

Histopathological Alterations and DNA Content Changes in Brain, Liver, and Kidney Tissues Induced by Tomtom Candy-Lacasera Drink Mixture in Wistar Rats

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Abstract

This study investigated the potential histological effects of a mixture of Tomtom candy and Lacasera drink (LTT) and its individual components on brain, liver, and kidney tissues of Wistar rats. Four groups of rats (n=4 per group) were used: a control group (Group A) received distilled water; Group B received LTT; Group C received Lacasera drink (LC); and Group D received Tomtom candy in water (TTW). The treatments were administered orally for 28 days. Results revealed that all treatment groups showed histological alterations in the brain, liver, and kidney. The most pronounced effects were observed in the TTW group (Group D), which showed moderate axon clumping and dendrite loss in the brain, suggesting significant neuronal impairment. In the liver, all treatment groups exhibited some degree of hepatic necrosis (HN). The kidney tissue of the TTW group (Group D) showed tubular adhesion (TA) and slight tubular necrosis (TN), indicating the most severe renal damage among the groups. The LTT mixture (Group B) also induced notable effects, including moderate Kupffer cell hyperplasia (KH) in the liver and slight glomerular and tubular necrosis in the kidneys. Feulgen staining of brain tissue showed a paradoxical increase in DNA content in the TTW group, despite the structural abnormalities. This may indicate a cellular stress response, such as DNA repair mechanisms or the proliferation of support cells, to counter the toxic insult. The findings suggest that both the LTT mixture and its individual components can induce histological alterations in the vital organs of Wistar rats. The TTW mixture appeared to cause more pronounced effects in the brain and kidneys than the LTT or LC drink alone. Further studies with larger sample sizes and long-term exposure are needed to fully elucidate the underlying mechanisms and potential long-term consequences of these effects.

Keywords: Wistar rats; Histopathology; Neurotoxicity; Hepatotoxicity; Nephrotoxicity.

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1. Introduction

Consuming various food and beverage combinations is a common practice worldwide, often driven by cultural preferences, convenience, or perceived health benefits. While some combinations are indeed beneficial, others may pose potential health risks due to interactions between their components. In Nigeria, the combination of Tomtom candy, a popular menthol candy, and Lacasera drink, a widely consumed fruit drink, warrants scientific investigation [1]. This improvised psychoactive mixture, known as 'Lacatomtom' (LTT), has gained popularity as a substitute for more traditional drug abuse among Nigerian youths, with its abuse linked to psychosocial deficits, including memory impairment [2, 3]. This study aims to investigate the potential histological effects of this mixture on the brains, livers, and kidneys of Wistar rats, providing crucial insights into its safety profile.

Tomtom candy (TT) contains menthol and eucalyptus oil, which, while generally safe in low concentrations, can be toxic in high doses [4, 5]. In addition to these ingredients, TomTom candy contains a significant amount of sugar. Excessive sugar consumption is a well-established risk factor for numerous health problems, including metabolic disorders, cardiovascular diseases, and inflammation [6]. The detrimental effects of high sugar intake are often mediated by increased oxidative stress and the production of advanced glycation end products (AGEs) [7].

Lacasera drink (LC) is a carbonated fruit drink characterized by its high sugar content, artificial flavours, and preservatives. High sugar content contributes significantly to the global epidemic of obesity and its associated comorbidities [8]. The rapid absorption of sugars can also lead to drastic spikes in blood glucose levels, increasing the risk of insulin resistance [9]. Concerns have also been raised about the potential toxicity of some of these additives, with certain artificial colors and preservatives implicated in allergic reactions, hyperactivity in children, and carcinogenic effects in animal studies [10].

The rationale for studying the combined effect of TT and LC is compelling, given the potential for synergistic or antagonistic interactions between their diverse components. Understanding these interactions is crucial

for a comprehensive assessment of the safety of this common consumption practice. The samples used in this study were prepared to match the dosage used by "Lacatomtom" drink users [5].

