Journal of Advances in Medical and Pharmaceutical Sciences

21(4): 1-8, 2019; Article no.JAMPS.52574
ISSN: 2394-1111

The Immuno-modulatory and Thrombocytopaenic Effects of the Varying Concentrations of the Aqueous Leaf Extract of *Moringa Oleifera* in Male Albino Wistar Rats

Ojeka Sunday Ogbu[1*] and Zabbey Victor Zigabelbari[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OSO conceived the study, designed the protocol and coordinated the experiment while the animal feeding, laboratory procedures, manuscript writing, statistical analysis and data interpretation were performed by author ZVZ. Both authors read through and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2019/v21i430141
Editor(s):
(1) Dr. Julius Olugbenga Soyinka Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
Reviewers:
(1) Maria Bintang, Bogor Agriculture University (IPB University), Indonesia.
(2) Nduka, Florence Obiageli, Federal College of Dental Technology and Therapy, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52574

Original Research Article

Received 05 September 2019
Accepted 09 November 2019
Published 19 November 2019

ABSTRACT

Introduction: *Moringa oleifera* and related species are commonly used in folk medicine for various human diseases.
Aim: The study was undertaken to establish the thrombocytopenic effect of the aqueous leaf extract of *moringa oleifera* and to find the utilization of platelet parameters in determining the cause of the thrombocytopenia.
Methodology: Fresh leaves of moringa were dried and extracted with water. Thirty (30) male albino Wistar rats, weighing between 150-250 g, which were kept under uniform laboratory conditions, were randomly divided into five (5) groups (A-E), based on their weights. The control group (group A) was orally given 0.5 ml of distilled water while the treatment groups (groups B to E) were orally given 250 mg/kg, 450 mg/kg,650 mg/kg and 850 mg/kg body weight respectively of the extract, once a day, for 56days and then sacrificed. At the end of the administration, blood samples were

**Corresponding author: E-mail: sunday.ojeka@uniport.edu.ng;*

collected from each rat and examined for platelet indices. The effects of treatment with aqueous leaf extract of *moringa oleifera* on the platelet parameters were compared with the control group.
Result: The rats treated with the extract, showed a decrease in platelet count and platelet crit while there was a significant increase in the platelet distribution width, mean platelet volume and immature platelet fraction, concerning the control.
Conclusion: The aqueous leaf extract of *moringa oleifera* is therefore shown to modulate the immune system and cause thrombocytopaenia, through platelet destruction.

Keywords: Moringa; platelet; thrombocytopaenia; blood; immuno-modulatory.

1. INTRODUCTION

Thrombocytopenia (TCP) refers to a disorder in which there is a relative decrease of platelets, present in the blood [1]. A normal human platelet count ranges from 150,000 to 450,000 platelets per microliter of blood and thrombocytopenia is said to be a platelet count below 50,000 per microliter [2]. The causes of thrombocytopenia include decreased platelet production, increased plateletdestruction and splenic sequestration/ abnormal pooling, based upon the causative process [3,2,4]. It is one of the common causes of abnormal bleeding and characterized by spontaneous bleeding from the skin, arms, nose, gums and other mucous membranes [2,4]. In many cases of thrombocytopenia, large platelet was seen in the peripheral smear, this size and other platelet parameters were suggested to help in deciding the category of thrombocytopaenia [5]. The present study was undertaking to establish the thrombocytopenic effect of the aqueous extract of *moringa oleifera* leaf and to find the utility of platelet parameters in determining the cause of the thrombocytopenia.

2. MATERIALS AND METHODS

Thirty male albino Wistar rats weighing between 120g-250 g were used for the experimental work. The animals were obtained from the animal care facility at the University of Portharcourt. The animals were housed in a wooden cage made of five (5) different compartments and the rats were placed in the cage and grouped into five (5) groups. The animals were allowed to acclimatize for fifteen days, to observe for any signs of illness before the experiment started. They were kept under standard laboratory conditions in a well ventilated standard housing condition and clean wooden rat cage with proper bedding (sawdust). The animals were properly fed with tap water and standard rat feed that contains groundnut, wheat brand, maize grains, palm kernel and fish meal, bought from the animal feed store in Choba. The feeding and water troughs were thoroughly cleansed daily to ensure proper hygiene and healthy living condition. The animal bedding was prepared with sawdust particles, obtained from a sawmill. These beddings were changed regularly to ensure a healthy environment for the animals.

