

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

## ABSTRACT

1. This study was carried out to determine the histomorphological effects of molly on the heart using adult wistar rats as the **animal** model. (Mammalian model)
2. The study was done on twenty-five adult albino wistar rats **whih**
3. the rats were put under light chloroform **anaesthesia** (spelling error)
4. and the Heart harvested for histological processing. Heart sections were obtained from the samples and processed histologically.  
The experimental animals were separated into five groups. Each group contained five rats each using 5 big cages to house them. (Reframe it) A-----E mention dose groups
5. The mean weight of rats in all groups before the period of molly administration **were significant**.(what significant?)

## INTRODUCTION

6. **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.**  
(Avoid repetition)

## MATERIALS AND METHODS

### Research Design

7. 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**, (after completing experiment)
8. the **rats were put under light chloroform anaesthesia** and the Heart harvested for histological processing.
9. **Heart tissues were collected and fixed in 10% formal saline for 24 hours.** **Heart sections were obtained from the samples.** (mention HE staining protocol summary)

**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. (3 mm or 3 nm.....?)

10. The study was done on twenty-five adult albino wistar rats and were sacrificed after 21 days of molly administration. (Avoid repetition)
11. After the administration, the rats were put **under light chloroform** (Avoid repetition)  
**anaesthesia** and the **Heart harvested for histological processing.** **Heart tissues were collected and fixed in 10% formal saline for 24 hours.** (Avoid repetition)
12. **Brain sections** .....

**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. (Avoid repetition)

### 13. 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. (that paragraph should be at the start of methodology)

### 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. (that paragraph should be second of methodology)

### Substance Preparation and Administration

14. The drug (molly) was purchased from a government approved pharmacy and diluted (how much diluted, mention concentration, dose)

15. 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. (Mention dose and avoid repetition)

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.

16. 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. (justify dose variation)

17. For the acute phase, two (2) adult albino wistar rats were sacrificed after ten days (not mentioned in abstract)

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.

### 18. 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. (avoid such detail in paper, it is thesis requirement not for paper)

### Sample Collection and Analysis

19. 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. (How many time that statement is repeated)

### Histological Processing

20. The tissues were been processed using automatic tissue processor according to the standard processing schedule used in the Histology Laboratory. (That protocol add with previous portion)...once

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 1 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.

21. 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**

22. 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. ....do you use Canada balsam

**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

23. WBMA: weight before marijuana administration (that name not mentioned in abstract and material and methods)

WAMA: weight after marijuana administration / may be copy paste error

Values are mean ± standard deviation

n: Number of sample

s: significant

n/s: not significant

24. Figs not clear, magnify at 1000X if possible, use coraldraw for labelling

25.

I. Fig 1a label parts with same size and color of arrow

II. Fig 2a, 3a are not 100X, it is 400X .....compare its size of nuclei with 1b (400x)

III. Fig 2b, 3b are 100X, not 400X .....compare its size of nuclei with 1a (100x)

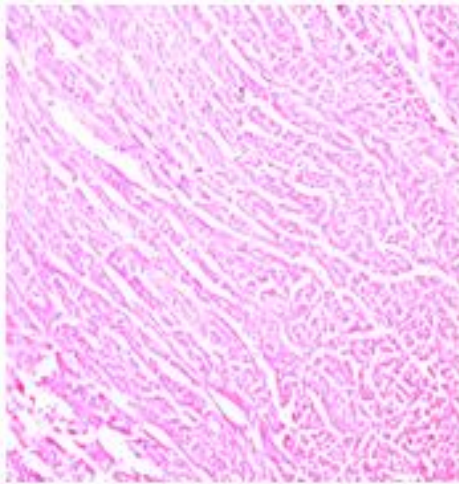


Figure 1a: Control Group showing normal cardiac muscle (H/E x100)