The brain, liver, and kidneys are highly vulnerable to the toxic effects of various substances due to their metabolic activity and roles in detoxification [11, 14, 16]. Histological alterations in the brain, such as neuronal degeneration and gliosis, can impair function [12, 13]. Similarly, histopathological changes in the liver, such as hepatic necrosis and Kupffer cell hyperplasia, are indicative of injury [15]. In the kidneys, abnormalities such as glomerular and tubular necrosis can impair function and lead to renal failure [17]. While previous studies have investigated the effects of individual components of Tomtom (TT) and Lacasera (LC) on various tissues [6, 10], there is a significant knowledge gap regarding their combined effects. This study aims to address this gap by examining the histological changes in these organs. Using Wistar rats, a well-established animal model, the findings will provide valuable insights into the potential health risks associated with the consumption of the Tomtom-Lacasera mixture [18]. The specific objectives of this study were to prepare and administer the Tomtom-Lacasera drink mixture (LTT) and the Tomtom-water mixture (TTW), and then to examine histological changes in brain, liver, and kidney tissues. We hypothesized that administering LTT and TTW will induce significant histological alterations in these organs compared with the control and Lacasera drink groups, including neuronal damage, hepatic necrosis, and renal tubular necrosis.

2. Materials and Methods

2.1. Chemicals and Reagents

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

2.2. Sample Preparation

The samples were prepared based on the typical consumption of the Lacasera-Tomtom drink mixture [5].

Group A (Control) received distilled water.

Group B (LTT) received a mixture of three Tomtom candies (13.16g) dissolved in 350 mL of Lacasera drink, administered at 1 mL/body weight.

Group C (LC) received 1 mL of Lacasera drink per body weight.

Group D (TTW) received a solution of three Tomtom candies (13.16 g) in 350 mL of water, administered at 1 mL/body weight.

2.3. Animal Treatment

Twenty-four (24) male Wistar rats, aged 8-10 weeks and weighing 150-200g, were obtained from the College of Health Science, Prince Abubakar Audu University, Anyigba, Nigeria. The rats were housed in standard laboratory conditions (specify conditions in cages under a 12-hour light/dark cycle at room temperature ($25\pm 2^{\circ}\text{C}$) with free access to standard rat chow and water. The rats were randomly divided into four groups ($n=4$ per group): Group A (control), Group B (LTT), Group C (LC), and Group D (TTW). The treatments were administered orally (specify administration method: oral gavage daily for 28 days). The dosage was 1 mL per Kg body weight (b.w) for groups B, C, and D. Group A received an equivalent volume of distilled water.

2.4. Ethical approval for animal handling and experiment

All experimental procedures were approved by the College of Health Ethical Committee (CHREC-CHS/PAAU/2023/0011) and followed the guidelines of the International Laboratory Animal Use and Care [18]. The rats were euthanised using chloroform vapour.

2.5. Tissue Preparation and Histology

After the treatment period, the rats were euthanised using the chloroform vapour Method. The brain, liver, and kidneys were harvested from each rat. The tissues were fixed in 10% buffered formalin and processed for paraffin embedding. Sections of 5 μm thickness were prepared using a microtome. The tissue sections were stained with Hematoxylin and Eosin (H&E) for general histological examination. Silver stain (SIL) to visualize axons in the brain. Feulgen stain to assess DNA content in the brain. The stained sections were examined under a light microscope (Olympus BX41) at a magnification of

x400. Photomicrographs were taken to document the histological findings. A blinded pathologist performed histological analysis.

2.6. Histopathological Assessment

The histopathological assessment involved evaluating the following parameters:

2.6.1. Hematoxylin and Eosin staining methods:

Following the procedures of Sheehan and Hrapchak [19], the brain (cerebral cortex), liver and Kidney were processed and stained with Hematoxylin and Eosin. The H and E staining techniques involved hydrating the tissue sections in descending grades of alcohol: 100 %, 95 %, 90 %, and 70 %. Each of these steps involved two changes lasting 30 minutes each, and the sections were then washed in running tap water. The sections were then stained with hematoxylin for 25 minutes, washed with water, and differentiated in acid alcohol. The sections were counterstained with eosin, blued in Scott's water, dehydrated in ascending grades of alcohol, and cleared in xylene for 3 changes of 20 minutes each. The sections were then mounted with cover slips using mounting media.

