

Original Research Article

Efficacy of *Garcinia kola* Seed Ethanolic Extract on Haematological Parameters in Male Wistar Rats

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ABSTRACT

The efficacy of *Garcinia kola* seed ethanolic extract on selected haematological parameters of male Wistar rats was investigated. Thirty (30) male Wistar rats weighing 150.42 ± 3.98 g 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 for 21 days. 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, carbohydrates, 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, white blood cell (WBC) count, platelet count and mean cell hemoglobin concentration (MCHC) in the plasma of the animals. The extract significantly ($p < 0.05$) decreased the mean cell volume (MCV) and mean cell hemoglobin (MCH) in the plasma of the animals. Therefore, the ethanolic extract of *Garcinia kola* seed through its antioxidant property showed hematopoietic, stimulating and enhancing effects.

Keywords: *Garcinia kola*, Haematological Parameters, Ethanolic Extract, Antioxidant property, Hematopoietic.

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

On haematology, studies by [19] have reported the effect of ingestion of *Garcinia kola* seed on erythrocytes in rabbits while [20] investigated the hematological effect of chronic administration of ethanolic extract of *Garcinia conruana* seed on rat, while [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 efficacy of the ethnobotanically acclaimed doses of 100 and 200 mg/kg body weight on haematological parameters. Therefore, this study presents information on the efficacy of *Garcinia kola* seed ethanolic extract 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) used throughout the experimental period.

2.2.2 Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardiac glycosides and carbohydrates were carried out by adopting the procedures described by [22, 23, 24, 25, 26, 27, 28, 29], respectively.

2.2.3 Acute Toxicity Study (LD₅₀)

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

Stage I: Nine rats were completely randomized into 3 groups (A-C) 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. Three groups (A-C) of one rat each were 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 (30) male Wistar 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 g 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 for 21 days using polystyrene. 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 Plasma

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. Plasma was obtained in this process.

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, carbohydrates, 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	++
Carbohydrates	++
Anthraquinones	-
Steroids	+
Cardiac glycosides	-

Key:

- Absent

+ Present

++ Significantly present

+++ Abundantly present

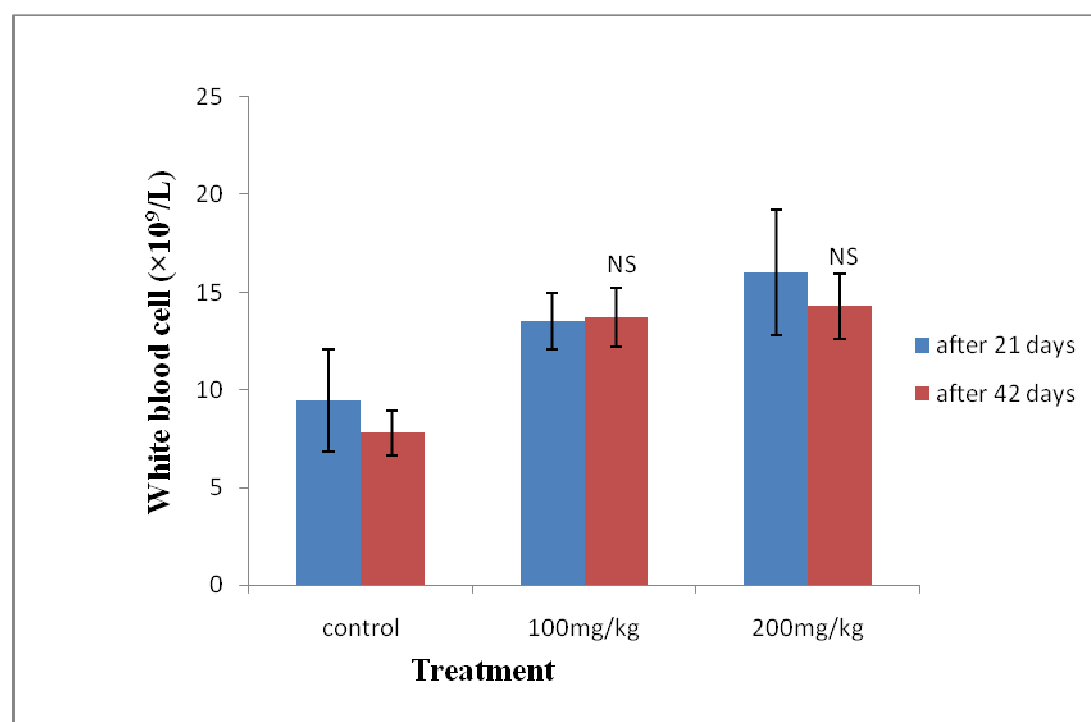
For acute toxicity study, all the graded doses of the ethanolic extract of *Garcinia kola* seed administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD₅₀ of ethanolic extract of *Garcinia kola* seed was found to be safe up to 5000 mg/kg body weight.

