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Antianaemic property of *Ficus capensis* leaves and its combination with *Cnidoscolus aconitifolius* leaves in phenylhydrazine-induced anemic rats

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ABSTRACT

Introduction: Anemia is a common health condition caused by a decrease in red blood cells. Some medicinal plants are used as a remedy to treat anemia. This study compares the anti-anemic properties of different doses of the aqueous extracts of *Ficus capensis* (AEFC) and its combination with the aqueous extract of *Cnidoscolus aconitifolius* (AECA) in phenylhydrazine-induced anemic rats.

Methods: Anemia was induced by intraperitoneal injection of 20 mg/kg phenylhydrazine for five consecutive days. Graded doses of the extracts were given by oral gavage once a day continuously for 30 days. At the end of the treatment, blood was collected for hematological analysis.

Results: The antianemic effects of AEFC and its combination with AECA were demonstrated by significant increases ($P<0.05$) in the hemoglobin (HGB), packed cell volume (PCV) and red blood cell (RBC) count of the extract-treated groups compared to the anemic control group. There was a better increase in the HGB levels of a combination of 400 mg/kg AEFC + AECA (13.97 ± 2.53) compared to 400 mg/kg AEFC (12.06 ± 0.02). The PCV increased more in 400 mg/kg combination of AEFC + AECA (41.94 ± 0.37) compared to 400 mg/kg AEFC (36.31 ± 1.51). A significant ($P<0.05$) increase was observed in the RBC count of a combination of 400 mg/kg AEFC + AECA (6.36±0.51) compared to 400 mg/kg AEFC (4.75 ± 0.46).

Conclusion: Although AEFC improved the haematological parameters of the animals when administered alone, its combination with AECA yielded a far much better result by totally restoring the haematological parameters of the phenylhydrazine-induced anemic rats to normal.

Implication for health policy/practice/research/medical education:
Ficus capensis and *Cnidoscolus aconitifolius* are phytomedicines endowed with antianemic potentials. This research shows the promising anti-anemic prospects of *F. capensis* and *C. aconitifolius* in rats. The combination of the extracts might be used for anemia due to the probable synergistic effects of the extracts.

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Introduction

Anemia is induced by iron deficiency and is indicated by dizziness, tiredness, headache, inability to breathe, and lack of concentration. This could result from insufficient iron in the diet or the inability of the body cell to absorb iron (1). Other causes of anemia include the presence of chronic diseases like kidney or liver failure, blood loss, an imbalance between the production and destruction of red blood cells (RBCs), certain medications, and alcoholism. Anemia can also be indicated when there is a decrease

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in RBCs, which transport an iron-rich protein called hemoglobin (HGB) (2). HGB carries oxygen attached to its iron molecule around the body while at the same time transporting the carbon dioxide from respiration out of the body through the lungs. Anemia is diagnosed using blood tests to determine the level of HGB. The levels below 13.5 g/dL in men and 12.0 g/dL in women indicate anemia. The levels of HGB in children vary with age (3).

A study carried out by World Health Organization (WHO) between 1993 and 2005 globally showed that 24.8% of the world population is affected by anemia with the highest prevalence in preschool children, while the lowest was in men (3). The prevalence of anemia in pregnancy is also high and has also become a health problem. The prevalence of anemia in pregnant women is shown to be 56% worldwide occurring highest in low- and medium-income countries. It has been reported that the highest prevalence is among pregnant women in sub-Saharan Africa (4). The prevalence in pregnant women is also high in eastern Nigeria (5).

Traditionally, strategies have been identified to help alleviate anemia in society with the help of foods fortified with iron, (Called blood boosters). Most of these foods are plants, which have been reported to have a high content of iron. The water extracts of these plants can help the patient's recovery within a short time. Examples of such plants are *Ficus capensis* (figplant), *Solanum aethiopicum* (eggplant), and *Cucurbita maxima* (pumpkin) (6).

Ficus capensis is referred to as Akokoro (Igbo), Obada (Edo), Uwaryara (Hausa), and Opoto (Yoruba). Its leaves are used as vegetable and medicine. The leaves are also used in the treatment of anemia and have been reported to enhance hematological parameters in anemic conditions (7).

