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Original Article

Pre-clinical protective potentials of *Carica papaya* constituents in experimentally induced anemia

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Abstract: Objective: Anemia is a pathological condition characterized by reduced oxygen bioavailability and/or changes in hematological parameters. This study investigated the anti-anemic activities of *Carica papaya* (CP) phytoconstituents in aluminium-chloride-induced anemic rats. Method: Twenty-seven rats were randomized into nine groups of three rats as follows; group 1 was the normal (non-induced) group, 2-9 were anemic rats administered 1 mL distilled water, standard drug (3 mg/kg body weight (bw) ferrous sulphate), 100, 300 and 500 mg/kg bw of crude methanolic extract of CP (CMECP) of the leaf and 100, 300 and 500 mg/kg bw of CMECP of the seed respectively in the first stage of the study. In the second stage, thirty-three rats were randomized into eleven groups of three rats as follows; group 1 was the normal group, 2-11 were anemic rats treated with 1 mL distilled water, standard drug, 75 mg/kg bw, 150 mg/kg of alkaloid fraction of CP seed, 75 mg/kg bw, 150 mg/kg bw of flavonoid fraction of CP seed, 75 mg/kg bw and 150 mg/kg of alkaloid fraction of CP leaf, 75 mg/kg bw and 150 mg/kg bw of flavonoid fraction of CP leaf respectively. Results: Treatment of anemic rats with CP extracts and fractions of the seed and leaf significantly reversed the hematological parameters and body weight of anemic rats in a dose independent fashion. The CMECP leaf at 100 and 500 mg/kg gave PCV of 42.50±0.50 and 47.00±0.50, while the seed gave 49.50±0.50 and 42.50±0.50 respectively after 2 weeks of treatment. However, the alkaloid and flavonoid fraction of CP presented better anti-anemic properties probably due to constituents' synergism. Conclusion: This study concluded that CP possesses phytoconstituents which potentiates it as a safe anti-anemic drug candidate.

Keywords: Anti-anemic, aluminium-chloride, *Carica papaya*, phytoconstituents, hematological parameters

Introduction

Anemia is a pathological and hematological condition resulting in reduction in blood parameters [1]. This condition may arise from lack of oxygen supply to epithelial tissues for various physiological body functions. Pathological conditions such as morphological or mechanical defects lead to reduction in the oxygen-carrying capacity of hemoglobin (Hb) with signs and symptoms including weakness, irregular heartbeat, pale skin, and nail bed and headache [2, 3]. At the early stage, mild symptoms might be observed, however as the anemia progresses, its symptoms become severe leading to conditions such as insomnia, hemolysis, renal impairments, and hypertension [4].

Anemic conditions can be induced externally with chemicals like phenyl-hydrazine, dapsone, hydroxylamine, divicine, and aluminum chloride [5]. Aluminum (Al) is a trivalent metallic cation that contributes to hemolytic free radical production causing damage to various proteins,

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DNA, membrane lipids, and prevents the binding of iron to transferrin. Aluminum also produces peroxidative changes in the erythrocyte's membrane causing hemolysis [6].

In 2021, 1.92 billion (24.3%) global cases of anemia were reported. However, the age, sex, and geographical distribution vary. For example, children below 5 years, females, sub-Saharan Africa, and south Africa are more affected by anemia [7]. Although, anemia is not listed as a disease of global concern, in Africa it causes significant morbidity and mortality; moreover, it decreases the already limited resources in less developed countries, contributing to social effects such as reduced quality of life and depression [8]. In Nigeria, more than 40% of the population is anemic with children and women being the most impacted [9].

The strategies used in the management and treatment of anemia, especially iron deficiency induced anemia are oral or intravenous administration and supplementation with iron (ferrous) [10]. Blood transfusion and bone marrow transplantation can also be used to treat anemia; however, they are cost intensive. Anti-anemic drug such as fersolates presents risk of iron overdose and mutagenicity [4]. Haemochromatosis (iron overload disorder) can also result from the use of anti-anemic drugs which can lead to cancer and heart diseases if left untreated. Excess iron can also get stored in the liver, heart, and pancreas, resulting in other pathological conditions [11]. Therefore, it becomes pertinent to explore other safer treatment options.

With the growing exploration of medicinal plants as alternative drug candidates, there is high demand for probes into their constituents to generate information for safer and effective drug development purposes. *Moringa oleifera, Ipomoea batata, Mormodica charantia* are among plants of reported anti-anemic properties [12], however, a large number of plants are yet to be reported for their anti-anemic potential. *Carica papaya* (family: *Caricaceae*; common name 'pawpaw') is widely cultivated for its nutritional and medicinal values [13]. This plant has several traditional uses such as treatment of fibroid, kidney, dental, and heart diseases, it is also used to boost memory and low sperm count [14]. It leaves have been reported to possess anticancer, anti-plamodial, anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects [15]. Due to well documented reports implicating the role of oxygen-based free radicals Al-induced anemia and presence of a large array of phytoconstituents in *C. papaya*, this study investigated the anti-anemic potential of selected phytochemicals of *C. papaya* leaf and seed extracts in aluminium chloride-induced ($AlCl_3$)-induced anemia in an animal model, with a view to formulating new cost-effective drugs that are easily accessible, non-toxic, and anti-anemic.

Materials and methods

Fresh leaves of *C. papaya* were collected from the biological garden of Federal University of Technology (FUT), Minna, Niger State in November 2018, while the seeds were obtained from fruit sellers in Bosso Local Government Minna, Niger State in November 2018. The plant materials were identified by Mr. Dangana, M. C. of Biological Science Department, FUT, Minna, Nigeria.

Reagents, chemicals, and equipment

All the chemicals and reagents used in this study were products of Sigma chemical Co, USA while the equipment used was provided by the Department of Biochemistry, FUT Minna, Nigeria.

Experimental animals

A total of sixty rats (weighing 125-160 g) were obtained from the animal breeding unit of the National Veterinary Research Institute, Vom, Nigeria. The animals were housed in standard plastic cages (temperature maintained at 22°C±3°C, relative humidity of 50%±5%, and a 12-hour dark and 12-hour light cycle) and fed a commercial rat diet with free access to water.

Ethical approval

This study was approved by the Animal Use and Research Committee of Federal University of Technology, Minna, Nigeria (BCHFUTMINNA-012019). All the procedures performed in the animal experiments were in accordance with the ethical standards of this institution.

