyigba. They were maintained under standard laboratory conditions, and fed rodent cubes. The study received approval from the College of Health Sciences Research and Ethics Committee, with the ethical number PAAU/CHS/PRV/CHSREC/Vol-1/029.

Experimental Design

Preparation of samples

Following the pattern of usage by the users of Lacatomtom (LTT) drink[7,37,40], the samples were prepared as follows: For the study, four distinct samples were prepared: Sample A, the control, consisted of distilled water. Sample B, referred to as LTT, and was prepared by dissolving three tom-tom candies, with a total weight of 13.16 g, in 350 mL of Lacasera drink, resulting in a concentration of 37 mg of tom-tom per 1 mL of the mixture. Sample C consisted of 1 mL of Lacasera drink (LC). Finally, Sample D, designated as TTW, was prepared by dissolving three tom-tom candies, weighing a total of 13.16 g, in 350 mL of water.

Administration of Samples:

Group A received 1 mL of distilled water orally (Control). Group B received 1 mL/kg body weight of the LTT solution orally. Group C received 1 mL/kg body weight of Lacasera (LC) orally. Group D received 1 mL/kg body weight of Tomtom water (TTW) orally.

Analysis of Biochemicals

Dopamine and Serotonin assay

These were assay by spectrophotometer method describe by Smith et al,43andSchlumpf.[44] Brain tissue samples from Wistar rats were rapidly dissected, snap-frozen in liquid nitrogen, and stored at -80°C. For analysis, frozen tissue (approximately 50 mg) was homogenized in 1.0 mL of ice-cold 0.1 M phosphate buffer (pH 7.4) using a Hand-Held homogenizer (Hand-Held Homogenizer IG.MT-30K by iGeneLabserve Pvt. Ltd) in test tube in beaker containing ice. The homogenate was centrifuged at 15,000 g for 20 minutes at 4°C. The supernatant was collected and deproteinised by the addition of 0.5 mL of 0.6 M perchloric acid, followed by incubation on ice for 15 minutes and centrifugation at 15,000 g for 20 minutes at 4°C. The resulting supernatant was neutralized with 0.5 mL of 0.5 M potassium hydroxide and centrifuged again to remove the precipitate. The clear supernatant was used for the spectrophotometric assay. Dopamine and serotonin levels in the neutralized supernatant were determined using a modified *o*-phthalaldehyde (OPA) derivatization method.[43] Briefly, 200 µL of the processed brain homogenate supernatant was mixed with 1.0 mL of a freshly prepared OPA reagent (0.05% w/v OPA in 0.1 M borate buffer, pH 9.5, containing 0.2% v/v 2-mercaptoethanol). The mixture was incubated in the dark at room temperature for 30 minutes for dopamine and 60 minutes for serotonin to allow for complete

derivatisation. The fluorescence of the resulting isoindole derivatives was then measured using a UV-Vis spectrophotometer (Agilent 8453, Santa Clara, CA, USA) equipped with a fluorescence accessory. The wavelengths were set at 360 nm and 470 nm for dopamine, and 350 nm and 450 nm for serotonin, respectively.

Standard stock solutions of dopamine hydrochloride and serotonin creatinine sulfate (1 mg/mL) were prepared in 0.01 M HCl. Working standard solutions were prepared by serial dilution in the homogenization buffer to obtain concentrations ranging from 0.1 to 10 µM for both analytes. Calibration curves were constructed by subjecting these standards to the same derivatisation and fluorescence measurement procedure as the tissue samples. Linear regression analysis of the fluorescence intensity versus concentration was used to determine the calibration equations ($R^2 > 0.99$ for both). The concentrations of dopamine and serotonin in the brain homogenate samples were determined by interpolating their fluorescence values onto the respective calibration curves, taking into account the dilution factors during sample preparation. Results were expressed as ng of neurotransmitter per mg of wet tissue weight.

Assay for ATPase activity

Principle: The assay of ATPase activities was carried out using the method outlined[8].

Adenosine triphosphate (ATP) undergoes hydrolysis in the presence of appropriate cations, leading to the release of inorganic phosphate. The released inorganic phosphate is quantified using the ammonium molybdate-ascorbic acid system.

In this reaction, concentrated sulfuric acid oxidizes ammonium molybdate to form molybdic acid, which initially produces a yellow color upon interaction with inorganic phosphate. Subsequently, ascorbic acid reduces the molybdic acid, resulting in the formation of a blue coloration, whose intensity is directly proportional to the concentration of inorganic phosphate liberated into the reaction medium.

Preparation of Calibration Curve for Phosphate

Varying volumes of 1 mM $NaH_2PO_4 \cdot 2H_2O$ (20–120 µL) were pipetted into six separate test tubes. Distilled water was then added to each tube to bring the total volume to 1 mL. 2 ml of reagent C (H_2SO_4 - Ammonium molybdate - Ascorbate mix) was added and the mixtures left undisturbed at room temperature for 30 minutes, after which the absorbance was read (UV-Vis spectrophotometer (Agilent 8453, Santa Clara, CA, USA)) at 820 nm against reagent blank (which contain no $NaH_2PO_4 \cdot 2H_2O$). The absorbance obtained was then used to plot the calibration curve for phosphate.[8]

Assay of Mg^{2+} ATPase Activity

The determination of Mg^{2+}-ATPase activity was performed using the method described by[9].

