

## Original Research Article

HAEMATOLOGICAL AND HISTOPATHOLOGICAL OBSERVATIONS IN ADULT WISTAR RATS ADMINISTERED WITH *Momordica charantia* (Bitter Melon) ~~AQUEOUS-AQUEOUS~~ LEAF EXTRACT

Comment [MB1]: wouldn't it be interesting to say it's acute toxicity?

### ABSTRACT

This research work was carried out to investigate the effect of the aqueous leaf extract of *Momordica charantia* on some ~~haematological~~~~hematological~~ parameters and ovaries of adult Wistar rats. Twenty five adult Wistar rats were grouped in to five (Control and A,B,C,D) and fed with saline, 100mg/kg, 200mg/kg, 400mg/kg and 800mg/kg of aqueous leaf extract of the plant ~~respectivbely~~~~respectively~~ for 30 days. Blood was collected for ~~haematological~~~~hematological~~ parameters and ovaries harvested for histopathological studies. This study was carried out partly at the Department of Medical Laboratory Science, University of Benin, Edo State, Nigeria between October and December 2020 with ethical approval number v.1041/55. The results of the work showed that there was significant decrease in packed cell volume (PCV), and red blood cell count (RBC) compared with the control ( $p < 0.05$ ) while dilation of blood vessels and thickening of tunica media of arteries were observed in ovaries of the rats. It was concluded that aqueous leaf extract of *Momordica charantia* has ~~haematologica~~~~hematologica~~ and histomorphological effects on adult Wistar rats which could lead to ~~anaemia~~~~anemia~~ and malnourishment of ovarian cells.

Comment [MB2]: what parameters?

**Keywords:** *Momordica charantia*, packed cell volume, red blood cell count, blood vessel, histopathology

## 1. INTRODUCTION

*Momordica charantia* commonly called bitter melon or bitter gourd is a one of the plants with wide application in complementary medicine and widely distributed in the tropics and subtropics (1, 2). It is also widely available in Asian countries (3). The plant was reported to be rich in nutritional elements and prevents various forms of diseases (4, 5).

Comment [MB3]: Which countries?

Comment [MB4]: Which diseases?

*Momordica charantia* was reported to have cytotoxic effect of *Plasmodium falciparum* trophozoites thus inhibiting their development to the schizont stage (6). Earlier investigations had confirmed the larvicidal property of *M. Charantia* against three mosquito species, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (7).

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

The weight reduction effect of *Momordica charantia* has also been investigated (8). This was attributed to reduction in metabolic activities said to be induced by inhibition of enzyme activities (9).

Comment [MB5]: at what concentrations? What was tested? leaf extract? Seed extract? attention to the taxonomic norms for the species, always in italics.

*Momordica charantia*'s effects on certain organs of the body has been investigated and found to have some toxicity effect on organs like liver and kidney of adult Wistar rats (10). Seeds of the plant have also been reported to have antireproductive effect on adult Wistar rats (11).

Comment [MB6]: ??

While several studies have reported the effect of aqueous leaf extract of *M. Charantia* on some haematological parameters and some organs, there exist paucity of information on its effect on packed cell volume, red blood cell count and ovaries. This study therefore wants to add to knowledge on the physiological and histopathological effects of aqueous leaf extract of the plant on the named blood parameters and ovaries of adult Wistar rats.

Comment [MB7]: Dear authors, I believe that you can improve the introduction, bringing more data about the bibliographic review. The work has a lot of potential, but needs to be better introduced.

## 2. MATERIAL AND METHODS

### 2.1 PLANT COLLECTION

Fresh leaves of *Momordica charantia* were harvested in a local garden at Alagbado area, off Eyenkorin, Ilorin, Kwara State and taken to the Herbarium unit of the Department of Plant Biology, University of Ilorin, for identification and authentication with number UILH/001/963/2020. The leaves were dried away from direct sun for one week.

