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Dose-Dependent Impact of Laca-tomtom Mixture on Cerebellar Histomorphology in Wistar Rats

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ABSTRACT

Twenty (20) Wistar rats, each weighing between 180 and 200 grammes, were divided into five (5) groups of five rats for this study. The control group was designated as Group 1. Group 2 only received 2 millilitres of Lacasera. A laca-tomtom mixture of 1 ml and 1.6 ml was given to groups 3 and 4, respectively. For 30 days, the medication was administered orally. In a chamber saturated with chloroform, the rats were put to sleep. They were then dissected on a dissecting board, and the cerebellum was removed for histological analysis. The results showed that the laca-tomtom mixture at graded doses had a detrimental effect on the cerebellum, causing a decrease in motor function, motor coordination, and balance as demonstrated by the neurobehavioural test. The cerebellum's histological examination revealed that the laca-tomtom mixture is detrimental to the cerebellum after the experimental groups' purkinje cell size decreased.
Keywords: Laca-tomtom**;** neurobehavioural**;** cerebellum**;** histology

INTRODUCTION

When certain substances that are normally non-toxic are consumed in combination, they can have a toxic effect on the body and its systems. Higher concentrations of these mixtures can cause symptoms of the central nervous system, including mood swings or personality changes, such as depression or aggression, drowsiness, slowed reflexes, vomiting, and rapid unconsciousness and death from respiratory failure in severe cases. Peer pressure, parental neglect, reducing physical and emotional pain, inducing sleep, boredom, and other factors may all contribute to the use of these non-toxic substance mixtures [1],[2],[3]. "Lacatomtom" is a consumer-made solution made from the combination of La casera drink and Tomtom candies; it is dark-brown in colour, has a sharp minty smell primarily from the tomtom component, and has a fizzy appearance due to the reaction between the two components, specifically the quick nucleation of carbon dioxide gas aided by menthol, carbonated water, sodium benzoate, and aspartame [4]. The cytotoxicity of the Lacatomtom mixture and its impact on Lactate dehydrogenase (LDH) in experimental albino wistar rats were examined in a prior study by [4]. It demonstrates that following the oral administration of the mixture containing varying ratios of LaCasera to Tomtom and the collection of sera for the LDH test. After twenty-four hours of exposure to the Lacatomtom drink, the results indicated a medium toxicity to brine shrimp with an LC50 of 103.915µg/ml. It was also discovered that the test groups' serum levels of LDH had significantly increased in comparison to the control. The toxic effect of Lacatomtom mixture at different concentrations on the liver function enzyme profile of experimental albino wistar rats was examined in a more recent study by [5]. Following oral administration, enzymatic activity was activated in the collected sera. The test groups' levels of liver enzymes, such as alkaline phosphatase, alanine transaminase, and aspartate transaminase, were found to have significantly increased in comparison to the control group. Additionally, rats given the test sample showed a significant decrease in weight and liver-to-body weight ratio in comparison to the control group. According to these results, consuming Lacatomtommixture may be harmful and result in liver damage, which could have an impact on human health. Lacatomtom has also been studied as a potential replacement for the well-known intoxicant "codeine," which has pharmacological and chemical effects on the user. [6], conducted this study. The experimental subjects were divided into three groups: control, acute, and chronic (T1, T2, and T3). The acute groups received 0.01 mg/g of lacatomtom mixture orally for 14 days, while the chronic groups received it for 42 days and were weighed and recorded on a regular basis. The animals were sacrificed, and their organs were harvested in accordance with ethical guidelines for animal killing. Following chemical pathology analysis to evaluate the liver and kidney functions, the blood and tissues of the harvested organs (blood, lungs, liver, and kidney) under investigation underwent histopathological examination using formalin-fixed paraffin processed methods with both routine and special stains. Oxidative stress biomarkers tests were performed on the liver, lungs, and kidney homogenate (MDA, SOD, Gpx, GSH, and Catalase). All of the organs under investigation had significant