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 in the plasma of the animals; as shown in Figure 1.

173 However, there was no significant increase in groups II and III as compared with control
174 ($p=0.324$ 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) in the plasma of the animals, and in group III,
177 there was also an increase to 14.28 ± 1.64 ; as shown in Figure 1. There was significant increase in
178 groups II and III as compared 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.



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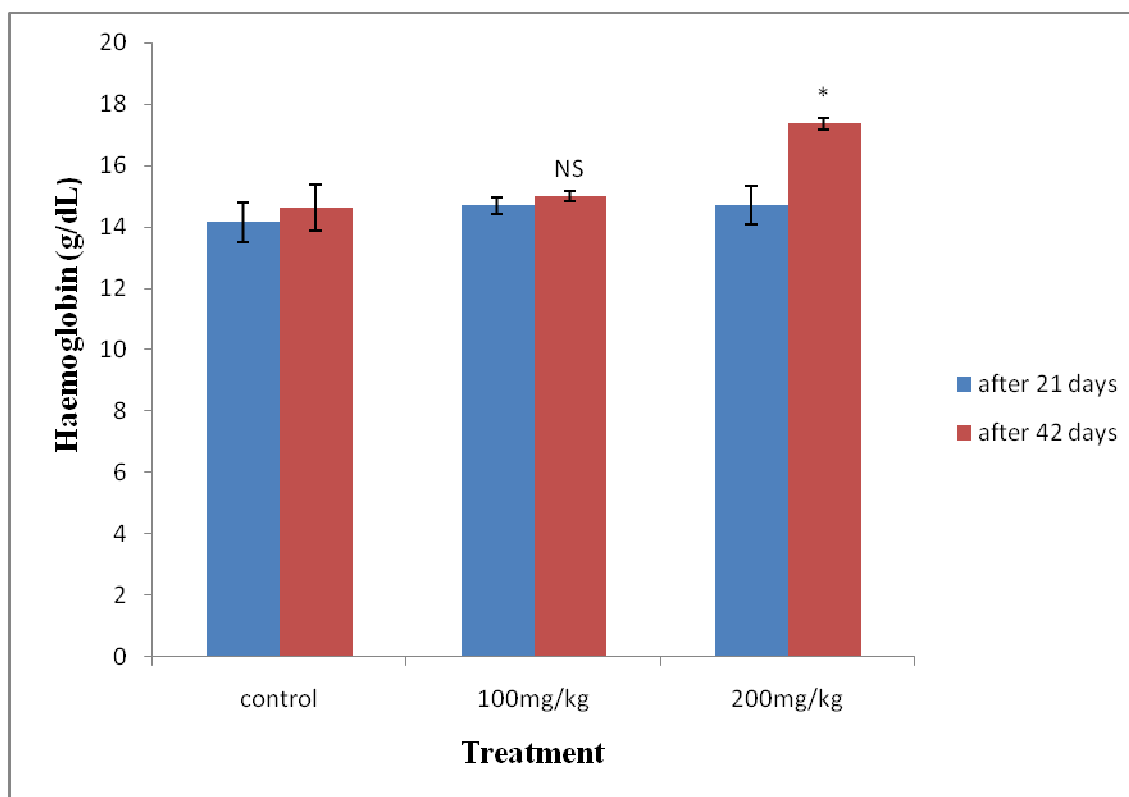
196 NS = Not significant

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 in the plasma of the animals; as shown in Figure 2. However,
202 there was no significant increase in groups II and III as compared with control ($p=0.440$ and
203 $p=0.225$ respectively).