The rats were randomly grouped into five (5) groups (groups A-E), comprising six rats in each group. A calculated amount of the aqueous extract of moringa was constituted in 20 mls of distilled water to give doses of 250 mg/kg to 850 mg/kg body weight. Administration of the aqueous leaf extract of *moringa oleifera* was performed orally once daily, between 7.30 am and 9.30 am, using a 2 ml syringe. The various groups were administered as follows:

A) Group A served as the control, with no extract being administered; instead, 2ml of distilled water was given

B) Groups B, C, D and E received 2 ml of the moringa extract, using a syringe from a 250 mg/kg for group B,450 mg/kg for group C,650 mg/kg for group D and 850mg/kg for group E.

These administrations were carried out in the space of 56 days after which the animals were sacrificed and the blood samples collected in an EDTA bottle. The blood samples were collected, using the method of cardiac puncture, after each rat has been anaesthetized in a desiccator, using diethyl ether.

The *Moringa* leaves were shaded, dried at a warm temperature (not directly under the sun), before tasking it for the preparation of the extract. The leaves were separately rinsed in clean water to remove dirt, dried at room temperature for 14 days. 500 g of the plant material was introduced into an extraction jar. 1.2 litres of sterile distilled water was added into it and corked, kept at room temperature and shaken at an interval of 30 minutes (with a mechanical shaker). It was filtered after 24 hours, the discarded material and

the filtrate concentrated using the rotary evaporator in a vacuum. The paste collected and air-dried and weighed. The percentage yield was calculated and the extract stored at -4°c in the refrigerator for photochemical studies on the animals. Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures, to identify the constituents as described by Sofowara (1993), Trease and Evans (1919) and Harborne (1973).

2.1 Statistical Analyses

The results were subjected to statistical analysis using statistical package for social sciences (SPSS) version 20.0. Data are presented as mean ± SEM. The difference of means was considered significant at P value less than 0.05.

3. RESULTS

The results of the qualitative phytochemical analysis indicate that alkaloids were most abundantly present while tannins, saponins, Salkowski, free anthraquinone, flavonoids were moderately present. Steroids, Phlobatanins, combined anthraquinone, Lierberman's and Keller kiliani were only slightly present while Cyanogenetic glycosides were observed to be absent (Table 1).

The results of the quantitative phytochemical analysis indicate the presence of Polyphenols, flavonoids, tannins, alkaloids and glycosides in the following percentages: 2.70, 4.10, 8.00, 15.00, and 2.50%, respectively (Table 2). The effects of the graded doses of *moringa oleifera* on platelet indices were also determined (Table 3).

The result of M. oleifera extract on platelet indices shows a significant ($P < 0.05$) decrease in the level of platelet count for the high dose administered when compared with the control. A non-significant reduction in the level of platelet count was seen for the 250, and 450 and 650 mg/kg doses administered when compared with the control (Fig. 1).

Table 1. Qualitative phytochemical analysis of aqueous leaf extract of *Moringa oleifera* leaf in Wistar rats

Phytochemical	Observation	Inference
Alkaloid	+++	Heavily present
Tannins	++	Moderately present
Saponins	++	Moderately present
Flavonoids	++	Moderately present
Steroids	+	Slightly present
Phlobatanins	+	Slightly present
Combined anthraquinone	+	Slightly present
Free anthraquinone	++	Moderately present
Cyanogenetic glycosides	-ve	Absent
Salkowski	++	Moderately present
Lierbernnans	++	Slightly present
Keller kiliani	+	Slightly present

*+, slightly present; ++, moderately present; +++, heavily present; −ve, absent; *, Significant at P < 0.05 when compared to control*

Table 2. Quantitative phytochemical analysis of aqueous leaf extract of *Moringa oleifera* leaf in Wistar rats

Phytochemical	Percentage abundance (%)
Polyphenols	2.70
Flavonoids	4.10
Tannins	8.00
Alkaloids	15.00
Glycoside	2.50
Polyphenols	2.70

**, Significant at P < 0.05 when compared to control.*

Table 3. Effects of the graded doses of *Moringa oleifera* on platelet indices

Groups	PLT(x10^{11}/l± sem)	PCT (ml/L ± sem)	MPV (fl ± sem)	PDW (%± sem)	IPF (%± sem)
Control	7.44 ± 0.33	5.20 ± 0.15	7.02 ± 0.22	15.08 ± 0.09	1.48 ± 0.36
250 mg/kg	7.07 ± 0.56	5.20 ± 0.44	7.25 ± 0.10	15.17 ± 0.08	1.70 ± 0.21
450 mg/kg	6.27 ± 0.31	4.77 ± 0.22	7.50 ± 0.10*	15.22 ± 0.07	2.08 ± 0.18
650 mg/kg	6.20 ± 0.50	4.80 ± 0.33	7.37 ± 0.12	15.13 ± 0.06	2.35 ± 0.21*
850 mg/kg	6.16 ± 0.42*	5.05 ± 0.28	7.83 ± 0.02*	15.12 ± 0.04	2.28 ± 0.15

