

Original Research Article

Effect of Ethanolic Extract of *Garcinia kola* Seed on Some Haematological Parameters of Male Wistar Rats

ABSTRACT

The ethanolic extract of *Garcinia kola* seed was investigated for its activity on selected haematological parameters of male wistar rats. Thirty (30) male wistar rats weighing 150.42 ± 3.98 were divided into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of the extract respectively once daily. After 21 days of extract administration, 5 rats from each group were sacrificed. Same was done after 42 days. Preliminary phytochemical screening of ethanolic extract of *Garcinia kola* seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. The lethal dose (LD₅₀) of the extract was found to be safe up to 5000 mg/kg body weight. The extract significantly ($p < 0.05$) increased the red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, platelet count and mean cell hemoglobin concentration (MCHC). The extract significantly ($p < 0.05$) decreased the white blood cell (WBC) count, mean cell volume (MCV), mean cell hemoglobin (MCH). Therefore, ethanolic extract of *Garcinia kola* seed through its antioxidant property showed hematopoietic, stimulating and enhancing effects. However, alterations in these haematological parameters by the extract may have deleterious effect on red blood cell constituents of the animals.

Keywords: *Garcinia kola*, Haematological Parameters, Ethanolic Extract, Hematopoietic, Deleterious Effect.

1. INTRODUCTION

Garcinia kola, a dicotyledonous plant belonging to the Clusiaceae or Guttiferae family is commonly found in the subtropical and tropical forests of some countries of West and Central Africa such as Benin, Cameroon, Democratic Republic of Congo, Ivory Coast, Ghana, Liberia, Nigeria, Senegal and Sierra Leone, Asia and Europe [1,2]. It is a medium-sized tree growing up to 12 m tall and 1.5 m wide and usually found in the rain forest of Nigeria [3]. The seed is popularly known as bitter kola or male kola in English. In Nigeria, the seed is commonly called “*Namiji goro*” in Hausa, “*Akilu*” in Igbo and “*Orogbo*” in Yoruba [4]. It is a brown nut-like

seed, used in traditional medicine [5]. The seeds when chewed have a bitter astringent taste. The seed extract and dry powdered seed of *G. kola* plants have been made into various forms including tablets, cream, and toothpaste [6]. It is used in traditional medicine for various therapeutic purposes based on the pharmacological effects of the active components (flavonoid) in the seed and other parts of the plant [5].

Previous studies by Farombi *et al* [7] reported the antioxidant and scavenging activities of flavonoid extract (kolaviron) of *Garcinia kola* seeds *in vitro* while Braide [8] examined the effect of *Garcinia kola* seed alkaloid extracts on levels of gonadal hormone and pituitary gonadotrophins in rat serum. Akintonwa and Esseini [9] reported the protective effects of *Garcinia kola* seed against paracetamol-induced hepatotoxicity in rats while Okunji *et al* [10] worked on the preparative isolation and identification of tyrosinase inhibitors from the seeds of *Garcinia kola* by high-speed counter-current chromatography. Orie and Ekon [11] reported the bronchodilator effects of *Garcinia kola* while Ositelu *et al* [12] demonstrated the antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in Lagos. Blaide [13] worked on the effects of chronic ingestion of *Garcinia kola* seeds in the rats. Ajayi *et al* [14] investigated the microstructural effects of aqueous extract of *Garcinia kola* on the hippocampus and cerebellum of malnourished mice while Udenze *et al* [15] examined the effects of gavage treatment with *Garcinia kola* seeds on biochemical markers of liver functionality in diabetic rats. Adejumo *et al* [16] reported the antisickling activities of extracts of leaf, seed and seed pod of *Garcinia kola* Heckel while Adefule–Ositelu *et al* [17] reported the antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in Lagos. Adaramoye and Adeyemi [18] Worked on the hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin induced diabetes mellitus rats.

On haematology, studies by Unigwe and Nwakpu [19] have reported the effect of ingestion of *Garcinia kola* seed on erythrocytes in rabbits while Ekpenyong *et al* [20] investigated the hematological effect of chronic administration of ethanolic extract of *Garcinia conruana* seed on rat, while Dada and Ikuerowo [21] evaluated the effects of ethanolic extracts of *Garcinia kola* seeds on growth and haematology of catfish (*Clarias gariepinus*) broodstock. Although, there have been several reports on the haematological activity of *Garcinia kola* seeds, there is still paucity of information on the 100 and 200 mg/kg body weight. Therefore, this study presents

information on the effect of ethanolic extract of *Garcinia kola* seed on selected haematological parameters using male wistar rats as model.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Plant Materials and Authentication

Garcinia kola seeds which were purchased from Karu market, in the Federal Capital Territory, Abuja and were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher specimen number (F.H.I. 10847).

2.1.2 Experimental Animals

Male Wistar rats (*Rattus norvegicus*) weighing 150.42 ± 3.98 g were obtained from the Animal House of Federal College of Animal Husbandry, Kuru, Jos, Plateau State, Nigeria.

2.1.3 Other Reagents

All other chemicals and reagents used which were of analytical grade were products of sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water except otherwise stated.

2.2 METHODS

2.2.1 Preparation of Ethanolic Extract of *Garcinia kola* Seed

Dried seeds of *Garcinia kola* were peeled to remove the testa. This was cut into smaller sizes and thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil) to obtain smooth powder. A known weight (200 g) of the powder was extracted in 1000 ml of ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator. The mixture was further transferred into steam bath where it was evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required doses (100 and 200 mg/kg body weight) in the study.

2.2.2 Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardiac glycosides and carbohydrate were carried out by adopting the procedures described by Harborne [22], Walls *et al.* [23], Odebiyi and Sofowora [24], Awe and Sodipo [25], El-Olemy *et al.* [26], Mainasara *et al.* [27], Sofowora [28] and Trease and Evans [29] respectively.

2.2.3 Acute Toxicity Study (LD₅₀)

The method described by Lorke [30] was adopted to determine lethal dose (LD₅₀). The experiment was carried out in two stages:

Stage I: Here, nine rats were involved. They were divided into 3 groups of 3 rats each. The animals in group A received ethanolic extract of *Garcinia kola* seed at a dose of 10 mg/kg body weight. Group B received 100 mg/kg body weight. Group C received 1000 mg/kg body weight. The animals were observed/monitored for 24 hours. The number of deaths in each group was noted.