Preparation of extracts

The plant materials were rinsed, air-dried, and pulverized using an electronic blending ma-

chine. The powdered samples were extracted with methanol in the ratio 1:8, refluxed for 2 hours in a distillation flask mounted on a heating mantle. The mixture was filtered and concentrated to give the crude methanolic extracts of *C. papaya* (CMECP). The extracts obtained were weighed for percentage yield estimation using the equation:

$$\text{Percentage yield} = \frac{\text{Weight (g) of extract}}{\text{Weight (g) of pulverized sample}} \times 100$$

Extraction of total flavonoid

The extraction of total flavonoid was carried out as described by Jouad et al. [16]. Briefly, 350 g each of powdered *C. papaya* seed and leaf were extracted with methanol in a Soxhlet extractor. The extracts were concentrated under reduced pressure using rotary evaporator. Subsequently, 41 g of the methanol extracts were dissolved in 400 mL of distilled water and extracted with n-butanol saturated with distilled water. This was then concentrated, and the butanol extract was subjected to column chromatography on silica gel eluted with n-hexane and methanol.

$$\% \text{ Yield} = \frac{\text{Weight of the flavonoid}}{\text{Weight of the powdered } \textit{C. papaya}} \times 100$$

Extraction of total alkaloids

The Soxhlet method as described by Gonzales et al. [17] was used for the alkaloid extraction. Briefly, 300 g of powdered *C. papaya* leaf and seed was weighed separately and packed in a cheese cloth bag which served as an extraction thimble. 95% ethanol was added to each sample in the thimble to moisten it before the addition of ammonia test solution (T.S) The extraction was done in a Soxhlet extractor for 24 hours after which 95% ethanol was then placed in the solvent flask and further extracted for another 3-4 hours. The mixture obtained was filtered and concentrated at 60°C. The resulting crude alkaloid extract obtained was treated with 1.0 N HCl and filtered. Ammonia T.S was then added to the filtrate. Afterwards, chloroform was added to the mixture and thoroughly shaken in a separating funnel to form two layers where the lower chloroform layer contained the alkaloid, and the aqueous portion formed the upper layer. The chloroform layer was carefully collected and concentrated using a rotary evaporator. The percentage alkaloid yield was calculated as:

$$\% \text{ Yield} = \frac{\text{Weight of the alkaloidal residue}}{\text{Weight of the powdered } \textit{C. papaya}} \times 100$$

Acute toxicity test of crude extract of C. papaya

This test was conducted using Lorke's method [18]. Three groups comprising three animals were administered 10, 100 and 1000 mg/kg of the extract per group in the first phase. Behavioral changes and mortality were observed for 24 hours. In the second phase, three animals were administered 1600, 2900 and 5000 mg/kg of the extract respectively and observed for behavioral changes and mortality for 24 hours. Then the lethal dose (LD_{50}) was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Mortality at the highest dose; D_{100} = Mortality at the lowest dose.

Experimental model for anti-anemic study

The protective potential effect of crude methanolic extracts and crude alkaloids and flavonoid fractions of *C. papaya* were evaluated in aluminium-chloride ($AlCl_3$)-induced anemic rats. The anemic condition was induced with 0.5 mg/kg for ten minutes followed by a 14-day oral treatment period. The study was carried out in two stages as follows:

The first stage involved treatment with CMECP leaf and seed. Nine groups comprising 3 rats each were treated as follows: group 1 was non-anemic (normal control), groups 2 and 3 were anemic rats administered distilled water (1 mL) and standard drug [(ferrous sulphate (3 mg/kg)] respectively, groups 4-6 were anemic rats treated with 100, 300 and 500 mg/kg extract of *C. papaya* leaf respectively while groups 7-9 were anemic rats treated with 100, 300 and 500 mg/kg of *C. papaya* seed respectively.

The second stage involved the treatment of anemic rats with crude alkaloid fraction of *Carica papaya* (CAFCP) and crude flavonoid fractions of *Carica papaya* (CFFCP) leaf and seed. Nine groups comprising 3 rats each were treated as follows: group 1 was non-anemic (normal control), groups 2 and 3 were anemic

Table 1A. Percentage yield of crude methanol extract, alkaloid and flavonoid fractions of *C. papaya* leaf and seed

Plant Samples	% Yield		
	Methanolic extract	Leaf	Seed
C. papaya leaf	17.51±1.93*	-	-
C. papaya seed	11.59±0.78	-	-
Crude *C. papaya* alkaloid fraction	-	2.65±0.03[a]	2.97±0.04[a]
Crude *C. papaya* flavonoid fraction	-	7.05±0.65[b]	7.96±0.29[b]

Data are presented in Mean ± standard error of one paired determination. *Significantly higher as compared to crude (seed) extract at P<0.05. Values with superscript alphabet b is significantly higher than value with superscript alphabet a.

Table 1B. Quantitative phytochemicals composition of crude methanolic extract of *C. papaya* leaf and seed

Parameters	Leaf (mg/100 g)	Seed (mg/100 g)
Phenols	261.34±1.07[a]	171.45±0.91[b]
Flavonoid	34.89±0.66[a]	25.80±0.99[b]
Tannins	45.25±0.46[a]	40.67±0.50[b]
Alkaloids	67.75±1.06[a]	35.42±0.50[b]
Saponins	1249.83±13.05[a]	723.65±0.39[b]

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabets (a and b) are significantly different across a row at P<0.05.

rats administered distilled water (1 mL) and ferrous sulphate (3 mg/kg) respectively, groups 4 and 5 were anemic rats treated with 75, and 150 mg/kg of the CAFCP seed. Similarly, groups 6 and 7 were treated with 75, and 150 mg/kg of CFFCP seed. Groups 8 and 9 were treated with 75, and 150 mg/kg of CAFCP leaf, while groups 10 and 11 were treated with 75, and 150 mg/kg of CFFCP leaf.

Blood sample collection and determination of hematological parameters

During the 2 weeks treatment period, the animals' body weight was recorded within an interval of 3 days before they were finally sacrificed on the 15th day of treatment [19]. Blood sample (3 mL) was collected by cardiac puncture into a well-labeled heparinized sample bottle and analyzed immediately for hematological parameters using the SYSMEX KX21 (SYSMEX, Co, Japan) automated analyzer [20].

Data analysis

Data generated were analyzed using Statistical Package for Social Science (SPSS v20). All the data in the results are expressed as mean ± standard error of three replicate determinations. One Way ANOVA (analysis of variance) was used for comparison between groups where a *P*-value <0.05 was considered significant. Values or bars with different letters of the alphabet are significantly (P<0.05) different.

