

# HEART HISTOMORPHOLOGICAL CHANGES OF ADULT WISTAR RATS EXPOSED TO 3, 4 METHYLENEDIOXYMETHAMPHETAMINE PILLS

## ABSTRACT

The Ecstasy pill sold with the trade name 'molly' which has 3,4-methylenedioxyamphetamine (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 animal 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 anaesthesia 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. Heart sections were obtained from the samples 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 significant. 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, Methylenedioxyamphetamine, Heart, Wistar, Rat

## INTRODUCTION

Molly (Ecstasy), with its active component 3, 4-methylenedioxyamphetamine (MDMA), is one of the most popular recreational drugs. Prevalence of lifetime use in the adult population is low (<5%) [1, 2] although in specific groups (e.g. clubbers) last year prevalence of use increases up to 50–60% [3, 4]. As a (psycho) stimulant, MDMA affects the central nervous system by increasing brain concentrations of monoamines, particularly of serotonin, but also norepinephrine and dopamine [5, 6]. These increases are due to several mechanisms of action. Firstly, MDMA inhibits the intracellular uptake of extracellular monoamines by inhibiting monoamine reuptake transporters. In particular the serotonin reuptake transporter (SERT) is inhibited, but also the norepinephrine and dopamine reuptake transporter (NET and DAT). Secondly, MDMA increases the cytosolic concentration of monoamines by decreasing their intracellular vesicular storage. Thirdly, cytosolic monoamines can be transported outwards, because MDMA reverses the transport direction of monoamine reuptake transporters from outside-inside (reuptake), to inside-outside (transporter mediated release), further increasing brain concentrations of monoamines [6]. Subsequently these extracellular monoamines can activate receptors. High extracellular monoamine concentrations, as a result of MDMA use, can overstimulate these receptors. In addition, MDMA itself also modulates receptor function, although mostly at a higher MDMA concentration than necessary for transporter inhibition. However, MDMA does modulate serotonin receptor function at sub-micro molar concentrations [6-8]. Due to these biochemical changes, MDMA increases alertness and also causes entactogenic effects such as warm, euphoric and loving feelings, and an increased closeness to others [9].

However, adverse effects can also occur following MDMA exposure, including an increase in heart rate (tachycardia), blood pressure (hypertension) and body temperature (hyperthermia). Adverse effects range from mild to life-threatening effects and occasionally deaths are reported [10-11]. Based on the prevalence of MDMA use and a registry of MDMA-related health incidents, the risk for moderate to severe effects is estimated at 0.11% (1 in 900 pills) [12]. Although the risk appears low, MDMA is the third most common stimulant drug reported in drug-related emergency department (ED) visits in Europe [1]. Integral worldwide or European figures on MDMA-related incidents are absent. However, the Euro-DEN Plus network, consisting of 31 sentinel centers, reported thousands of MDMA-related ED visits from 2014 to 2017 [13-14], indicating a substantial impact on health care facilities.

The heart is a hollow muscular organ that pumps blood throughout the blood vessels to various parts of the body by repeated, rhythmic contractions. It is found in all animals with a circulatory system, which includes the vertebrates. The vertebrate heart is principally composed of connective tissue and cardiac muscle. Cardiac muscle is an

involuntary striated muscle tissue specific to the heart and is responsible for the heart's ability to pump blood [15].

MDMA users frequently combine MDMA and ethanol; 91% of Australian clubbers (experienced MDMA users) reported concurrent use [16]. Also, the combination of MDMA and ethanol was the second most prevalent combination reported among past year Dutch clubbers, after the combination of ethanol and cannabis [3].