Stage II: This stage was carried out based on the results of the first stage. Here, another three groups of one rat each was used. The animal in Group A received 1000 mg/kg body weight of the extract. The animal in Groups B and C received 3000 and 5000 mg/kg body weight of the extract respectively. The animals were monitored for another 24 hours during which the number of deaths or abnormal reaction/behaviour was noted.

2.2.4 Animal Grouping and Extract Administration

A total of thirty male albino rats, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31⁰C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. The thirty (30) male Wistar rats weighing 150.42 ± 3.98 were completely randomized into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml

of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of the extract respectively once daily. After 21 days of extract administration, 5 rats from each group were sacrificed. Same number was sacrificed after 42 days. Extract administration was done daily using polystyrene. This research was carried out in Physiology Department of Bingham University, according to the rules in Nigeria (Revised Helsinki Declaration, 2008) governing the care and use of laboratory animals as acceptable internationally.

2.2.5 Preparation of Serum

Under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scapel blade and an aliquot (2 ml) of the blood was collected into EDTA sample bottles for the haematological analysis. This was done by running it down the side of the bottle, covered and rolled gently to mix with EDTA so as to avoid clotting.

2.2.6 Determination of Haematological parameters

Haematological Autoanalyzer (Beckman Coulter, Inc. Fullerton, CA, USA) was used for the determination of haematological parameters: red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell (WBC) count, platelet count and mean cell volume (MCV).

2.2.7 Statistical Analysis

Results were expressed as the mean $\pm$ SEM of ten determinations. Means were analyzed using Duncan's Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at $p < 0.05$. All these analyses were done using SPSS 16.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

3. RESULTS

Preliminary phytochemical screening of the ethanolic extract of *Garcinia kola* seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. Anthraquinones and cardiac glycosides were not detected (Table 1).

Table 1: Phytochemical constituents of ethanolic extract of *Garcinia kola* seed

Phytochemical constituents	Inference
Alkaloids	++
Flavonoids	+++
Saponins	++
Tannins	++
Carbohydrate	++
Anthraquinones	-
Steroids	+
Cardiac glycosides	-

Key:

- Absent

+ Present

++ Significantly present

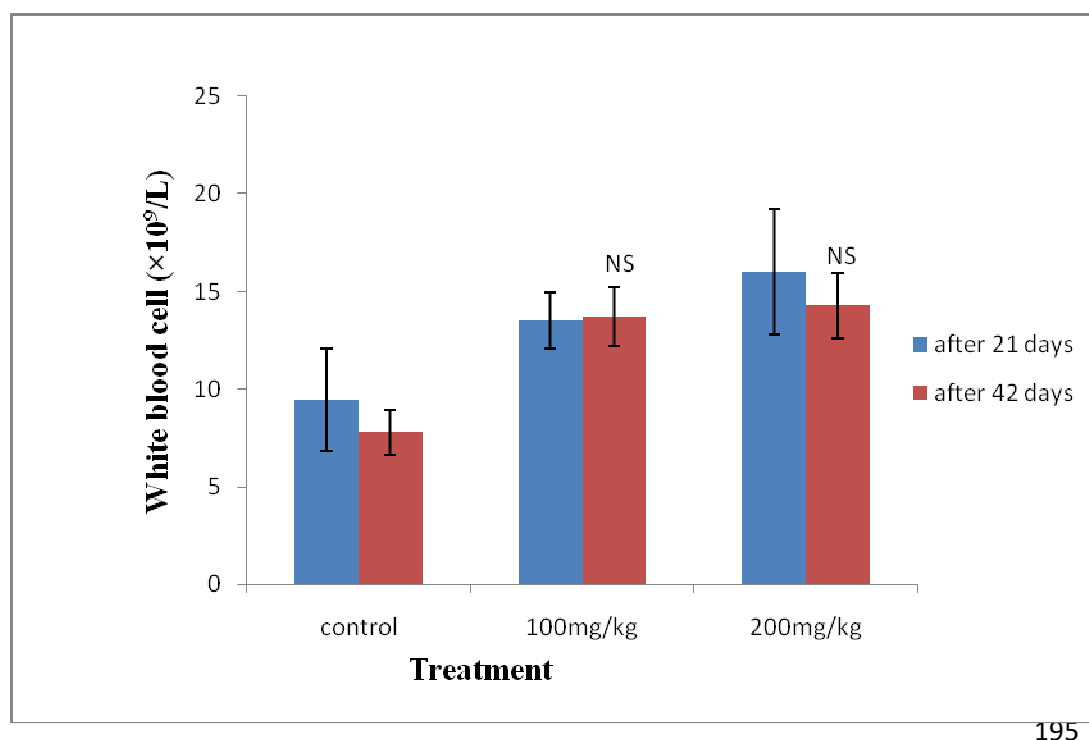
+++ Abundantly present

For acute toxicity study, all the graded doses of the ethanolic extract of *Garcinia kola* seed administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD₅₀ of ethanolic extract of *Garcinia kola* seed was found to be safe up to 5000 mg/kg body weight. 

After 21 days of administration, the ethanolic extract of *Garcinia kola* seed increased the white blood cell (WBC) count from 9.46±2.64 (control) to 13.54±1.46 (group II), and in group III, there was also an increase to 16.02±3.22; as shown in Figure 1. However, there was no significant increase in groups II and III as compared with control (p=0.324 and p=0.131 respectively).

175 After 42 days of administration, the ethanolic extract of bitter kola increased the WBC count
 176 from 7.82 ± 1.15 (control) to 13.72 ± 1.53 (group II), and in group III, there was also an increase to
 177 14.28 ± 1.64 ; as shown in figure 1. There was significant increase in groups II and III as compared
 178 with control ($p=0.034$ and $p=0.017$ respectively).

179 Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia*
 180 *kola* seed increased the WBC count in group II from 13.54 ± 1.46 after 21 days to 13.72 ± 1.53
 181 after 42 days but in group III, there was a decrease from 16.02 ± 3.22 after 21 days to 14.28 ± 1.64
 182 after 42 days. There was no significant increase in groups II and III after comparing 21 days and
 183 42 days ($p=0.887$ and $p=0.678$ for groups II and III respectively); as shown in Figure 1.