Cnidoscolus aconitifolius is another plant whose leaves are commonly eaten in different parts of Nigeria as vegetable and medicinal plant. Its nutrients have been reported to be greater than most leafy green vegetables (8). It is commonly referred to as Chaya or tree spinach and is used to ameliorate anemic conditions traditionally thus, referred as "hospital too far" or "Ogwu obala" in Niger Delta. Previous studies have successfully demonstrated that the extracts of *F. capensis* and *C. aconitifolius* possess huge haematinic effects when used individually. However, the recorded effect cannot be compared with that of the highly expensive orthodox alternatives, synthetic blood-boosting multivitamin syrups and tablets. Thus, it is our hypothesis that the combination of these nutritional haematinic plants may act synergistically to give a relatively better effect. In our earlier studies, we have been able to establish that the aqueous extract of *F. capensis* leaves and its combination with *C. aconitifolius* leaves may promote liver function parameters, maintain the normal serum electrolyte level and kidney function indices, reduce "bad cholesterol", increase "good cholesterol", and reduce oxidative stress in phenylhydrazine-induced anemic rats (9). This study was designed to compare the anti-anemic properties of graded doses of the aqueous extracts of *F. capensis* with different doses of combined aqueous extracts of *F. capensis* and *C. aconitifolius* in phenylhydrazine-induced anemic rats.

Materials and Methods

Study area

This research was carried out between the months of July to September 2019 at the Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Plant material and aqueous extraction

The leaves of *Ficus capensis* were collected at Ibeagwa Nike, Enugu East Local Government Area, Enugu State at a temperature of 32°C. The leaves of *C. aconitifolius* were collected at Umueze town, Nkanu West Local Government Area, Enugu State at a temperature of 30°C. The sample identification was performed in the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. The voucher code numbers of *C. aconitifolius* and *F. capensis* deposited in the herbarium unit of the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, are 168 and 164, respectively.

The leaves were hand-picked, thoroughly washed, and dried at room temperature to constant weight. The dried leaves were pulverized into powder using a Corona manual grinding machine. Three hundred grams of the pulverized leaves powder of *F. capensis* and *C. aconitifolius* were respectively soaked in 1 L of distilled water for 24 hours for complete extraction. The aqueous extractions were sieved with muslin cloth and filtered using Whatman no. 1 filter paper (Whatman International Ltd, Madison, UK). The filtrates were Freeze dried by a freeze dryer. Then, the aqueous extract combination of *C. aconitifolius* and *F. capensis* were reconstituted at a ratio of 1:1 and solubilized with distilled water before administration.

Experimental animals, ethics statement, and treatment

Male rats of Wistar strain of 12 weeks old with body weight of 130-140 g were supplied by Chris Animal Farms and Research Laboratory, Awka, Anambra State. The handling of animals was performed in agreement with the principles of laboratory animal care and use, as approved by the Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka, Nigeria, in line with the recommendations for Animal Care and Use in Research, Education and Testing (ACURET).

The rats were grouped and housed in cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. The laboratory temperature was 28 ± 1°C. They were allowed to acclimatize with the environment for one week prior to the experiment. Rats were fed with vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state. Food and

water were available *ad libitum*.

After weighing and labeling, 35 rats were randomized into seven equal groups. Animals were induced anemia with phenylhydrazine and treated for fourteen days. Then, the blood samples were collected for hematological analysis through orbital plexus sinus after the induction of anesthesia by pentobarbital. The rats were divided into the following groups: Group A – Normal control, Group B – Anemic control (without treatment), Group C – Standard drug (Anaemic and treated with vitamin B_{12}), Groups D and E – Anaemic rats treated with 200 and 400 mg/kg b.w. aqueous extract of *F. capensis,* respectively, Group F – Anaemic rats treated 200 mg/kg b.w. of a combination of aqueous extract of *F. capensis* and *C. aconitifolius,* and Group G – Anaemic rats treated 400 mg/kg b.w. of a combination of aqueous extract of *F. capensis* and *C. aconitifolius*.

Induction of anemia

Anemia was induced by intraperitoneal injection of 20 mg/kg phenylhydrazine for five consecutive days. The animals' blood samples were collected before and after the induction of anemia for hematological analysis to evaluate anemia induction before treatment commencement (9).

Hematological analysis

Hematological parameters including HGB, white blood cells (WBCs), RBC, packed cell volume (PCV), platelets (PLT), neutrophils, lymphocytes, monocytes, eosinophils, basophils, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were analyzed using an automated haematology analyzer (Mindray-BC-28000).

Statistical analysis of the results

All the data were expressed as mean ± SEM (standard error of the mean) and analyzed by SPSS (the Statistical Package for Social Sciences) version 25, using ANOVA and post hoc tests to determine the differences between the means of the test and control groups. $P<0.05$ was considered significant.

Results

The induction of anemia resulted in a significant ($P<0.05$) decrease in the HGB concentration of the various study groups induced with phenylhydrazine, which was confirmed on day 6 compared with the normal control group. The observed decrease in the HGB concentration was significantly reversed on day 20 of the study (Table 1). The HGB concentration increased in all the treatment groups within one week and two weeks of treatment. The group of rats treated with a combination of 400 mg/kg of aqueous extract of *F. capensis* (AEFC) and aqueous extract of *C. aconitifolius* (AECA) showed the highest increase in HGB concentration compared to other treatment groups.