While the risk of combining MDMA and ethanol is mostly unknown, an early review already reported interactions that affected the pharmacokinetic and dynamic properties of MDMA [17]. Also, intoxication and mortality data indicate that combining MDMA and ethanol could worsen health outcome [18-19]. For example, in 70% of MDMA-related ED visits in Europe, ethanol was also used [14]. There are indications that MDMA-related health incidents with concurrent ethanol exposure are more severe compared to lone MDMA incidents [19]. Furthermore, a higher proportion of MDMA and ethanol intoxicated patients at a Belgium dance event required hospital care, compared to lone MDMA intoxicated patients [20]. In addition to recreational MDMA use, its therapeutic potential for ethanol use disorder is under investigation [21]. Consequently, additional populations could be at risk for exposure to a combination of MDMA and ethanol. In view of its, the present study was designed to determine the histomorphological effects of molly on the heart using adult wistar rats as the animal model. The aim of this study is to determine the histomorphological effects of molly on the heart using adult wistar rats as the animal model..

## **MATERIALS AND METHODS**

### **Research Design**

The study was a double blinded experimental study carried out using twenty-five adult albino wistar rats and were sacrificed after 21 days of molly administration. After the administration, the rats were put under light chloroform anaesthesia and the Heart harvested for histological processing. Heart tissues were collected and fixed in 10% formal saline for 24 hours. Heart sections were obtained from the samples. Samples were cut with thickness of 3mm in the cutting- up room. The selected tissues were placed in tissue baskets carefully labeled and processed histologically.

The study was done on twenty-five adult albino wistar rats and were sacrificed after 21 days of molly administration. After the administration, the rats were put under light chloroform anaesthesia and the Heart harvested for histological processing. Heart tissues were collected and fixed in 10% formal saline for 24 hours. Brain sections were obtained from the samples. Samples were cut with thickness of 3mm in the cutting- up room. The selected tissues were placed in tissue baskets carefully labeled and processed histologically.

### **Ethical Consideration**

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].

### **Experimental Animals/Housing Condition**

Twenty-five (25) adult albino Wistar rats having a mean weight of 110.10g were procured from the animal farm, and transferred to the experimental Laboratory at the Histology laboratory of the Department of Medical Laboratory Science where they were allowed two (2) weeks of acclimatization. They were kept in wire mesh cages with tripod that separates the animal from its faeces to prevent contamination. During this period of acclimatization, the rats were fed with growers' mash and water provided ad libitum. The animals were maintained and utilized in accordance with the standard guide for the care and use of Laboratory animals.

### **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.

### **Substance Preparation and Administration**

The drug (molly) was purchased from a government approved pharmacy and diluted to appropriate concentrations

using commercially produced normal saline. Oral consumption of the drug was prepared and given to the animals. The drugs were administered orally using 1.0ml standard syringe for 21 days. Molly was grounded and dissolved as well as diluted to approximate concentration using normal saline. Each of the animals in group B to E were picked at a time with a hand towel and appropriate volumes of the drugs were administered into the animals orally. 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 21 days.

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.

The study was done in two phases; acute and chronic phase.

For the acute phase, two (2) adult albino wistar rats were sacrificed after ten days of molly administration while the remaining three (3) animals were sacrificed after 21 days of molly administration. After the administration, the rats were put under light chloroform anaesthesia and the heart harvested for histological processing.

### **Study Duration**

The preliminary studies, animal acclimatization, ingredient procurement (molly preparation and production), actual animal experiment and evaluation of results, lasted of a period of five month (from August, 2015 to December, 2015). However, the actual administration of molly to the test animals lasted for 21 days.

### **Sample Collection and Analysis**

The weights of the animals were measured before and after acclimatization and similar weight measurements were done at the end of each week and the average weight recorded accordingly. The heart of each rat was obtained at the end of each stage (10 days for acute phase and 21 days for chronic phase) under chloroform anaesthesia and fixed in 10% formalin for histological processing.