cellular damage, according to the histopathology results, which were first compared to the normal control. The biochemistry and histology results were correlated with increased MDA, decreased SOD, Gpx, GSH, and catalase, and a significant elevation in certain enzymes, including AST, ALP, and ALT, which are also signs of liver damage. One of the many substances that young people in Nigeria mix in their desperate attempt to get drunk is "lacatomtom." In contrast to other components of mixtures that have been outlawed or are solely based on prescriptions, such as methylated spirit with Cola drink, a combination of Lacasera drink and codeine, Refnol, Tramadol, and cannabis, this mixture is one of the most popular among young people because it is easily obtained and is not prohibited from standard supermarket shelves. Youths benefit from this by feeling more energised and strong, better able to handle psychological disorders like depression, anxiety, grief, and trauma, performing better in school, forgetting physical or emotional pains, or just having fun. Many innocent young people are using it despite known harm to some of their vital organs, and it is regarded as one of the main causes of abuse in society. The literature that is currently available indicates that the consumption of certain Lacatomtom mixture constituents is linked to histopathological alterations in lipid profiles, liver enzymes, and certain kidney parameters. These days, young people frequently suffer from these illnesses. The impact of lacatomtom and its components on the nervous system, however, has received little to no attention. The main motivation for conducting this study is the significant knowledge gap. Therefore, the purpose of this study is to assess how the Lacatomtom mixture affects the adult male wistar rats' cerebellar histoarchitecture.

Materials & Methods

COLLECTION OF LACASERA AND TOMTOM

The LaCasera drink and Tomtom candy were purchased from Remy supermarket in Elele, Rivers state.

FORMULATION OF LACATOMTOM MIXTURE

For Group 2, 500ml of Lacasera alone was utilised as the solution in order to further understand its distinct components. For the remaining groups, the mixtures were prepared by mixing different weights of ground tomtom (Group 3 = 37.20g, Group 4 = 74.40g) with 500ml of Lacasera to create the mixture that was given orally to the rats in Groups 3, 4. For the duration of the administration, the mixtures were then kept in a refrigerator between 2 and 80 degrees Celsius.

Experimental Animals

From the Department of Physiology at the University of Nigeria, Enugu Campus, twenty (20) adult male wistar rats weighing 180–200g were acquired. Two weeks, or fourteen days prior to the start of the experiment, the animals were acclimated to standard laboratory conditions at the Animal House of Basic Medical Sciences, Madonna University, Elele Campus, for feeding schedules and handling techniques. They were given water and commercial rat food. Every animal was cared for in accordance with Nigeria's legislation on laboratory animal experimentation, which is based on the National Institutes of Health's recommendations in the United States.

Table1: Experimental Animal Groupings

GROUPS	N=5	MIXTURE OF LACATOMTOM
Group 1 (Control)	5	Feed + Water
Group 2	5	Feed + **2ml** of LaCasera drink
Group 3	5	Feed + **1ml** of Lacatomtom mixture
Group 4	5	Feed + **1.6ml** of Lacatomtom mixture

Following two weeks of acclimatisation, test substance administration began and continued for four weeks, with weekly neurobehavioral testing.

NEUROBEHAVIOURAL TEST

Neurobehavioral studies were conducted by following the protocols of [7],[8],[9],[10],[2].

ROTAROD TEST

The rotarod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test measures parameters such as riding time (seconds) or endurance. Some of the functions of the test include evaluating balance, grip strength and motor coordination of the subjects; especially in testing the effect of experimental drugs or after traumatic brain injury. In the test, a rodent is placed on a horizontally oriented, rotating cylinder (rod) suspended above a cage floor, which is low enough not to injure the animal, but high enough to induce avoidance of fall. Rodents naturally try to stay on the rotating cylinder, or rotarod, and avoid falling to the ground. The length of time that a given animal stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning. The speed of the rotarod is mechanically driven, and may either be held constant, or accelerated.

HANG GRIP TEST

The purpose of this test is to evaluate the limb motor or muscular functions in rodents. The grip strength meter test allows the study of neuromuscular functions in rodents by determining the maximum force displayed by an animal. This test is included in the Functional Observational Battery (FOB) to screen for neurobehavioral toxicity. In this context, changes in grip strength are interpreted as evidence of motor neurotoxicity. Basically, the grip strength meter is positioned horizontally, and the subjects are held by the tail and lowered towards the apparatus. The animals are allowed to grasp the metal grid or triangular pull bar and are then pulled backwards in the horizontal plane. The force applied to the grid or to the bar just before it loses grip is recorded as the peak tension.

BEAM WALKING TEST

The 'beam walking test' or 'balance beam test' is used to analyse rodent gait in a testing environment that challenges their ability to maintain balance given that the animals must cross an elevated beam with a narrow diameter. This test is used for the assessment of motor coordination, particularly of the hindlimb. Firstly, animals are placed in one corner of the narrow beam and allowed to walk across the narrow beam from one end to the other for at least three times. The narrow

beam measures 1-3 cm wide and is elevated between a pole and their home cage (to attract the rat to the finish point). This training step can be useful to achieve a stable baseline measurement. The number of foot slips encountered, and time taken to cross the beam in each trial are recorded. If animals hesitate to cross the beam, walking can be stimulated by tapping on the tail. However, this must be performed in all animals to keep testing sessions comparable. What's more, a ledge can be installed next to the narrow beam to be used as a crutch, on which the impaired limb can be placed.