2.6.2. Feulgen staining method

The sections of the cerebral cortex were stained according to the procedure of Pearse [20]. The sections were brought to water using xylene and ethanol, then briefly rinsed with cold 1N hydrochloric acid. The sections were placed into prewarmed hydrochloric acid for the appropriate time at 60°C , and briefly rinsed with cold 1N hydrochloric acid and distilled water. The sections were placed in Schiff's reagent for 30-60 minutes at room temperature, then given three sulphite rinses of about 1 minute each, washed well with water, and finally counterstained with light green for 1 minute. Each section was dehydrated in ethanol, cleared in xylene, and mounted in a resinous medium.

2.6.3. Silver staining method

The sections of the cerebral cortex were stained according to the procedure of Crookham and Dapson [21]. The sections were hydrated in distilled water for 3

changes. 20% silver nitrate solution was preheated in a Coplin jar for 35 seconds in the microwave, then slides were added, and the jar was placed in a 37°C oven for 15 minutes. Washed in distilled water for 3 changes. The slides were placed in ammoniacal silver solution for 10 minutes in a 37°C oven. The slides were placed in a working developer solution for 1 to 5 minutes, checked under a microscope, and washed in fresh ammonia water, followed by 3 changes in distilled water. The slides were placed in 5% Hypo for 2 minutes and then washed in distilled water for 3 changes. The slides were then dehydrated, cleared and mounted.

3. Results and Discussion

H&E staining (**Figure 1**) showed normal features in the control group (Group A). Group B (LTT) showed slight neuronal damage and gliosis. Group C (LC) showed slight neuronal necrosis, while Group D (TTW) presented slight neuronal atrophy and necrosis.

Silver staining (**Figure 2**) confirmed normal axons and astrocytes in the control group (Group A). Group B (LTT) showed slight axon loss, while Group C (LC)

displayed slight axon loss and clumping. Group D (TTW) showed the most significant damage, with moderate axon clumping and loss of dendrites.

H&E staining (**Figure 3**) showed normal hepatocytes in the control group (Group A), though slight hepatic necrosis was observed. Group B (LTT) showed moderate Kupffer cell hyperplasia (KH) and slight hepatic necrosis (HN). Group C (LC) showed moderate HN, while Group D (TTW) showed slight HN and slight hyperplasia of inflammatory cells.

H&E staining (**Figure 4**) revealed normal glomeruli and tubules in the control group (Group A). All treatment groups showed some degree of damage. Group B (LTT) exhibited slight glomerular necrosis (GN) and tubular necrosis (TN). Group C (LC) showed slight GN and TN, while Group D (TTW) showed the most damage, with slight TN and tubular adhesion (TA).

Feulgen staining (**Figure 5**) showed normal DNA content in Group A. Groups B (LTT) and C (LC) showed decreased DNA content compared to the control. Interestingly, Group D (TTW) showed a high amount of DNA content despite the observed structural abnormalities.

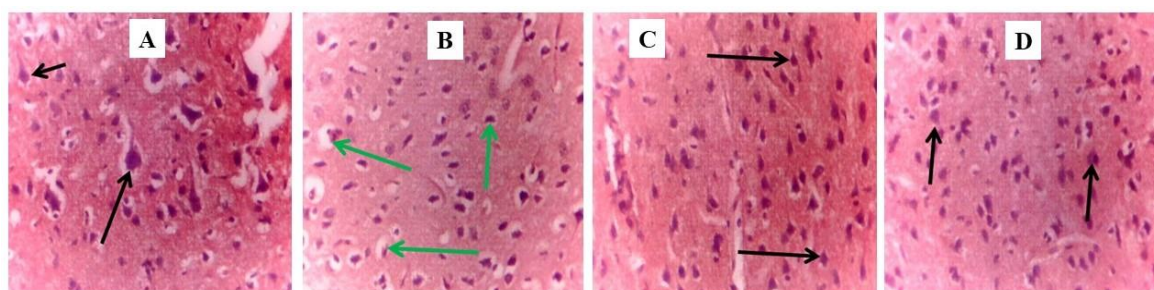


Figure 1: Photomicrograph of the brain (cerebral cortex) histology. (A) Control rats that received only water showed normal neurons (black arrows). (B) Rat administered mL/kg b.w of LTT showed slight neuronal damage (green arrows) and gliosis. (C) Rat administered mL/kg b.w of LC, showing normal neurons (black arrows) (D) Rat administered mL/kg b.w of TTW, showing normal neurons (black arrows). (H & E, x 400).

