

1 **THE IMMUNO-MODULATORY AND THROMBOCYTOPAENIC EFFECTS OF THE**
2 **VARYING CONCENTRATIONS OF THE AQUEOUS LEAF EXTRACT OF MORINGA**
3 **OLEIFERA IN MALE ALBINO WISTAR RATS.**

Comment [U1]: IMMUNOMODULATORY AND THROMBOCYTOPENIC

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4
5 **Abstract**

6 **Introduction:** *Moringa oleifera* and related species are commonly used in folk medicine for
7 various human diseases.

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8 **Aim:** The study was undertaken to establish the thrombocytopaenic effect of the aqueous leaf
9 extract of *moringa oleifera* and to find the utilization of platelet parameters in determining the
10 cause of the thrombocytopaenia.

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11 **Methodology:** Fresh leaves of moringa were dried and extracted with water. Thirty (30) male
12 albino wistar rats, weighing between 150-250g, which were kept under uniform laboratory
13 conditions, were randomly divided into five (5) groups (A-E), based on their weights. The control
14 group (group A) was orally given 0.5ml of distilled water while the treatment groups (groups B
15 to E) were orally given 250mg/kg, 450mg/kg, 650mg/kg and 850mg/kg body weight respectively
16 of the extract, once a day, for 56 days and then sacrificed. At the end of the administration, blood
17 samples were collected from each rat and examined for platelet indices. The effects of treatment
18 with aqueous leaf extract of *moringa oleifera* on the platelet parameters were compared with the
19 control group.

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20 **Result:** The rats treated with the extract, showed a decrease in platelet count and plateletocrit
21 while there was a significant increase in the platelet distribution width, mean platelet volume and
22 immature platelet fraction, with respect to the control.

23 **Conclusion:** The aqueous leaf extract of *moringa oleifera* is therefore shown to modulate the
24 immune system and cause thrombocytopaenia, through platelet destruction.

25
26 **Keywords:** *Moringa*, Platelet, Thrombocytopaenia, Blood and immuno-modulatory.

Comment [U6]:
Keywords are usually arranged in alphabetical.

27
28 **INTRODUCTION**

29 Thrombocytopenia (TCP) refers to a disorder in which there is a relative decrease of platelets,
30 present in the blood^[5]. A normal human platelet count ranges from 150,000 to 450,000 platelets
31 per microliter of blood and thrombocytopenia is said to be a platelet count below 50,000 per
32 microliter^[8]. The causes of thrombocytopenia include decreased platelet production, increased
33 platelet destruction and splenic sequestration/abnormal pooling, based upon the causative
34 process^[7, 8, 11]. It is one of the common causes of abnormal bleeding and characterized with
35 spontaneous bleeding from the skin, arms, nose, gums and other mucous membranes^[8,11]. In
36 many cases of thrombocytopaenia, large platelet are seen in peripheral smear, this size and other
37 platelet parameters were suggested to help in deciding the category of
38 thrombocytopaenia^[15]. The present study was undertaken to establish the thrombocytopenic

39 effect of the aqueous extract of *moringa oleifera* leaf and to find the utility of platelet parameters
40 in determining the cause of the thrombocytopenia.

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41 **MATERIALS AND METHOD**

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

54 The rats were randomly grouped into five (5) groups (groups A-E), comprising six rats in each
55 group. A calculated amount of the aqueous extract of moringa was constituted in 20mls of
56 distilled water to give doses of 250mg/kg to 850mg/kg body weight. Administration of the
57 aqueous leaf extract of *moringa oleifera* was performed orally once daily, between 7.30am and
58 9.30am, using a 2ml syringe. The various groups were administered as follows:

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

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

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

67 The *Moringa* leaves were shaded, dried at a warm temperature (not directly under the sun),
68 before tasking it for the preparation of the extract. The leaves were separately rinsed in clean
69 water to remove dirt, dried at a room temperature for 14 days. 500g of the plant material was
70 introduced into an extraction jar. 1.2 litres of sterile distilled water was added into it and corked,
71 kept at room temperature and shaken at an interval of 30 minutes (with a mechanical shaker). It
72 was filtered after 24 hours, the discarded material and the filtrate concentrated using the rotary
73 evaporator in vacuum. The paste collected and air dried and weighed. The percentage yield was
74 calculated and the extract stored at -4°C in the refrigerator for photochemical studies on the
75 animals. Chemical tests were carried out on the aqueous extract and on the powdered specimens
76 using standard procedures, to identify the constituents as described by Sofowara (1993), Trease
77 and Evans (1919) and Harborne (1973).