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

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



*P < 0.05 = statistically significant

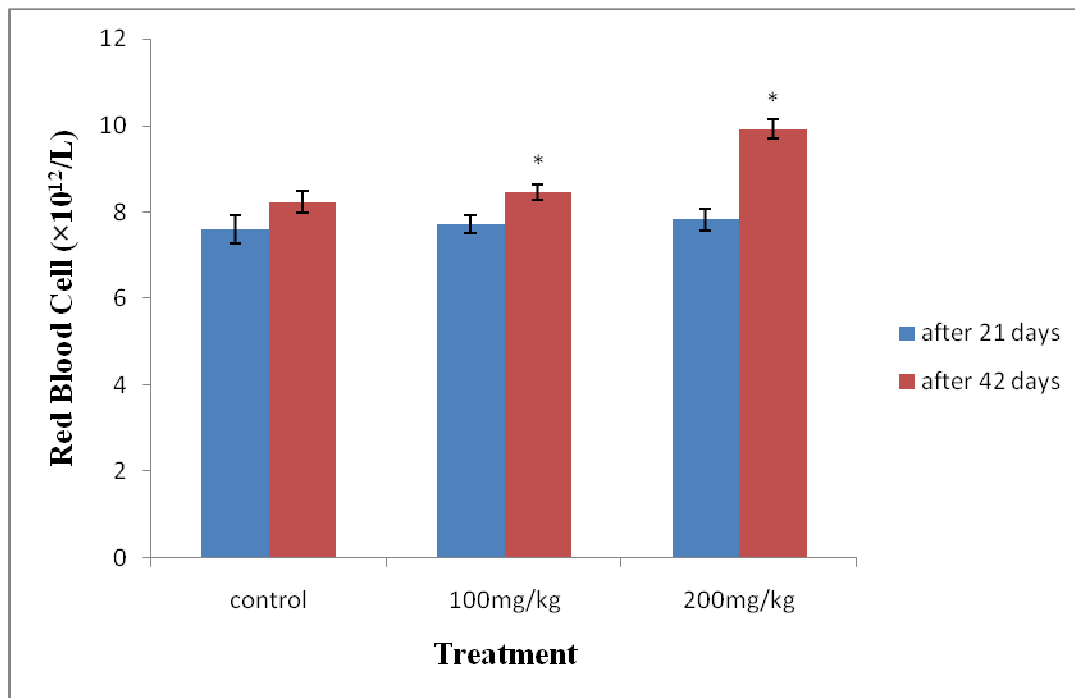
NS = Not significant

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

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

After 42 days of administration, the ethanolic extract of bitter kola increased the RBC count from 8.23 ± 0.26 (control) to 8.45 ± 0.18 (group II), and in group III, there was also an increase to 9.91 ± 0.22 , as shown in Figure 3. There was no significant increase in group II as compared with control, but there was significant increase in group 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 red blood cell count in group II from 7.71 ± 0.2 after 21 days to 8.45 ± 0.18 after 42 days, and in group III, there was also an increase from 7.82 ± 0.26 after 21 days to 9.91 ± 0.22 after 42 days. There was significant increase in groups II and III after comparing 21 days and 42 days; as shown in Figure 3. ($p=0.010$ and $p=0.002$ for groups II and III respectively).



* $P < 0.05$ = 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) in the plasma of the animals, 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 21 days and 42 days, but there was significant increase in group III after comparing 21 and 42 days; as shown in Figure 4 (p=0.052 and p=0.002 for groups II and III respectively).