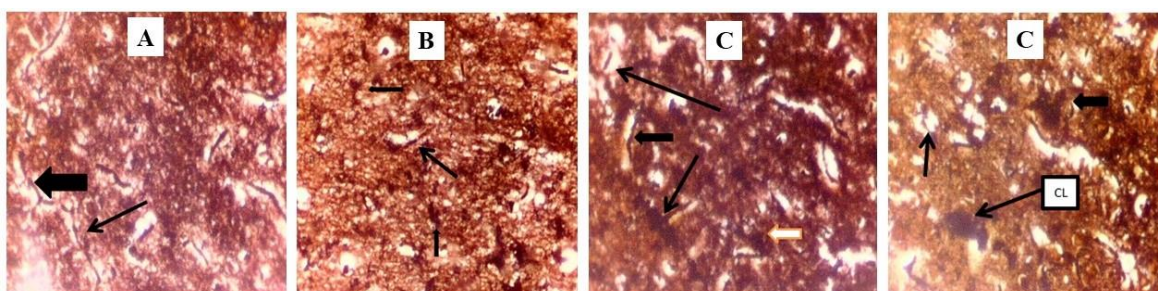


Figure 2: Photomicrograph of the brain (cerebral cortex) histology. (A) Control rats received water only and showed normal axons (thick and light arrows). (B) Rat administered mL/kg b.w of LTT showed slight axon loss (light arrows) with slight neuronal degeneration and gliosis (short arrows). (C) Rat administered mL/kg b.w of LC showing normal axons (thick and light arrows) (D) Rat administered mL/kg b.w of TTW, showing normal axons (thick and light arrows). (Silver stain, x 400).

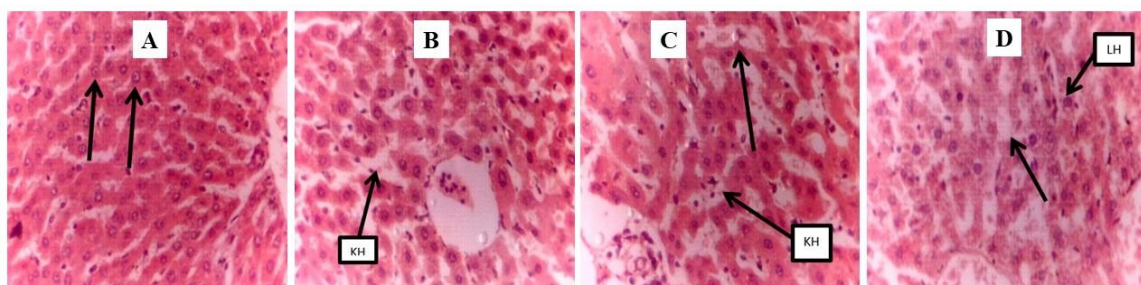


Figure 3: Photomicrograph of Liver histology. (A) Control rats (animals that received Water only) showed normal hepatocytes (arrows). (B) A rat administered mL/kg b.w of LTT showed Kupffer cell hyperplasia (KH). (C) A rat administered mL/kg b.w of LC showed Kupffer cell hyperplasia (KH) (4). Rat administered mL/kg b.w of LW, showed slight hyperplasia of inflammatory cells (LH). (H & E, x 400).