*All values are expressed as Mean ± S.E.M. n = 6, p < 0.05. *=statistically significant when compared to the control*

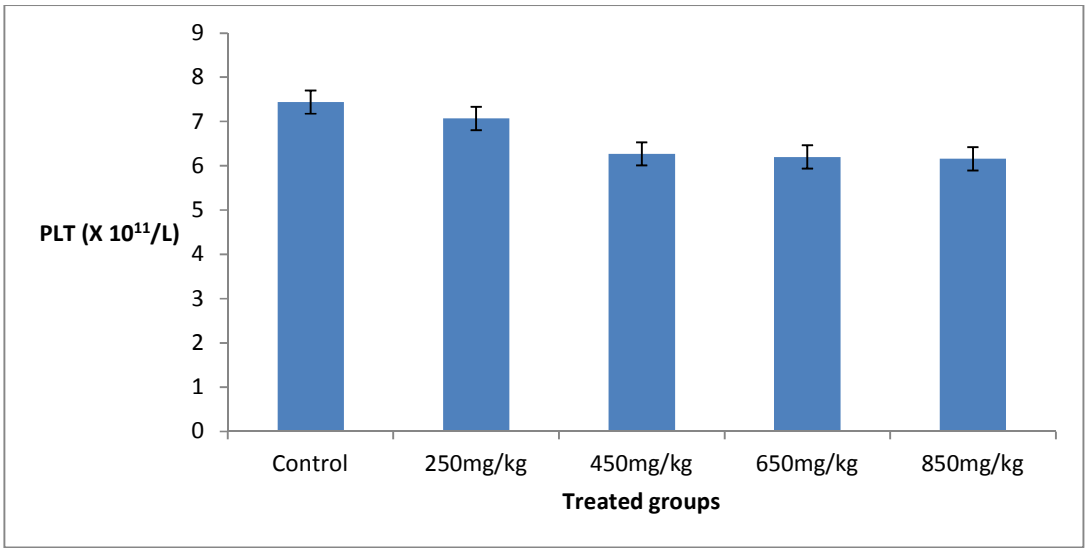

Fig.1. Effect of *Moringa oleifera* leaf extract on platelet count (PLT)

A dose-dependent non-significant (*P* > 0.05) decrease in the level of Plateletocrit (PCT) was observed following the administered doses when compared with the control. Following the administration of the extract, a non-significant decrease was recorded in the level of PCT for the doses of 450, 650 and 850 mg/kg when compared to the control (Fig. 2). A significant (*P* < 0.05) increase in the level of mean platelet volume (MPV) for the 450 mg/kg and 850 mg/kg administered dose when compared to the control. However, a dose-dependent but non-significant increase in the level of mean platelet volume (MPV) was observed for the 450 mg/kg and 850 mg/kg doses administered (Fig. 3).

A dose-dependent non-significant (P > 0.05) increase in the level of Platelet distribution width (PDW) was observed following the administered doses when compared with the control (Fig. 4). A significant (P < 0.05) increase in the level of immature platelet fraction (IPF) for the 650 mg/kg administered dose when compared to the control. However, a dose-dependent but non-significant increase in the level of immature platelet fraction (IPF) was observed for the 250 mg/kg, 450 mg/kg and 850 mg/kg doses administered (Fig. 5).

4. DISCUSSION

Table 1 above, reveals that the extract contains various types of phytochemicals in different concentrations. Table 2 shows that Alkaloids are the most abundant phytochemicals (have the highest percentage abundance) while Glycosides are the least abundant (in terms of percentage abundance). The level of alkaloids in the aqueous leaf extract of M. oleifera (Tables 1 and 2), may suggest that the extract has immunomodulatory activity, since some bitter alkaloids (tropane alkaloids) are metabolized in the liver into dimethylxanthine and finally methyl uric acid by cytochrome p_{450} oxygenase systems. Methyl uric acid in the liver stimulates the expression of tumour necrosis factor (in the

endothelial cells of the liver by macrophages) which modulates the immune system [6]. Also, saponins, which was moderately present, are implicated in the modulation of the immune system by serving as an adjuvant (saponins – cholesterol – phospholipid complexes) at low concentrations that stimulate the cell-mediated immune system by inducing the production of interleukins, especially by the antigen-presenting cells in most cells [7,8]. The presence of phenolic compounds in the extract may help among others, in preventing oxidative stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver.