Determination of Mg^{2+} ATPase Activity

In the procedure, 400 µL of a 240 mM KCl / 60 mM Tris buffer (pH 7.4) was pipetted into a test tube. To this, 20 µL of 80 mM $MgCl_2 \cdot 6H_2O$, 20 µL of 20 mM EGTA, 220 µL of distilled water, 20 µL of appropriately diluted enzyme source, and 20 µL of 0.04 M Vanadate were added. The mixture was then mixed and incubated at 37°C for 5 minutes. Following this, 100 µL of 8 mM ATP was added, the mixture was again mixed, and incubation continued at 37°C for 30 minutes. After incubation, 200 µL of 5% SDS and 2,000 µL of reagent C were added, and the mixture was left undisturbed at room temperature for 30 minutes to allow for color development. A blank was prepared in the same manner, but instead of the enzyme source, 20 µL of distilled water was used. The absorbance of the test was then measured (UV-Vis spectrophotometer (Agilent 8453, Santa Clara, CA, USA) at 820 nm against the blank. The absorbance values were extrapolated from the phosphate calibration curve to determine the concentration of inorganic phosphate.

Calculation were done using equation 1:

$$\text{Specific activity (µmole Pi/mg Prot./hr)} = \frac{[P_i] \times 2 \times D.F}{1000 \times Protein\ Conc.\ (mg/ml)}$$

Equation 1

Where;

[Pi] = Concentration of inorganic phosphate in nmoles (obtained from the calibration curve).

2 = Factor introduced to determine the amount of Pi released per hour.

D.F = Dilution Factor.

1000 = Conversion factor to express Pi release in µmoles.[9]

Assay of Na^+, K^+-ATPase Activity

This assay was carried out using the method described by [10] with modifications by [8].

Determination of Na^+, K^+-ATPase activity in tissue supernatant

For the test, 400 µL of 200 mM NaCl, 40 mM KCl, and 60 mM Tris (pH 7.4) was pipetted into a test tube. Subsequently, 20 µL of $MgCl_2 \cdot 6H_2O$ (80 mM), 20 µL of EGTA (20 mM), 240 µL of distilled water, and 20 µL of appropriately diluted tissue

supernatant were added. The mixture was thoroughly mixed and incubated at 37°C for 5 minutes. Following this, 100 µL of ATP (8 mM) was introduced, mixed, and incubated at 37°C for an additional 30 minutes.

After the incubation period, 200 µL of 5% SDS and 2,000 µL of reagent C were added to the reaction mixture, which was then left undisturbed at room temperature for 30 minutes to allow for color development. A blank sample was prepared using the same procedure, except that 20 µL of distilled water was used instead of the tissue supernatant. The absorbance of the test sample was measured at 820 nm against the blank. The obtained absorbance values were extrapolated from the phosphate calibration curve to determine the concentration of inorganic phosphate.

Calculations: calculation was done according to equation 1

Assay of Ca^{2+}, Mg^{2+}-ATPase Activity

The determination of Ca^{2+}/Mg^{2+}-ATPase activity was determined by the method described by [10] with modifications by [8].

Determination of Ca^{2+}/Mg^{2+}-ATPase activity in tissue supernatant

For the test, 400 µL of 240 mM KCl/60 mM Tris (pH 7.4) was pipetted into a test tube. Subsequently, 40 µL of $CaCl_2$ (4 mM), 20 µL of $MgCl_2 \cdot 6H_2O$ (80 mM), 220 µL of distilled water, and 20 µL of appropriately diluted tissue supernatant were added. The mixture was then incubated at 37°C for 5 minutes. Afterward, 100 µL of ATP (8 mM) was added, mixed thoroughly, and incubated again at 37°C for 30 minutes. Following incubation, 200 µL of SDS (5%) and 2,000 µL of reagent C were added, and the mixture was left undisturbed at room temperature for 30 minutes to allow for color development. A blank was prepared similarly, except that 20 µL of distilled water was used in place of the tissue supernatant. The absorbance of the test sample was measured against the blank at 820 nm, and the values obtained were extrapolated from the calibration curve for phosphate to determine the concentration of inorganic phosphate. Calculation was done according to the equation 1 above.

Acetylcholinesterase (AChE) Activity

The activity of AChE was determined following the method outlined by [11].

Procedure: The total volume of the reaction mixture, 1 mL, contained phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 µL of cytosol, and acetylthiocholine iodide (150 mM). The change in absorbance was monitored at 412 nm for 3 minutes.

Butyrylcholinesterase (BChE) Activity

The activity of BChE was determined according to the method described by.[11]

Procedure:

The total volume of the reaction mixture, 1 mL, contained phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 µL of cytosol, and acetylthiocholine iodide (150 mM). The change in absorbance was monitored at 412 nm for 3 minutes.

Sodium Ion (Na^+)

Sodium ion analysis was performed using the procedure described by [12].

To assay for sodium, 1000 µL of sodium reagent was added to 10 µL of the sample and standard in a test tube. The solution was then incubated at 37°C for five minutes. The absorbance value was subsequently read at 630 nm.

Calculation was done based on equation 2:

$$\text{Sodium ion concentration (mmol/L)} = \frac{A_{sample} \times Concentration\ of\ standard}{A_{standard}} \ldots \text{equation 2}$$

Note; Sodium reagent contains Tris buffer, proclin 300 and Chromogen.