Anemia caused a marked decrease in the PCV of the experimental subjects. The treatment of various groups showed a significant ($P<0.05$) increase in PCV on days 13 and day 20 compared to the anemic untreated control and the standard drug group. However, administration of the extracts as a combination at a dose of 400 mg/kg b.w. caused a much better increase in PCV compared to other test groups (Table 2).

Induction of anemia caused a significant ($P<0.05$) reduction in the RBC of the rats. Treatment with *F. capensis* and its combination at a dose of 200 and 400 mg/kg b.w. restored the RBCs to normal (Table 3). However, a better improvement in the RBCs of the treated groups was observed in the group administered a combination of aqueous extract of *F. capensis* and *C. aconitifolius* at a dose of 400 mg/kg b.w.

Anemia caused a significant ($P<0.05$) increase in the platelet count of all anemic groups induced with phenylhydrazine compared to the normal control group. Treatment with 200 and 400 mg/kg b.w. of aqueous extract of *F. capensis* significantly ($P<0.05$) decreased the platelet count on the thirteenth and twentieth days of treatment compared to the anemic untreated control (Table 4). However, administration of the aqueous extract

Table 1. Hemoglobin (HGB) concentration of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Hemoglobin (g/dL)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	12.30 ± 0.81	12.75 ± 0.90	13.26 ± 0.51	12.93 ± 0.05
Group B - Anemic Control	12.10 ± 0.05	7.20 ± 0.02[b,h]	7.91 ± 0.04[h]	8.13 ± 0.01[h]
Group C - Standard drug	11.6 ± 0.26	7.50 ± 0.01[b,h]	10.21 ± 0.31[c,e,h]	12.63 ± 0.09[c,e]
Group D - AEFC (200 mg/kg)	12.3 ± 0.10	6.98 ± 0.03[b,h]	9.65 ± 0.60[b,c,e,h]	11.40 ± 0.92[c,e]
Group E - AEFC (400 mg/kg)	11.80 ± 0.67	7.53 ± 0.60[b,h]	11.30 ± 1.05[c,e]	12.06 ± 0.02[c,e]
Group F - AEFC+AECA (200 mg/kg)	12.36 ± 0.80	7.57 ± 0.03[b,h]	10.51 ± 0.68[c,e,h]	12.75 ± 1.02[c,e]
Group G - AEFC+AECA (400 mg/kg)	12.13 ± 0.25	8.13 ± 0.05[b,h]	12.56 ± 3.91[c,e]	13.97 ± 2.53[a,c,e]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*

[a] Significant increase with respect to day 0; [b] Significant reduction with respect to day 0; [c] Significant increase with respect to day 6; [e] Significant increase with respect to anemic untreated; [h] Significant decrease.

Table 2. Packed cell volume (PCV) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Packed cell volume (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	37.90 ± 2.61	38.42 ± 2.37	39.65 ± 1.03	38.82 ± 0.29
Group B - Anemic Control	36.60 ± 2.40	21.60 ± 1.51[b]	23.98 ± 1.52[b]	24.53 ± 2.00[b,c]
Group C - Standard drug	35.11 ± 3.43	22.70 ± 2.60[b]	30.72 ± 2.72[b]	37.95 ± 2.63[c,e]
Group D - AEFC (200 mg/kg)	37.6 ± 2.10	21.06 ± 2.50[b]	28.97 ± 0.56[b,c]	35.86 ± 3.95[c,e]
Group E - AEFC (400 mg/kg)	36.5 ± 2.71	22.65 ± 0.53[b]	33.90 ± 1.09[c]	36.31 ± 1.51[c,e]
Group F - AEFC+AECA (200 mg/kg)	37.11 ± 3.82	23.71 ± 2.65[b]	32.61 ± 0.83[b,c]	38.53 ± 0.96[c,e]
Group G - AEFC+AECA (400 mg/kg)	37.30 ± 0.56	24.65 ± 3.93[b]	37.97 ± 1.13[c]	41.94 ± 0.37[c,e,g]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[b] Significant reduction with respect to day 0; [c] Significant increase with respect to day 6; [e] Significant increase with respect to anemic untreated; [g] Significant increase with respect to normal control.