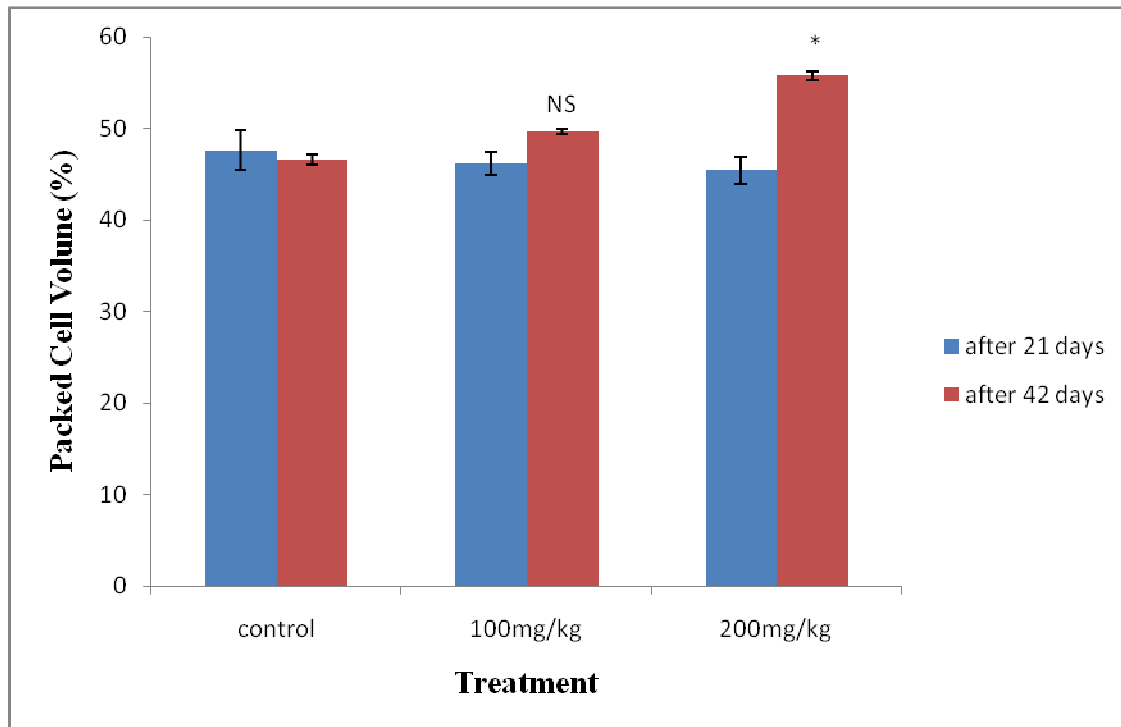


Figure 4: Effect of ethanolic extract of *Garcinia kola* seed on packed cell volume (PCV) (%) after 21 and 42 days; *P < 0.05 = statistically significant; NS = Not significant

After 21 days of administration, the ethanolic extract of *Garcinia kola* seed decreased the mean cell volume (MCV) from 62.74±1.4 (control) to 61.04±0.88 (group II) in the plasma of the animals, and in group III, there was also a decrease to 58.2±0.42, as shown in Figure 5.

However, there was no significant increase in group II as compared with control ($p=0.354$), but there was significant increase in group III as compared with control ($p=0.036$).

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

Comparing the effect of 21 days and 42 days of administration, the ethanolic extract of *Garcinia kola* seed decreased the mean cell volume in group II from 61.04 ± 0.88 after 21 days to 58.78 ± 0.47 after 42 days, but in group III, there was an increase from 58.2 ± 0.42 after 21 days to 66.14 ± 0.54 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 5 ($p=0.126$ and $p=0.001$ for groups II and III respectively).