196 NS = Not significant

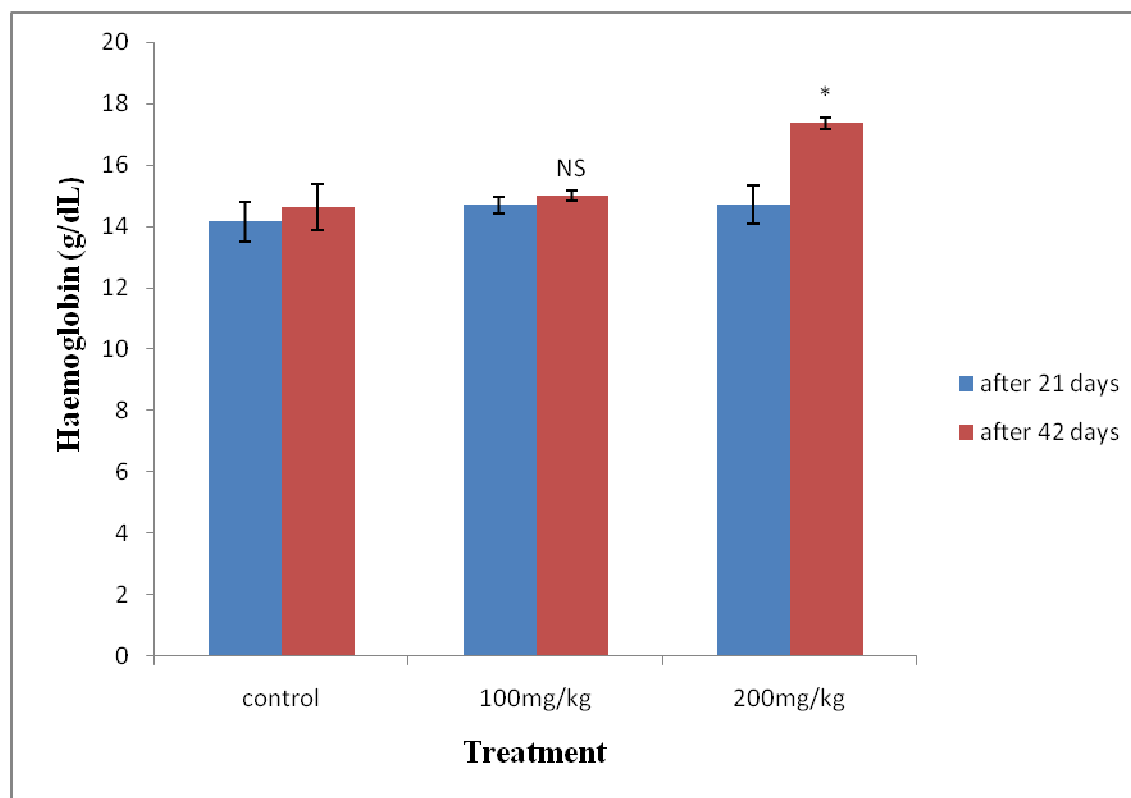
197 **Figure 1:** Effect of ethanolic extract of *Garcinia kola* seed on white blood cell (WBC) count
 198 ($\times 10^9/L$) after 21 and 42 days.

199 After 21 days of administration, the ethanolic extract of *Garcinia kola* seed increased the
 200 hemoglobin (Hb) level from 14.16 ± 0.64 (control) to 14.7 ± 0.27 (group II), and in group III, there

201 was also an increase to 14.7 ± 0.62 ; as shown in Figure 2. However, there was no significant
202 increase in groups II and III as compared with control ($p=0.440$ and $p=0.225$ respectively).

203 After 42 days of administration, the ethanolic extract of *Garcinia kola* seed increased the Hb
204 level from 14.64 ± 0.75 (control) to 15.0 ± 0.18 (group II), and in group III, there was also an
205 increase to 17.36 ± 0.20 ; as shown in Figure 2. There was no significant increase in group II as
206 compared with control, but there was significant increase in group III as compared with control
207 ($p=0.100$ and $p=0.001$ respectively).

208 Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of bitter
209 kola increased the white blood cell count in group II from 14.7 ± 0.27 after 21 days to 15.0 ± 0.18
210 after 42 days, and in group III, there was also an increase from 14.7 ± 0.62 after 21 days to
211 17.36 ± 0.20 after 42 days. However, there was no significant increase in group II after comparing
212 21 and 42 days, but there was significant increase in group III after comparing 21 and 42 days; as
213 shown in Figure 2. ($p=0.376$ and $p=0.025$ for groups II and III respectively).



214
215 * $P < 0.05$ = statistically significant

216 NS = Not significant

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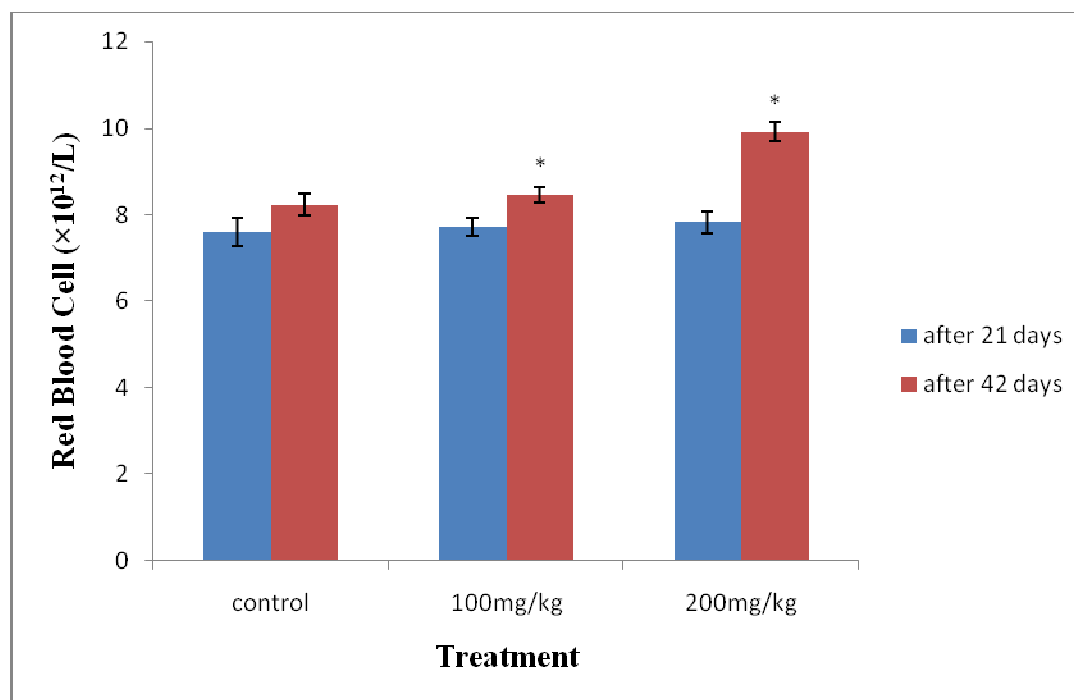
218 **Figure 2:** Effect of ethanolic extract of *Garcinia kola* seed on Hemoglobin (Hb) concentration
219 (g/dL) after 21 and 42 days.