#### 2.1.1 PLANT EXTRACTION

Soaking method was used for the extraction (12). *Momordica charantia* dry leaves were soaked for 48hr in a conical flask plugged with cotton wool. The mixture was filtered, evaporated over water bath, and kept inside hot air oven at 30°C-30°C until a semi solid substance was obtained.

## 2.2 EXPERIMENTAL ANIMALS

### 2.2.1 PROCUREMENT AND TREATMENT

Wistar rats weighing between 180-200g body weights were purchased from the Animal House of the Department of Anatomy, University of Benin, Benin City. The Wistar rats were allowed to acclimatize for 2 weeks where feed and water were administered without restrictions. The International Guidelines For Animals handling was observed in the course of this study.

Comment [MB8]: Please, more details.

### 2.2.2 PREPARATION AND ADMINISTRATION OF EXTRACT

The extract of *Momordica charantia* was weighed by using a digital balance which measured in grams. The weighed extract was transferred to well labeled, small sized plastic bottles. The bottles were labeled according to the predetermined dosage of *Momordica charantia*. The corresponding weights were placed in each bottle with matching labels and reconstituted in 1ml of distilled water. The rats were subsequently administered with the extract of *M. charantia* through oral gavage while giving them their normal feed and drinking water ad libitum.

### 2.2.3 ACUTE TOXICITY

This was done in two phases. In the first phase, nine Wistar rats were grouped into three comprising three each. They were administered 10, 100 and 1000 mg/kg of *M. charantia* leaves extract respectively and placed under observation for 24 hr. Their behaviours were observed as well as mortality. No mortality was recorded at the end of this phase.

Formatted: Highlight

In the second phase, three Wistar rats were distributed into three groups of one animal each and administered 1600, 2900 and 5000 mg/kg of *M. charantia* leaves extract and then observed for 24 hr for behavioural changes and mortality (13, 14). No mortality was observed at the end of this phase.

Comment [MB9]: Has this methodology already been reported?

### 2.2.4 EXPERIMENTAL DESIGN

Twenty five Wistar rats were randomly distributed into 5 groups including the Control and experimental groups after 2 weeks acclimatization as follows:

Control group: Consisting of 5 rats were given 1 ml of normal saline daily through oral gavage. The rats in this group were sacrificed at the end of the experiment (30 days).

Group A: Consisted of 5 rats and were given 100 mg/kg body weight of aqueous extract of *Momordia charantia* and sacrificed at the end of one month.

Group B: Consisted of 5 rats and were given 200 mg/kg body weight of aqueous extract of *Momordia charantia* and sacrificed at the end of one month.

Group C: Consisted of 5 rats and were given 400 mg/kg body weight of aqueous extract of *Momordia charantia* and sacrificed at the end of one month.

Group D: Consisted of 5 rats and were given 800 mg/kg body weight of aqueous extract of *Momordia charantia* and sacrificed at the end of one month.

### **2.3 COLLECTION OF SPECIMENS**

Cardiac puncture under anesthesia was employed to collect blood samples into Ethylene Diamine Tetraacetic Acid (EDTA) specimen bottles for the evaluation of Full Blood Count (FBC). Ovaries were removed at necropsy and fixed immediately in 10% neutral buffered formalin (NBF).

### **2.5 TISSUE PROCESSING**

All organs were examined for gross lesions. Small pieces of tissues were taken at necropsy. The tissues were processed in an automatic tissue processor (LEICA, 2000 Frankfurt, Germany). Tissues were dehydrated through ascending grades of alcohol, cleared in xylene, impregnated in molten paraffin wax and finally embedded in molten paraffin wax. They were trimmed at 0 $\mu$  and sectioned at 5 $\mu$  using a Rotary Microtome (LEICA RM 2125). The sections were stained by Mayer's haematoxylin and eosin (H&E) staining technique for microscopic assessment. The stained sections were examined microscopically using Olympus Microscope.