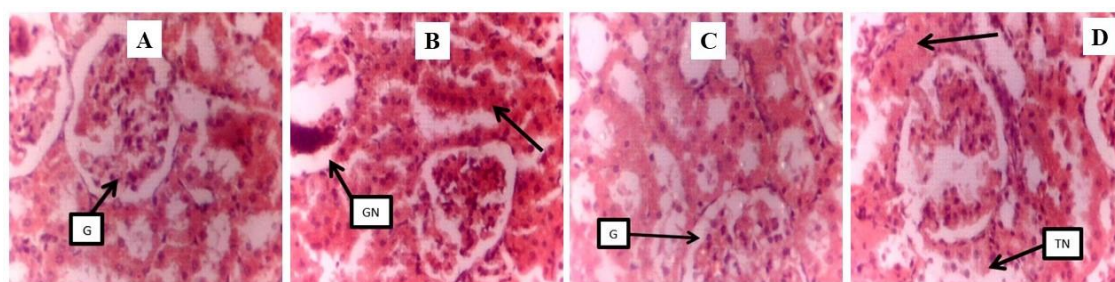


Figure 4: Photomicrograph of Kidney histology. (A) Control rats, animals that received Water only, showed normal glomeruli (G). (B) Rat administered mL/kg b.w of LTT, slight glomerular necrosis (GN). (C) Rat administered mL/kg b.w of LC, showed normal glomeruli (G). (4) Rat administered mL/kg b.w of TTW, showed tubular necrosis (TN). (H & E, x 400).

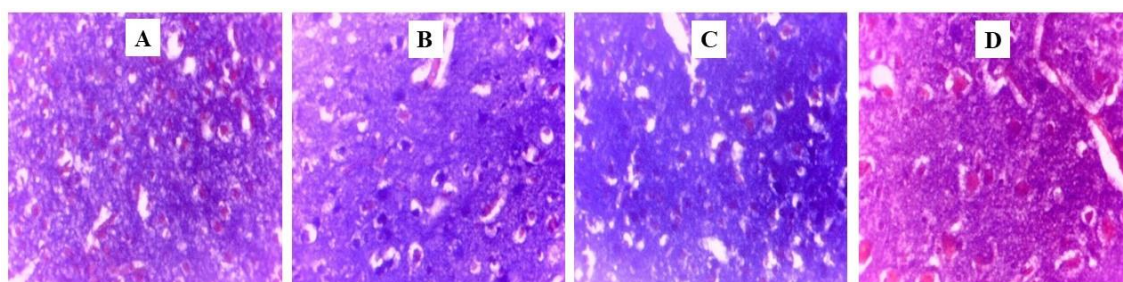


Figure 5: Photomicrograph of Brain (cerebral cortex) histology with Feulgen stain. (A) Control rats, animal that received Water only, (B) Rat administered mL/kg b.w of LTT, (C) Rat administered mL/kg b.w of LC, (4) Rat administered mL/kg b.w of TTW, (Feulgen stain, x 400).

Plate 5 details brain histology stained with Feulgen. It shows DNA sites by bright pink (DNA +) to dark red (DNA ++ or ++++) stained cells. Group A (Control) exhibited normal DNA content and distribution, as revealed by Feulgen staining. In contrast, Feulgen staining of Group B (LTT) showed a decrease in DNA content compared to Group A, while Group C (LC) also demonstrated a decrease in DNA content compared to Group A, but notably, its DNA content was higher than that of Group B. Finally, Group D (TTW) surprisingly displayed a high amount of DNA despite the observed structural abnormalities.

3.1. Discussion

This study investigated the histological effects of a Tomtom candy-Lacasera drink mixture (LTT), Lacasera drink (LC), and Tomtom candy in water (TTW) on the brain, liver, and kidney of Wistar rats. The findings reveal that both the mixture and its individual components can induce histological alterations in these organs, with varying degrees of severity [5]. The finding that Tomtom candy in water (TTW) caused more pronounced effects than the mixture (LTT) or Lacasera drink (LC) alone may be due to the absence of other

compounds in the drink that could reduce the bioavailability or toxicity of the candy's components.

The most significant brain alterations were observed in Group D (TTW), which showed moderate axon clumping and dendrite loss, suggesting a major impairment of neuronal structure [5, 6, 7]. This finding implies that Tomtom candy components, when administered in water, may have a more pronounced neurotoxic effect compared to the LTT mixture or Lacasera drink alone. This could be due to several factors. Firstly, the absence of other compounds present in the Lacasera drink might increase the bioavailability or toxicity of certain components of TomTom candy. For instance, the acidity or other constituents of Lacasera might interact with Tomtom components, potentially reducing their absorption or modifying their metabolism.