Comment [U9]: phytochemical

78 STATISTICAL ANALYSES

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

82 RESULTS

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

88 **Table 1:** Qualitative phytochemical analysis of aqueous leaf extract of *M. oleifera* leaf in
89 Wistar rats.

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Phytochemical	Observation	Inference
Alkaloid	+++	Heavily present
Tannins	++	Moderately present
Saponins	++	Moderately present
Flavonoids	++	Moderately present
Steoids	+	Slightly present

Phlobatanins	+	Slightly present
Combined anthraquinone	+	Slightly present
Free anthraquinone	++	Moderately present
Cyanogenetic glycosides	-ve	Absent
Salkowski	++	Moderately present
Liebermanns	++	Slightly present
Keller kiliani	+	Slightly present

91 +, slightly present; ++, moderately present; +++, heavily present; -ve, absent; *, Significant
 92 at P < 0.05 when compared to control.

93
 94 The results of the quantitative phytochemical analysis indicate the presence of Polyphenols,
 95 flavonoids, tannins, alkaloids and glycosides in the following percentages: 2.70, 4.10, 8.00,
 96 15.00, and 2.50%, respectively (Table 2). The effects of the graded doses of moringa oleifera on
 97 platelet indices were also determined (Table 3)

98 **Table 2: Quantitative phytochemical analysis of aqueous leaf extract of *M. oleifera* leaf in**
 99 **Wistar rats.**

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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.

100 **Table 3: Effects of the graded doses of *m. oleifera* on platelet indices**

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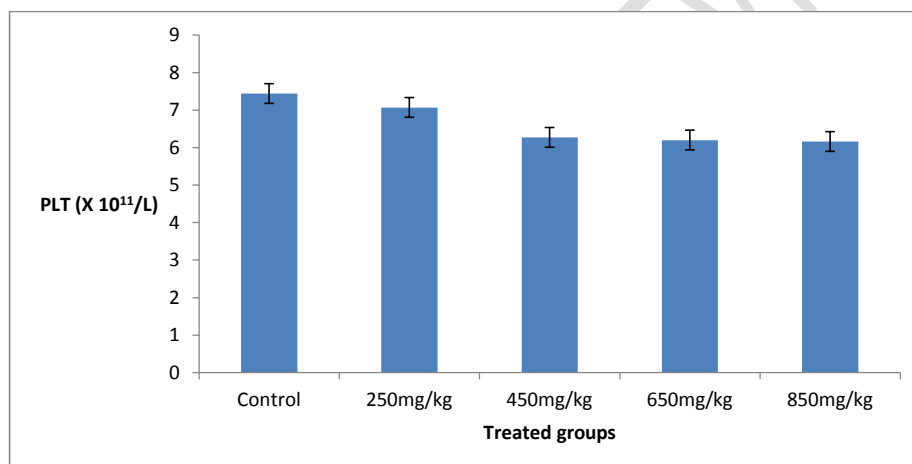
Groups	PLT($\times 10^{11}$ /L \pm sem)	PCT (ml/L \pm sem)	MPV (fl \pm sem)	PDW (% \pm sem)	IPF (% \pm sem)
Control	7.44 \pm 0.33	5.20 \pm 0.15	7.02 \pm 0.22	15.08 \pm 0.09	1.48 \pm 0.36
250mg/kg	7.07 \pm 0.56	5.20 \pm 0.44	7.25 \pm 0.10	15.17 \pm 0.08	1.70 \pm 0.21

450mg/kg	6.27 ± 0.31	4.77 ± 0.22	7.50 ± 0.10*	15.22 ± 0.07	2.08 ± 0.18
650mg/kg	6.20 ± 0.50	4.80 ± 0.33	7.37 ± 0.12	15.13 ± 0.06	2.35 ± 0.21*
850mg/kg	6.16 ± 0.42*	5.05 ± 0.28	7.83 ± 0.02*	15.12 ± 0.04	2.28 ± 0.15

101 All values are expressed as Mean ± S.E.M. n = 6, p < 0.05. *=statistically significant when
 102 compared to the control
 103

