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Cardiac Histomorphological Alterations in Adult Wistar Rats Following Exposure to 3,4-Methylenedioxymethamphetamine (MDMA) Pills

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IIC and BJK contributed to the conception and design of the work/idea for the manuscript. Authors AE and OA collected the data and obtained the results for the manuscript. Authors IKO and OKO wrote the manuscript. Authors OJ and LSB critically revised the manuscript. Authors IKO, OKO, and LSB conducted the final review of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The Ecstasy pill sold with the trade name 'molly' which has 3,4-methylenedioxymethamphetamine (MDMA) as the active component, is one of the most popular recreational drugs in parts of Africa. This study was carried out to determine the histomorphological effects of molly on the heart using adult wistar rats as the mammalian model. The study was done on twenty-five adult albino wistar rats which were sacrificed after 21 days of molly administration, the rats were put under light chloroform anesthesia and the Heart harvested for histological processing. The experimental animals were separated into five groups. Each group contained five rats each using 5 big cages to house them. The administration of molly was given orally as follows: Group A (Control) received only normal feed (growers' mash) and distilled water daily for 21days. Group B-E (Test groups) received growers mash and graded quantities of molly (B, 0.12 mg/kg body weight each; C, 0.18 mg/kg body weight each; D, 0.32 mg/kg body weight each and E, 0.4 mg/kg body weight each) and water was given ad libitum for 21 days. Heart sections were obtained from the rats and processed histologically. The results showed that the weight of the control increased during the period of the study. The mean weight of rats in all groups before the period of molly administration were significantly increased. Group A (Control), group B and C showed normal cardiac muscle, while Group D and E showed section of cardiac muscle showing fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm and features of Myolysis. This study revealed that consumption of molly could result in the distortion and disruption of the microanatomy of the cardiac muscle showing fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Molly consumption may be the cause of the various deleterious distortions which occurred in the heart and a necessary reduction in its consumption is required to avoid these effects.

Keywords: Molly; drug; methylenedioxymethamphetamine; heart; Wistar; rat.

1. INTRODUCTION

As one of the most widely used recreational drugs, molly (Ecstasy) contains 3, 4-methylenedioxymethamphetamine (MDMA), which is its primary ingredient. Although the prevalence of usage increased to 50–60% in some categories last year (such as clubbers), the prevalence of lifetime use in the adult population is still low (<5%) [1,2,3]. Reducing brain concentrations of monoamines, especially serotonin, but also norepinephrine and dopamine, is how MDMA acts as a (psycho) stimulant on the central nervous system [4,5,6]. There are multiple mechanisms at play for these increases. To begin with, MDMA blocks monoamine reuptake transporters, which prevents extracellular monoamines from being absorbed into cells. SERT, as well as the dopamine and norepinephrine reuptake transporters (NET and DAT), are specifically inhibited. Subsequently, MDMA reduces the intracellular vesicle storage of monoamines, hence increasing their cytosolic concentration. Lastly, because MDMA changes the direction of transport of monoamine reuptake transporters from outside-inside (reuptake) to inside-outside (transporter mediated release), cytosolic monoamines can be carried outwards, which raises the concentration of monoamines in the brain even further [6]. Receptors may then be activated by these extracellular monoamines. The use of MDMA can cause these receptors to become overstimulated by high amounts of extracellular monoamine. Not only that, but MDMA itself also affects receptor function—albeit mostly at doses greater than those required to inhibit transporters. Yet, at sub-micro molar doses, MDMA does alter serotonin receptor function [6–8]. The physiological alterations that result from MDMA usage lead to heightened awareness as well as entactogenic effects, which include feelings of warmth, euphoria, and love, as well as a closer sense of connection with people [9].