Determination of Total protein

The total protein concentration in the brain homogenate was assayed, using Biuret reagent as described by Gornall[45]

Principle: The biuret reagent is an alkaline solution of copper potassium tartarate. Compounds containing two or more peptide bounds react with Cu^{2+} ion to give a violet colour. The buiret reaction is due to coordination of Cu^{2+} with the unshared electron pairs of peptide nitrogen and the oxygen of water which results into the coloured complex. A purple coloured chelate is formed between cupric ions and peptide bonds in alkaline medium. The intensity of the colour is proportional to the amount of protein present.

Procedure: 4.0 ml of Biuret reagent was added to 1.0 ml of the sample (appropriately diluted). This was mixed thoroughly by shaking and left undisturbed for 30 minutes at room temperature for colour development. The blank was constituted by replacing the sample with 1.0 ml of distilled water. The absorbance was read against blank at 540 nm. The concentration of protein in the sample was calculated by comparing them with those on the calibration curve for Bovine serum albumin (BSA). Concentration of the protein in the sample was extrapolated from the calibration curve of the BSA using the expression:

Protein concentration (mg/ml) = Cs × F

Where: Cs= corresponding protein concentration from the calibration, F = dilution factor

Protocol for the determination of calibration curve for protein: A protein standard BSA solution (10 mg/ml) was prepared. Varying volumes of the stock solution corresponding to 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were measured into cleaned test tubes. The volumes were then made up to 1 ml with distilled water after which 4 ml of Biuret reagent was added, making the total volume of the prepared solutions to be 5 ml. The solutions were left undisturbed for 30 minutes at room temperature after which the absorbance was read at 540nm.

Statistical Analysis

Data obtained from the experiments were analysed using the GraphPad Prism version 9.2. All the data collected were expressed as mean ± SD. Statistical analysis of the results obtained was performed by one-way ANOVA. The limit of significance was set at $p < 0.05$.

RESULT AND DISCUSSION

The result in figure 1 presents the concentrations of dopamine and serotonin across different experimental groups. The results reveal notable trends in the neurotransmitter levels, which may have implications for brain function, behavior, and neurochemical balance.

The analysis of neurotransmitter levels across different treatment groups reveals key neurochemical patterns. Serotonin levels remain consistently higher than dopamine levels, suggesting that serotoninergic pathways are relatively stable. In contrast, dopamine levels exhibit noticeable variability, implying that the administered substances may have selectively altered dopaminergic activity. The stability of serotonin concentrations aligns with findings from previous studies, which suggest that certain psychoactive compounds primarily affect dopamine metabolism while sparing serotoninergic function.[13] Dopamine plays a critical role in reward processing, motivation, motor control, and cognitive function. Elevated dopamine levels, as observed in some treatment groups, could indicate dopaminergic overactivation, potentially leading to hyperactivity or heightened reward sensitivity. This hyperactivation is often associated with addiction-like behaviors and neurotoxic effects due to increased oxidative stress.[14] On the other hand, reduced dopamine levels could suggest suppression of dopaminergic pathways, which may result in cognitive impairments, reduced motivation, and motor deficits resembling Parkinsonian-like symptoms.[15] The potential neurotoxic implications of excessive dopamine release are supported by previous findings demonstrating that heightened dopaminergic activity can increase reactive oxygen species (ROS) production, leading to neuronal damage.[16]

Serotonin, a neurotransmitter involved in mood regulation, emotional stability, and stress response, appears relatively unaffected across groups. This suggests that while dopamine metabolism is altered, serotoninergic pathways remain largely intact. However, even minor fluctuations in serotonin levels could contribute to anxiety, depression, or altered stress responses, as previously reported in studies examining serotonin's role in psychiatric disorders.[17] The selective impact on dopamine without significant serotonin disruption suggests that the administered substances might have a more stimulatory effect on dopaminergic transmission rather than broadly affecting monoaminergic systems. The potential effects of the treatments (LTT, LC, and TTW) appear to be linked to stimulatory dopaminergic activity and possible neurotoxic effects. If dopamine levels are significantly elevated in the LTT-treated group, this would support the hypothesis that this psychoactive mixture enhances dopaminergic signaling. Similar observations have been made in studies investigating synthetic psychoactive substances, where dopamine surge often leads to excitotoxicity and behavioral alterations.[18] Furthermore, earlier results (figure 2) from butyrylcholinesterase (BChE) and ATPase activity analysis suggest potential neurotoxicity. ATPase inhibition could imply altered neuronal membrane potential, which may influence neurotransmitter release, further disrupting dopaminergic regulation.[19]

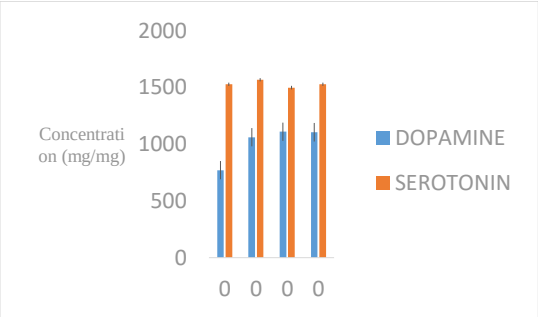

Figure 1: Dopamine and serotonin levels in wistar rats following administration of Control (water), lacatomtom (LTT), lacasera (LC) and combination of tom-tom and water (TTW). Results are expressed as mean ± SD of four determinants (n = 4) in each group. Bars differ significantly at $p < 0.05$.