Table 3. Red blood cells (RBCs) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Red blood cell (g/dL)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	5.58 ± 0.22	5.62 ± 0.51	5.47 ± 0.01	5.39 ± 0.63
Group B - Anemic Control	5.64 ± 0.04	3.76 ± 0.03[b,h]	3.75 ± 0.93[b,h]	3.82 ± 0.08[b,h]
Group C - Standard drug	5.97 ± 0.01	4.06 ± 0.25[b,h]	4.67 ± 0.10[e]	5.70 ± 0.20[c,e]
Group D - AEFC (200 mg/kg)	6.12 ± 0.02	3.61 ± 0.20[b,h]	4.27 ± 0.35[c,e,h]	5.40 ± 0.02[c,e]
Group E - AEFC (400 mg/kg)	5.43 ± 0.33	3.75 ± 0.05[b,h]	4.82 ± 0.21[c,e]	4.75 ± 0.46[c,e]
Group F - AEFC+AECA (200 mg/kg)	5.98 ± 0.61	3.17 ± 0.28[b,h]	5.25 ± 0.15[c,e]	5.96 ± 0.32[c,e]
Group G - AEFC+AECA (400 mg/kg)	5.21 ± 0.50	3.97 ± 0.56[b,h]	5.49 ± 0.02[c,e]	6.36 ± 0.51[c,e]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[b] Significant reduction with respect to day 0; [c] Significant increase with respect to day 6; [e] Significant increase with respect to anemic untreated; [h] Significant decrease with respect to normal control.

Table 4. Platelets (PLT) count of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Platelets ($\times 10^9$/L)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	527.9±2.08	591.2±3.65	620.7±5.32	636.1±4.70
Group B - Anemic Control	583.1±2.01	795.9±7.25[a,g]	836.3±3.29[a,c,g]	752.1±3.86[a,g]
Group C - Standard drug	596.4±2.01	842.5±3.92[a,g]	652.3±2.73[d,f]	598.6±2.11[d,f]
Group D - AEFC (200 mg/kg)	675.3±4.67	725.4±6.75[a,g]	753.7±7.34[c,f,g]	638.2±7.20[d,f]
Group E - AEFC (400 mg/kg)	696.4±4.21	861.5±5.63[a,g]	795.6±3.70[a,d,f,g]	721.9±5.40[a,d,f,g]
Group F - AEFC+AECA (200 mg/kg)	582.7±7.27	825.2±3.05[a,g]	598.2±3.08[d,f,h]	659.7±3.56[a,d,f,g]
Group G - AEFC+AECA (400 mg/kg)	625.2±2.55	739.3±6.72[a,g]	619.7±7.83[d,f]	673.2±6.13[a,d,f,g]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[a] Significant increase with respect to day 0; [c] Significant increase with respect to day 6; [d] Significant reduction with respect to day 6; [f] Significant decrease with respect to anemic untreated. [g] Significant increase with respect to normal control; [h] Significant decrease with respect to normal control.

of *F. capensis* and *C. aconitifolius* as a combination at the doses of 200 and 400 mg/kg b.w. significantly ($P<0.05$) decreased the platelet count more compared to the groups treated with only *F. capensis* extract.

Anemia caused a significant increase ($P<0.05$) in the white blood cell counts of all the groups induced with phenylhydrazine compared to the normal control group. Treatment with 200 and 400 mg/kg b.w. of aqueous extract of *F. capensis* significantly ($P<0.05$) decreased the white blood cell count on the thirteenth and twentieth days of the treatment compared to the anemic untreated group (Table 5). However, the administration of the aqueous extract

of *F. capensis* and *C. aconitifolius* as a combination at the doses of 200 and 400 mg/kg b.w. significantly ($P<0.05$) decreased the white blood cell count more compared to the groups treated with only *F. capensis* extract. A better reduction in the white blood cell count was recorded in the group treated with a combination of *F. capensis* and *C. aconitifolius* at a dose of 400 mg/kg b.w. (Table 5).

Anemia caused a significant ($P<0.05$) increase in the neutrophil of all the groups induced with phenylhydrazine compared to the normal control group. Treatment with 200 and 400 mg/kg b.w. of aqueous extract of *F. capensis* significantly ($P<0.05$) decreased the neutrophil on the thirteenth and twentieth days of treatment compared to the anemic untreated group (Table 6). However, the administration of the aqueous extract of *F. capensis* and *C. aconitifolius* as a combination at 200 and 400 mg/kg b.w. significantly ($P<0.05$) decreased the neutrophil more compared to the groups treated with only *F. capensis* extract. A better reduction in the neutrophil was recorded in the group treated with a combination of *F. capensis* and *C. aconitifolius* at a dose of 400 mg/kg b.w. (Table 6).

Anemia caused a significant ($P<0.05$) decrease in the lymphocyte of the experimental subjects. The treatment of various groups showed a significant ($P<0.05$) increase in the lymphocyte on day 13 and day 20 compared to the anemic untreated control and the standard drug group. However, the administration of the extracts as a combination at a dose of 400 mg/kg b.w. caused a much better increase in the lymphocyte compared to other test groups (Table 7).