104 The result of *M. oleifera* extract on platelet indices shows a significant ($P < 0.05$) decrease in the
 105 level of platelet count for the high dose administered when compared with the control. A non-
 106 significant reduction in the level of platelet count was seen for the 250, and 450 and 650 mg/kg
 107 doses administered when compared with the control (Fig. 1).
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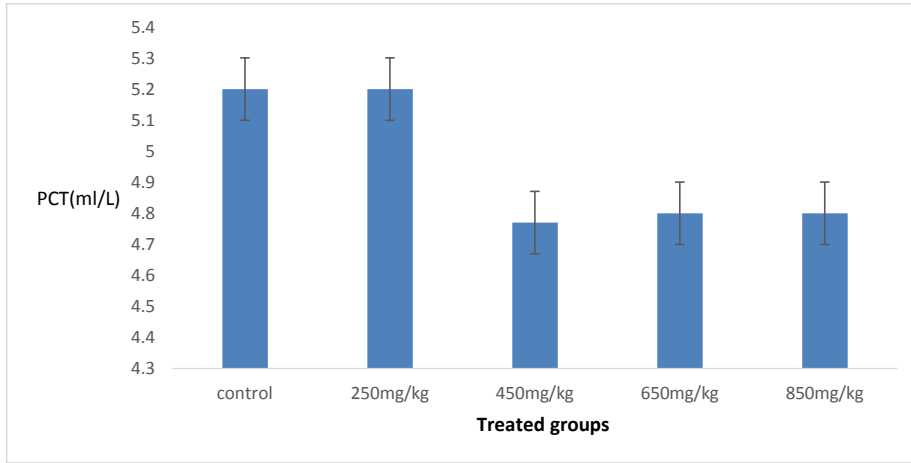
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109
 110 Fig.1: Effect of *moringa oleifera* leaf extract on platelet count (PLT)
 111

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112 A dose-dependent non-significant ($P > 0.05$) decrease in the level of Plateletocrit (PCT) was
 113 observed following the administered doses when compared with the control. Following the
 114 administration of the extract, a non-significant decrease was recorded in the level of PCT for the
 115 doses of 450, 650 and 850 mg/kg when compared to the control (Fig 2). A significant ($P < 0.05$)
 116 increase in the level of mean platelet volume (MPV) for the 450 mg/kg and 850mg/kg
 117 administered dose when compared to the control. However, a dose-dependent but non-significant
 118 increase in the level of mean platelet volume (MPV) was observed for the 450mg/kg and
 119 850mg/kg doses administered (Fig 3).

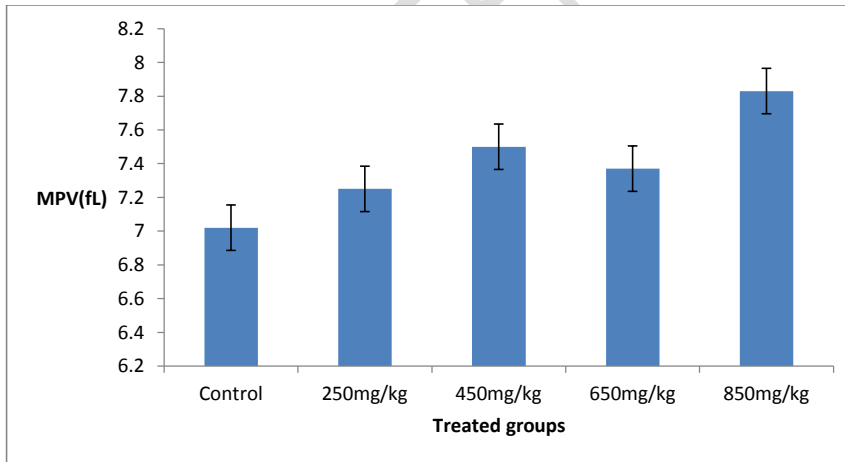


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121 Fig.2: Effect of *moringa oleifera* leaf extract on plateletocrit (PCT)