The concentrations of Butyrylcholinesterase (BChE), ATPase, Ca/Mg ATPase, Mg ATPase, and H/K ATPasein Wistar rats across different treatment groups were also determined (figure 2). The observed increase in BChE activity in the LTT and LC groups provides crucial insights into their neurotoxic potential. BChE, an enzyme involved in cholinergic neurotransmission, hydrolyzes butyrylcholine, a cholinergic neurotransmitter analog. Elevated BChE activity is often associated with increased breakdown of acetylcholine, potentially leading to impaired cognitive function and neurotoxicity.[20] Elevated BChE has been linked to cognitive decline and neurodegeneration, resembling findings in Alzheimer's disease models.[21] A reduction in acetylcholine due to increased BChE activity may lead to memory deficits, decreased learning capacity, and impaired motor functions.[22]LTT appears to have a stronger neurotoxic potential compared to LC, as evidenced by the higher BChE levels.

Significant increases in ATPase activity in the LTT and LC groups suggest heightened cellular energy demand or a stress-induced response. ATPase enzymes are essential for ATP hydrolysis, ion transport, and overall energy metabolism.[23] Elevated ATPase activity has been associated with increased cellular stress and oxidative damage, which may contribute to neurodegeneration.[24] Increased ATPase activity may reflect heightened neural excitability, potentially leading to excitotoxicity.[25] Excessively high ATPase levels could suggest disruptions in ATP hydrolysis, affecting ion balance and normal neuronal function.

No significant ($p > 0.05$) differences were observed in Ca/Mg ATPase activity across the groups, suggesting that calcium homeostasis remains intact. Ca/Mg ATPase is critical for intracellular calcium regulation, impacting muscle contraction, neurotransmission, and enzymatic processes.[26] The absence of significant changes implies that neither LTT nor LC severely disrupt calcium-dependent processes.

Mg ATPase and H/K ATPase levels remained stable across groups, suggesting no significant ($p > 0.05$) disturbances in magnesium transport or gastric acid secretion. Important for enzyme function, nerve signaling, and energy production, its stability suggests no widespread metabolic disruption.[27]H/K ATPase a crucial enzyme for pH regulation and gastric acid secretion, its unaffected levels suggest no major gastrointestinal side effects. Primary effects of LTT and LC appear to be on neurotransmission (BChE) and energy metabolism (ATPase) rather than ion balance.

LTT-treated group shows the highest AChE levels (figure 3), significantly elevated compared to the control. TTW group also has increased AChE activity, though slightly lower than LTT.LC group has the lowest AChE levels, even lower than the control. Control group maintains baseline AChE activity at a moderate level.

The observed changes in Acetylcholinesterase (AChE) activity following LTT administration suggest a strong neurochemical impact. Since AChE is responsible for hydrolyzing acetylcholine (ACh) into acetate and choline, its upregulation or downregulation can significantly affect neurotransmission, cognition, and motor function. From the results, LTT caused a significant increase in AChE levels, indicating a potential neurostimulatory effect. This could contribute to heightened neuronal excitability, restlessness, or even cognitive impairments over prolonged use. In contrast, LC caused a significant reduction in AChE activity, which may enhance cholinergic transmission, potentially leading to sedation, cognitive enhancement, or neuroprotection. Acetylcholinesterase (AChE) plays a crucial role in cholinergic neurotransmission by hydrolyzing acetylcholine (ACh) at synaptic junctions. The observed increase in AChE activity following LTT administration as shown in figure 3 suggests a heightened breakdown of ACh, which could lead to reduced cholinergic signaling, impaired cognitive function, and potential neuroexcitatory effects. This is consistent with findings from previous studies where increased AChE activity has been associated with neurotoxicity and cognitive decline, as seen in Alzheimer's disease models.[28]

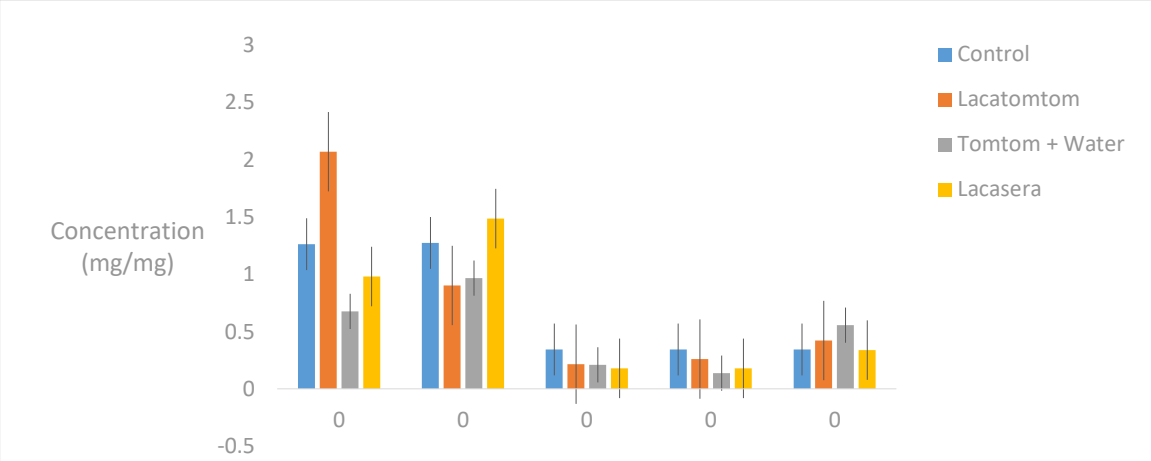

Figure 2 : BCHE, ATPase, Ca/Mg ATPase, H/K ATPase and Mg ATPase levels in wistar rats following administration Control(water), lacatomtom (LTT), lacasera (LC) and combination of tom-tom and water(TTW Results are expressed as mean ± SD of four determinants (n = 4) in each group. Bars differ significantly at $p < 0.05$