Results

Phytochemicals compositions of C. papaya leaf and seed

The percentage yield of crude methanolic extract, alkaloid, and flavonoid fractions of *C. papaya* leaf and seed are presented in **Table 1**. The percentage yields of crude *C. papaya* leaf extract (17.51±1.93%) was significantly (P< 0.05) higher than the that of the crude seed extract (11.59±0.78%) (**Table 1A**). Upon crude alkaloid and flavonoid fractionation of the leaf and seed, a higher flavonoid yield was recorded in the leaf and seed fractions when compared to their alkaloid yields (**Table 1A**). The CMECP possessed different secondary metabolites in different concentrations (**Table 1B**). For the leaf and seed respectively, saponins had the highest concentrations; 1249.83±13.05 and 723.65±0.39 mg/g, followed by phenols (261.34±1.07 and 171.45±0.91 mg/g), alkaloids (67.75±1.06 and 35.42±0.50 mg/g), tannins (45.25±0.46 and 40.67±0.50 mg/g) and flavonoids (34.89±0.66 and 25.80±0.99 mg/g).

Acute oral toxicity test for CMECP leaf and seed

The result of the oral toxicity of CMECP is presented in **Table 2**. The lethal dose (LD_{50}) of CMECP seed and leaf in rats was >5000 mg/kg while the safe doses were 1600 and 2900 mg/kg bw, respectively. However, animals

Table 2. Safe dose of methanol seed and leaf extract of *C. papaya*

Phases	Plant part/ groups	Dose (mg/kg)	Number of rats	Number of deaths after 24 hours	% Mortality	Sign of toxicity
PHASE 1				Seed		
	Group 1	10	3	0	0	Nil
	Group 2	100	3	0	0	Nil
	Group 3	1000	3	0	0	Nil
				Leaf		
		10	3	0	0	Nil
		100	3	0	0	Nil
		1000	3	0	0	Nil
PHASE 2				Seed		
	Group 1	1600	3	0	0	Nil
	Group 2	2900	3	0	0	Rubbing of the mouth on the surface of the cage
	Group 3	5000	3	0	0	Hyperactivity/restlessness
				Leaf		
	Group 1	1600	3	0	0	Nil
	Group 2	2900	3	0	0	Nil
	Group 3	5000	3	0	0	Hyperactivity/restlessness

Table 3. Effect of crude methanol extract of *C. papaya* leaf and seed on body weight of $AlCl_3$-induced anemic rats

Group	Day 0	Day 3	Day 7	Day 10	Day 14
Normal Control	191.50±10.50^{a,b}	196.50±23.50^{a}	199.25±36.45^{b}	203.00±39.00^{a}	209.75±37.25^{b}
Untreated anemic rats	107.00±33.00^{b}	102.00±30.00^{a}	94.05±11.35^{a,b}	81.50±9.50^{a}	71.00±11.00^{a,b}
3 mg/kg bw $FeSO_4$	133.50±19.50^{a}	137.75±12.75^{a}	142.00±17.00^{a}	148.00±26.00^{a}	152.50±11.50^{a}
100 mg/kg bw CMECP leaf	163.25±2.75^{a,b}	154.75±0.25^{a}	162.50±1.50^{a,b}	171.50±6.50^{a}	185.00±5.50^{a,b}
300 mg/kg bw CMECP leaf	196.25±23.75^{a,b}	180.50±30.20^{a}	201.50±22.50^{a,b}	190.00±37.00^{a}	206.75±39.75^{a,b}
500 mg/kg bw CMECP leaf	161.50±10.50^{a,b}	168.50±9.50^{a}	175.75±7.25^{a,b}	169.50±6.50^{a}	177.80±4.20^{a,b}
100 mg/kg bw CMECP seed	202.50±10.50^{a,b}	209.50±14.50^{a}	222.00±19.00^{a,b}	226.00±33.00^{a}	230.25±12.75^{a,b}
300 mg/kg bw CMECP seed	156.25±0.25^{a,b}	173.50±10.50^{a}	186.350±3.50^{a,b}	186.00±12.00^{a}	191.50±15.00^{a,b}
500 mg/kg bw CMECP seed	134.00±38.00^{a}	143.00±36.00^{a}	151.35±34.35^{a}	151.50±37.50^{a}	168.25±28.25^{a}

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabets (a and b) are significantly different between groups at $P<0.05$.

administered 2900 mg/kg and 5000 mg/kg bw demonstrated behavioral signs including rubbing of mouth on the surface of the cage, hyperactivity/restlessness but no death was recorded.

Body weight and PCV changes in $AlCl_3$-induced anemic rats treated with CMECP seed and leaf

The changes in weight of $AlCl_3$-induced anemic rats treated with CMECP seed and leaf over a period of 2 weeks are presented in **Table 3**. A decrease in the weight of untreated anemic rats relative to the normal and treatment groups was observed. However, treatment with CMECP led to an increase in the body weights of the animals.

The effect of 2 weeks CMECP treatment on the packed cell volume (PCV) of $AlCl_3$-induced anemic rats is presented in **Table 4**. The reduced PCV count of the untreated group relative to other groups was observed. However, the extract groups had higher PCV counts than the normal control.

Changes in body weight and PCV of $AlCl_3$-induced anemic rats treated with alkaloids and flavonoid fractions of C. papaya seed and leaf

The changes in body weight of $AlCl_3$-induced anemic rats treated with alkaloids and flavonoid fractions of *C. papaya* seed and leaf are presented in **Table 5**. Aluminium-chloride caused a decrease in the body weight of the

Table 4. Changes in Packed Cell Volume (PCV) in aluminium chloride-induced anemic rats treated with methanol extracts of *C. papaya* for two weeks

GROUPS	DAY 0	DAY 4	DAY 8	DAY 12	DAY 15
Normal Control	38.37±0.43^{a,b}	40.51±0.45^{b}	41.03±0.09^{c}	41.63±0.36^{d}	43.00±2.00^{a,b}
Untreated anemic rats	40.50±1.81^{d}	36.43±0.97^{c}	31.36±2.19^{a}	28.70±1.30^{a}	26.50±3.50^{a}
3 mg/kg bw $FeSO_4$	38.21±0.39^{a,b}	37.75±0.38^{a}	38.43±0.25^{b}	38.69±0.42^{b}	40.00±0.00^{b}
100 mg/kg bw CMECP leaf	37.87±0.14^{a}	37.81±0.41^{a}	39.10±0.32^{b}	40.48±0.33^{c}	42.50±0.50^{b}
300 mg/kg bw CMECP leaf	37.43±0.43^{a}	37.30±0.85^{a}	36.92±0.45^{b}	39.16±0.22^{b,c}	40.50±0.50^{b}
500 mg/kg bw CMECP leaf	40.43±0.27^{b}	42.58±0.29^{c}	43.94±0.04^{d}	45.78±0.09^{e}	47.00±0.50^{c,d}
100 mg/kg bw CMECP seed	42.72±0.43^{c}	43.95±0.04^{c}	46.22±0.40^{d}	47.72±0.26^{f}	49.50±0.00^{d}
300 mg/kg bw CMECP seed	36.87±0.46^{a}	37.52±0.37^{a}	38.56±0.29^{b}	38.00±0.58^{b}	40.00±0.00^{b}
500 mg/kg bw CMECP seed	38.00±0.57^{a}	38.43±0.58^{a}	38.22±0.39^{b}	39.66±0.33^{b,c}	42.50±2.50^{a,b}

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabets (a, b, c, d, e, and f) are significantly different between groups at P<0.05.