In contrast, dissolution in water might facilitate a more rapid or complete absorption of the candy's constituents. Secondly, the observed axon clumping and dendrite loss are critical indicators of neuronal damage. Axons transmit signals between neurons, and dendrites receive these signals; thus, their disruption can severely impair brain function [12]. These findings are consistent with studies showing that certain food additives or high concentrations of sugars can induce neurodegenerative changes [6, 10]. It is important to note that the brain is particularly vulnerable to toxic insults due to its high metabolic rate and limited regenerative capacity [11].

The Feulgen staining results, showing increased DNA content in Group D despite severe structural damage, are particularly noteworthy. This paradoxical finding could be a reflection of a cellular stress response, where the tissue attempts to cope with the toxic insult, and this could be as a result of cellular proliferation; the increase in DNA content may be attributed to gliosis, a process where glial cells proliferate in response to neuronal injury [6, 7]. This compensatory proliferation increases the number of support cells, thereby increasing the tissue's overall DNA content. It could also be due to DNA repair mechanisms. The toxic compounds may have caused direct DNA damage, triggering a robust DNA repair response. The synthesis of new DNA strands during the process could contribute to the observed increase in DNA content.

This finding suggests that, while the body is attempting to repair the damage, the neurons' structural

integrity remains severely compromised, leading to significant functional impairment [7]. Groups B (LTT) and C (LC) also showed some degree of neuronal damage, including slight axon loss and clumping [5, 6]. This suggests that both the Tomtom-Lacasera mixture and Lacasera drink alone have the potential to exert neurotoxic effects, although to a lesser extent than Tomtom candy in water. The presence of slight neuronal degeneration and gliosis in these groups further supports this notion [6]. Gliosis, an increase in glial cells, is a common response to neuronal injury and inflammation in the central nervous system [13].

The liver, as the primary site of detoxification, is particularly susceptible to damage from xenobiotics [14]. In this study, all treatment groups showed some level of hepatic necrosis (HN), indicating liver injury [5, 6]. Hepatic necrosis is the death of liver cells and can be caused by various factors, including toxins, drugs, and infections [15]. Group B (LTT) exhibited moderate Kupfer cell hyperplasia (KH) in addition to slight HN [5, 6]. Kupfer cells are resident macrophages in the liver that play a crucial role in the immune response; an increase in Kupfer cells suggests an inflammatory response to the LTT mixture, potentially due to components the liver perceives as foreign or harmful [15]. Group C (LC) also showed moderate HN, but with slight KH and hyperplasia of inflammatory cells (LH) [5, 6]. This suggests that Lacasera alone can induce liver damage and trigger an inflammatory response. The presence of artificial additives and high sugar content in the Lacasera drink might contribute to these effects. High sugar intake has been linked to non-alcoholic fatty liver disease (NAFLD) and other liver pathologies [6]. Group D (TTW) showed slight HN and LH, indicating that Tomtom candy components can also contribute to liver damage and inflammation [5, 6]. The specific components responsible for this effect require further investigation, but potential candidates include menthol, eucalyptus oil, and other flavorings present in the candy [4, 5].

All treatment groups showed some degree of liver and kidney damage, highlighting the potential hepatotoxic and nephrotoxic effects of these products. The moderate Kupffer cell hyperplasia in the LTT group indicates a significant immune and inflammatory response to the mixture. Previous biochemical studies by the authors

have corroborated these histological findings. In a 2024 study, chemical profiling and *in vivo* toxicity assessments of LTT revealed elevated plasma levels of liver enzymes (ALP, ALT, and AST) and creatinine, further supporting the observed hepatic and renal damage. These results are also consistent with earlier findings by the authors, Emmanuel [3, 5, 7], which demonstrated hepatotoxicity.

The observation that all treatment groups showed some degree of liver damage highlights the potential hepatotoxic effects of both the Tomtom-Lacasera mixture and its individual components. This finding is particularly concerning given the widespread consumption of these products. Previous chemical profiling of LTT identified 47 compounds, and *in silico* investigations predicted that some top-docked compounds, such as HP and propionylcodeine, could induce human hepatotoxicity and drug-induced liver injury [3]. Furthermore, some compounds in LTT showed inhibitory activity against cytochrome P450 enzymes, which could adversely affect phase I drug metabolism [3, 8]. *In vivo* studies also revealed elevated plasma levels of liver enzymes, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as creatinine, total cholesterol, and LDL-cholesterol, further supporting the observed hepatic damage [3].