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

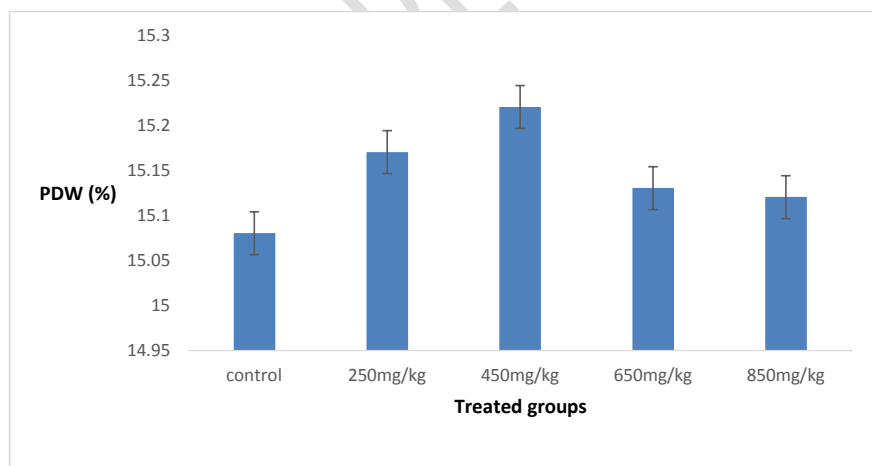
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125 A dose-dependent non-significant ($P > 0.05$) increase in the level of Platelet
126 distribution width (PDW) was observed following the administered doses when
127 compared with the control (Fig 4). A significant ($P < 0.05$) increase in the level of
128 immature platelet fraction (IPF) for the 650 mg/kg administered dose when
129 compared to the control. However, a dose-dependent but non-significant increase
130 in the level of immature platelet fraction (IPF) was observed for the 250mg/kg,
131 450mg/kg and 850mg/kg doses administered (Fig 5).

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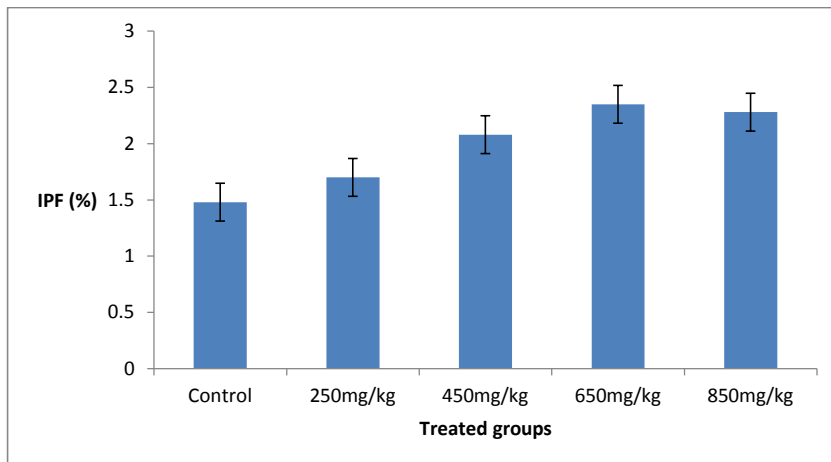
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136 Fig.4: Effect of moringa oleifera leaf extract on platelet distribution width (PDW)

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

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139 **DISCUSSION**

140 Table 1 above, reveals that the extract contains various types of phytochemicals in different
 141 concentrations. Table 2 shows that Alkaloids are the most abundant phytochemicals (have the
 142 highest percentage abundance) while Glycosides are the least abundant (in terms of percentage
 143 abundance).The level of alkaloids in the aqueous leaf extract of *M. oleifera* (tables 1 and 2), may
 144 suggest that the extract has immuno-modulatory activity, since some bitter alkaloids (tropane
 145 alkaloids) are metabolized in the liver into dimethylxanthine and finally methyl uric acid by
 146 cytochrome p₄₅₀ oxygenase systems. Methyl uric acid in the liver stimulates the expression of
 147 tumour necrosis factor (in the endothelia cells of the liver by macrophages) which modulates the
 148 immune system^[16].Also, saponins, which was moderately present, are also implicated in the
 149 modulation of the immune system by serving as adjuvant (saponins – cholesterol – phospholipid
 150 complexes) at low concentrations that stimulate cell mediated immune system by inducing the
 151 production of interleukins, especially by the antigen – presenting cells in most cells^[13,17]. The
 152 presence of phenolic compounds in the extract may help among others, in preventing oxidative
 153 stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver

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154 Phenolic compounds are also known to scavenge directly nitric oxide molecule, thereby
 155 preventing the oxidation of LDL-C and tissue oxidative damage^[14]. Nitric oxide is constitutively
 156 produced in endothelial cells to maintain the dilation of blood vessels and relaxation of smooth

157 muscles ^[9]. Flavonoids were reported to decrease also, the immobilization and adhesion of
158 leukocytes to endothelial walls, and degranulation of neutrophils without affecting superoxide
159 production, thereby regulating inflammatory responses in tissue injury and immune responses ^[6].