220 After 21 days of administration, the ethanolic extract of *Garcinia kola* seed increased the red
221 blood cell (RBC) count from 7.6 ± 0.33 (control) to 7.71 ± 0.2 (group II), and in group III, there
222 was also an increase to 7.82 ± 0.26 , as shown in Figure 3. However, there was no significant
223 increase in groups II and III as compared with control ($p=0.745$ and $p=0.232$ respectively).

224 After 42 days of administration, the ethanolic extract of bitter kola increased the RBC count from
225 8.23 ± 0.26 (control) to 8.45 ± 0.18 (group II), and in group III, there was also an increase to
226 9.91 ± 0.22 , as shown in Figure 3. There was no significant increase in group II as compared with
227 control, but there was significant increase in group III as compared with control ($p=0.561$ and
228 $p=0.018$ respectively).

229 Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia*
230 *kola* seed increased the red blood cell count in group II from 7.71 ± 0.2 after 21 days to 8.45 ± 0.18
231 after 42 days, and in group III, there was also an increase from 7.82 ± 0.26 after 21 days to
232 9.91 ± 0.22 after 42 days. There was significant increase in groups II and III after comparing 21
233 days and 42 days; as shown in Figure 3. ($p=0.010$ and $p=0.002$ for groups II and III
234 respectively).

235



*P < 0.05= Statistically significant

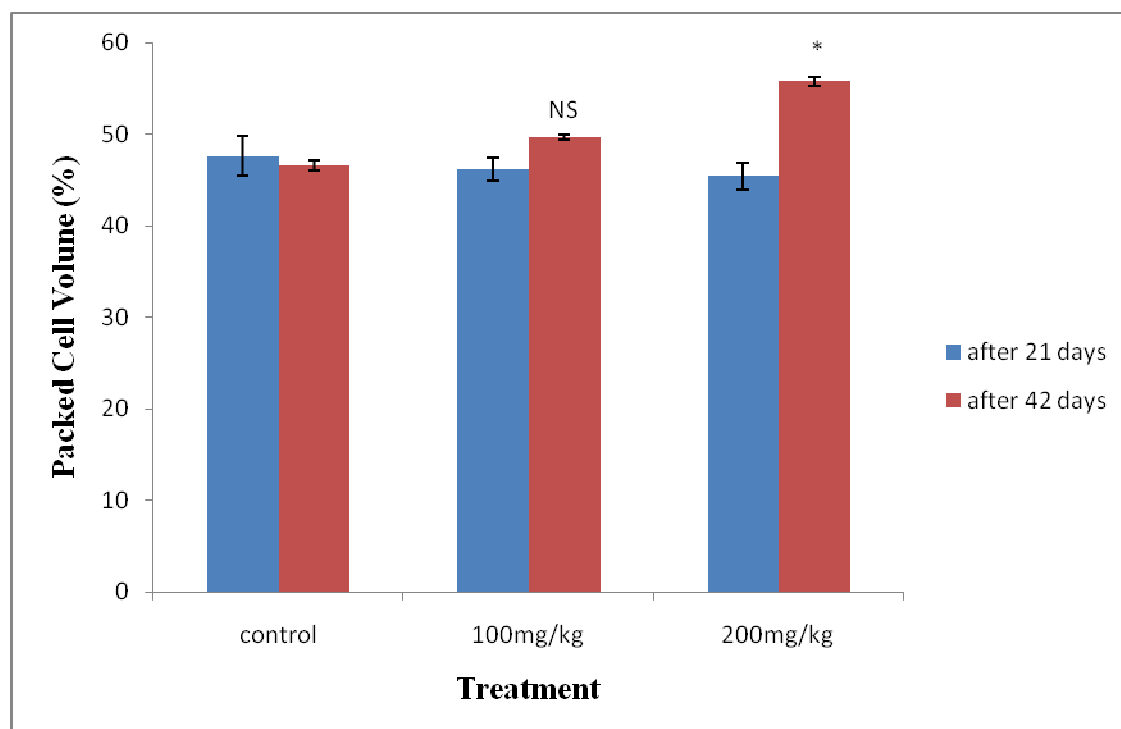
Figure 3: Effect of ethanolic extract of *Garcinia kola* seed on red blood cell (RBC) count ($\times 10^{12}/L$) after 21 and 42 days.

After 21 days of administration, the ethanolic extract of *Garcinia kola* seed decreased the packed cell volume (PCV) from 47.6 ± 2.19 (control) to 46.2 ± 1.29 (group II), and in group III, there was also a decrease to 45.44 ± 1.48 ; as shown in Figure 4. There was no significant increase in groups II and III as compared with control ($p=0.469$ and $p=0.095$ respectively).

After 42 days of administration, the ethanolic extract of bitter kola increased the PCV from 46.54 ± 0.57 (control) to 49.68 ± 0.30 (group II), and in group III, there was also an increase to 55.76 ± 0.54 ; as shown in Figure 4. There was significant increase in groups II and III as compared with control ($p=0.561$ and $p=0.018$ respectively).

Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia kola* seed increased the packed cell volume in group II from 46.2 ± 1.29 after 21 days to 49.68 ± 0.30 after 42 days, and in group III, there was also an increase from 45.44 ± 1.48 after 21 days to 55.76 ± 0.54 after 42 days. There was no significant increase in group II after comparing

252 21 days and 42 days, but there was significant increase in group III after comparing 21 and 42
 253 days; as shown in Figure 4 ($p=0.052$ and $p=0.002$ for groups II and III respectively).