PREPARATION OF FIXATIVE

The fixative (10% formal saline) was used to fix the collected tissue and was prepared thus: 90mI of distilled water was mixed with 10ml of formalin.

Collection of Sample

The rats were anesthetized in a chloroform saturated chamber and the rats were then dissected on the dissecting board using dissecting kit and the cerebellum harvested and fixed in 10% formal saline. [10], [11],[12], [13], [14], [15],[16].

Preparation of Fixative

The fixative (10% formal saline) was used to fix the collected tissue and was prepared thus:
90ml of distilled water was mixed with 10ml of formalin.

Histological Analysis

To assess the histoarchitecture of the cerebellum, the cerebellum of both the control and experimental groups were harvested, and the tissues processed. The purpose of this tissue processing was to provide a solid support medium for tissue during section cutting and to aid microscopic examination [17], [18], [19].

Statistical Analysis of Results

Data among the groups with different concentrations of the treatment agents were analysed using one way analysis of variance (ANOVA). Thereafter, the post-hoc test of multiple comparisons (Newman Keuls test) was used to test the individual groups against each other.

RESULTS

Table 1 – Result of motor function test using Rotarod test after exposure of experimental animals to their respective diets.

Groups	Trial 1 Time (s)	Trial 2 Time (s)	Trial 3 Time (s)
Group 1 (control)	19.60±2.94	23.00±9.04	25.00±6.33
Group 2 (2ml of lacasera alone)	23.40±0.6	24.20±3.307	33.40*±0.8
Group 3 (1ml of lacatomtom mixture)	7.800±3.28	11.20±3.00	10.40±3.2*
Group 4 (1.6ml of lacatomtom mixture)	8.800±3.28	10.20±3.00	8.40±3.2*

Values are presented in mean ± sem, n=5* means values are statistically significant ($p \leq 0.05$) when compared to the control

Table 2 – Result of motor coordination test using Hand grip test after exposure of experimental animals to their respective diets.

Groups	Trial 1 Time (s)	Trial 2 Time (s)	Trial 3 Time (s)
Group 1 (control)	116.80±25.87	136.40±8.95	125.80±3.82
Group 2 (2ml of lacasera alone)	134.20±1.95*	144.60±1.98*	150.40±2.42*
Group 3 (1ml of lacatomtom mixture)	13.00±1.73*	11.40±1.50	8.80±.9695
Group 4 (1.6ml of lacatomtom mixture)	42.00±2.00*	25.44±3.50*	22.12±4.50

Values are presented in mean ± sem, n= 5. * means values are statistically significant ($p \leq 0.05$) when compared to the control

Table 3 – Result of motor balance and equilibrium test using Beam walking test after exposure of experimental animals to their respective diets

Groups	Trial 1 Time (s)	Trial 2 Time (s)	Trial 3 Time (s)
Group 1 (control)	57.40±13.02	38.60±18.17	34.60±12.50
Group 2 (2ml of lacasera alone)	34.80±8.22	50.40±61.11	54.60±59.71
Group 3 (1ml of lacatomtom mixture)	211.20±57.09	200.40±42.33*	270.00±18.37*
Group 4 (1.6ml of lacatomtom mixture)	213.20±57.09*	205.40±42.33*	270.00±16.32*

Values are presented in mean ± sem, n= 5. * means values are statistically significant ($p \leq 0.05$) when compared to the control.