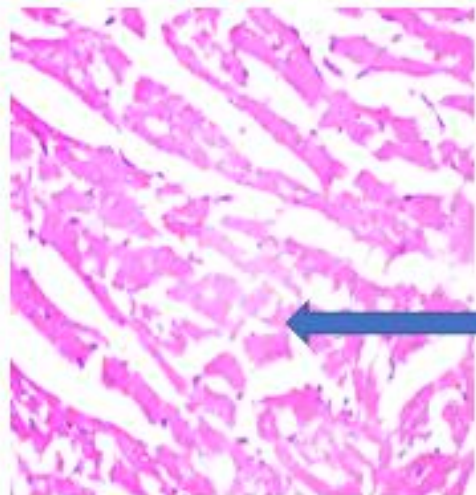


Figure 1b: Control Group showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

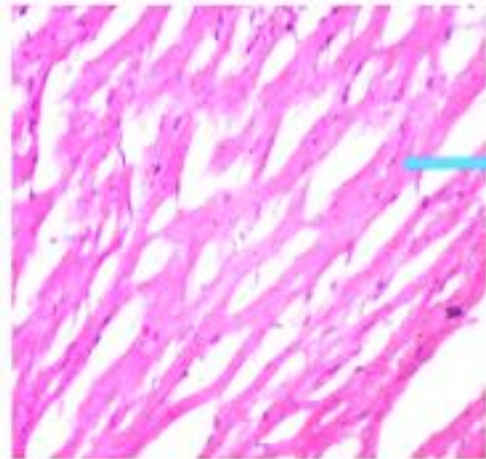


Figure 2a: Group B showing normal cardiac muscle (H/E x100)

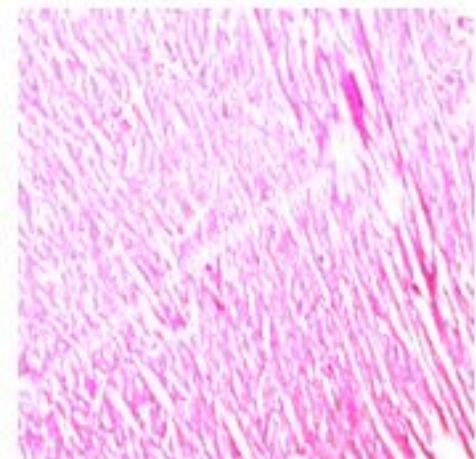


Figure 2b: Group B showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

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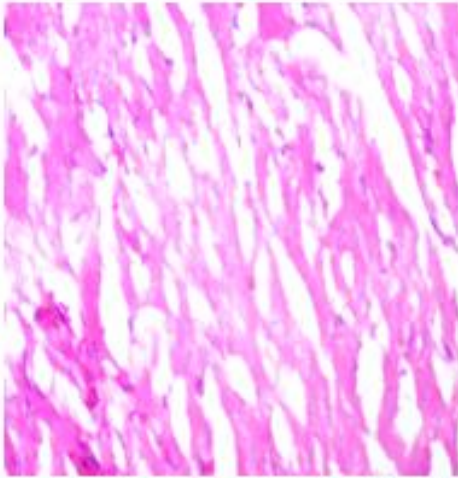


Figure 3a: Group C showing cardiac muscle (H/E x100)

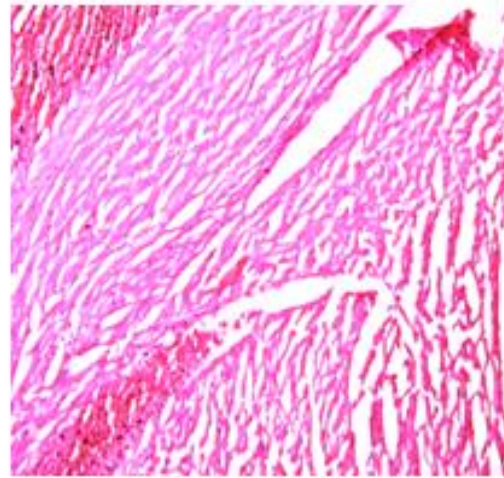


Figure 3b: Group C showing section of cardiac muscle shows myocytes (arrow) with normal peripherally placed nuclei surrounded by eosinophilic cytoplasm. Normal Heart Muscle (H/E x400)