255 **Figure 4:** Effect of ethanolic extract of *Garcinia kola* seed on packed cell volume (PCV) (%)
 256 after 21 and 42 days; * $P < 0.05$ = statistically significant; NS = Not significant

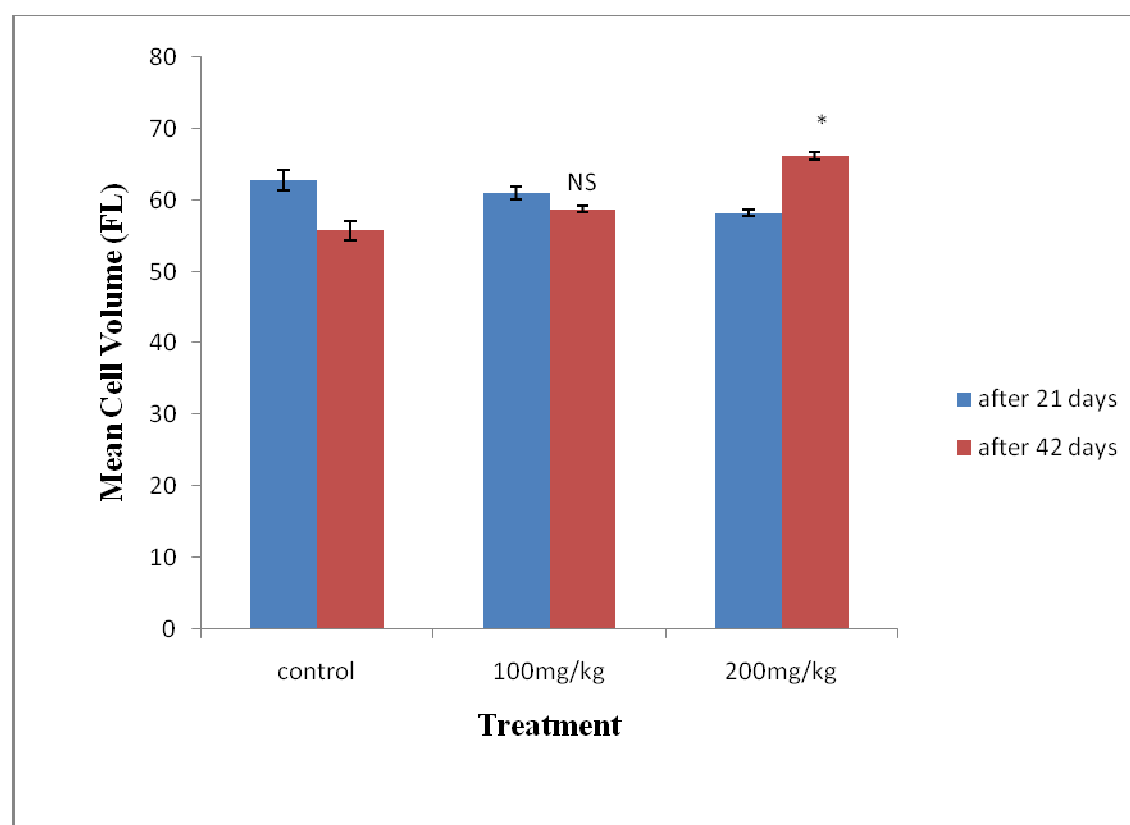
257
 258 After 21 days of administration, the ethanol extract of *Garcinia kola* seed decreased the mean
 259 cell volume (MCV) from 62.74 ± 1.4 (control) to 61.04 ± 0.88 (group II), and in group III, there
 260 was also a decrease to 58.2 ± 0.42 , as shown in Figure 5. However, there was no significant
 261 increase in group II as compared with control ($p=0.354$), but there was significant increase in
 262 group III as compared with control ($p=0.036$).

263 After 42 days of administration, the ethanolic extract of bitter kola increased the MCV from
 264 55.78 ± 1.34 (control) to 58.78 ± 0.47 (group II), and in group III, there was also an increase to
 265 66.14 ± 0.54 ; as shown in Figure 5. There was no significant increase in group II as compared
 266 with control, ($p=0.113$), but there was significant increase in group III as compared with control
 267 ($p=0.003$).

268 Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia*
 269 *kola* seed decreased the mean cell volume in group II from 61.04 ± 0.88 after 21 days to
 270 58.78 ± 0.47 after 42 days, but in group III, there was an increase from 58.2 ± 0.42 after 21 days to
 271 66.14 ± 0.54 after 42 days. There was no significant increase in group II after comparing 21 days
 272 and 42 days, but there was significant increase in group III after comparing 21 and 42 days; as
 273 shown in Figure 5 ($p=0.126$ and $p=0.001$ for groups II and III respectively).

274

275



276

277 **Figure 5:** Effect of ethanolic extract of *Garcinia kola* seed on mean cell volume (MCV) (FL)
 278 after 21 and 42 days; * $P < 0.05$ = statistically significant; NS = Not significant.

279

280 After 21 days of administration, the ethanolic extract of *Garcinia kola* seed increased the mean
 281 cell hemoglobin (MCH) from 18.58 ± 0.46 (control) to 19.32 ± 0.21 (group II), but in group III,

there was a decrease to 18.72 ± 0.29 ; as shown in Figure 6. There was no significant increase in groups II and III as compared with control ($p=0.252$ and $p=0.835$ respectively).

After 42 days of administration, the ethanolic extract of bitter kola increased the mean cell hemoglobin from 17.27 ± 0.16 (control) to 17.78 ± 0.25 (group II), and in group III, there was also an increase to 19.34 ± 0.15 ; as shown in Figure 6. There was no significant increase in group II as compared with control ($p=0.228$), but there was significant increase in group III as compared with control ($p=0.001$).

Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia kola* seed decreased the MCH in group II from 19.32 ± 0.21 after 21 days to 17.78 ± 0.25 after 42 days, but in group III, there was an increase from 18.72 ± 0.29 after 21 days to 19.34 ± 0.15 after 42 days. There was significant increase in groups II and III after comparing 21 days and 42 days respectively; as shown in Figure 6 ($p=0.005$ and $p=0.015$ for groups II and III respectively).