Table 5. Effect of alkaloids and flavonoid fractions of *C. papaya* seed and leaf on body weight changes in $AlCl_3$-induced anemic rats

Group	Day 0	Day 3	Day 7	Day 10	Day 14
Normal Control	105.67±5.78^{a,b}	109.33±6.64^{a,b}	115.00±4.93^{a,b,c}	117.67±3.48^{a,b,c}	119.33±3.18^{a,b,c}
Untreated anemic	99.66±34.48^{a}	96.67±9.24^{a,b}	93.33±9.52^{a,b}	92.00±10.12^{a}	87.00±7.81^{a}
$AlCl_3$ + 3 mg/kg bw $FeSO_4$	116.33±2.91^{a,b}	120.00±2.08^{a,b,c}	123.33±2.40^{a,b,c}	128.00±2.65^{a,b,c}	129.33±1.86^{a,b,c}
$AlCl_3$ + 75 mg/kg bw CAFCP seed	95.33±16.15^{a}	104.67±19.38^{a,b}	108.00±19.86^{a,b}	112.00±16.46^{a,b}	114.00±16.16^{a,b}
$AlCl_3$ + 150 mg/kg bw CAFCP seed	98.66±10.68^{a}	110.67±14.89^{a,b}	106.67±12.45^{a,b}	108.67±16.46^{a,b}	109.33±11.72^{a,b}
$AlCl_3$ + 75 mg/kg bw CFFCP seed	81.00±7.21^{a}	87.67±5.78^{a,b}	90.67±6.33^{a}	95.33±6.57^{a}	98.00±6.43^{a}
$AlCl_3$ + 150 mg/kg bw CFFCP seed	72.66±4.67^{a}	76.67±4.91^{a}	81.33±4.25^{a}	87.67±6.01^{a}	89.33±5.93^{a}
$AlCl_3$ + 75 mg/kg bw CAFCP leaf	95.33±16.15^{a}	104.67±19.38^{a,b}	108.00±19.86^{a,b}	112.00±16.46^{a,b}	114.00±16.16^{a,b}
$AlCl_3$ + 150 mg/kg bw CAFCP leaf	98.66±10.68^{a}	110.67±14.89^{a,b}	106.67±12.45^{a,b}	108.67±16.46^{a,b}	109.33±11.72^{a,b}
$AlCl_3$ + 75 mg/kg bw CFFCP leaf	81.00±7.21^{a}	87.67±5.78^{a,b}	90.67±6.33^{a}	95.33±6.57^{a}	98.00±6.43^{a}
$AlCl_3$ + 150 mg/kg bw CFFCP leaf	72.66±4.67^{a}	76.67±4.91^{a}	81.33±4.25^{a}	87.67±6.01^{a}	89.33±5.93^{a}

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabets (a, b, and c) are significantly different between groups at P<0.05.

rats (**Table 5**). However, treatment with the fractions brought about improvement in the body weights of the animals at the end of the 2 weeks treatment except in the 150 mg/kg bw CAFCP leaf group.

The changes in PCV of $AlCl_3$-induced anemic rats treated with alkaloids and flavonoid fractions of *C. papaya* seed and leaf are presented in **Table 6**. The consistent reduction observed in the PCV of the untreated anemic rat was reversed in the alkaloid and flavonoid fractions of *C. papaya* seed and leaf treated groups over the treatment period (**Table 6**).

Anti-anemic activity of CMECP leaf and seed in $AlCl_3$-induced anemic rats

The effect of CMECP leaf and seed in $AlCl_3$-induced anemic rats are presented in **Figure 1**. The untreated anemic rats showed reduced PCV, Hgb and RBC counts relative to the normal and extract treated groups (**Figure 1A**). The CMECP treated groups showed dose dependent increase in RBC relative to the normal and reference drug treated groups. However, PCV and Hgb in the extract treated groups at all tested concentrations were different from the non-induced group. **Figure 1B** shows the white blood cell (WBC), lymphocytes (L), neutrophils (N) and red blood cell wide counts (RDWC) in $AlCl_3$-induced anemic rats. A decrease in the WBC and lymphocytes of the untreated group was improved upon treatment with the extracts to levels comparable to the normal. Interestingly, the neutrophil counts in the CMECP treated groups were higher than the normal group. The mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell

Table 6. Effect of alkaloids and flavonoid fractions of *C. papaya* seed and leaf on the changes in packed cell volume (PCV) in aluminium chloride-induced anemic rats

Group	Day 0	Day 4	Day 8	Day 12	Day 15
Normal Control	36.56±9.85^{a}	34.56±0.54^{a}	37.46±0.49^{b}	38.13±0.35^{b}	40.00±2.00^{b}
Untreated anemic	40.90±0.35^{b}	38.20±0.42^{b}	35.06±0.33^{a}	27.56±0.34^{c}	26.50±3.50^{c}
$AlCl_3$ + 3 mg/kg bw $FeSO_4$	35.66±0.35^{a}	35.96±0.27^{a}	29.06±0.42^{c}	33.50±0.50^{a}	37.00±0.00^{b}
$AlCl_3$ + 75 mg/kg bw CAFCP seed	36.36±11.68^{a}	28.30±0.37^{c}	30.90±0.32^{a,b}	33.86±0.38^{a,b}	36.00±0.58^{a,b}
$AlCl_3$ + 150 mg/kg bw CAFCP seed	40.90±0.06^{a,b}	38.13±0.44^{b}	32.63±0.50^{a,b}	34.86±0.15^{a,b}	36.00±3.06^{b}
$AlCl_3$ + 75 mg/kg bw CFFCP seed	34.16±0.33^{d}	34.53±0.29^{d}	27.80±0.55^{b,c}	25.06±0.22^{b,c}	23.67±3.28^{b,c}
$AlCl_3$ + 150 mg/kg bw CFFCP seed	36.96±0.09^{a}	27.90±0.05^{c}	31.36±0.37^{b,c}	26.13±0.33^{b,c}	23.67±1.86^{b,c}
$AlCl_3$ + 75 mg/kg bw CAFCP leaf	42.10±0.55^{b}	38.86±1.00^{a,b}	41.36±0.72^{b}	37.23±1.07^{a}	39.33±2.40^{a,b}
$AlCl_3$ + 150 mg/kg bw CAFCP leaf	40.73±0.13^{b}	37.33±0.48^{a}	35.10±0.38^{a}	36.53±0.29^{a}	37.66±0.88^{a}
$AlCl_3$ + 75 mg/kg bw CFFCP leaf	35.20±0.61^{a}	37.03±0.28^{a}	32.00±0.58^{a,b}	28.20±0.42^{d}	29.66±2.40^{d}
$AlCl_3$ + 150 mg/kg bw CFFCP leaf	38.26±0.37^{a}	37.830.12^{a}	31.46±0.12^{a}	33.50±0.55^{b,c}	34.33±5.17^{b,c}