160 The result of the effect of the extract on platelet count (PLT) can be seen in figure 1. The figure
161 shows a significant dose-dependent decrease in platelet count, with respect to the control. There
162 was a statistically significant decrease in platelet count at the 850mg/kg dose of the extract, with
163 respect to the control. This result corroborates with other works which report a decrease in
164 platelet count with the leaf extract of *moringa oleifera* ^[11]. This decrease in platelet parameters
165 suggests that the extract contains some phytoconstituents which might have destroyed the
166 blood platelets (thrombocytopenia) and consequently causing excessive bleeding from wounds
167 and negatively interfere with the normal coagulation process. A normal human platelet count
168 ranges from 150,000 to 450,000 platelets per microliter of blood and thrombocytopenia is said to
169 be a platelet count below 50,000 per microliter

170 Figure 2 shows the effect of the varying concentrations of the extract on Plateletcrit. It shows
171 that the 250mg/kg concentration of the extract has no effect on the value of the parameter, with
172 respect to the control but there was an observable sharp decrease in the parameter at higher
173 concentrations of the extract. Plateletcrit is a measure of the total platelet mass. Its value
174 depends on the mean platelet volume resulting in overlap between normal platelets,
175 thrombocytopenia and thrombocytosis ^[15].

176 The effect of the varying concentrations of the extract on the mean platelet volume (MPV) can
177 be seen in figure 3. There was an observable (though irregular) increase in the value of the mean
178 platelet volume, with respect to the control. There was a statistically significant increase
179 ($P < 0.05$) in the value of the parameter at 450mg/kg and 850mg/kg concentrations of the extract.
180 The mean platelet volume is a machine-calculated measurement of the average size of platelets
181 found in the blood and is typically included in the blood tests as part of the complete blood count
182 (CBC). The normal range in Humans is given as 7.5fL-11.5fL. It reflects the average size of
183 platelets in a person's sample of blood. Larger platelets are usually relatively young and more
184 recently released from the bone marrow, while smaller platelets may be older and have been in
185 the circulation for a few days. The mean platelet volume test results can be used to make
186 inferences about platelet destruction problems; it is generally higher when there is destruction of
187 platelets, as seen in inflammatory bowel disease ^[12]. Abnormally low MPV values has been found

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188 to correlate primarily with thrombocytopaenia, when it is due to impaired production as in
189 aplastic anaemia^[12]. A large number of large platelets (a large MPV) in a person with a low
190 platelet count therefore suggests that the bone marrow is producing platelets and releasing them
191 into the circulation rapidly.

192 Figure 4 show that the varying concentrations of the extract cause a dose-dependent increase in
193 the Platelet distribution width (PDW) of the experimental animals. The platelet distribution
194 width measures the heterogeneity of platelet volume; it reflects how uniform the platelets are in
195 size. The heterogeneity of platelet volume is considered to be due to ageing of platelets or due to
196 the heterogeneous demarcation of megakaryocytes. It has been found that increase in platelet
197 distribution width (increased platelet heterogeneity) is associated with thrombocytopaenia caused
198 by platelet destruction^[2].

199 The effect of the varying concentrations of the extract on the immature platelet fraction (IPF) is
200 shown in figure 5. There was an observable dose-dependent increase in the immature platelet
201 fraction, with respect to the control. The immature platelet fraction (IPF, %) is a measure of
202 reticulated platelets (RPs), which represents the state of thrombopoiesis^[10]. It is obtained from an
203 automated haematology analyzer as one of the platelet parameters. It is an index of
204 thrombopoiesis and can help to determine the mechanism of thrombocytopenia. An increased
205 IPF in the presence of thrombocytopenia is indicative of platelet destruction or consumption,
206 while a decreased or low IPF value is indicative of a decreased bone marrow production of
207 platelets^[3]. Patients with decreased platelet production, including those undergoing cytotoxic
208 chemotherapy, have been found to have IPF either in the low or normal range^[3]. There has also
209 been found a significant inverse correlation of platelet count with IPF, such that the lower the
210 platelet count, the higher the IPF^[3].