On the other hand, there are potential side effects from MDMA consumption, such as

elevated blood pressure, body temperature, and heart rate (hypertension and hyperthermia). There are sporadic reports of deaths along with a spectrum of adverse effects from minor to fatal [10–11]. A registry of health occurrences linked to MDMA use and the incidence of MDMA usage have led to an estimate of 0.11% (1 in 900 pills) of moderate to severe side effects [12]. In Europe, MDMA ranks third among stimulant drugs in terms of reported drug-related ED visits, despite the seemingly minimal risk [1]. Global statistics on incidents related to MDMA are missing. Thousands of MDMA-related ED visits were reported by the 31 sentinel sites that make up the Euro-DEN Plus network between 2014 and 2017 [13–14], indicating a significant impact on healthcare institutions.

The heart is a hollow muscular organ that contracts repeatedly and rhythmically to pump blood across the blood arteries to different regions of the body. All animals with a circulatory system, including vertebrates, have it. The main components of the vertebrate heart are cardiac muscle and connective tissue. The heart's involuntary striated muscle, or cardiac muscle, is what gives the organ its capacity to pump blood [15].

It is common for MDMA users to mix their drug with ethanol; 91% of Australian clubbers, or seasoned MDMA users, reported doing so [16]. Furthermore, behind the combination of ethanol and cannabis, the combination of MDMA and ethanol was the second most common combination among Dutch clubbers in the previous year [3].

The pharmacokinetic and dynamic features of MDMA were found to be influenced by interactions with ethanol, however the exact risk of mixing the two substances is still mostly unknown [17]. Additionally, research on mortality and intoxication suggest that mixing MDMA and ethanol may impair health outcomes [18–19]. For instance, ethanol was also used in 70% of MDMA-related ED visits in Europe [14]. According to certain reports, health occurrences linked to MDMA that occur concurrently with ethanol exposure appear to be more serious than incidents involving MDMA alone [19]. Additionally, compared to patients who were only drunk by MDMA, a greater percentage of patients who were intoxicated by ethanol and MDMA at a dance event in Belgium needed hospital treatment [20]. Apart from its recreational application, MDMA is also being studied for its potential as a treatment for ethanol consumption disorder [21]. As a result, exposure to ethanol and MDMA together may put other populations at danger. Given this, adult wistar rats were used as the mammalian model in the current investigation, which aimed to ascertain the histomorphological effects of molly on the heart.

2. MATERIALS AND METHODS

A total of twenty-five (25) adult albino Wistar rats weighing an average of 110.10g were obtained from the animal farm and brought to the Department of Medical Laboratory Science's Histology laboratory for two (2) weeks of acclimation. To avoid infection, they were housed in wire mesh cages with tripods that kept the animal and its waste apart. The rats were fed growers' mash and given unlimited water during this phase of acclimation. According to the standard guide for the care and use of laboratory animals, the animals were maintained and used.

2.1 Research Design

Twenty-five adult albino wistar rats were used in the investigation; they were slaughtered after being given molly for 21 days. Following the experiment, the rats were given a little chloroform anesthesia, and their hearts were removed for histological examination. After being collected, heart tissues were preserved for 24 hours in 10% formal saline. Rats were used to acquire heart slices. In the cutting-up room, samples were cut to a thickness of 3 nm. The chosen tissues underwent histological processing and were arranged in tissue baskets with meticulous labeling.

2.2 Animal Grouping

The experimental animals were separated into five groups (A – E). Each group contains five rats each (n = 5) using 5 big cages to house them. Group A served as the control, while groups B - E served as the test groups.

Group B – E received graded doses of molly prepared accordingly and weighed to determine the quantity to be administered. Group A received only the normal feed (grower’s mash) and water with no administration of molly.

2.3 Substance Preparation and Administration

The medication (molly) was bought from a government-approved pharmacy and diluted using commercially produced normal saline to the proper concentrations (B, 0.12 mg/kg body weight each; C, 0.18 mg/kg body weight each; D, 0.32 mg/kg body weight each, and E, 0.4 mg/kg) needed for the investigation. The medication was prepared for oral administration and administered to the animals. For 21 days, the medications were given orally via a 1.0 ml standard syringe. Molly was powdered, dissolved, and then diluted with regular saline to a roughly concentration. Using a hand towel, each animal in groups B through E was selected one at a time, and the proper dosages of the medications were given orally to the animals. They were divided into five groups of five rats each. The rats were weighed before the administration of molly and before they were sacrificed. The administration of molly was given orally as follows:

Group A (Control) received only normal feed (growers' mash) and distilled water daily for 21days.