Histological Examination

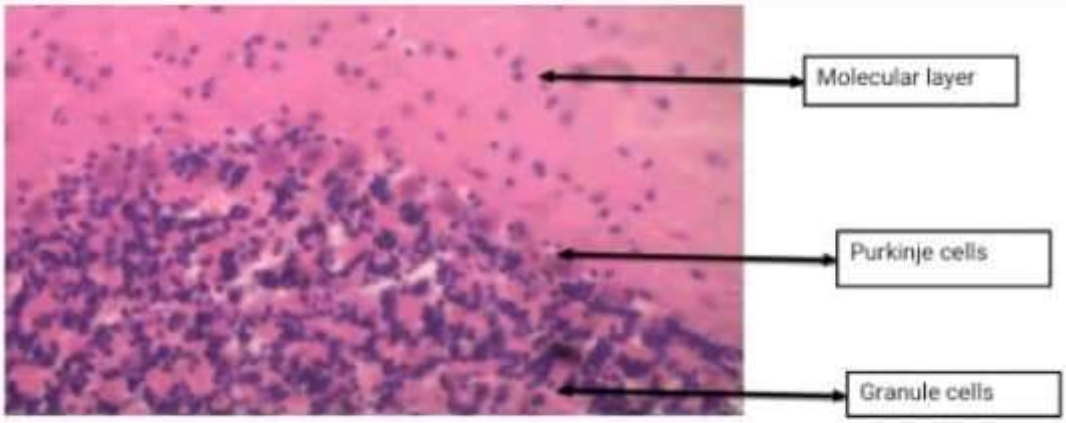

Plate 1: Photomicrograph of the cerebellum of Group

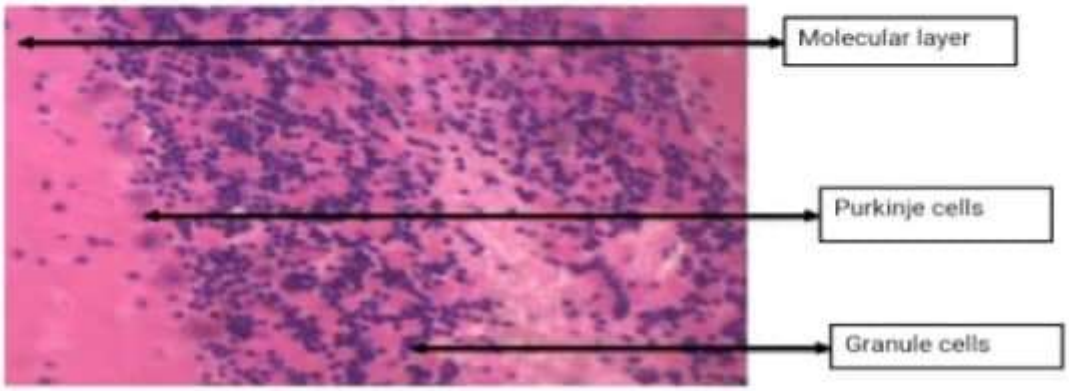

Plate 2: Photomicrograph of the cerebellum of Group 2

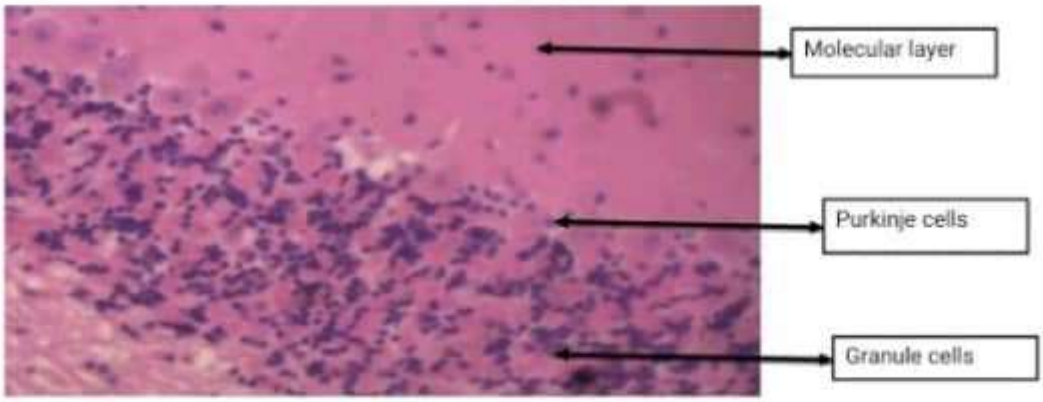

Plate 3: Photomicrograph of the cerebellum of Group

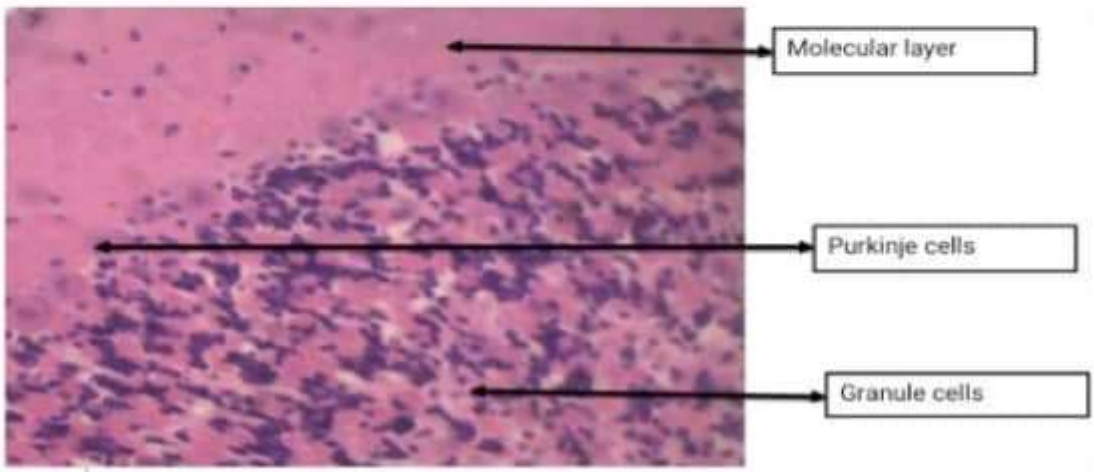

Plate 4: Photomicrograph of the cerebellum of Group

DISCUSSION OF FINDINGS

A rotarod test was used to assess the motor coordination between the groups, and the results of the rotarod test revealed a significant increase in riding time among Group 2 fed LaCasera only. These neurobehavioural studies examined the effects of Lacatomtom mixture on the histoarchitecture of the cerebellum of adult male wistar rats. This suggests that LaCasera drinks have a positive effect on motor coordination in adult male wistar rats. The hand grip test was used to measure motor function among the groups, and the results showed a significant increase in duration of hand grip among the Group 2 fed with LaCasera alone. It was found that the riding time of Groups 3 and 4 significantly decreased when fed with different concentrations of Lacatomtom mixture, at 1 ml and 1.6 ml, respectively. This suggests that the Lacatomtom mixture impaired motor coordination and balance in adult male wistar rats. This suggests that in adult male wistar rats, LaCasera improved motor function. Groups 3 and 4 were fed the Lacatomtom mixture at 1 ml and 1.6 ml, respectively, and it was found that their hand grip duration significantly decreased. This notable reduction in hand grip duration among the groups fed varying concentrations of Lacatomtom mixture suggested that the Lacatomtom mixture may be the cause of motor impairment. The purpose of the beam walking test was to evaluate the groups' balance and motor coordination. When compared to the control, the test result indicated a marginally significant increase in the amount of time needed to reach the finish line. The test results also revealed a markedly significant increase in the amount of time that Groups 3 and 4 spent feeding with varying concentrations of Lacatomtom mixture (1 ml and 1.6 ml, respectively). While walking on the beam, every rat in Groups 3 and 4 was dragging its limbs. These neurological symptoms suggest that adult male wistar rats may experience motor deficits as a result of the lacatomtom mixture. Researchers looked at how the Lacatomtom mixture