The kidneys, which filter waste products from the blood, are also vulnerable to toxic injury [16]. In this study, all treatment groups exhibited some degree of renal damage, as evidenced by glomerular necrosis (GN) and tubular necrosis (TN) [5]. Glomerular necrosis involves damage to the glomeruli, the kidney's filtering units. In contrast, tubular necrosis refers to damage to the tubules, which reabsorb essential substances and secrete waste products [17]. Group D (LW) showed the most severe kidney damage, with tubular adhesion (TA) observed in addition to TN [5, 7]. Tubular adhesion can impair kidney function by obstructing the flow of filtrate and leading to further damage. This finding suggests that Tomtom candy components, when administered in water, may have a more pronounced nephrotoxic effect than the LTT mixture or Lacasera drink alone. The mechanisms underlying the observed kidney damage are not fully elucidated in this study. However, they may involve direct toxicity of the compounds or their

metabolites on renal cells, as well as indirect effects such as inflammation and oxidative stress. Further studies are needed to investigate the specific mechanisms of nephrotoxicity induced by Tomtom candy and Lacasera drink. The systemic elevation of creatinine levels in LTT-treated rats further corroborates kidney dysfunction [3].

This study confirms that the Tomtom-Lacasera mixture and its individual components can induce histological alterations in the brains, livers, and kidneys of Wistar rats. The most significant findings were the severe brain and kidney damage observed in the TTW group. The moderate axon clumping and dendrite loss in this group suggest a major impairment of neuronal structure and function. While the specific mechanisms underlying these effects require further investigation, several potential factors may be involved, such as oxidative stress, inflammation, direct toxicity, and synergistic effects of the mixture. Many food additives and high sugar concentrations can induce oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defences [7]. ROS can damage cellular components, including DNA, proteins, and lipids, leading to cellular dysfunction and tissue damage. Inflammation is a complex biological response to harmful stimuli, such as toxins and pathogens. While acute inflammation is a protective mechanism, chronic inflammation can contribute to tissue damage and disease [15]. The observed hyperplasia of inflammatory cells in the liver and the gliosis in the brain suggest that inflammation plays a role in the toxicity of the Tomtom-Lacasera mixture and its components [6, 13]. Some components of TomTom candy and Lacasera drink may have direct toxic effects on cells. For example, certain artificial sweeteners or preservatives might interfere with cellular metabolism or damage cell membranes [10]. The combination of different compounds in the Tomtom-Lacasera mixture might lead to synergistic effects, where the combined toxicity exceeds the sum of the individual toxicities. This could explain why the LTT mixture showed some toxicity despite the individual components also being toxic. *In-silico* studies also indicated that some identified compounds could permeate the blood-brain barrier and inhibit hERG channels, raising concerns about potential cardiotoxicity, and the

prediction of AMES mutagenicity for some compounds further highlights their genotoxic potential [3, 8].

The implications of these findings are significant, given the widespread consumption of the TomTom candy and Lacasera drink mixture, particularly among young people. The observed histological alterations in vital organs raise concerns about the potential long-term health consequences of consuming these products, especially in combination. Further research is needed to: identify the specific compounds responsible for the observed toxicity; investigate the dose-response relationship and determine safe consumption levels; examine the long-term effects of chronic exposure to the Tomtom-Lacasera mixture; and assess the potential for inter-individual variability in susceptibility to these toxic effects.

4. Conclusion

This study demonstrates that the Tomtom candy-Lacasera drink mixture and its individual components can induce histological alterations in the brains, livers, and kidneys of Wistar rats. The Tomtom candy in water group exhibited the most pronounced toxic effects, particularly in the brain and kidneys, raising concerns about potential health risks associated with their consumption. These findings, supported by the authors' previous biochemical investigations, underscore the need for further research to elucidate the toxicological profile of these common consumables fully.

Study Limitations

A key limitation of this study is the small sample size (n=4 per group), which may limit the statistical power and the ability to generalize the findings. Future studies with larger sample sizes and longer-term exposure are recommended to further validate these findings.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

All data are available from the corresponding author upon reasonable request

Authors Contributions

All authors contributed to all aspect of the research.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

Authors' Declaration

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

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