Group B-E (Test groups) received growers mash and graded quantities of molly (B, 0.12 mg/kg body weight each; C, 0.18 mg/kg body weight each; D, 0.32 mg/kg body weight each and E, 0.4 mg/kg body weight each) and water was given ad libitum for 21 days.

2.4 Sample Collection and Analysis

Before and after acclimatization, the animals' weights were recorded. Comparable weight measurements were also taken at the conclusion of each week, and the average weight was noted. Following each phase (10 days for the acute phase and 21 days for the chronic phase), the hearts of the rats were removed while they were still under chloroform anesthesia and preserved in 10% formalin in preparation for histological examination.

2.5 Histological Processing

Using an automatic tissue processor, the tissues were treated in accordance with the Histology Laboratory's regular processing schedule. Fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through various grades of alcohol for two hours: two hours with 70% alcohol, two hours with 80% alcohol, two hours with 90% alcohol, two hours with 90% alcohol, two hours with 95% alcohol, and two hours with absolute alcohol. Next came two hours with Xylene 1 and two hours with Xylene II, and finally two hours with Molten paraffin wax I and II. The tissues were taken out of their plastic cassettes following the final time, put in the middle of the metallic tissue mold, and then filled with melted paraffin wax. Additionally, they were allowed to firm before spending 15 minutes at 5 ^{o}C in the refrigerator. The blocks were taken out of the metallic case with a knife and the paraffin wax at the side was removed once they had cooled in the refrigerator for the previously mentioned fifteen minutes.

A rotary microtome was then used to trim and cut serial pieces of the blocks at a thickness of 3 nm. The portion was lifted using a clean, frosted end slide after floating in a water bath at 55 degrees Celsius. After the frosted end slides were properly attached to the slides by placing them on the hot plate for forty minutes, the sections were dewaxed, hydrated, allowed to air dry, and then kept in a slide box in preparation for staining.

2.6 Staining Procedure

Sections for general tissue structure was stained by Haematoxylin and Eosin technique as follows:

The sections were dewaxed in 2 changes of xylene (5 minutes) after which the sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%). The sections were then stained in Harris haematoxylin (5 minutes) followed by rinsing in running tap-water to remove excess stain and then differentiated in 1% acid alcohol (3 seconds). The sections were blued in running tap water for 10 minutes and then counterstained with 1% eosin for 1 minute. The sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80, 95% and absolute) and then cleared in xylene, air-dried and mounted with dibuthylphthalate in xylene (DPX). The slides were examined under a light microscope and photomicrographs were taken.

2.7 Photomicrography

The sections were examined under a light microscope and photomicrographs of each group were taken. The photomicrographs are then used to interpret the results of all the groups.

2.8 Data Analysis

The obtained data were then subjected to statistical analysis using SPSS (version 25). The test groups' values were compared with the values of the control group using ANOVA (Scheffe) at 95% level of confidence.

3. RESULTS

On observation of the body weight changes of rats fed with graded dose of molly at various intervals, the weight values of rats in group B, C, D and E were lower before administration of molly when compared to the weight after administration of molly. When the weight of all the groups (B, C, D and E) fed with molly were compared with the control group (A), there was statistically significant difference. But the weight of the control group (A) was higher than the weight of the test group rats (B, C, D and E) after administration of molly. This could signify that the molly actually made the rats feed more.