Phenolic compounds are also known to scavenge directly nitric oxide molecule, thereby preventing the oxidation of LDL-C and tissue oxidative damage [9]. Nitric oxide is constitutively produced in endothelial cells to maintain the dilation of blood vessels and relaxation of smooth muscles [10]. Flavonoids were reported to decrease also, the immobilization and adhesion of leukocytes to endothelial walls, and degranulation of neutrophils without affecting superoxide production, thereby regulating inflammatory responses in tissue injury and immune responses [11].

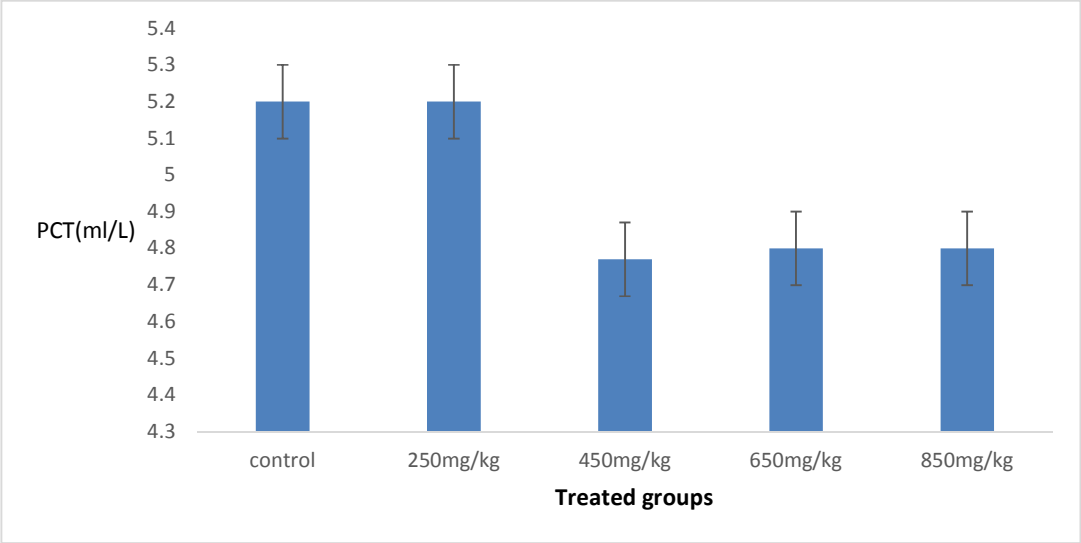

Fig. 2. Effect of *Moringa oleifera* leaf extract on platelet crit (PCT)

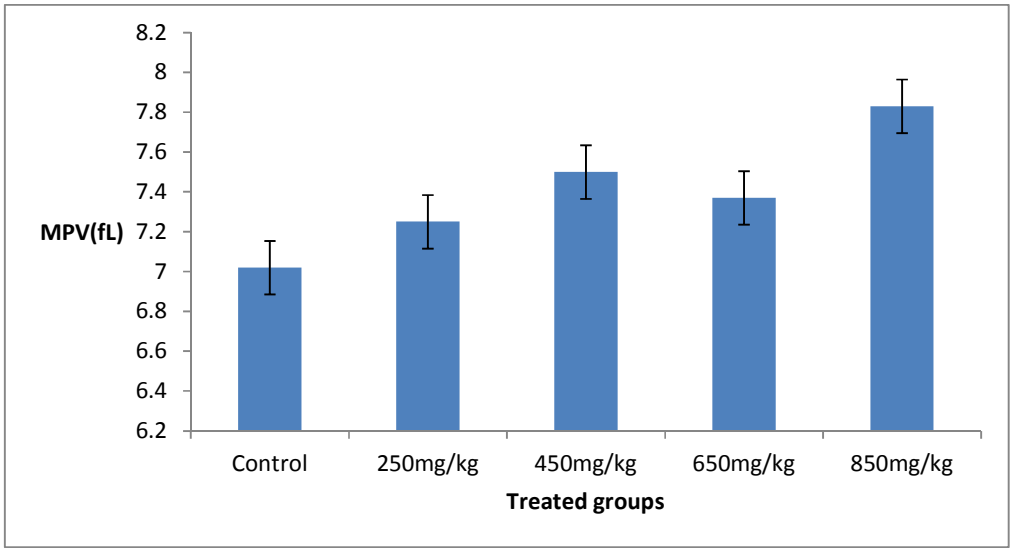

Fig. 3. Effect of *Moringa oleifera* leaf extract on the mean platelet volume (MPV)