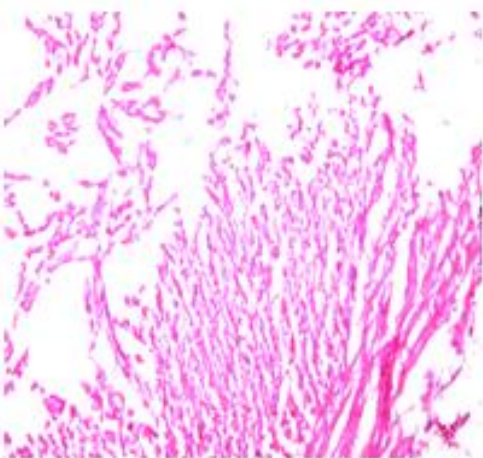


Figure 4a: Group D showing cardiac muscle disarray (H/E x100)

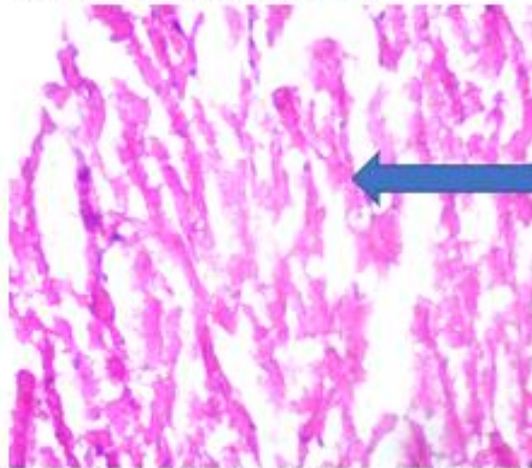


Figure 4b: Group D showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

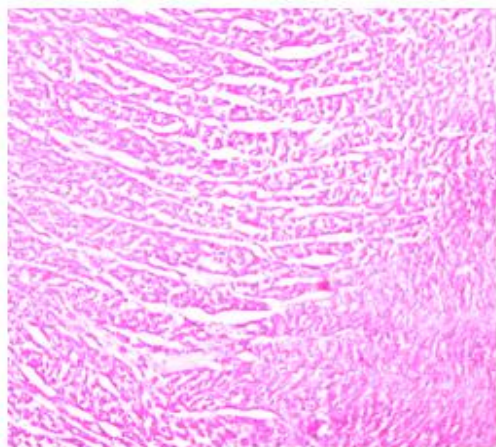


Figure 5a: Group E showing cardiac muscle disarray (H/E x100)

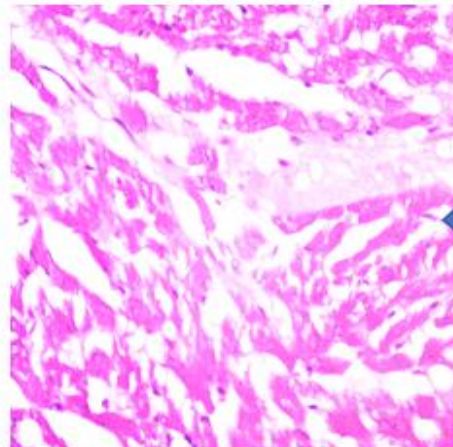


Figure 5b: Group E showing section of cardiac muscle shows fragmented myocytes with karyolysis of nuclei surrounded by eosinophilic cytoplasm. Features of Myolysis (H/E x400)

## DISCUSSION

26. One human study/? investigated the acute biochemical and cardiovascular effects of concurrent MDMA-ethanol exposure.

27. two mice studies/? reported potentiation of the MDMA-induced increase in biomarkers indicative for cardiotoxicity following concurrent ethanol exposure,

28. Add list of abbreviation

29. Add recent reference from 2020 to 2023

## REFERENCE

Schürmann L, Croes E, Lameijer M, Valkenberg H. Monitor drugsincidenten 2018 [Monitor drugs incidents 2018]. Trimbo Institute. Dutch, 2019. Page no, volume no

UNDER PEER REVIEW