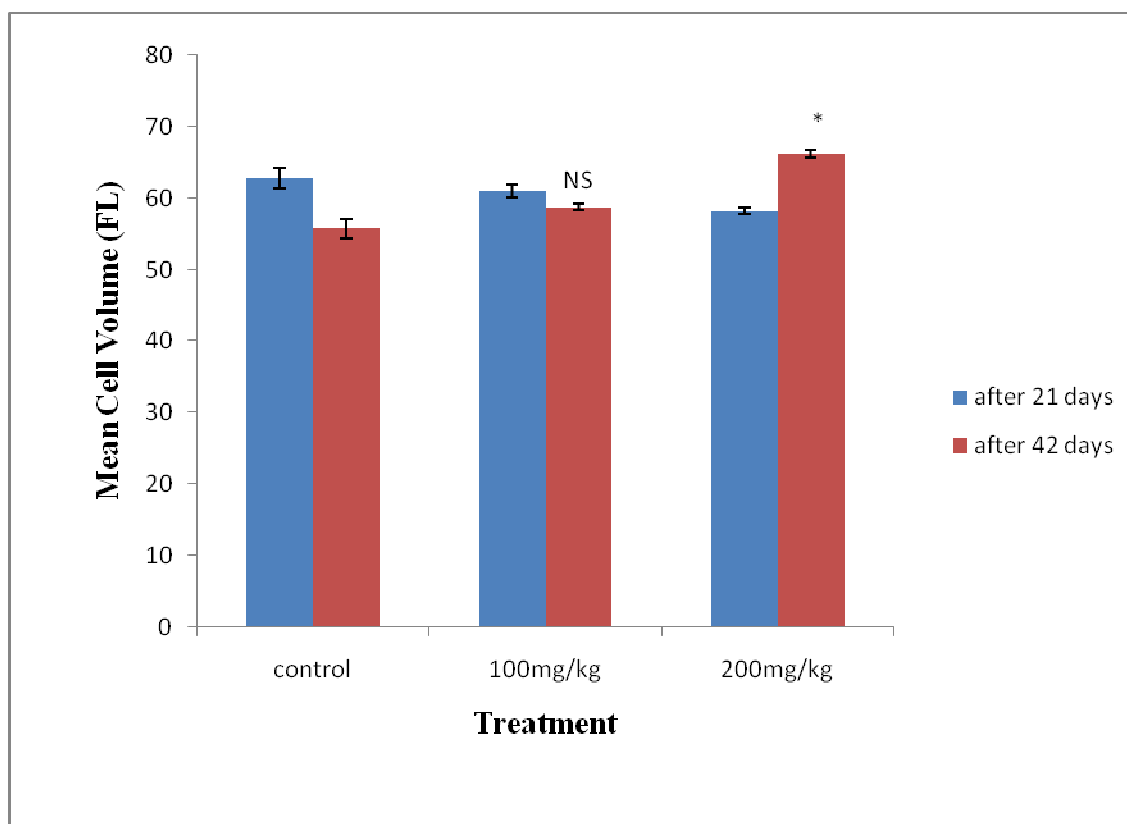


Figure 5: Effect of ethanolic extract of *Garcinia kola* seed on mean cell volume (MCV) (FL) after 21 and 42 days; *P < 0.05 = statistically significant; NS = Not significant.