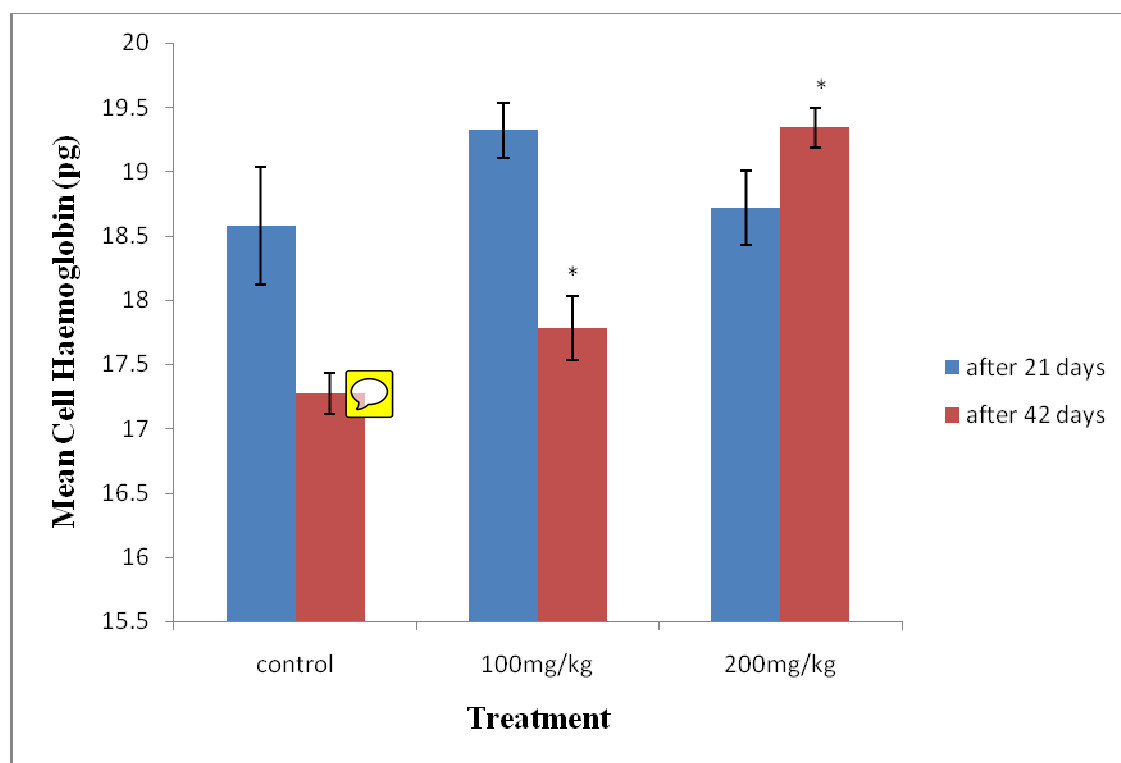


Figure 6: Effect of ethanolic extract of *Garcinia kola* seed on mean cell hemoglobin (MCH) (pg) after 21 and 42 days; * $P < 0.05$ = statistically significant; NS = Not significant.

After 21 days of administration, the ethanolic extract of *Garcinia kola* seed increased the mean cell hemoglobin concentration (MCHC) from 29.72 ± 0.35 (control) to 31.8 ± 0.36 (group II), and in group III, there was also an increase to 32.28 ± 0.43 ; as shown in Figure 7. There was significant increase in groups II and III as compared with control ($p=0.001$ and $p=0.008$ respectively).

After 42 days of administration, the ethanolic extract of bitter kola increased the mean cell hemoglobin concentration from 29.46 ± 0.75 (control) to 32.62 ± 0.39 (group II), and in group III, there was also an increase to 35.62 ± 0.27 ; as shown in Figure 7. There was significant increase in groups II and III as compared with control ($p=0.001$ and $p=0.001$ respectively).

Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia kola* seed increased the MCHC in group II from 31.8 ± 0.36 after 21 days to 32.62 ± 0.39 after 42 days, and in group III, there was also an increase from 32.28 ± 0.43 after 21 days to 35.62 ± 0.27 after 42 days. There was no significant increase in group II after comparing 21 days and 42 days, but there was significant increase in group III after comparing 21 and 42 days; as shown in Figure 7 ($p=0.297$ and $p=0.002$ for groups II and III respectively).