The result showed a significant reduction of lymphocyte percentage on day 6 with respect to day 0. At the end of the study, the percentage of lymphocytes was increased in the treated groups with respect to the untreated group to day 6. Induction of anemia caused a slight increase in the monocyte of the experimental animals. The monocyte level had an insignificant decrease within the first week of treatment (Table 8). However, a significant decrease ($P<0.05$) was observed in the groups treated with a combination of the extracts after the second week of treatment.

Induction of anemia caused a significant ($P<0.05$) increase in the eosinophils of the experimental animals. The eosinophil level had an insignificant decrease within the first week of treatment (Table 9). A significant ($P<0.05$) decrease was observed in the groups treated

Table 5. White blood cells (WBCs) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	White blood cell (g/dL)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	21.6 ± 0.35	18.4 ± 1.30	25.1 ± 2.38	23.4 ± 1.14
Group B - Anemic Control	23.3 ± 0.20	43.9 ± 3.25[a,g]	45.3 ± 3.16[a,g]	48.9 ± 1.67[a]
Group C - Standard drug	29.8 ± 2.32	37.2 ± 2.91[a,f,g]	29.6 ± 2.23[d,f,g]	27.3 ± 2.85[d,f]
Group D - AEFC (200 mg/kg)	26.5 ± 1.45	46.8 ± 1.85[a,f,g]	31.5 ± 0.5[1a,d,f,g]	25.7 ± 3.57[d,f]
Group E - AEFC (400 mg/kg)	26.8 ± 3.42	43.1 ± 3.62[a,f,g]	33.4 ± 2.90[a,d,f,g]	28.9 ± 2.68[d,f,g]
Group F - AEFC+AECA (200 mg/kg)	19.6 ± 2.85	42.3 ± 2.73[a,f,g]	28.5 ± 2.20[d,f,g]	26.2 ± 3.37[d,f,g]
Group G - AEFC+AECA (400 mg/kg)	25.3 ± 2.17	39.7 ± 1.20[a,f,g]	26.4 ± 3.82[d,f]	22.5 ± 1.56[d,f]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[a] Significant increase with respect to day 0; [d] Significant reduction with respect to day 6; [f] Significant decrease with respect to anemic untreated. [g] Significant increase with respect to normal control.

Table 6. Neutrophils of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Neutrophil (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	23.8 ± 0.67	25.3 ± 1.02	23.5 ± 0.68	21.7 ± 0.05
Group B - Anemic Control	25.1 ± 0.81	53.6 ± 1.82[a,g]	51.7 ± 1.64[a,g]	46.0 ± 1.91[a,g]
Group C - Standard drug	23.6 ± 1.72	55.2 ± 1.35[a,g]	37.3 ± 1.03[a,d,f,g]	24.1 ± 1.56[d,f,g]
Group D - AEFC (200 mg/kg)	21.3 ± 0.17	51.3 ± 0.26[a,g]	46.1 ± 0.60[a,d,f,g]	25.9 ± 1.01[d,f,g]
Group E - AEFC (400 mg/kg)	25.9 ± 1.78	49.8 ± 1.61[a,g]	32.9 ± 3.57[a,d,f,g]	26.4 ± 0.82[d,f]
Group F - AEFC+AECA (200 mg/kg)	23.4 ± 1.50	52.5 ± 1.89[a,g]	38.3 ± 0.37[a,d,f,g]	27.6 ± 1.20[d,f]
Group G - AEFC+AECA (400 mg/kg)	23.7 ± 1.87	45.9 ± 0.83[a,g]	29.2 ± 0.91[a,d,f,g]	21.5 ± 1.23[d,f]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[a] Significant increase with respect to day 0; [d] Significant reduction with respect to day 6; [f] Significant decrease with respect to anemic untreated. [g] Significant increase with respect to normal control.

Table 7. Lymphocytes of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Lymphocytes (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	67.2 ± 1.32	63.8 ± 1.80	69.1 ± 0.60	67.4 ± 1.02
Group B - Anemic Control	69.8 ± 2.89	43.6 ± 2.65[b,h]	45.1 ± 0.65[b,h]	48.8 ± 1.26[b,h]
Group C - Standard drug	53.2 ± 2.13	45.2 ± 1.02[b,h]	57.5 ± 1.06[c,e,h]	63.8 ± 1.54[c,e]
Group D - AEFC (200 mg/kg)	64.7 ± 1.81	42.9 ± 1.73[b,h]	52.3 ± 2.26[b,c,e,h]	65.5 ± 1.42[c,e]
Group E - AEFC (400 mg/kg)	65.5 ± 1.06	47.6 ± 1.50[b,h]	50.6 ± 2.15[b,c,e,h]	59.2 ± 2.23[c,e,h]
Group F - AEFC+AECA (200 mg/kg)	61.3 ± 1.99	43.1 ± 0.12[b,h]	48.3 ± 0.85[b,h]	63.5 ± 1.17[c,e]
Group G - AEFC+AECA (400 mg/kg)	69.7 ± 1.71	46.3 ± 1.59[b,h]	54.7 ± 1.21[b,e,h]	68.2 ± 1.53[c,e]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[b] Significant reduction with respect to day 0; [c] Significant increase with respect to day 6; [e] Significant increase with respect to anemic untreated; [h] Significant decrease with respect to normal control.