Data are presented in Mean ± standard error of triplicate determinations. Values with different superscript alphabets (a, b, c, and d) are significantly different between groups at P<0.05.

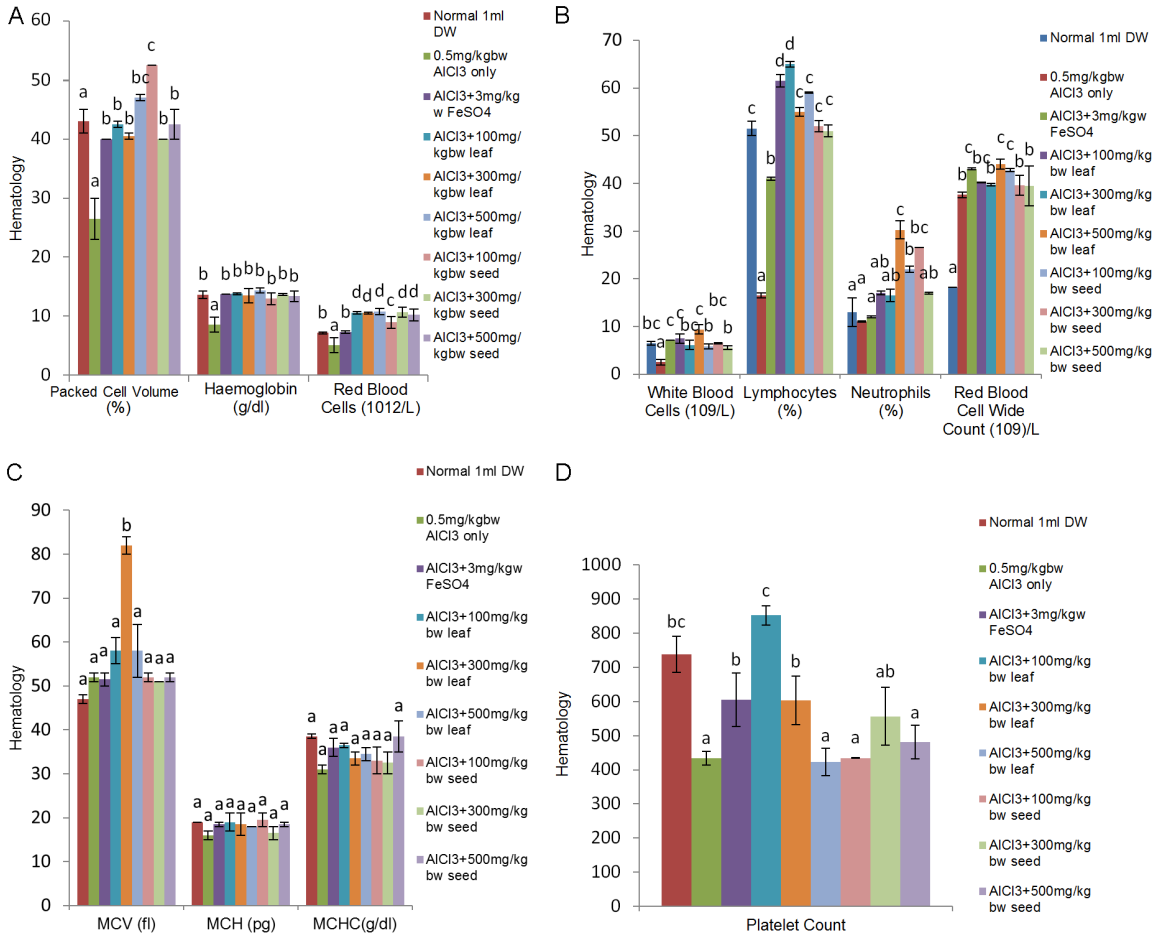

Figure 1. Effect of leaf and seed extracts of *C. papaya* on (A) packed cell volume, hemoglobin, and red blood cells (B) white blood cell, lymphocytes, neutrophils, and red blood cell wide counts in $AlCl_3$-induced anemic rats (C) mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin (D) platelet counts in $AlCl_3$-induced anemic rats. Bars are presented in Mean ± standard error of triplicate determinations. Bars with different superscript alphabets (a, b, c, and d) are significantly different between groups at P<0.05.