### **2.6 HAEMATOXYLIN AND EOSIN STAINING TECHNIQUE**

Sections of testes and ovaries were taken to water, hydrated in descending grades of alcohol and water. Hydrated sections were stained in Mayer's haematoxylin for 10 min, rinsed in water and differentiated in 1% acid alcohol. Sections were rinsed in water, allowed to blue for 10 min and counterstained in 1% aqueous eosin for 2 min. Stained sections were rinsed in water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in Dibutylphthalate Polystyrene Xylene (DPX). Stained sections were examined for histopathological changes using Olympus Microscope.

### **2.7 FULL BLOOD COUNT**

The blood samples were collected through cardiac puncture into EDTA bottles for each rat and analysed using the Sysmex automated machine (Sysmex Autoanalyzer, Koba, Japan) for Haematocrit, Platelets, Red blood cell (RBC) and White blood cell (WBC).

### **2.8 STATISTICAL ANALYSIS**

The data obtained from these studies were analyzed with Statistical Products and Service Solution (SPSS) software, Version 20. One-way analysis of variance (ANOVA) was used to compare mean difference between and within the groups complemented with post-hoc test. The differences were considered statistically significant at  $p < 0.05$ . Data were expressed as mean and  $\pm$ SEM (standard error of mean). Data were presented in tables.

### 3. RESULTS AND DISCUSSION

#### 3.1 HAEMATOLOGICAL PARAMETERS

Results showed that there was a significant effect of *M. charantia* on haematocrit (PCV) value of Wistar rats, red blood cell (RBC) count and white blood cell (WBC) count when compared with the control ( $p < 0.05$ ). There was no significant effect on platelets count. There was a significant reduction in the haematocrit (PCV) and red blood cell count (RBC) of Wistar rats treated with aqueous extract of *Momordica charantia* for 30 days at the dosage of 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg respectively when compared with the control ( $P < 0.05$ ) (Table 1).

There was a dose dependent increase in the white blood cell count (WBC) of Wistar rats at 100, 200, 400 and 800 mg/kg administered with extract of *Momordica charantia*. The increase observed for 100 mg/kg and 800 mg/kg was however statistically significant while that of 200 mg/kg and 400 mg/kg was not significant when compared with the control ( $P < 0.05$ ) (Table 1). There was also an observed reduction in platelet count for rats administered 100, 200, 400 and 800 mg/kg of *Momordica charantia* but the reduction was not statistically significant ( $P > 0.05$ ) as shown in Table 1. )

**Table 1: Dose –dependent Impact of *Momordica charantia* leaf extracts on some Haematological parameters of Wistar rats.**

Groups	PCV (%)	W.B.C. ( $10^9/L$ )	R.B.C. ( $10^{12}/L$ )	Platelets ( $10^9/L$ )
Control (1ml of saline)	49.66	10.06	8.55	1008
(*P-values)	0.00	0.00	0.02	0.051
A (100mg/kg)	39.44 $\pm$ 2.1	10.60 $\pm$ 0.2	6.95 $\pm$ 0.2	537.80 $\pm$ 70.5

Comment [MB10]: Table formatting could be improved

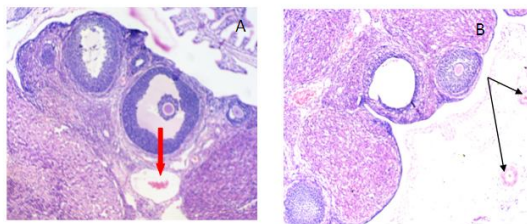
B (200mg/kg)	42.02 ± 1.3	11.68 ± 0.2	7.55 ± 0.4	472.80 ± 219.5
C (400mg/kg)	42.08 ± 0.5	14.24 ± 0.5	7.63 ± 0.1	417.20 ± 9.9
D (8000mg/kg)	41.24 ± 1.2	16.86 ± 0.1	7.34 ± 0.1	458.60 ± 190.6

\*P-values were expressed as Mean ± SEM for five animals (n=5) in each group after treatment with the extract of *Momordica charantia* for one month.