Table 8. Monocytes of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Monocytes (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	1.08 ± 0.01	1.03 ± 0.01	1.27 ± 0.00	1.25 ± 0.01
Group B - Anemic Control	1.13 ± 0.02	1.15 ± 0.01	1.51 ± 0.02	1.57 ± 0.01
Group C - Standard drug	1.06 ± 0.01	1.20 ± 0.00	1.18 ± 0.01	1.22 ± 0.01
Group D - AEFC (200 mg/kg)	1.28 ± 0.02	1.26 ± 0.00	1.18 ± 0.00	1.22 ± 0.01
Group E - AEFC (400 mg/kg)	1.00 ± 0.01	1.06 ± 0.01	1.56 ± 0.01	1.21 ± 0.02
Group F - AEFC+AECA (200 mg/kg)	1.30 ± 0.00	1.34 ± 0.01	1.31 ± 0.02	0.91 ± 0.00[b,d,f,h]
Group G - AEFC+AECA (400 mg/kg)	1.01 ± 0.01	1.16 ± 0.01	1.23 ± 0.00	0.61 ± 0.01[b,d,f,h]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[b] Significant reduction with respect to day 0; [d] Significant reduction with respect to day 6; [f] Significant decrease with respect to anemic untreated. [h] Significant decrease with respect to normal control.

Table 9. Eosinophils of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Eosinophils (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	0.85 ± 0.01	0.91 ± 0.01	0.84 ± 0.00	0.93 ± 0.00
Group B - Anemic Control	0.97 ± 0.00	1.83 ± 0.01	1.76 ± 0.01	1.81 ± 0.00
Group C - Standard drug	0.86 ± 0.01	1.72 ± 0.01	1.63 ± 0.01	0.99 ± 0.00[d,f]
Group D - AEFC (200 mg/kg)	0.92 ± 0.00	1.76 ± 0.00	1.60 ± 0.00	1.02 ± 0.00
Group E - AEFC (400 mg/kg)	0.65 ± 0.01	1.06 ± 0.01	1.25 ± 0.00	0.98 ± 0.00[d,f]
Group F - AEFC+AECA (200 mg/kg)	0.75 ± 0.01	1.65 ± 0.01	1.32 ± 0.00	0.79 ± 0.00[d,f]
Group G - AEFC+AECA (400 mg/kg)	0.73 ± 0.01	1.65 ± 0.01	1.32 ± 0.00	0.79 ± 0.00[d,f]

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[d] Significant reduction with respect to day 6; [f] Significant decrease with respect to anemic untreated.

with the graded doses of a combination of the extracts after the second week of treatment.

The result showed an increase in the percentage of basophil of phenylhydrazine-induced anemic rats on day 6 with respect to day 0 in groups B, D, and G, while the other groups showed a decrease (Table 10). At the end of the study, the percentage of basophil increased in groups C, E, and G with respect to day 6, while a decrease was observed in groups B and D; G remained the same.

The result showed a reduction in MCV of phenylhydrazine-induced anemic treated rats on day 6 with respect to day 0. The MCV of the treated groups on day 20 increased with respect to day 6 (Table 11).

Induction of anemia did not cause a significant

difference in the MCH of phenylhydrazine-induced anemic rats. Treatment of the rats with *F. capensis* and its combination with *C. aconitifolius* did not significantly increase or decrease the MCH of the phenylhydrazine-induced anemia (Table 12).

Induction of anemia did not cause a significant difference in the MCHC of phenylhydrazine-induced anemic rats. Treatment of the rats with *F. capensis* alone at a dose of 200 and 400 mg/kg b.w. did not significantly increase or decrease the MCHC of the phenylhydrazine-induced anemia (Table 13). However, treatment with 200 mg/kg b.w. of a combination of *F. capensis* and *C. aconitifolius* caused a significant ($P<0.05$) increase in the MCHC on the 20th day of the experiment (Table 13).