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

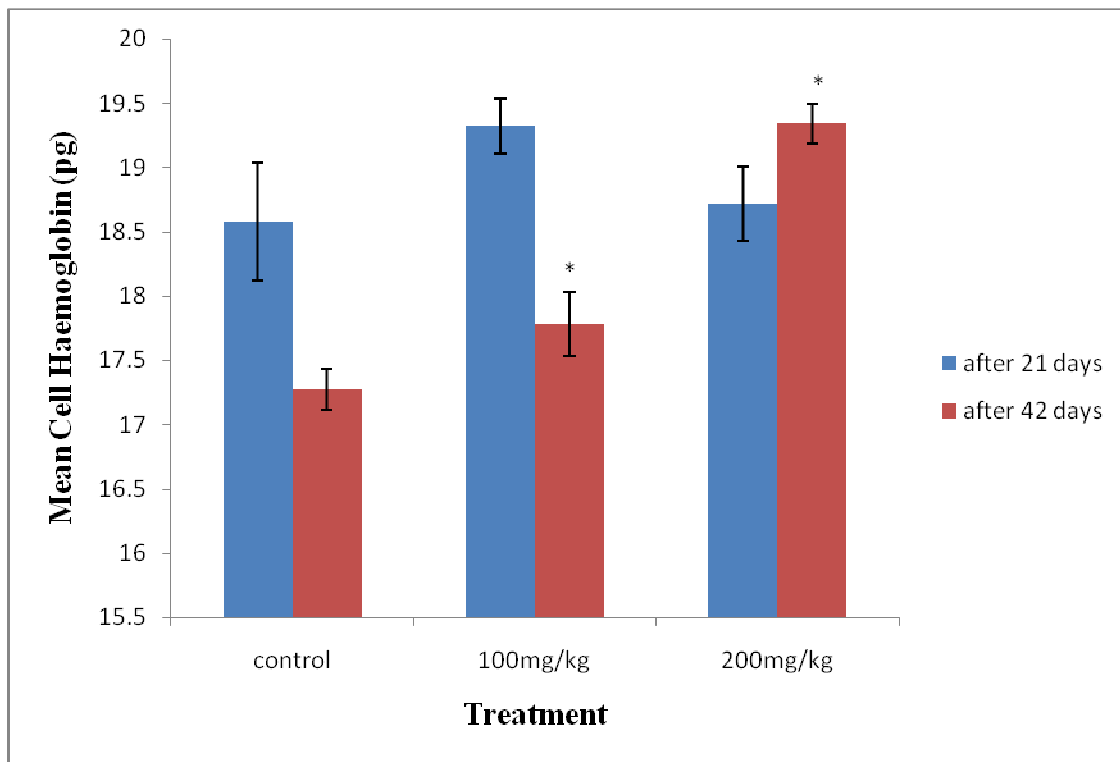


Figure 6: Effect of ethanolic extract of *Garcinia kola* seed on mean cell hemoglobin (MCH) (pg) after 21 and 42 days; * $P < 0.05$ = statistically significant; NS = Not significant.

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) in the plasma of the animals, 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).