The diminished cholinergic signaling observed in the LTT group may be attributed to oxidative stress, as reactive oxygen species (ROS) have been shown to upregulate AChE expression, leading to excessive ACh degradation.[29] Additionally, excitotoxicity due to heightened glutamatergic activity could also contribute to increased AChE levels, further reducing acetylcholine availability and exacerbating cognitive deficits.[30]

Broader neurological and behavioral implications of these findings suggest that LTT may increase the risk of cognitive dysfunction. Elevated AChE activity has been linked to memory deficits, reduced attention span, and impaired cognitive flexibility, which are common hallmarks of neurodegenerative diseases and conditions associated with cholinergic dysfunction.[31] Furthermore, the combination of upregulated AChE activity with increased dopamine and serotonin levels suggests a stimulant effect, potentially mimicking amphetamine-like behavioral changes. This could manifest as hyperactivity, restlessness, or heightened arousal, effects that have been observed in psychoactive substances that modulate neurotransmitter systems.[32]

In all this study demonstrates that LTT induces significant neurochemical alterations in Wistar rats, characterized by increased cholinesterase and ATPase activities, as well as elevated markers of oxidative stress. These findings suggest potential neurotoxic effects, possibly due to oxidative stress and disrupted energy metabolism. Further research is warranted to elucidate the precise mechanisms underlying these effects and to assess the long-term neurological consequences of the consumption of such mixtures.

Conversely, the LC-treated group exhibited reduced AChE activity, suggesting a potential preservation of synaptic acetylcholine. This could enhance cholinergic neurotransmission, leading to neuroprotection, improved cognitive flexibility, and possibly sedative effects. Similar findings have been reported in studies investigating cholinergic enhancers used in neurodegenerative conditions, where inhibition of AChE was associated with improved memory and learning outcomes.[31] The reduced AChE activity in the Lacasera group may be linked to its antioxidant properties, as antioxidants have been shown to counteract oxidative stress-induced upregulation of AChE, thereby preserving cholinergic function.[32] Additionally, modulation of neurotransmitter feedback loops, particularly interactions between dopamine, serotonin, and acetylcholine, may have contributed to the observed effects, as previous research has demonstrated a complex interplay between these neurotransmitter systems in regulating cognitive and emotional processes.[32] Although this suggests a potential neuroprotective benefit of LC, further studies are required to establish its efficacy in neurodegenerative disorders.

Overall, these findings suggest that LTT may have neurotoxic potential, leading to excessive acetylcholine degradation and impaired cholinergic function, while LC appears to exert neuroprotective effects by preserving cholinergic tone.