### **Histological Processing**

The tissues were been processed using automatic tissue processor according to the standard processing schedule used in the Histology Laboratory. The fixed plastic cassette tissues in 10% formalin was automatically processed by passing them through different grades of alcohol as follows: 70% alcohol for 2hrs followed by 80% alcohol for 2hrs, then 90% alcohol for 2hrs, 90% alcohol for 2hrs, 95% alcohol 2hrs and Absolute alcohol for 2hrs, followed by Xylene I for 2hrs, then Xylene II for 2hrs before passing through Molten paraffin wax 1 for 2hrs and Molten paraffin Wax II for 2hrs. After the last timing, the tissues was removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5°C for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15 minutes), the blocks were then removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed.

The blocks were then trimmed and cut serial sections at 3µm on a rotary microtome. The sections was floated in water bath at 55°C and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were dewaxed, hydrated, air dried and stored in a slide box ready for staining process.

### **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 dibutylphthalate in xylene (DPX). The slides were examined under a light microscope and photomicrographs were taken.

### 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.

### 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.

## RESULTS

**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 marijuana administration

WAMA: weight after marijuana administration

Values are mean ± standard deviation

n: Number of sample

s: significant

n/s: not significant

## Histomorphological Observations

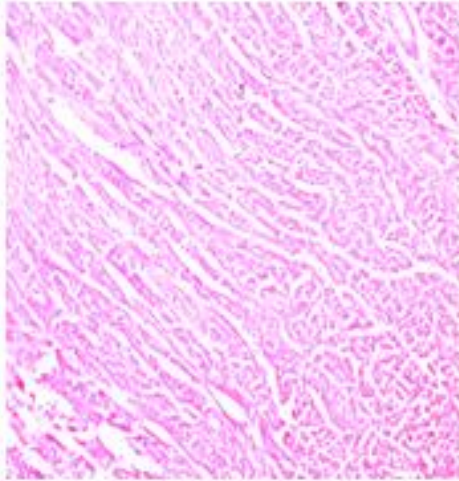


Figure 1a: Control Group showing normal cardiac muscle (H/E x100)

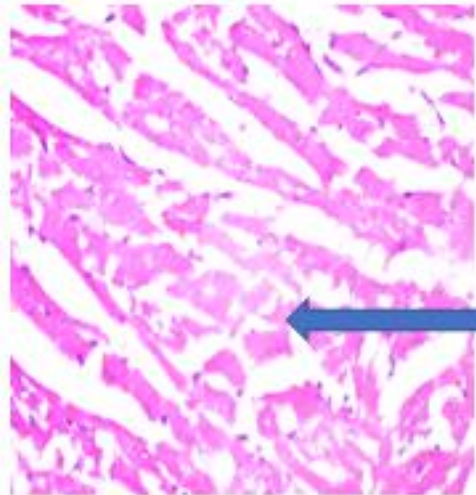


Figure 1b: Control Group showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

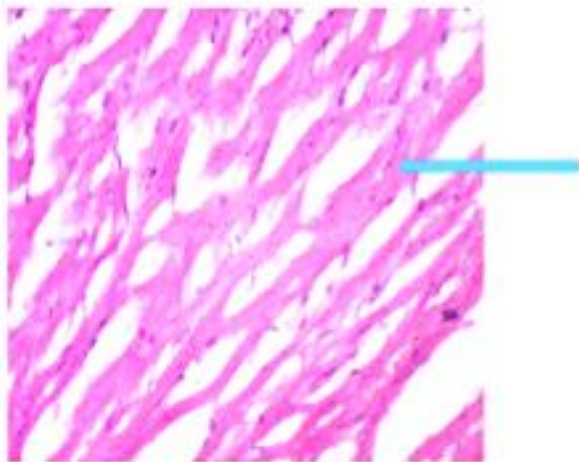


Figure 2a: Group B showing normal cardiac muscle (H/E x100)