Table 1. Body Weight Changes of Rats Fed with Graded Dose of Molly at Various Intervals

Weight (g)	A Control (n=5)	B (0.12mg/kg) (n=5)	C (0.18mg/kg) (n=5)	D (0.32mg/kg) (n=5)	E (0.4mg/kg) (n=5)	F value	P value
WBMA	252.5±3.53	277.5±3.53	252.5±3.53	272.5±3.53	215.5±2.12	29.30	0.001 (S)
WAMA	327.5 ± 3.53	310.0±14.14	273.0±2.32	295.0±7.07	277.0±3.53	18.18	0.004 (S)

KEY: P-value (p<0.05): significant; WBMA: weight before molly administration; WAMA: weight after molly administration; Values are mean ± standard deviation; n: Number of samples; s: significant; n/s: not significant

Histomorphological Observations:

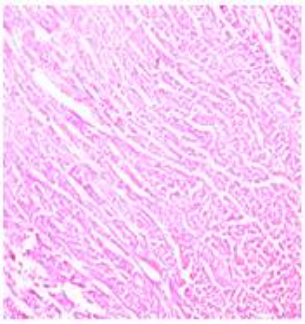

Fig. 1a. Control Group showing normal cardiac muscle (H/Ex100)

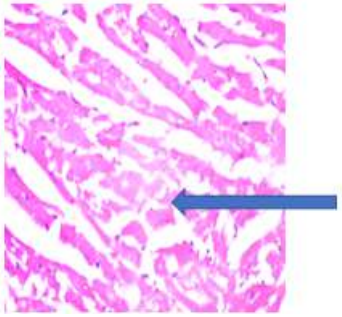

Fig. 1b. Control Group showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/Ex400)

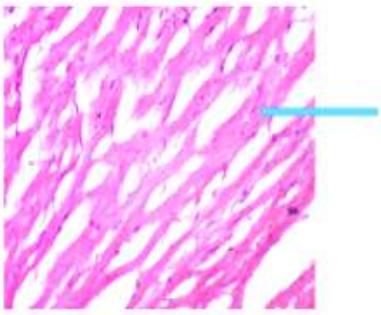

Fig. 2a. Group B showing normal cardiac muscle (H/E x100)

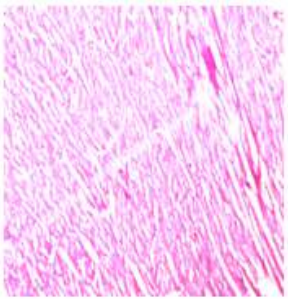

Fig. 2b. Group B showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/Ex400)

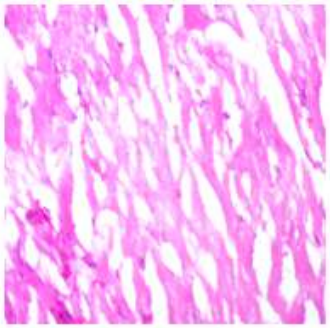

Fig. 3a. Group C showing cardiac muscle (H/E x100)

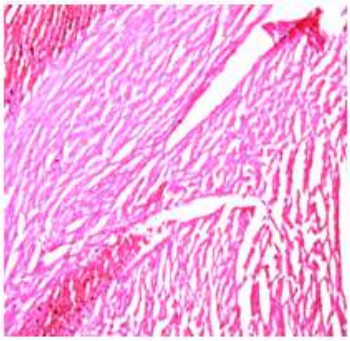

Fig. 3b. Group C showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

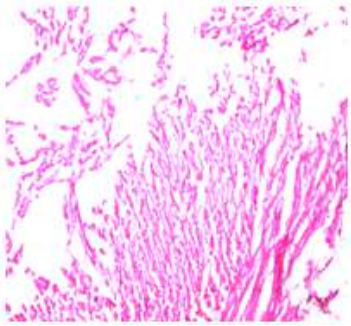

Fig. 4a. Group D showing cardiac muscle disarray (H/E x100)