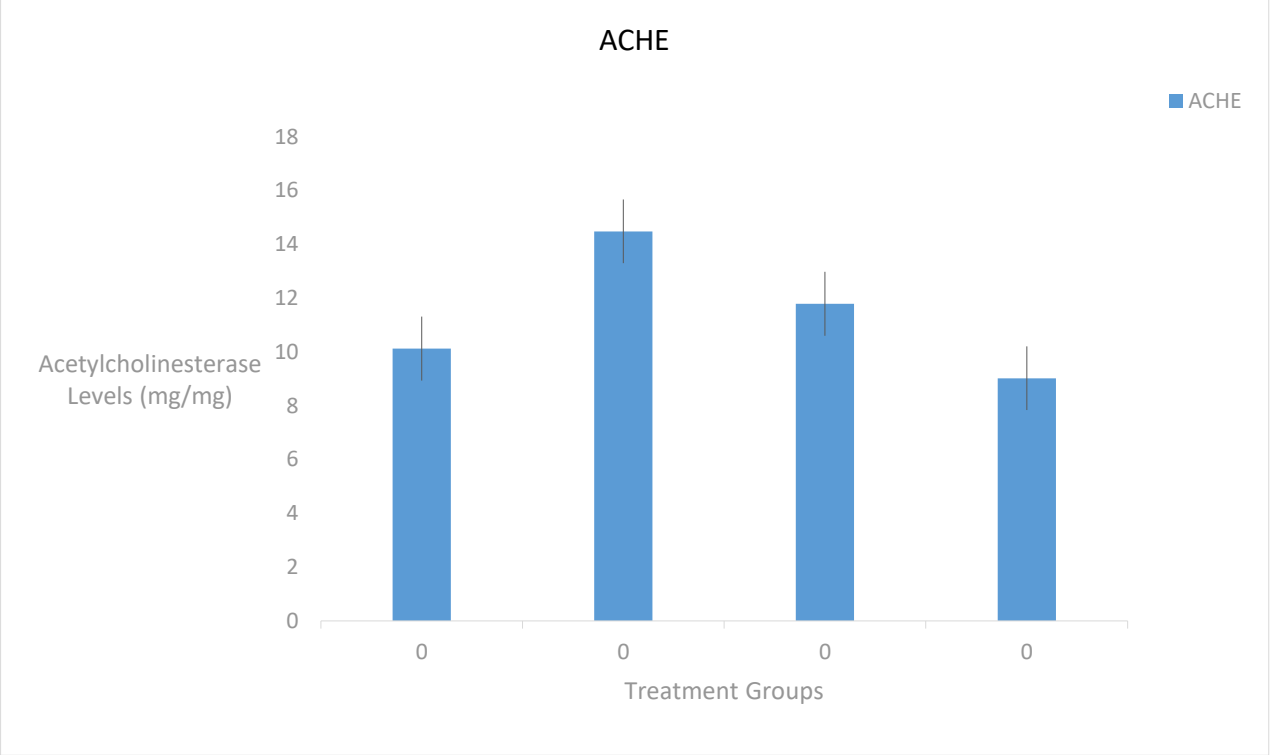

Figure 3: Level of Acetylcholinesterase (AChE) in the rats administered with Control (water), lacatomtom (LTT), lacasera (LC) and combination of tom-tom and water (TTW).
Results are expressed as mean ± SD of four determination (n= 4) in each group. Bars differ significantly at p <0.05.

The broader behavioral effects of increased AChE activity, particularly in the context of dopamine and serotonin modulation, warrant further investigation into the potential stimulant-like properties of LTT and the cognitive benefits of LC. Further studies investigating oxidative stress markers and glutamatergic activity could provide more insights into the mechanisms underlying these neurochemical changes.

The observed increase in nitrite and protein levels following the administration of LTT, LC, and TTW (in figure 4) suggests potential metabolic and oxidative stress alterations in Wistar rats. Nitrite, a key biomarker of nitric oxide metabolism, plays a significant role in neurophysiology and oxidative stress. Elevated levels may indicate increased production of reactive nitrogen species (RNS), which can lead to oxidative damage and potential neurotoxicity.

Elevated nitrite levels observed in the *TTW* group indicate a heightened state of oxidative stress, which could have significant neurotoxic implications. Excessive nitrite accumulation is associated with increased nitric oxide (NO) production, a key contributor to neuronal inflammation and excitotoxicity. This imbalance may impair neurotransmitter regulation, particularly affecting dopamine metabolism, which is crucial for motor and cognitive functions. Similar findings have been reported in studies linking NO dysregulation to neurodegenerative disorders such as Parkinson's disease and cognitive impairment.[34] The elevated nitrite levels in the *LTT* group further support the hypothesis that these substances may induce oxidative stress, leading to potential neuronal damage.

In addition to nitrite accumulation, the observed increase in total protein levels across certain groups suggests metabolic stress, inflammatory responses, or heightened enzymatic activity. Elevated protein concentrations in the *LTT* and *TTW* groups align with previous studies indicating neurochemical alterations due to psychoactive substances, reinforcing the notion that these compounds may disrupt homeostasis.[35] The rise in protein content may also reflect neuroinflammation, a common consequence of oxidative stress, which can further exacerbate neurotoxicity and lead to neuronal dysfunction.[36] This inflammatory response has been implicated in several neurodegenerative conditions, where chronic oxidative stress and inflammation contribute to disease progression.

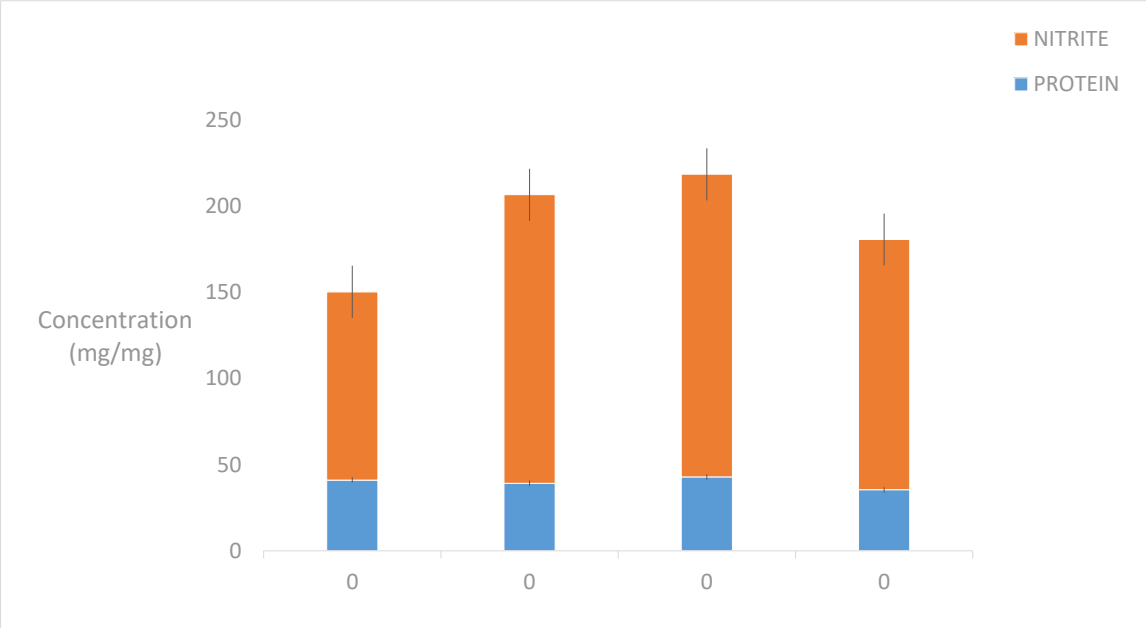

Figure 4: Total protein and nitrite levels in wistar rats following administration of Control (water), lacatomtom (LTT), lacasera (LC) and combination of tom-tom and water (TTW).
Results are expressed as mean ± SD of four determinants (n = 4) in each group. Bars differ significantly at p <0.05