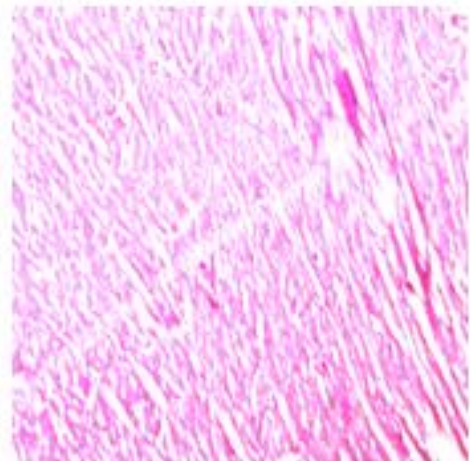


Figure 2b: Group B showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

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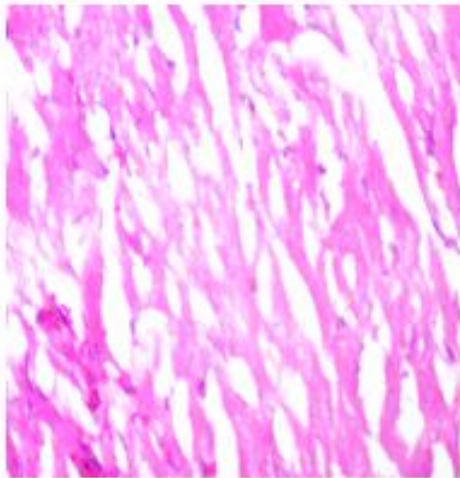


Figure 3a: Group C showing cardiac muscle (H/E x100)

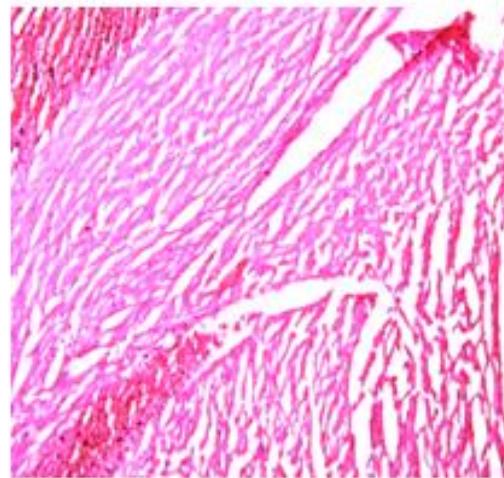


Figure 3b: Group C showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

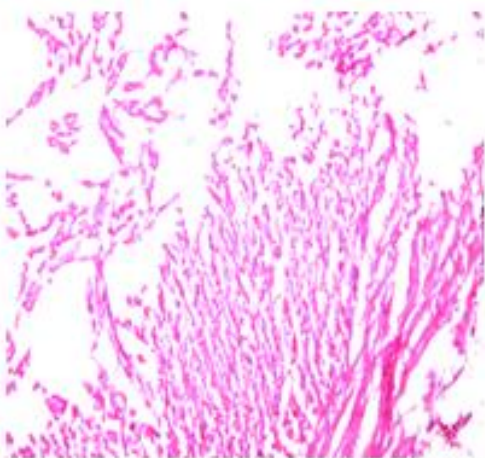


Figure 4a: Group D showing cardiac muscle disarray (H/E x100)