affected the adult male wistar rats' cerebellar histoarchitecture. The three layers of the surface grey matter of the cerebellum—the molecular layer, the purkinje cell layer, and the granular cell layer—were clearly visible in the photomicrograph of plate 2 that received 2 ml of LaCasera alone, which also displayed normal histoarchitecture of the cerebellum based on histological examination of haematoxylin and eosin sections as seen in the photomicrograph of plate 1 (the control group) at a magnification of x500. When compared to the control group, the photomicrograph of plate 3 that received 1 ml of Lacatomom mixture revealed a significant reduction in the size and quantity of purkinje cells. The purkinje cell size and quantity were significantly reduced in the photomicrograph of plate 4, which had been exposed to 1.6 ml of Lacatomom mixture. Cerebellar output is reduced when purkinje cells decline. Major cerebellar outputs like fastigial nucleus projections, which affect extensor muscles that maintain balance and posture, may be impacted by this. Additionally, this has an indirect effect on the dentate nucleus projections, which influence the motor cortex to control fine and skilled movements, and the interpositus nucleus, which corrects errors related to gross movement. The results of the neurobehavioural tests conducted in this study using the rotarod, hand grip, and beam walking test were in agreement with the histological findings. This is also in accordance with the results of [20], that reported decreased purkinje cells size and total volume of the white matter following exposure to tramadol. Both the current findings and earlier findings are inconsistent [21], [22], [23],[24].

CONCLUSION

Graded doses of the lacatomtom mixture clearly affected cerebellar health. According to the results of the neurobehavioural test, it led to a decline in their motor function, motor coordination, and balance. The results also show that the cerebellar output, which influences the areas of the cerebellum that influence spinal motor neurones that control the extensor muscles to maintain posture and balance, decreased. Histological analysis of the cerebellum's haematoxylin and eosin section confirms the neurobehavioural test's findings that the Lacatomtom mixture damages the cerebellum because it causes the purkinje cells' cell size to decrease in the experimental groups under study.

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