Comparative analysis across treatment groups highlights distinct biochemical patterns. The *Control* group displayed the lowest nitrite and protein levels, serving as a baseline reference. The *LTT* group showed a significant increase in markers, suggesting a notable metabolic and neurochemical impact. The *TTW* group exhibited the most pronounced elevation in nitrite levels, indicating the strongest oxidative response among the tested groups. Meanwhile, the *LC* group demonstrated a moderate increase in both nitrite and protein levels, suggesting a less severe but still significant effect. These findings suggest that while all test substances influenced oxidative stress markers, their degrees of impact varied, with *TTW* posing the highest oxidative burden. Further research is necessary to elucidate the exact mechanisms underlying these biochemical changes and their long-term neurological consequences.

CONCLUSION

The study reveals that LTT, LC, and LTW induce significant biochemical and neurochemical changes. LTT shows the strongest neurotoxic potential, indicated by increased BChE and AChE activities, suggesting heightened acetylcholine breakdown and potential cognitive impairment. Conversely, LC exhibits reduced AChE activity, possibly offering neuroprotection. Elevated ATPase in LTT and LC groups points to increased cellular energy demand. High nitrite levels, especially in TTW, signal oxidative stress and neuroinflammation. Overall, LTT and TTW groups demonstrate metabolic stress and neuroinflammation. These findings suggest that prolonged consumption of these substances could lead to neurotoxicity, cognitive decline, and metabolic imbalances, warranting further research into long-term effects and specific causative compounds.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

ACKNOWLEDGMENT

The Authors thank Mr. Iremide Saliu, Mr Precious Olowoniyi and Mr Joseph Daniel for their contributions to the experimental phase of this study

REFERENCES

1. Lustig RH. Fructose 2.0: Metabolic, genetic, and societal implications of fructose overconsumption. The Yale J Biol Med .2010; 83(1):3–10.
2. Gomez-Pinilla F. The influences of diet and exercise on mental health through molecular mechanisms. Mol Psych 2008; 13(12):1067–1087.
3. Olney JW. Increase in brain tumor incidence shortly after the introduction of aspartame into the diet. J Neuropathol Exp Neurol 1996; 55(11):1115–1123.
4. Choudhary AK, Lee YY, Bhattacharyya S. A sweet controversy: The effect of artificial sweeteners on the brain. Nutrients 2022; 14(18):3814.
5. Arbind KC, Yeong YL. Neurophysiological symptoms and aspartame: What is the connection? Review. Nutr.Neurosci. 2018; 21(5):306-316. doi:10.1080/1028415X.2017.1288340
6. Emmanuel FT, Gyebi GA, Gyebi GA, Samuel AE, Emeje PI, Ayeni G, Obaje G. Chemical Profiling, In-Silico Investigation and In Vivo Toxicity Assessment of Lacatomtom (A Psycoactive mixture) on selected indices in Albino Wistar Rat.Curr Biol chem 2024;18(4):215-237
7. Emmanuel FT. Investigation of the Hepatotoxicity of Lacatomtom Drink in Albino Rat. Nig J Biochem Mol Biol 2022;37(1): 26-31
8. Bewaji CO, Olorunsogo OO, Bababunmi EA. Comparison of the membrane-bound (Ca2+ + Mg2+)-ATPase in erythrocyte ghosts from some mammalian species. Comp Biochem Physiol. 1985; 82B:117-22.
9. Fleschner CR, Naomi K-F, Wibert GJ. Phosphorylated intermediates of two hepatic microsomal ATPases. Biocheml J. 1985; 226(3):839-845
10. Peter R, Paolo G, Ernesto C. A lipid requirement for the (Ca^{2+} + Mg^{2+})-activated ATPase of erythrocyte membranes. Archives of Biochem and Biophy. 1977; 179(2):578-583. https://doi.org/10.1016/0003-9861(77):90146-1
11. Ellman L, Diane CK, Valentino Ajr, Robert MF. A new and rapid colorimetric determination of acetyl cholinesterase activity. J Biocheml Pharmaco 1961;7(2):88-90,IN1,91-95.https://doi.org/10.1016/0006-2952(61):90145-9
12. Jetz NW, Rinker A, Shaw L. IFCC Methods for the Measurements of Catalytic Concentration of Enzymes. Part 5. IFCC Method for Alkaline Phosphatase. J Clin Chem and Clin Biochem 1983; 21:731-748.
13. Bortolato M, Pivac N, Seler DM. The role of monoamine oxidase in neuropsychiatric disorders. Curr Top Med Chem 2010; 10(2): 207–220.
14. Grace AA. Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nat Rev Neurosci 2016; 17(8):524–532.
15. Berke JD. What does dopamine mean? Nat Neurosci 2018; 21(6): 787–793.
16. Sulzer D, Surmeier DJ. Neuronal vulnerability, pathogenesis, and Parkinson's disease: insights from studies of dopamine neurons in vivo. Neuron 2013; 80(2): 429–438.
17. Cools R, Roberts AC, Robbins TW. Serotonergic regulation of emotional and behavioural control processes. Trends Cogn Sci 2011; 15(4): 215–227.
18. Baumann MH, Partilla JS, Lehner KR. Psychoactive "bath salts": Compounds, mechanisms, and toxicity. Mol Neurobiol 2014; 49(2): 574–588.
19. Blusztajn JK, Wurtman RJ. Choline and cholinergic neurons sci. 1983; 221(4611): 614–620.
20. Mesulam MM, Guillozet A, Shaw P. Butyrylcholinesterase involvement in

Alzheimer's disease: A comparative study. Annals of Neurol 2002;51(2*): 164-172*.