Discussion

Phenylhydrazine (PHZ) causes hemolytic anemia by reacting with the HGB leading to the formation of free radicals within the erythrocytes thereby causing oxidation of oxyhemoglobin to methemoglobin (10). A

Table 10. Basophils of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Basophils (%)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	0.56 ± 0.01	0.58 ± 0.00	0.61 ± 0.00	0.63 ± 0.00
Group B - Anemic Control	0.58 ± 0.00	0.66 ± 0.00	0.68 ± 0.00	0.57 ± 0.00
Group C - Standard drug	0.43 ± 0.00	0.37 ± 0.00	0.52 ± 0.00	0.50 ± 0.01
Group D - AEFC (200 mg/kg)	0.52 ± 0.01	0.65 ± 0.01	0.62 ± 0.00	0.57 ± 0.00
Group E - AEFC (400 mg/kg)	0.61 ± 0.00	0.35 ± 0.00	0.57 ± 0.00	0.62 ± 0.00
Group F - AEFC+AECA (200 mg/kg)	0.53 ± 0.01	0.48 ± 0.00	0.58 ± 0.01	0.59 ± 0.00
Group G - AEFC+AECA (400 mg/kg)	0.46 ± 0.01	0.62 ± 0.01	0.54 ± 0.00	0.62 ± 0.00

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*

Table 11. Mean corpuscular volume (MCV) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Mean corpuscular volume (fl)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	62.5 ± 1.40	63.8 ± 2.38	59.4 ± 1.52	64.3 ± 2.35
Group B - Anemic Control	58.4 ± 2.30	52.9 ± 1.55	54.5 ± 2.63	62.2 ± 1.68
Group C - Standard drug	60.9 ± 1.02	56.2 ± 1.02	61.3 ± 2.34	59.2 ± 1.44
Group D - AEFC (200 mg/kg)	61.7 ± 1.29	54.6 ± 1.12[b]	56.3 ± 1.02	55.4 ± 3.17
Group E - AEFC (400 mg/kg)	58.6 ± 2.26	57.3 ± 2.67	63.5 ± 1.06	59.0 ± 2.19
Group F - AEFC+AECA (200 mg/kg)	56.3 ± 1.27	51.1 ± 2.85	60.4 ± 1.64	69.8 ± 1.52[c]
Group G - AEFC+AECA (400 mg/kg)	62.2 ± 2.93	53.4 ± 0.26[b]	57.3 ± 2.71	58.2 ± 1.08

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[b] Significant reduction with respect to day 0; [c] Significant increase with respect to day 6; fl: femtolitres

Table 12. Mean corpuscular hemoglobin (MCH) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Mean corpuscular hemoglobin (pg)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	19.9 ± 0.10	18.7 ± 0.03	22.2 ± 0.23	19.4 ± 0.00
Group B - Anemic Control	20.6 ± 0.15	20.5 ± 0.01	20.9 ± 0.20	18.9 ± 0.05
Group C - Standard drug	19.3 ± 0.21	21.2 ± 0.02	18.9 ± 0.00	19.5 ± 0.05
Group D - AEFC (200 mg/kg)	22.1 ± 0.42	22.2 ± 0.41	21.0 ± 0.26	19.0 ± 0.03
Group E - AEFC (400 mg/kg)	20.3 ± 0.03	19.4 ± 0.25	18.8 ± 0.30	18.2 ± 0.25
Group F - AEFC+AECA (200 mg/kg)	19.3 ± 0.30	20.4 ± 0.03	20.5 ± 0.32	21.1 ± 0.12
Group G - AEFC+AECA (400 mg/kg)	19.7 ± 0.02	20.5 ± 0.16	21.0 ± 0.25	18.6 ± 0.19

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*

Table 13. Mean Corpuscular Hemoglobin Concentration (MCHC) of anemic rats treated with *F. capensis* or a combination of *F. capensis* and *C. aconitifolius* (mean ± SEM)

Groups	Mean corpuscular hemoglobin concentration (g/dL)			
	Before induction (day 0)	After 5th day of induction (day 6)	After 7th day of treatment (day 13)	After 14th day of treatment (day 20)
Group A - Control	30.9 ± 0.37	31.0 ± 0.10	31.8 ± 0.01	33.9 ± 0.15[d]
Group B - Anemic Control	34.4 ± 0.21[e]	33.0 ± 0.42[e]	33.8 ± 0.14[e]	34.5 ± 0.10[e]
Group C - Standard drug	33.3 ± 0.01[e]	35.6 ± 0.21[ae]	34.7 ± 0.23[e]	36.0 ± 0.20[a,e]
Group D - AEFC (200 mg/kg)	33.9 ± 0.22[e]	33.8 ± 0.19	34.0 ± 0.25[e]	33.9 ± 0.32
Group E - AEFC (400 mg/kg)	32.2 ± 0.01	33.4 ± 0.02[e]	32.4 ± 0.21	32.2 ± 0.04
Group F - AEFC+AECA (200 mg/kg)	31.8 ± 0.01	32.1 ± 0.01	31.7 ± 0.01	36.1 ± 0.03[a,d,e]
Group G - AEFC+AECA (400 mg/kg)	31.3 ± 0.02	34.1 ± 0.03[a,e]	32.1 ± 0.26	32.5 ± 0.25