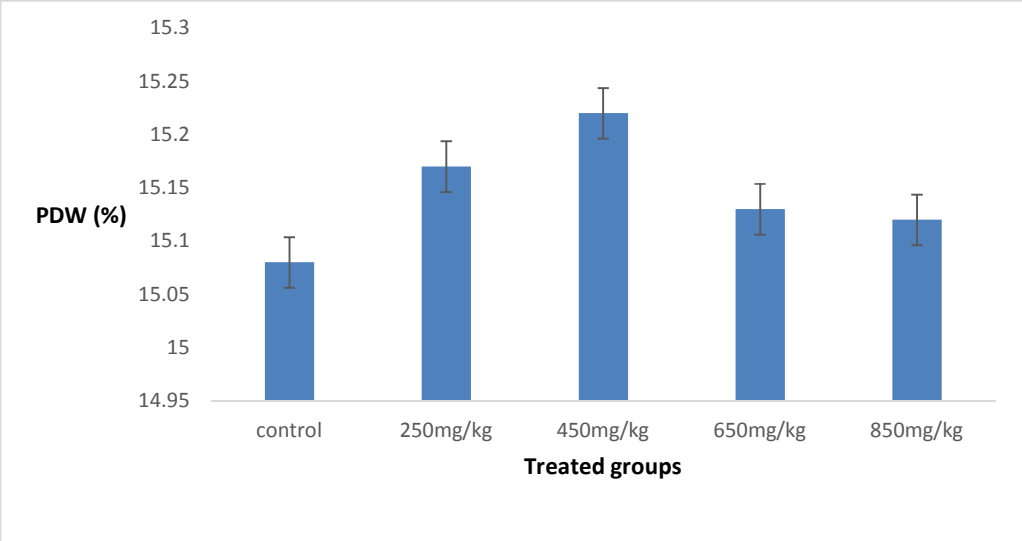

Fig. 4. Effect of *Moringa oleifera* leaf extract on platelet distribution width (PDW)

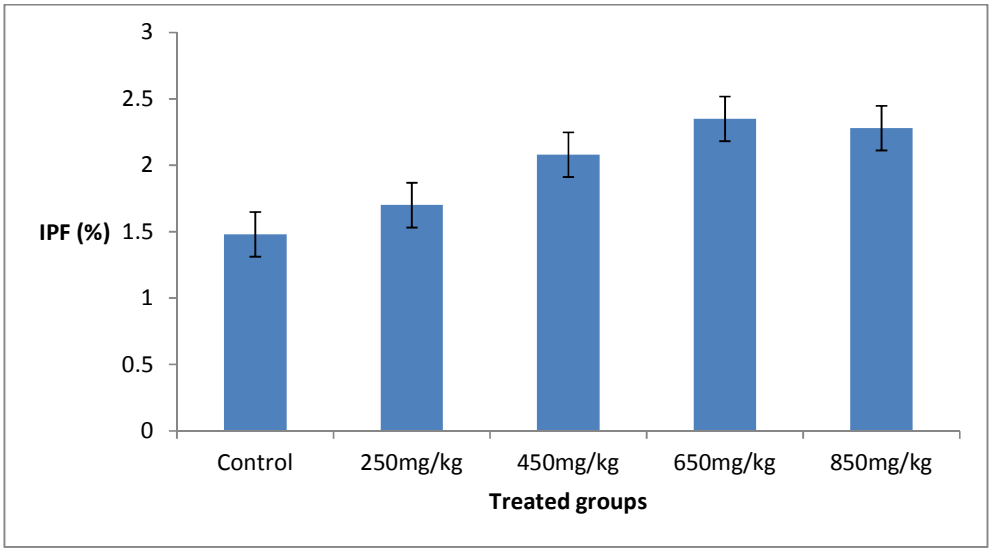

Fig. 5. Effect of *Moringa oleifera* leaf extract on immature platelet fraction (IPF)