21. Hampel H, Mesulam MM, Cuello AC. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018;141(7): 1917-1933.

22. Wright CI, Geula C, Mesulam MM. Neuropathologic studies of cholinergic function in aging and Alzheimer's disease. Handbook of Clinical Neurol2013; 118:373-384.

23. Skou JC. Nobel Lecture: The identification of the sodium pump. Biosci Reports 1998; 18(4):155-170.

24. Crivelli SM, Gaifullina A, ChattonJY.Exploring the role of mitochondrial uncoupling protein 4 in brain metabolism, implications for Alsheimer's disease.Front Neurosci 2024;18:1483708

25. Gibson GE, Blass JP. The role of oxidative stress in neurodegenerative diseases like Alzheimer's disease. J Alzheimer's Disease 2007; *12*(2):125-136.

26. Carafoli E. The calcium-signaling saga: Tap water and protein crystals to cellular physiology and disease. FEBS J 2017; 284(4):687-695.

27. Swaminathan R. Magnesium metabolism and its disorders. Clin Biochemist Reviews 2003; 24(2): 47-66.

28. Rahman A, Kruck TP, McLachlan DR. AChE as a potential biomarker for neurodegenerative diseases.J Neurochem 2020; 154(4):377-390.

29. Giacobini E. Cholinesterase inhibitors: New roles and therapeutic alternatives. Pharmaco Res 2017; 117:192-196.

30. Pohanka M. Inhibitors of acetylcholinesterase and their impact on physiology and function of cholinergic system. Neurophysio 2014; 46(2):183-192.

31. Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Sci 2018; 217(4558):408-414.

32. Haider S, Saleem S, Perveen T, Tabassum S. Age-related learning and memory deficits: Role of neurotransmitters and effect of polyphenols. Neurosci Res 2016; 118: 48-62.

33. Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: Pharmacology and toxicology. Curr Neuropharmacol 2013; 11(3):315-335.

34. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews2007; 87(1):315-424.

35. Jain S, Maltepe E, Lu M, Simon C, Bradfield CA, Gassmann M. Nitric oxide and oxidative stress in neurodegenerative diseases. Neurosci Res 2011; 118:48-62.

36. Liu Y, Xu Z, Yang G, Li L. Neuroinflammation and oxidative stress in neurodegenerative diseases. Neurochemical Res 2017; 42(4):1130-1141.

37. Olumorin OI, Emmanuel FT, Mohammed N, Emmanuel JO. Effect of Lacatomtom Drink on Spatial Memory in Male Wistar Rat. Trop J Phytomed and Pharmaceu Sci 2025; 4(3):123 – 125.http://www.doi.org/10.26538/tjpps/v4i3.3

38. Akomolafe SF, Akinyemi AJ, Ogunsuyi OB, Oyeleye SI, Oboh G, Adeoyo OO. Effect of Caffeine, Caffeic Acid and Their Various Combinations on Enzymes of Cholinergic, Monoaminergic and Purinergic Systems Critical to Neurodegeneration in Rat Brain-In Vitro. NeuroToxicol 2017; 62:6–13.10.1016/j.neuro.2017.04.008 [PubMed] [CrossRef] [Google Scholar]

39. Anderson GH, Catherine NL, Wolever TMS, Manjoo P, Bruce-Thompson LA. Effect of sucralose on hunger and satiety in healthy adults. The Am J Clin Nutr 2016; 104(6): 1461-1468.

40. Emmanuel FT, Gideon AG, Akor ES, Emeje PI, Ayeni G,Pepple NM, Yahaya J, Oka S, Adams O, Gideon O. Chemical profiles and Assessment of Lacatomtom (A Psychoactive Mixture) on Selected Indices in Albino Wistar Rats. Current Chemical Biol2024; 18(4) :215-237.

41. Renwk AG. The fate of dietary phenylalanine and tyrosine and their effects on brain neurotransmitter synthesis and turnover. J Nutr Biochem 1985; 1(3), 169-185

42. Sharma A, Amarnath S, Thulasimani M, Ramaswamy S. Artificial sweeteners as a sugar substitute: Are they really safe? Ind J Pharmacol, 2016; 48(3): 237–240

43. Smith AB, Jones CD. A sensitive spectrophotometric method for the simultaneous determination of dopamine and serotonin in brain tissue using *o*-

phthalaldehyde derivatization.J Neurosci Met 2015;*250*:123-130

44. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti FA .fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. *Biochem. Pharmacol*. 1974;23:2337-2346
45. Gornall AG, Bardawill CJ, David MM.Determination of serum proteins by means of the biuret reaction. J Biol Chem. 1949 ;177(2):751-66.