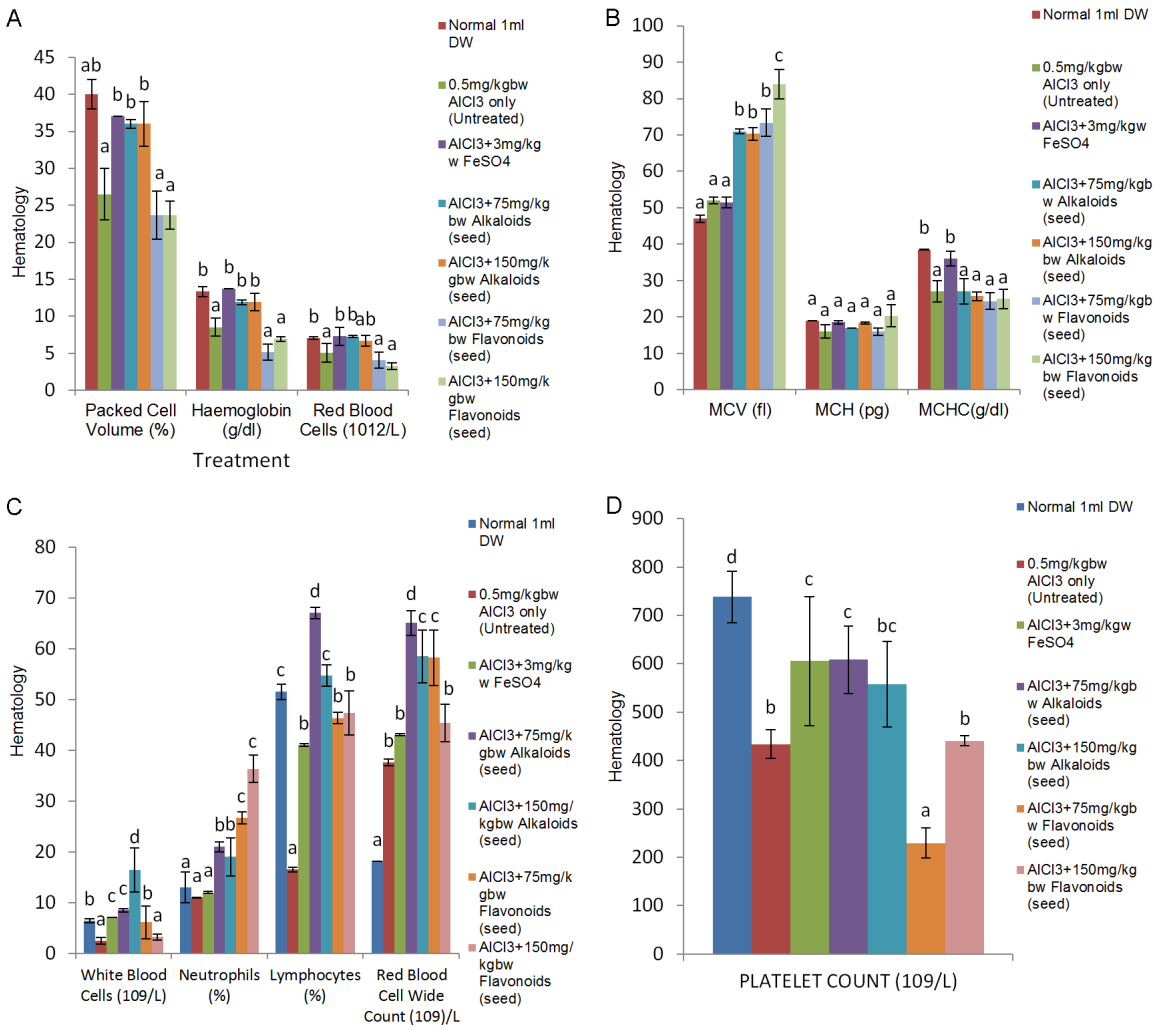

Figure 2. Effect of alkaloids and flavonoid of *C. papaya* seed fractions on (A) packed cell volume, hemoglobin, and red blood cells (B) mean corpuscular volume (MCV) mean cell hemoglobin (MCH), and mean cell hemoglobin counts (MCHC) (C) white blood cell, neutrophils, Lymphocytes, and red blood cell wide (D) platelet counts in $AlCl_3$-induced anemic rats. Bars are presented in Mean ± standard error of triplicate determinations. Bars with different superscript alphabets (a, b, c, and d) are significantly different between groups at $P<0.05$.

hemoglobin counts (MCHC) in $AlCl_3$-induced anemic rats are presented in **Figure 1C**. Except in the 300 mg/kg bw CMECP treated group, no appreciable difference was recorded in MCH and MCHC levels of the other CMECP treated groups relative to the untreated and normal groups. Similar trend was observed difference in the MCV counts of all the groups. There was an increase in the platelet counts of the groups treated with the standard drug, 100 and 300 mg/kg of CP leaf extracts when compared to the untreated. However, other treatment groups showed no appreciable difference in platelet counts from the anemic group (**Figure 1D**).

Anti-anemic activity of $AlCl_3$-induced anemic rats treated with alkaloid and flavonoid fractions of C. papaya seed

The anti-anemic activity alkaloid and flavonoid fractions of *C. papaya* seed in $AlCl_3$-induced anemic rats is displayed in **Figure 2**. The effects of crude alkaloids and flavonoid fractions of CP seed on PCV, Hgb and RBC on $AlCl_3$-induced anemic rats is presented in **Figure 2A**. The reduced PCV, Hgb and RBC counts in the anemic untreated rats were increased upon fractions treatment. **Figure 2B** shows the MCV, MCH and MCHC levels of anemic rats. Except in the untreated rats, the MCH level in