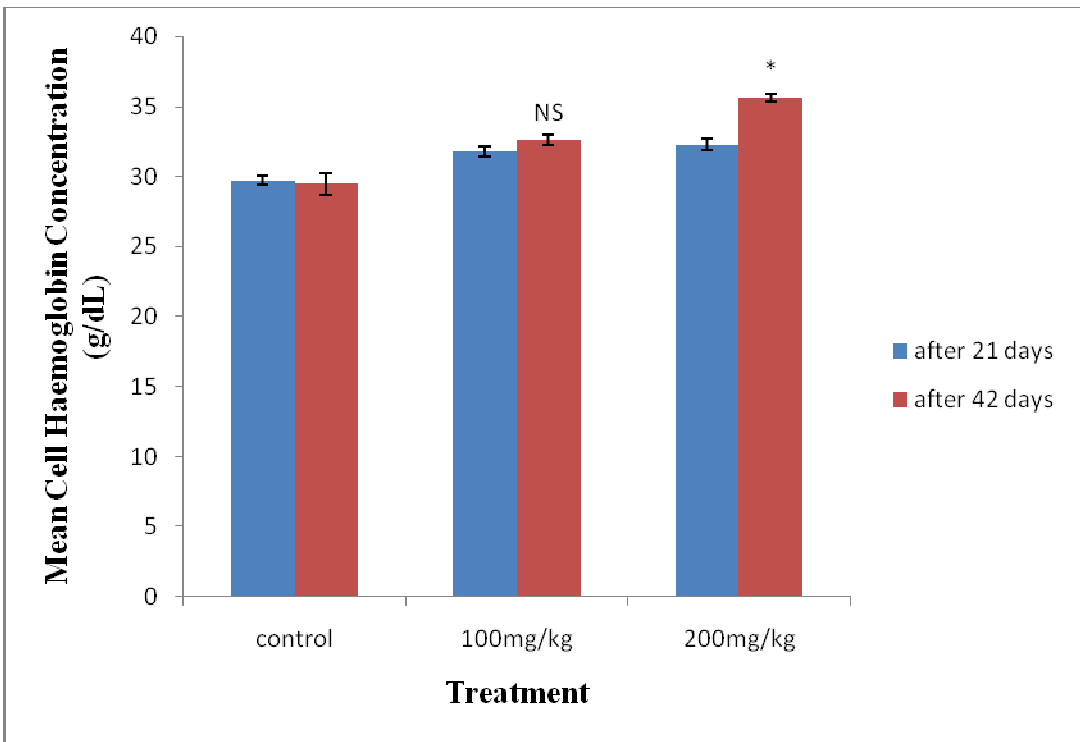


Figure 7: Effect of ethanolic extract of *Garcinia kola* seed on mean cell hemoglobin concentration (MCHC) (g/dL) after 21 and 42 days; *P < 0.05 = statistically significant; NS = Not significant

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) in the plasma of the animals, 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 659.0 ± 14.69 after 42 days. There was significant increase in groups II and III after comparing 21 days and 42 days respectively; as shown in Figure 8 ($p=0.001$ and $p=0.002$ for groups II and III respectively).

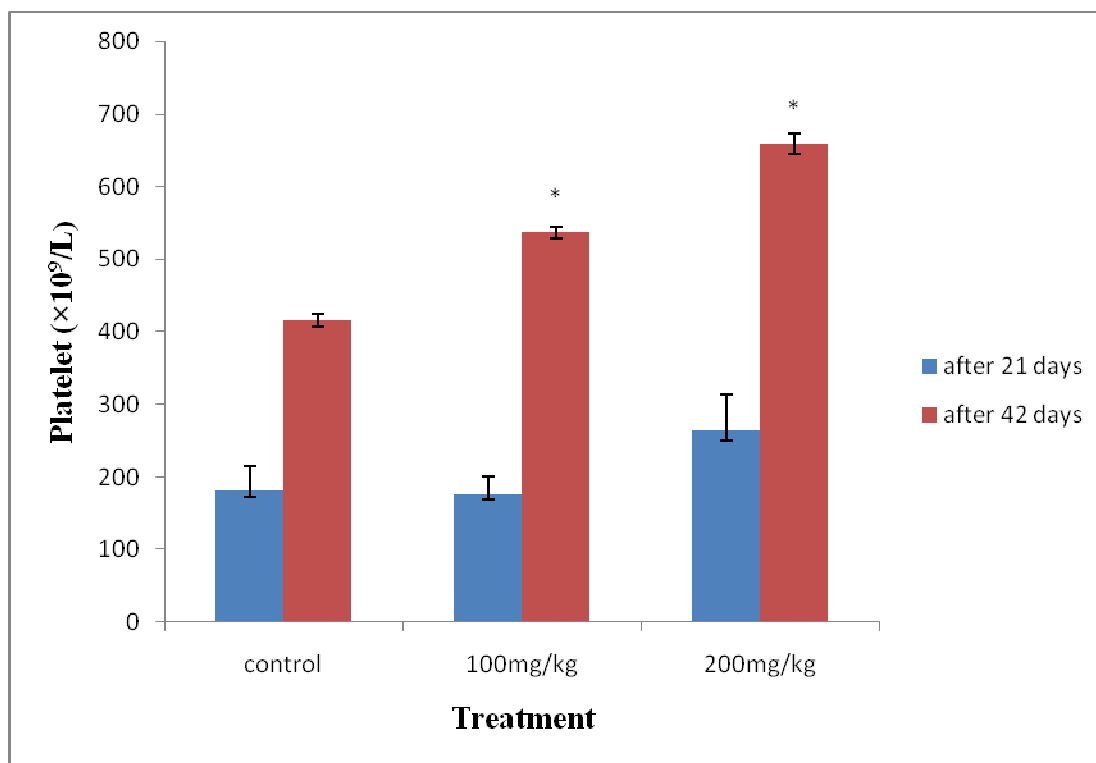


Figure 8: Effect of ethanolic extract of *Garcinia kola* seed on platelet count ($\times 10^9/\text{L}$) after 21 and 42 days; * $P < 0.05$ = statistically significant; NS = Not significant