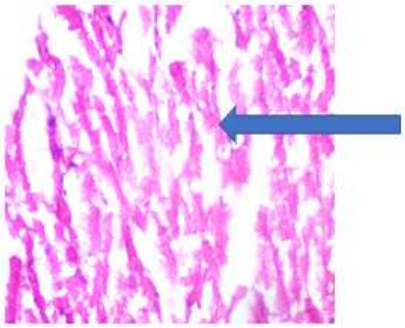

Fig. 4b. Group D showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

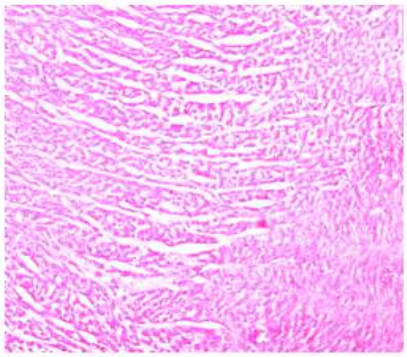

Fig. 5a. Group E showing cardiac muscle disarray (H/E x100)

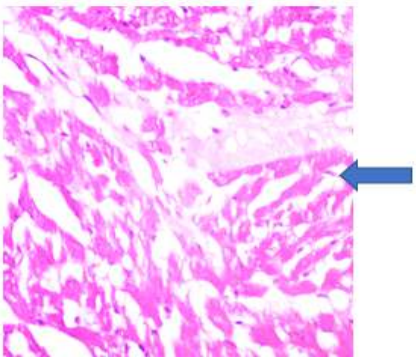

Fig. 5b. Group E showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

4. DISCUSSION

The heart is a hollow muscular organ that contracts repeatedly and rhythmically to pump blood through blood arteries to different regions of the body. Every animal with a circulatory system has it. Vertebrate hearts are mostly composed of cardiac muscles and connective components. The heart's involuntary striated muscle, or cardiac muscle, is what gives the organ its capacity to pump blood [15].

Group E has fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm and signs of myolysis, while groups B through D exhibit normal cardiac muscle. However, when compared to the H and E stains used to illustrate overall tissue architecture, this staining was correctly differentiated. The control group's histological examination showed that the heart's structure was normal and that its cardiac muscle was noticeably large.

In human volunteers, a recreational dose of MDMA slightly raises blood pressure and heart rate [22,23]. Increases in adrenaline and norepinephrine blood concentrations soon after MDMA consumption (307% and 23%, respectively) are primarily responsible for this [23]. High dosages of MDMA can cause myocardial infarction, cardiac arrhythmias, tachycardia, and hypertension [6, 10]. Acute exposure to ethanol is thought to have no effect on blood pressure, while larger amounts (more than five drinks) may cause a modest rise in blood pressure. Hypertension may arise from long-term alcohol usage [24–25].

The immediate biochemical and circulatory consequences of simultaneous MDMA-ethanol consumption were examined in one human investigation. Blood pressure, heart rate, and the amounts of adrenaline and norepinephrine did not increase further [23]. Furthermore, after concurrent ethanol administration, two mouse investigations found that MDMA increased biomarkers suggestive of cardiotoxicity, but it also increased protective variables [26–27].

The experiment's findings also suggest that the cytoarchitecture of the cardiac tissues is distorted, which may have had a negative impact on the animals' health due to functional alterations brought on by molly on the heart tissues. Nonetheless, it has been noted that a compound's capacity for toxicity on an organism's tissues determines how seriously it damages tissue when it acts as a toxicant. Moreover, different animal tissues and cells have varying degrees of vulnerability to chemical damage. In some animal groups, it can occasionally be higher. Furthermore, the chemical's method of action may help pinpoint the site of the main injury. Each poison's method of action, the tissue vulnerability pattern, and the toxic dose of each agent at which a comparatively uniform, recognizable pattern of tissue damage has been investigated have all been thoroughly characterized.

5. CONCLUSION

This study revealed that consumption of molly could result in the distortion and disruption of the microanatomy of the cardiac muscle showing fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis. It is probable that the heart function may be adversely affected. However, molly consumption may be the cause of the various deleterious distortions which occurred in the heart and a necessary reduction in its consumption is required to avoid these effects. No alternative to animal testing was done.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Approval for the study was obtained from the Research Ethics Committee of the College of Medical Sciences, and was carried out in strict accordance with the guideline for the care and use of animals for research committee which is in line with that set by WHO [22].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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