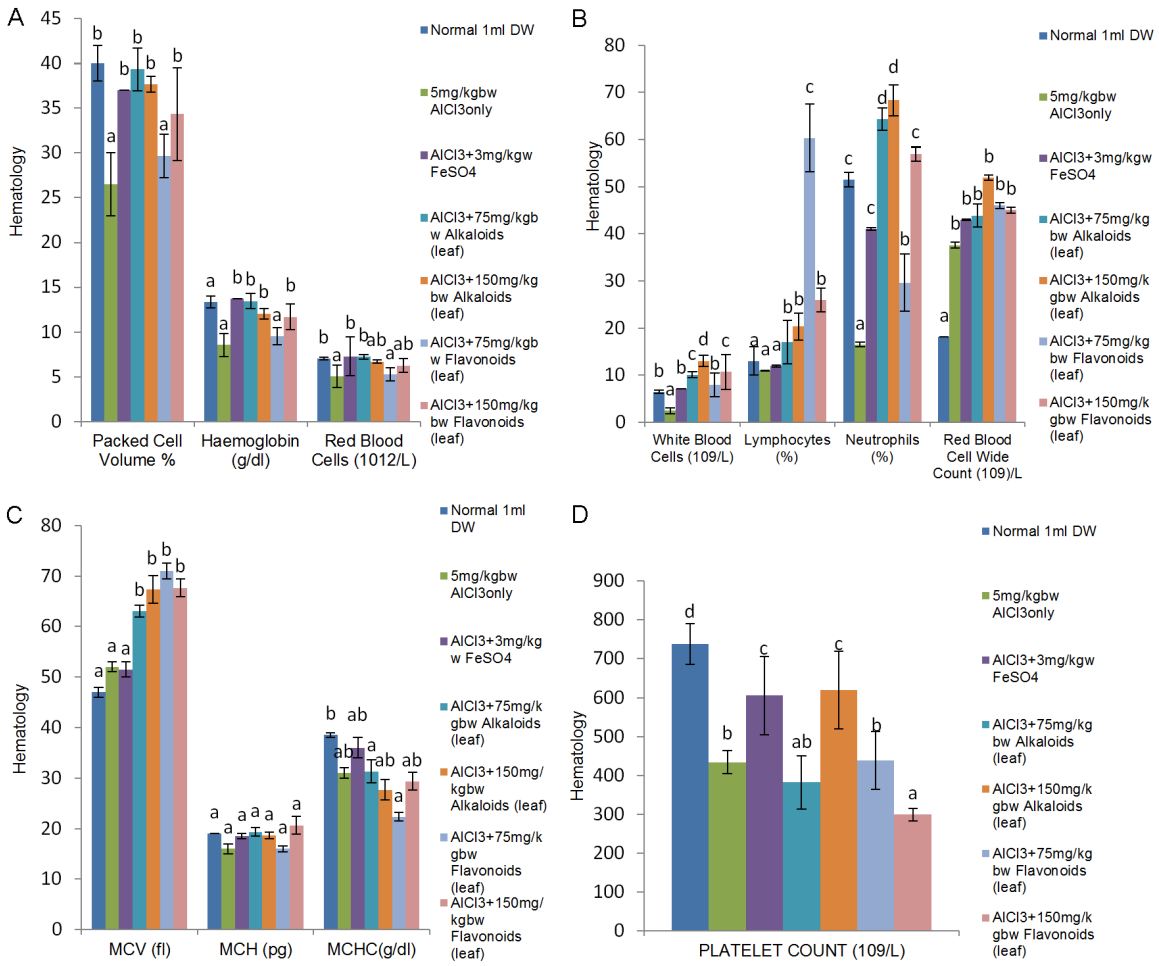

Figure 3. Effect of alkaloids and flavonoid of *C. papaya* leaf fractions on (A) packed cell volume, hemoglobin, and red blood cells (B) white blood cell, lymphocytes, neutrophils, and red blood cell wide (C) packed cell volume, hemoglobin, and red blood cells (D) platelet counts of $AlCl_3$-induced anemic rats. Bars are presented in Mean ± standard error of triplicate determinations. Bars with different superscript alphabets (a, b, c, and d) are significantly different between groups at $P<0.05$.

the groups compared well with the normal whereas the anemic untreated rats showed decreasing MCHC and MCV levels compared to the other groups.

The effect of alkaloids and flavonoid fractions of *C. papaya* seed extracts on WBC, N, L, and RDWC counts in $AlCl_3$-induced anemic rats are presented in **Figure 2C**. These counts were higher in both the alkaloid and flavonoid fractions of *C. papaya* seed relative to the untreated group. These fractions also compared well with the normal and reference drug groups. Except in the 150 mg/kg bw of CFFCP seed treated group, an increased WBC in the flavonoid fraction treatment group was recorded compared to the normal and untreated groups. Differences in N, L, and RDWC counts were seen in the flavonoid fraction treated rats relative to the untreated and normal groups. The platelet count of the anemic rats is presented in **Figure 2D**. A lowered platelet count in the 75 mg/kg body weight CFFCP seed compared with all other groups was observed. However, all the other treatment groups had higher platelet counts than the anemic rats.

Anti-anemic activity of $AlCl_3$-induced anemic rats treated with alkaloids and flavonoid fractions of C. papaya leaf

The anti-anemic activity alkaloid and flavonoid fractions of *C. papaya* leaf in $AlCl_3$-induced anemic rats is displayed in **Figure 3**. The effects of crude alkaloids and flavonoid fractions of CP leaf on PCV, Hb, and RBC in $AlCl_3$-induced ane-

mic rats are presented in **Figure 3A**. The lowered PCV, Hb and RBC counts in the anemic untreated group were increased upon fractions treatments which compared well to the normal and standard groups.

The WBC, L. N and RDWC in $AlCl_3$-induced anemic rats are presented in **Figure 3B**. Higher levels of WBC, L, N, and RDWC were observed when the rats were treated with CAFCP and CFFCP leaf at all the concentrations tested when compared to untreated anemic rats and normal group. The MCV, MCH and MCHC counts are presented in **Figure 3C**. MCV was improved upon treatment with alkaloids and flavonoid fractions of *C. papaya* leaf relative to normal control, standard drug, and untreated groups. MCH was not significantly ($P>0.05$) different in all extract treated groups relative to the normal group. However, MCHC level was higher in the normal group when compared to the extract treated and untreated groups.

The platelet counts of $AlCl_3$-induced anemic rats are presented in **Figure 3D**. Higher platelet counts were recorded in the normal and standard groups relative to the extract treated groups and untreated groups. However, decreased platelet counts were observed in the treatment groups when compared with the untreated except the 150 mg/kg bw alkaloids fraction of *C. papaya* leaf.

Discussion

Anemia is a condition of reduced hematological parameters constituting health, economic and social concerns particularly in the developing and less developed countries [21]. Due to the cost and safety issues associated with conventional medicine, plants are being explored as better alternatives especially since they are rich in phytochemicals of medicinal values. Therefore, our study investigated the protective effect of *C. papaya* phytochemicals in $AlCl_3$-induced anemic rats.

Phytochemicals

Plant phytochemicals serve protective, hormonal, growth, and developmental purposes to plant [22]. These metabolites are abundant in all plants in different quantities including flavonoids, saponins, alkaloids, dietary fibers, carotenoids, polyphenols, phytoseterols, tannins among others. Several studies have demonstrated the antimicrobial, antidiabetic, antioxidant, anti-dyslipidemia, antiallergic, anti-anemic, anti-obesity, anti-inflammatory, immune boosting, anticancer, gene, neurotransmitter and enzyme modulatory effects and lots more, these functions are attributable to the presence of these chemicals in plants [23-26]. Furthermore, quality and quantity of phytochemicals obtained in a sample are dependent on factors including season, climate, soil type. The choice of solvent and/or heat is also considered for their application in the fields of medicinal and nutraceuticals [27]. The use of compatible solvent medium and suitable method of extraction helps in the retainment of functional properties [28, 29].

Carica papaya methanolic extract contains varying quantities of alkaloids, flavonoid, saponins, phenols and tannins in both the leaf and seed. This observation is in line with several studies [14, 30] where they reported several phytochemicals in the plant. Saponins are glycosides with distinctive foaming characteristics and had higher contents in the CMECP leaf and seed than the other phytochemicals analyzed. Alkaloids are bioactive compounds of medicinal properties with the ability to intercalate with DNA [31]. Flavonoid and phenols are essential biological antioxidants having the ability to mediate against free radicals' deleterious effects. Low antioxidants to reactive oxygen species ratio may result in reactions that cause damage to the cells. Cellular damage results in various diseases. The flavonoid content of *C. papaya* in this study suggests its prevention and management against diseases resulting from oxidative stress such as cardiovascular diseases, atherosclerosis, chronic ulcer, arthritis, ischemic injury, and neurodegenerative diseases [32].