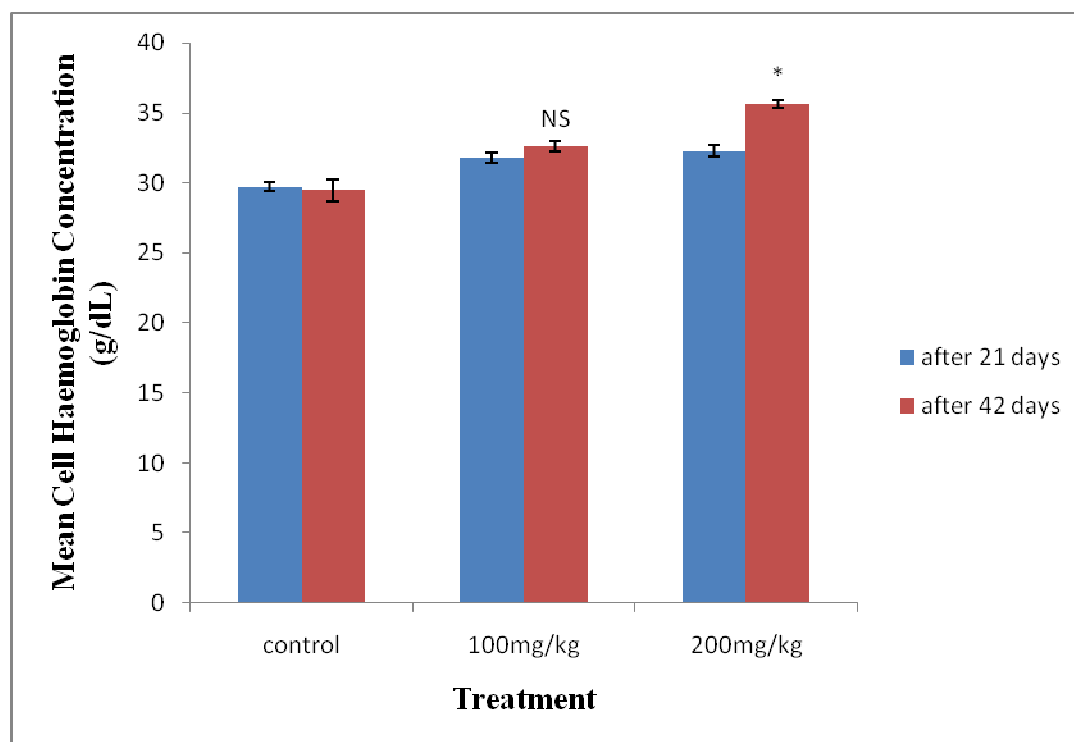


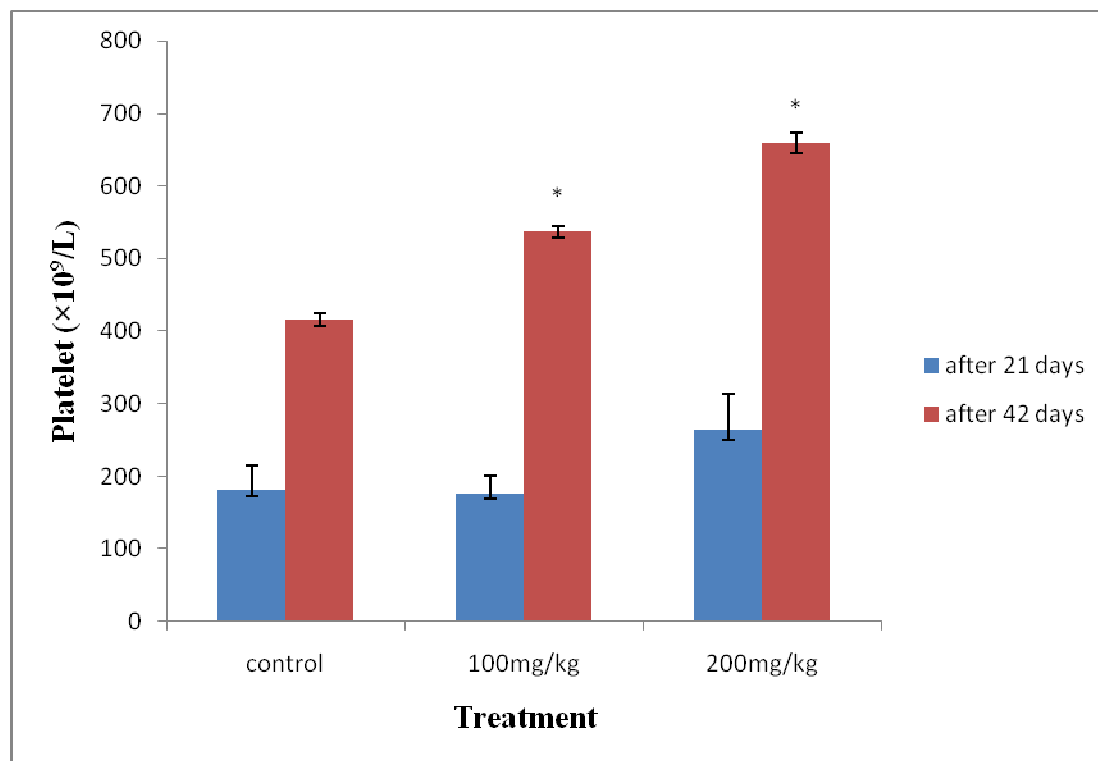
Figure 7: Effect of ethanol extract of *Garcinia kola* seed on mean cell hemoglobin concentration (MCHC) (g/dL) after 21 and 42 days; *P < 0.05 = statistically significant; NS = Not significant

After 21 days of administration, the ethanolic extract of *Garcinia kola* seed decreased the platelet count from 181.4 ± 32.98 (control) to 177 ± 24.25 (group II), but in group III, there was an increase to 264.8 ± 47.96 ; as shown in Figure 8. There was no significant increase in groups II and III as compared with control ($p=0.898$ and $p=0.106$ respectively).

After 42 days of administration, the ethanolic extract of bitter kola increased the platelet count from 416.20 ± 8.34 (control) to 537.40 ± 7.99 (group II), $p < 0.05$ and in group III, there was also an increase to 659.0 ± 14.69 , $p < 0.05$. There was significant increase in groups II and III as compared with control ($p=0.001$ and $p=0.001$ respectively).

Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia kola* seed increased the platelet count in group II from 177 ± 24.25 after 21 days to 537.40 ± 7.99 after 42 days, and in group III, there was also an increase from 264.8 ± 47.96 after 21 days to

333 659.0±14.69 after 42 days. There was significant increase in groups II and III after comparing 21
 334 days and 42 days respectively; as shown in Figure 8 (p=0.001 and p=0.002 for groups II and III
 335 respectively).



336
 337 **Figure 8:** Effect of ethanolic extract of *Garcinia kola* seed on platelet count (×10⁹/L) after 21
 338 and 42 days; * P < 0.05 = statistically significant; NS = Not significant



339 4. DISCUSSION

340 Medicinal plants such as *Garcinia kola* are believed to be an important source of new chemical
 341 substances with potential therapeutic benefits [31,32]. Knowledge of haematological
 342 characteristics is an important tool used as an effective and sensitive index of maintaining
 343 physiological and pathological changes in animal models [33]. The analysis of blood indices has
 344 proven to be a valuable approach for analyzing the health status of animal models as these
 345 indices provide reliable information on metabolic disorders, deficiencies, chronic stress status
 346 and blood relating functions before they are present in a clinical setting [34]. Alterations in blood
 347 parameters may be due to changes in cellular integrity, membrane permeability of cells or even
 348 due to exposure to toxic chemicals [35]



From the result of this experiment, *Garcinia kola* caused a dose dependent increase in white blood cell (WBC) count. Figure 1 showed an increase in control to group II to group III after 21 days and after 42 days. While the increase after 21 days is not statistically significant, the increase after 42 days is statistically significant at $p<0.05$. Also, from Figure 1, in low dose groups (100 mg/kg body weight), there was an increase from 13.54 ± 1.46 to 13.72 ± 1.53 from 21 to 42 days, but in high dose groups (200 mg/kg body weight), there was a decrease from 16.02 ± 3.22 to 14.28 ± 1.64 from 21 to 42 days; however, both showed no statistical significance. Thus the effect on white blood cell count is more pronounced after 21 days and reduced a little after 42 days (comparing high dose groups of 21 and 42 days). The result is similar to the findings of Ahumibe and Braide [36] where an experiment was carried out on the effect of chronic administration of ethanolic extract of *Garcinia conruana* seed on rats and the white blood cell count did not show any significant enhancement in numbers.

Red blood cell (RBC) count showed an increase after 21 days and after 42 days of administration of the extract; as seen in Figure 3. The values were not significant except for group III as compared with control after 42 days from 8.23 ± 0.26 to 9.91 ± 0.22 .

Figure 3 shows a significant increase in low dose groups (100 mg/kg body weight) and high dose groups (200 mg/kg body weight) after comparing 21 days and 42 days.

Hemoglobin (Hb) concentration increased from control to group II to group III after 21 days and after 42 days; as seen in Figure 2. However, the values were not significant except for group III as compared with control after 42 days from 14.64 ± 0.75 to 17.36 ± 0.20 .