The result of the effect of the extract on platelet count (PLT) can be seen in Fig. 1. The Figure shows a significant dose-dependent decrease in platelet count, concerning the control. There was a statistically significant decrease in platelet count at the 850 mg/kg dose of the extract, for the control. This result corroborates with other works which report a decrease in platelet count with the leaf extract of *moringa oleifera* [12]. This decrease in platelet parameters suggests that the extract contains some phytoconstituents which might have can destroyed the blood platelets (thrombocytopenia) and consequently causing excessive bleeding from wounds and negatively interfere with the normal coagulation process. A normal human platelet count ranges from 150,000 to 450,000 platelets per microliter of blood and thrombocytopenia is said to be a platelet count below 50,000 per microliter.

Fig. 2 shows the effect of the varying concentrations of the extract on Plateletocrit. It shows that the 250 mg/kg concentration of the extract has no effect on the value of the parameter, for the control but there was an observable sharp decrease in the parameter at

higher concentrations of the extract. Plateletocrit is a measure of the total platelet mass. Its value depends on the mean platelet volume resulting in the overlap between normal platelets, thrombocytopenia and thrombocytosis [5].

The effect of the varying concentrations of the extract on the mean platelet volume (MPV) can be seen in Fig. 3.There was an observable (though irregular) increase in the value of the mean platelet volume, for the control. There was a statistically significant increase ($P<0.05$) in the value of the parameter at 450 mg/kg and 850 mg/kg concentrations of the extract. The mean platelet volume is a machine-calculated measurement of the average size of platelets found in the blood and is typically included in the blood tests as part of the complete blood count (CBC). The normal range in Humans is given as 7.5fL-11.5Fl. It reflects the average size of platelets in a person's sample of blood. Larger platelets are usually relatively young and more recently released from the bone marrow, while smaller platelets may be older and have been in the circulation for a few days. The mean platelet volume test results can be used to make inferences about platelet destruction problems; it is generally higher when there is the destruction of platelets, as seen in inflammatory bowel disease [13]. Unusual low MPV values have been found to correlate primarily with thrombocytopenia when it is due to impaired production as in aplastic anaemia [13]. A large number of large platelets (a large MPV) in a person with a low platelet count, therefore, suggests that the bone marrow is producing platelets and releasing them into the circulation rapidly.

Fig. 4 showed that the varying concentrations of the extract cause a dose-dependent increase in the Platelet distribution width (PDW) of the experimental animals. The platelet distribution width measures the heterogeneity of platelet volume; it reflects how uniform the platelets are in size. The heterogeneity of platelet volume is considered to be due to ageing of platelets or due to the heterogeneous demarcation of megakaryocytes. It has been found that the increase in platelet distribution width (increased platelet heterogeneity) is associated with thrombocytopenia caused by platelet destruction [14].

The effect of the varying concentrations of the extract on the immature platelet fraction (IPF) is shown in Fig. 5. There was an observable dose-dependent increase in the immature platelet fraction, for the control. The immature platelet fraction (IPF, %) is a measure of reticulated platelets (RPs), which represents the state of thrombopoiesis [15]. It is obtained from an automated haematology analyzer as one of the platelet parameters. It is an index of thrombopoiesis and can help to determine the mechanism of thrombocytopenia. An increased IPF in the presence of thrombocytopenia is indicative of platelet destruction or consumption, while a decreased or low IPF value is indicative of a decreased bone marrow production of platelets [16]. Patients with decreased platelet production, including those undergoing cytotoxic chemotherapy, have been found to have IPF either in the low or normal range [16]. There has also been found a significant inverse correlation of platelet count with IPF, such that the lower the platelet count, the higher the IPF [16].

5. CONCLUSION

Based on this study, it is therefore concluded that the aqueous extract of *moringa oleifera* leaf is immuno-modulatory and thrombocytopenic in action. Since platelets in addition to other functions have been implicated in boosting the immune system, the extract likely enhances the immune system through other mechanisms and not through an increase in platelet production. Again, the thrombocytopenic action of the extract is probably mediated through the actual destruction of the platelets and not an interruption of the platelet production at the bone marrow. This is because the increase in platelet distribution width (PDW), Mean platelet volume (MPV) and Immature platelet fraction (IPF) observable from the study have been associated with actual platelet destruction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

The authors would like to express warm appreciation to Professors DV Dapper and IM Siminialayi of the Department of Human Physiology and Pharmacology respectively,

College of Health Sciences, University of Port Harcourt, Nigeria for their immense technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/52574