With the knowledge that each phytochemical has its own function, we proceeded to fractionate alkaloid and flavonoid from the leaf and seed of *C. papaya*. The findings that the crude methanolic extract outperformed the fractions in terms of anti-anemic and weight increment are suggestive of the possible synergistic effects of the phytochemicals in the plant while the fractions displayed an individual effect. Improved pharmacological effects are reportedly obtained with combinations of compounds [33].

Toxicity test for CMECP

In testing for the safety of potential drug candidates, any substance that does not result to mortality at 5000 mg/kg bw is considered safe and non-lethal [18]. In this study, crude extract of *C. papaya* leaves, and seed showed high safety margin since no mortality was recorded within 24 hours post extract treatments except for rubbing of the mouth on the surface of the cage and hyperactivity suggesting the relative safety of the extracts. This finding agrees with the findings of [34-36] where they reported that *C. papaya* did not show any toxic effects at 5000 mg/kg bw and at higher doses used traditionally. However, LD_{50} may vary due to biological, environmental, and social differences in organisms and may hence not be used to make a general assumption [37].

Hematopoietic effect of CMECP

The administration of CMECP improved the effect of aluminium induced toxicity. The observed body weight increases in the treated groups relative to the anemic untreated group might be adduced to the nutrients present in *C. papaya* [38]. This agrees with the findings of other researchers [39]. The significant loss in weight could also be linked to aluminium-induced oxidative stress which may result in free radical mediated cytotoxicity and reduced cholinergic function, decrease in disaccharidases (sucrase and lactase enzymes that catalyzed the last stage of carbohydrate digestion), or inhibition of enzymes such as hexokinase, acid and alkaline phosphatase, phosphodiesterase and phosphooxidase activities and consequently results in tissue wasting [40, 41].

The pathological, nutritional, and physiological status of organisms can be examined via the blood [42]. Examination of hematological parameters could also indicate the effect of xenobiotics in animals [43]. The significant decrease in PCV, Hb and RBC levels in the anemic group could be due to toxicity caused by $AlCl_3$. Aluminium chloride toxicity reportedly inhibits heme synthesis, inhibition of iron utilization, destruction of matured RBC and enzyme activity resulting in a decrease in PCV and RBC [44, 45]. However, aluminum-induced hematological alterations in MCV, MCH and MCHC might be adduced to microcytic hypochromic anemia.

The reduction observed in RBC, Hgb and PCV levels of the anemic rats was probably due to the oxidation of Fe^{2+} to form Fe^{3+} by aluminium, thus causing alterations in RBC, Hgb and PCV. However, the reversal of this effect upon extract administration might be due to the haemopoietic promoting factors such as iron, potassium, calcium, vitamins, and fiber content of the plant [46, 47]. The detoxifying function of saponins which allows for the lysing of RBC [48] could have been circumvented by the extract availing iron for the synthesis of new RBCs thus improving the Hb, PCV and RBC of the extract treated groups. The increase in treated groups lymphocyte counts may suggest the inhibition of possible lymphocytosis that could have occurred in the anemic rats [49]. Again, $AlCl_3$ probably caused a decrease in the immune system predisposing animals to infection. The immune boosting potential of the extracts could have led to elevation in the WBC levels [50]. Interestingly, the high platelet counts in the extract treated groups could be due to their ability to produce stimulating effects on platelet production, suggesting their role as a clot-formation agent [51].

According to the study of Osman et al. [52] reduced MCV, MCH and MCHC counts are reported in cases of aluminium toxicity. However, crude extracted groups could not reverse the reduced levels observed in the anemic group while the increase in the fraction treated groups could imply that the induction of anemia was reversed, and the fraction might probably prevent other pathological conditions like liver cirrhosis and hemolytic anemia [53]. The increase in RBC, WBC, Hb, and PCV levels of the extract treated groups might be due to the high flavonoid and alkaloid levels present in the plant.

Hematopoietic effect of alkaloids and flavonoid seed and leaf fractions of C. papaya

The reduction in weight of anemic rats was reversed in the treatment groups. The significant weight loss could arise from the deleterious effect of the reactive oxides induced by administration of aluminium chloride [54]. The destruction of matured RBC by aluminium could have resulted in the reduced hematological parameters such as RBC, PCV, and Hb observed in the untreated anemic rats. This fact is also supported by the findings of Muhammad and Oloyede [45] and Ogbonnia et

al. [55] that heme biosynthesis is impaired in aluminium induced anemia. The reduction in RBC count and PCV may also be due to the hemolytic action of aluminium resulting from the replacement of Fe^{3+} by Al^{3+} during hemoglobin formation [56]. Treatment with alkaloids and flavonoid fractions of *C. papaya* leaf prevented the occurrence of anemia in the experimental groups as significantly higher RBC count, hemoglobin and PCV were obtained as compared to the non-treated group ($AlCl_3$ only administered rats). Flavonoid and alkaloids are antioxidants that have direct influence on blood formation with the ability to inhibit free radical-induced blood cell damage [46].

The flavonoid fraction of *C. papaya* seed showed no positive effect on hematopoietic system of anemic rats suggesting that flavonoid fractions of *C. papaya* seed are not a major hematopoietic stimulatory bioactive metabolite. However, the ability of the alkaloid fraction to improve the hematological status to levels comparable to the normal rats is suggestive of alkaloids as a hematopoietic stimulatory bioactive metabolite in *C. papaya* seed. The fractions also brought about improvement in immune response as observed in the elevated WBC levels whereas, the anemic rats could have compromised immune system that have overwhelmed the WBC defense mechanism thus the reduced WBC level in the anemic untreated rat [57, 58].

Conclusion

Carica papaya leaves and seeds are rich in phytocompounds, this makes it a promising in phytotherapy and could be used in the protective and management of diseases resulting from oxidative stress such as anemia. Due to its phytoconstituents, *C. papaya* had shown the ability to protect and restore the integrity of hemoglobin, PCV and RBC and other hematological parameters that were compromised by $AlCl_3$-induction, therefore it may possess haemato-protective and hematinic potentials. The general observation of improved anti-anemic capability and weight gain effect of the crude methanolic extract is suggestive of the combinatory effect of the phytoconstituents. However, further studies should be carried out to investigate the mode of anti-anemic action of *C. papaya*, its effects iron deficiency anemia as well as metabolomic profiling of *C. papaya* constituents.

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Disclosure of conflict of interest

None.

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