Figure 2 shows no significant increase in low dose groups (100 mg/kg body weight), but there was significant increase in high dose groups (200 mg/kg body weight) after comparing 21 and 42 days.

Packed cell volume (PCV) showed decreased values from control to group II to group III after 21 days that were not significant; as shown in Figure 4, but there was an increase after 42 days from 46.54 ± 0.57 (control) to 49.68 ± 0.30 (group II) to 55.76 ± 0.54 (group III) that was statistically significant. Figure 4 shows no significant increase in low dose groups (100 mg/kg body weight), but there was significant increase in high dose groups (200 mg/kg body weight) after comparing 21 and 42 days.

Mean cell volume (MCV) decreased from control to group II to group III after 21 days and increased from control to group II to group III after 42 days. For both 21 and 42 days, group II showed no statistical significance. However, for both 21 and 42 days, group III showed statistical significance from 62.74 ± 1.4 (control) to 58.2 ± 0.42 (group III) after 21 days and from 55.78 ± 1.34 (control) to 66.14 ± 0.54 (group III) after 42 days; as shown in Figure 5. Figure 5 shows no significant increase in low dose groups (100 mg/kg body weight), but there was significant increase in high dose groups (200 mg/kg body weight) after comparing 21 and 42 days.



Mean cell hemoglobin (MCH) increased from control to group II but decreased in group III after 21 days and increased from control to group II to group III after 42 days. However, the values were not significant except for group III as compared with control after 42 days from 17.27 ± 0.16 to 19.34 ± 0.15 as shown in Figure 6. Figure 6 shows a significant increase in low dose groups (100 mg/kg) and high dose groups (200 mg/kg body weight) after comparing 21 days and 42 days.

Mean cell hemoglobin concentration increased from control to group II to group III after 21 days and after 42 days; as shown in Figure 7. All the values were significant. Figure 7 shows no significant increase in low dose groups (100 mg/kg body weight), but there was significant increase in high dose groups (200 mg/kg body weight) after comparing 21 and 42 days.

The values for red blood cell count, hemoglobin concentration and mean cell hemoglobin concentration increased as the dosage increased and the period of administration increased, i.e. they were dose-dependent and duration-dependent. The values for packed cell volume were mostly duration-dependent; they increased with increase in the period of administration.

These findings are similar to that of Ahumibe and Braide [36], Unigwe and Nwakpu [19] and Esomonu *et al* [4].  here there was a steady increase in the red blood cell count, packed cell volume, hemoglobin concentration and mean cell hemoglobin concentration. There was a decrease in the values of mean cell volume and mean cell hemoglobin.

The steady rise in the values of red blood cell count, packed cell volume and hemoglobin concentration as the bitter kola seed concentration increased is said to be due to the antioxidant

activity of flavonoid (Wang *et al.*, 2000) which was present in the blood due to *Garcinia kola* seed extract administration, thereby elevating the total antioxidant activity of the blood [19]. Studies have shown that polyphenols particularly flavonoid supplements are likely to be beneficial for long term health since they act as potent antioxidants [38].

The increase in the erythrocyte level is said to be due to an increase in the stimulant cytokine erythropoietin [36]. This increase in erythrocyte count is also said to have alternately led to the increase in the packed cell volume and hemoglobin concentration values since there is a direct relationship between erythrocytes, packed cell volume and hemoglobin concentration [39].

After the 21 day administration, there was an initial decrease in hemoglobin concentration before it increased after 42 days. This could be due to complexes which flavonoid forms with reactive metals such as iron, zinc and copper thereby reducing their nutrient absorption [40]. These complexes might have affected hemoglobin synthesis and erythropoiesis since iron deficiency is characterized by deficient hemoglobin synthesis with red blood cells containing less than the usual concentration of hemoglobin [41]. Though this is compensated as ageing erythrocytes are destroyed, some of the iron is salvaged and transported to the erythroid cells of the marrow for new hemoglobin synthesis. This was seen in the subsequent weeks as the hemoglobin concentration values increased significantly.

There was a clear decrease in the mean cell volume after 21 days of administration with an increase in the *Garcinia kola* dose, implying that the cell size decreases significantly as dosage was increased (from 100mg/kg to 200 mg/kg body weight). This decrease can possibly be accounted for by the increase of mature erythrocytes relative to the larger immature forms (erythroblasts) due to the protective antioxidant action of *Garcinia kola* seed constituents, especially the flavonoid. The interplay of protective actions such as modulation of erythropoietin production, absence of anemia and delayed release of reticulocytes into peripheral blood, ensures an aggregate decrease in mean cell volume [42].

The decrease in MCV can also be attributed to possible functional deficiency of elemental iron, facilitated by accelerated mobilization of iron stores without supplementation in the diet (Sezer *et al.*, 2002). This supports the observed significant rise in MCHC at a dose of 100 mg/kg body

weight; considering the decreases in MCH at doses of 100mg/kg and 200mg/kg, signifying the consequence of depreciating iron stores. This depreciation was not low enough to reverse increases in PCV and hemoglobin concentration at the said doses of *Garcinia kola* seed.

From the result of this experiment, *Garcinia kola* caused an increase in platelet count. Platelet count initially decreased from control to group II, then increased in group III after 21 days, these values showed no significance; as shown in figure 8. It then increased from 416.20 ± 8.34 (control) to 537.40 ± 7.99 (group II) to 659.0 ± 14.69 (group III) after 42 days, these values were statistically significant. Figure 8 shows a significant increase in low dose groups (100 mg/kg body weight) and high dose groups (200 mg/kg body weight) after comparing 21 days and 42 days. The effect of *Garcinia kola* seed extract on platelet count were dose – related; and were observed to be more intense at higher doses. It was also duration–dependent as values increased significantly from 21 to 42 days. This was contrary to the work of Ekpenyong *et al* [20] and Ahumibe and Braide [36], where there was no significant enhancement in platelet numbers due to flavonoids being said to cause an inhibition of thromboxane A₂ formation that inhibits platelet aggregation. The high level of platelet count observed in the experiment could be attributed to increased platelet aggregation, resulting in influx of platelet from the bone marrow. This could result from allergies or inflammation [44].

5. CONCLUSION

Findings of this study have shown that the ethanolic extract of *Garcinia kola* seed possess hematopoietic, stimulating and enhancing effects. However, following the alterations in these haematological parameters by the extract, caution should be exercised in its usage, as it may not be completely ‘safe’ as oral remedy at the doses investigated in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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