### 3.2 OVARIES

Sections of ovary of rats given 100 mg/kg body weight of *Momordica charantia* extract showed secondary follicles suspended in a dense connective tissue stroma, composed mainly of collagen connective tissues. The ovarian stroma surrounding a mature Graafian follicle is composed of spindle –shaped, fibroblast-like cells and delicate collagen fibres admixed with ground substance. The oocyte of the Graafian follicle was seen detached from the zona granulosa by a thick layer of cumulus oophorus but closely knitted by the corona radiata. The zona pellucida was clear and distinct. The follicular antrum was wide and amorphous.

Sections also showed mature corpus luteum with eosinophilic cytoplasm and large nuclei. There were numerous vesicular spaces between the luteal cells. A wide and oedematous blood vessel with few red blood cells was identified between two corpus luteum (Fig. A). Sections of ovary of rats given 200 mg/kg body weight of *Momordica charantia* extract showed many corpus luteum with vesicular spaces. One of the follicles seen presented with a large cystic space. Arteries with thick tunica intima and few red blood cells were also identified (Fig. B).



A: Ovary at 100mg/kg showing dilated blood vessel (red arrow) and B: Ovary at 200 mg/kg showing arteries with thick tunica media (black arrows). Mg X 100

Figure A & B. Ovaries of adult Wistar rats showing blood vessel response to *M. Charantia* administration.

*Momordica charantia* has become attractive as a source of complementary medicine especially for its antimalaria and ~~abortifacient~~ abortifacient effect among rural dwellers in sub Saharan Africa where malaria is endemic and sexual activity

high especially among the young population which could have been a reason for its reported abuse (Umar IM, Unisersity of Benin, Nigeria, Unpublished results).

It has been proven that *Momordica charantia* has the capability to arrest the development of malaria parasites from the trophozoid to the schizont stage (10). One of the consequences of malaria disease is **anaemia** (2). Although oral consumption of *Momordica charantia* has been reported to be safe (14), the significant reduction of the red blood cell count (RBC) and packed cell volume (PCV) observed from our investigation raises concern as reduced PCV is an indication of anaemia (16). The low PCV and RBC observed in this study may be attributed to the inability of *M. Charantia* to stimulate erythropoietin production in the liver (8). Traditional practitioners should therefore endeavour to supplement treatment of malaria using *Momordica charantia* with **haematinics** and vitamins if studies in humans corroborate this finding of ours **so as to** boost the low values of PCV and RBC count.

Blood supplies to organs are limited when there is **anaemia** (16). The dilation of blood vessels and thickening of the tunica media observed in the ovaries in this study could have been an indirect implication of the low RBC and PCV previously observed. Organs and tissues respond to compensate for reduction in volume of blood. One of such ways is dilation of blood vessels and thickening of the tunica media of the arteries (16). When blood supply to the organ is reduced, nutrients available is also reduced which could lead to cellular dysfunction and hormonal imbalance. Cellular dysfunction in the ovaries could have negative reproductive implications (17).

The level of white blood cell count (WBC) was significantly elevated in this study especially at the dosage of 100mg/kg and 800 mg/kg. When WBC level is raised, stress, infection, inflammation and trauma are indicated. White blood cells are the body's first line of action against infection or foreign aggression justifying their roles in the immune system. They ensure that the invading substance is attacked and eliminated before it manifests into any disease (15).

#### 4. CONCLUSION

Consumption of *Momordica charantia* has reducing effect on the red blood cell count and packed cell volume of adult Wistar rats. It also has a dilating effect on blood vessels of ovaries of the rats. Care should therefore be exercised in its abusive use so as not to induce severe **anaemic** conditions with tissue effects especially ovaries.

**Comment [MB11]:** Why were the concentrations so high? I suggest that in the future, a minimum inhibitory concentration is performed.