211 **CONCLUSION**

212 Based on this study, it is therefore concluded that the aqueous extract of moringa oleifera leaf is
213 immuno-modulatory and thrombocytopaenic in action. Since platelets in addition to other
214 functions, have been implicated in boosting the immune system, it is likely that the extract
215 enhances the immune system through other mechanisms and not through an increase in platelet
216 production. Again, the thrombocytopaenic action of the extract is probably mediated through the
217 actual destruction of the platelets and not an interruption of the platelet production at the bone
218 marrow. This is because the increase in platelet distribution width (PDW), Mean platelet volume

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219 (MPV) and Immature platelet fraction (IPF) observable from the study have been associated with
220 actual platelet destruction.

221 **COMPETING INTEREST / CONFLICT OF INTEREST:**

222 The authors have declared that no competing interests exist.

223 **REFERENCES**

- 224 1 Ajibade T.O., Olayemi F.O., and Arowolo R.O.A The haematological and biochemical
225 Effects of methanol extract of the seeds of moringa oleifera in rats.Journal of
226 medicinal plants research. (2012); 6(4):615-621
- 227 2 Babu, E and Basu, D Platelet large cell ratio in the differential diagnosis of abnormal platelet
228 counts. Indian journal of pathological microbiology. (2004); 47 (20): 202-205.
- 229 3 British journal of haematology, 2004. Volume 126.
- 230 4 Brown,R.D .A review of the last decade. Aust J Med Lab Sci. (1988); 9:35-41
- 231 5 Deutschman, Clifford S., Neligan and Patrick J. Evidence-based practice of critical care.
232 Elsevier health sciences. (2010):
- 233 6 Ferandiz M.L.,Gilead B. and Sanz M.J. Effect of bakuchiol on leucocyte function and some
234 inflammatory responses in mice. J. Pharm.Pharmacol. (1996): 48:975-980.
- 235 7 Greer,P.,Daniel, A.,Bertil,G.,Alan,F.,Robert, T.,Frixos,P. and George,M.:William's
236 haematology,chapter 49,thrombocytopaenia 7th edition MacGraw-Hill,2006;1101-
237 1102.
- 238 8 Houghton, Andrew R., Gray and David Chamberlain's symptoms and signs in clinical
239 medicine, 13th edition. (2010):
- 240 9 Huk I.,Brovkovich v.,Nanobash vili J.,Weigel G.,Patton S. and Malinski Bioflavonoid-
241 quercetin scavenges superoxide and increases nitric oxide concentration in ischemia
242 injury:Br. J. Surg. (1998): 85:1080-1085.
- 243 10 Jung,H.,JeonH.K.,Kim,H.J. and Kim,S.H. Immature platelet fraction:establishment of a
244 reference interval and diagnostic measure for thrombocytopenia.Korean journal of
245 laboratory medicine. (2010) :30(5):451-459.
- 246 11 Lee,G.R.,Forester,J. and Lukens,JWintrob's clinical haematology,12th edition,Lippincott
247 Williams and wilkins. (2009):

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- 248 12 Liu, S., Ren, J., Han, G., Wang, G., GU, G., Xia, Q. and Li, J “Mean platelet volume: a
249 controversial marker of disease activity in Cohn’s disease”. European journal of
250 medical research. (2012): 17:27.
- 251 13 Oda K.,Matsuda H.,Murakani T.,Kayayama S.,Ohgitan T and Yoshikawa M. Adjuvant and
252 haemolytic activities of 47 saponins derived from medicinal and food plants. Biol.
253 Chem. (2000): 381(1): 67-74
- 254 14 Van Acker S.A., Tromp M.N.,Haehen G.R.,Vandervijah J.W and Bast A. Flavonoids as
255 scavengers of Nitric oxide radical.Bioch. and Biophysical Research Communications.
256 (1995):214(3):755-759.
- 257 15 Vani Chaudrashekar Plateletocrit as a screening tool for detection of platelet quantitative
258 disorders. Journal of Haematology. (2013):.2(1):22-26.
- 259 16 Wickramasinghe,S.N. and Brain,B.JBlood and bone marrow. In: Symmers W St C:Systemic
260 pathology,3rd edition. .(1986):
- 261 17 Yoshida Y. Immunodulating activity of Chinese medical herbs. Int.J.Immunopharmacol.
262 .(1997): 19:353-370
- 263 18 Zahid I.R.,Song-hua H.U.,Chen-wen X. and Abdullah G.AAdjuvant effects of saponins on
264 animals immune responses.J. Zhe jang univ. Sc. B.8. (2007): (3):153-161

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