4. DISCUSSION

Medicinal plants such as *Garcinia kola* are believed to be an important source of new chemical substances with potential therapeutic benefits [31, 32]. Knowledge of haematological characteristics is an important tool used as an effective and sensitive index of maintaining physiological and pathological changes in animal models [33]. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of animal models as these indices provide reliable information on metabolic disorders, deficiencies, chronic stress status and blood relating functions before they are present in a clinical setting [34]. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even 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 in the plasma of the animals. The result is similar to the findings of [36]; where an experiment was carried out on the effect of chronic administration of ethanolic extract

357 of *Garcinia conruana* seed on rats where the WBC count did not show any significant
358 enhancement in numbers.

359 Red blood cell (RBC) count in the plasma of the animals showed an increase after 21 days and
360 after 42 days of administration of the extract as seen in Figure 3. The mechanism leading to the
361 increase in RBC count is probably mediated by the anti-oxidant property of the extract. This
362 gives the extract the haemopoietic, protective and stimulating potentials.

363
364 Packed cell volume (PCV) showed decreased values from control to group II to group III after 21
365 days that were not significant; as shown in Figure 4. Mean cell volume (MCV) decreased from
366 control to group II to group III after 21 days and increased from control to group II to group III
367 after 42 days. Mean cell hemoglobin (MCH) increased from control to group II but decreased in
368 group III after 21 days and increased from control to group II to group III after 42 days. These
369 alterations in mean cell volume (MCV) and mean cell hemoglobin (MCH) might have adverse
370 effect on blood related functions of the extract.

371 The values for red blood cell (RBC) count, hemoglobin (Hb) concentration and mean cell
372 hemoglobin concentration (MCHC) in the plasma of the animals increased as the dosage
373 increased and the period of administration increased, i.e. they were dose-dependent and duration-
374 dependent. The values for packed cell volume (PCV) were mostly duration-dependent; they
375 increased with increase in the period of administration. All these are alterations and would effect
376 on blood related function of the extract.

377 Similar findings where there was a steady increase in the red blood cell (RBC) count, packed cell
378 volume (PVC), hemoglobin (Hb) concentration, mean cell hemoglobin concentration (MCHC),
379 have been documented [4, 19, 36].

380 The steady rise in the values of red blood cell count, packed cell volume and hemoglobin
381 concentration in the plasma of the animals as the bitter kola seed concentration increased is said
382 to be due to the antioxidant activity of flavonoid which was present in the blood due to *Garcinia*
383 *kola* seed extract administration [37], 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 (Hb) concentration in the plasma of the animals 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. However, the non-significant effect of the extract at 100 mg/kg body weight on Hb concentration after 21 and 42 days may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not altered.

There was a clear decrease in the mean cell volume (MCV) in the plasma of the animals 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 decreased MCV and the elevated levels of PCV in the plasma of the animals by the extract further suggest selective toxicity of the extract and or its components.

The decrease in MCV in the plasma of the animals can also be attributed to possible functional deficiency of elemental iron, facilitated by accelerated mobilization of iron stores without supplementation in the diet [43]. 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 in the plasma of the animals. The effects 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. The high level of platelet count could be attributed to increase platelet aggregation, resulting in influx of platelet from the bone marrow due to increased thromboxane A2 biosynthesis [44]. This was contrary to the work of [20] and [36], where there was no significant enhancement in platelet number due to flavonoids being said to cause an inhibition of thromboxane A2 formation that inhibits platelet aggregation.

5. CONCLUSION

Findings from the present study have shown that the ethanolic extract of *Garcinia kola* seed, through its antioxidant property possess hematopoietic, stimulating and enhancing effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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