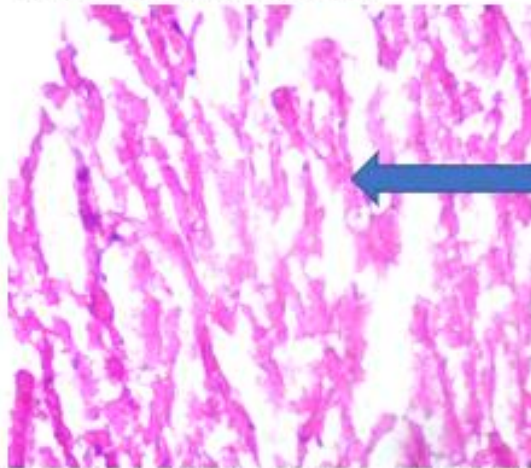


Figure 4b: Group D showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

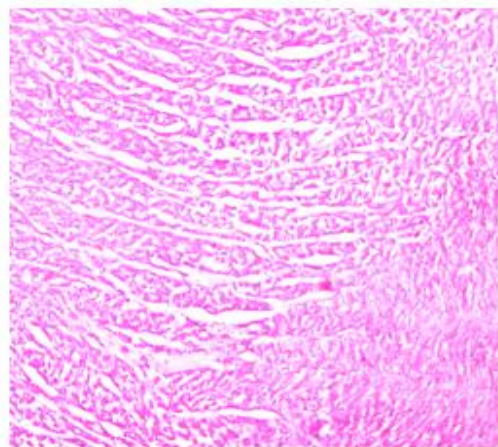


Figure 5a: Group E showing cardiac muscle disarray (H/E x100)

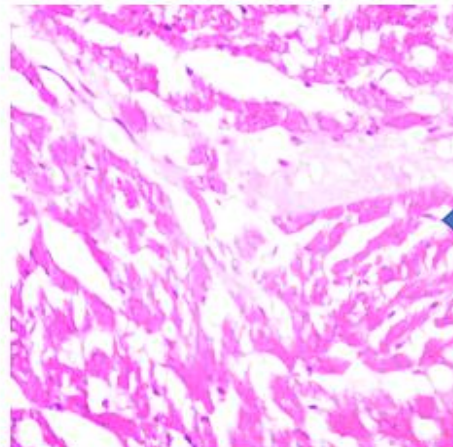


Figure 5b: Group E showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

## DISCUSSION

The heart is a hollow muscular organ that pumps blood through the blood vessels to various parts of the body by repeated, rhythmic contractions. It is found in all animals with a circulatory system. The heart of vertebrates principally made up of connective tissues and cardiac muscles. Cardiac muscle is an involuntary striated muscle tissue specific to the heart and is responsible for the heart's ability to pump blood [15].

The control group, group B to D shows normal cardiac muscle, while Group E shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm and features of Myolysis. However, this staining was properly differentiated with the H and E stains used in the demonstration of general tissue architecture. Histopathological analysis of the control group revealed that the heart had a normal structure, and the cardiac muscle were of significant size.

A recreational MDMA dose mildly increases heart rate and blood pressure in human volunteers [23]. This is largely mediated by increases in epinephrine and norepinephrine blood concentrations shortly after MDMA exposure (307% and 23% respectively) [23]. During high MDMA doses, tachycardia and hypertension can occur, as well as cardiac arrhythmias and myocardial infarction [6, 10]. Acutely, ethanol exposure presumably has a limited effect on blood pressure, although higher doses (>5 drinks) can slightly increase blood pressure. Chronic alcohol abuse can result in hypertension [24-25].

One human study investigated the acute biochemical and cardiovascular effects of concurrent MDMA-ethanol exposure. No additional increases were observed for epinephrine and norepinephrine blood concentrations, blood pressure and heart rate [23]. In addition, two mice studies reported potentiation of the MDMA-induced increase in biomarkers indicative for cardiotoxicity following concurrent ethanol exposure, but also an increase in protective factors [26-27].

It can also be inferred from the results of this experiment that the distortion of the cytoarchitecture of the heart tissues is associated with functional changes that may have been detrimental to the health status of the animals as a result of molly on the heart tissues. However, it has been reported that the extent of severity of tissue damage of a particular compound as toxicant depends on its toxic potentiality on the tissues of organisms. More so, susceptibility to chemical injury varies greatly in the tissues and cells of the same animal. It is sometimes greater in different animal groups. In addition, the location of the major damage may be determined by the mode of action of the chemical. The mode of action of each poison and the pattern of tissue vulnerability has been well defined and the toxic level of each agent at which a fairly standard distinctive pattern of tissue damage has been studied.

## 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.

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