**Comment [MB12]:** high

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

## REFERENCES

1. Sofowara A. Medicinal plants and traditional medicine in Africa. 2nd Edition. Spectrum. Books Limited, Sunshine House, Ibadan, Nigeria. 1993;289.
2. Adedeji GT, Ojulari SL. Effect of *Momordica charantia* (Bitter melon) Leaves on Haemoglobin Concentration in Male Albino rats. *International Blood Research and Reviews*. 2014;2(2):82-86.
3. Ahadina R, Shishir AS, Sabrina, HS, Hosne J.S, Pritesh R D. A comprehensive review of *Momordica charantia* L. (Karela). *International Journal of Botany Studies*. 2018; 3(1):95-102.
4. Namsa ND, Mandal M, Tangjang S, Mandal, SC. Ethnobotany of the Monpa Ethnic Group at Arunachal Pradesh. *Indian Journal of Ethnobiology and Ethnomedicine*. 2011; 17:31-37.
5. Farhan S., Muhammad A, Bushra N, Muhammad UA, Tabussam T, Muhammad BH, Ahsan J. Bitter melon (*Momordica charantia*). A natural healthy vegetable. *International Journal of Food Properties*. 2018;21(1):1270-1290.
6. Grace IO, Olusola O, Adegboyega OA, Obasola EF, Neena V, Isaac, O.. In vitro studies on the sensitivity pattern of *Plasmodium falciparum* to anti-malarial drugs and local herbal extracts. *Malaria Journal*. 2014;13:63 <https://www.malariajournal.com/content/13/1/63>
7. Singh RK, Dhiman RC, Mittal PK. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). *Journal of Vector Borne Diseases*. 2006;43:88-91
8. Husna NR, Nuriham HN, Azizah AH, Aman FO. Acute Oral Toxicity Effects of *Momordica Charantia* In Sprague Dawley Rats. *International Journal of Bioscience, Biochemistry and Bioinformatics*. 2013;3(4):408-410.
9. Okunji C, Komamytsky S, Fear G, Poulev A, Ribnicky DM, Awachie PI, Raskinn I. Preparatory isolation and identification of tyrosinase inhibitors from the seeds of *Garcinia kola* by high speed counter current chromatography. *Journal of Chromatograph*. 2007;1151:45-50.

10. Ampa L., Watchrar K., Pichet S. Effect Of *Momordica charantia* Linn. Leaf and fruit juice on fertility in male mice. *International Journal of Phytomedicine*. 2015;7:199-204.
11. AOAC. *Official Methods of Analysis*. 2000; 17th Edition, The Association of Official Analytical Chemists, Gaithersburg; MD, USA.
12. Lorke D. A new approach to practical acute toxicity testing. *Archive of Toxicology*. 1983;54:75-87.
13. Enevide C., David A., Fidelis S. A New Method for Determining Acute Toxicity in Animals Models. *Toxicology International*. 2013;20(3):224-226.  
[https://Doi:10.4103/0971-6580.121674](https://doi.org/10.4103/0971-6580.121674)
14. Nkechi E., Priscillia O., Ntekim O., Amos S. *Momordica Charantia* (Bitter melon): Safety and Efficacy During Pregnancy and Lactation. *International Journal of Studies in Nursing*. 2018; 3(2):140-145.
15. Umar IM. Effects of oral administration of *Momordica charantia* on haematological parameters of adult albino rats. *Bayero Journal of Pure and Applied Sciences*. 2017; 10(2):52-56.
16. Metivier F., Marchais S., Guerin A., Pannier B., London G. Pathophysiology of anaemia: Focus on the heart and blood vessels. *Nephrology, dialysis, transplantation official publication of the European Dialysis and Transplant Association - European Renal Association*. 2000; 15(3):14-18. [10.1093/oxfordjournals.ndt.a027970](https://doi.org/10.1093/oxfordjournals.ndt.a027970).
17. Seung JC, Zobia U, Mee-Sup Y. Premature Ovarian Insufficiency: Past, Present and Future, *Frontiers In Cell And Developmental Biology*. 2021;9(672890):1-13