AEFC: Aqueous extract of *Ficus capensis;* AECA: Aqueous extract of *Cnidoscolus aconitifolius.*
[a] Significant increase with respect to day 0; [d] Significant reduction with respect to day 6; [e] Significant increase with respect to anemic untreated.

marked reduction in Hb, PCV, and RBC after induction can be caused by the hemolytic effect of PHZ, which are the symptoms of anemia. Traditionally, *F. capensis* and *C. aconitifolius* are used in anemia due to their blood boosting effects (7,11). The plants are rich in minerals and phytochemicals, which has been reported to alleviate anemia (12).

The result revealed a significant decrease ($P < 0.05$) in HGB concentration after the induction of anemia with PHZ in all groups except the normal control, which was not induced. This is caused by the destruction of the globin in HGB leading to its denaturation (13) or the alteration of the absorption of iron by PHZ (2). At the end of the study, the groups treated with different doses of aqueous extracts of *F. capensis* and *C. aconitifolius* showed an increase in HGB concentration. The highest increase was observed in the group treated with the combination of 400 mg/kg of *F. capensis* and *C. aconitifolius*. Earlier studies by Ezeigwe et al (14) using a combination of ethanol extract of *F. capensis* and *C. aconitifolius* reported that the extracts are capable of ameliorating phenylhydrazine-induced anemia by increasing the HGB concentration in the test groups. These results are also in line with the previous reports (7,15). This could be due to the presence of iron (Fe) in the leave extracts, which is an important component of the HGB (16). The presence of vitamin C (17,18) in the extracts can be a contribution to the increase in HGB. Vitamin C has been reported to aid dietary iron absorption (19). The leaves of *F. capensis* and *C. aconitifolius* contain iron and substantial quantities of vitamin C (12).

Phenylhydrazine-induced anemia significantly ($P < 0.05$) reduced the PCV. This showed the hemolytic effect of phenylhydrazine in the rats. The groups treated with different doses of *F. capensis* had an increase in PCV percentage. This was also reported by Chikwendu et al (20). The group treated with 400 mg/kg of a combination of *F. capensis* and *C. aconitifolius* showed the highest increase in PCV with respect to other treated groups. This showed that the extracts might be used as a remedy for anemia. PCV is a measure of the percentage of RBCs contained in the blood. Since the RBC was increased due to the presence of various vitamins and components of the extracts (12), this may have led to the increase in PCV. The increase in PCV by *F. capensis* and *C. aconitifolius* has been reported previously (7,21), although in these studies the plants were used individually.

The reduction of RBC after induction of anemia with phenylhydrazine showed the presence of anemia. This could be caused by the effects of free radicals created by phenylhydrazine on RBC leading to the peroxidation of its membrane lipids (22). The treatment with the extracts at different doses increased the RBC with the highest increase observed in the group treated with 400 mg/kg b.w. of a combination of both extracts. The increase in RBC in the test groups can be due to the antioxidant effect of the extracts in reversing the oxidative stress created by the phenylhydrazine. The extracts have been reported to contain flavonoids and phenols, which have antioxidation properties (12,15). The presence of vitamins (A, B, C, and E) in the extracts, which also act as antioxidants can also help in increasing the RBC by protecting the membrane (23). The presence of carbohydrates, iron, copper, and cobalt in the extract can also help in the synthesis of RBC and Hb (12).

There was an increase in WBC after the induction of anemia with PHZ. This was also reported by Criswell et al (24). PHZ has been reported to cause anemia by increasing the number of circulating leukocytes leading to increase in WBC and activation of the immune system (25). The aqueous extracts of *F. capensis* and *C. aconitifolius* may have reduced WBC by decreasing the oxidative stress caused by PHZ thereby reactivating the immune system. An increase in WBC by the leave extract of *F. capensis* has previously been reported.

Phenylhydrazine caused a reduction in the number of lymphocytes. The treatment of the rats with the extracts at various doses caused an increase in the lymphocytes. The increase in lymphocyte levels in the test groups means that the extract can help in improving the immune system. The extracts of both *F. capensis* and *C. aconitifolius* have been

reported to contain Vitamins C, $B_{12,}$ and B_6. These vitamins can lead to an increase in lymphocytes. Vitamin B_6 can cause the proliferation of lymphocytes (26), vitamin C can cause improved lymphocyte development and function (27), and vitamin B_{12} has also been reported to increase lymphocyte expression (28).

The neutrophil concentration increased after the injection of PHZ. This could be because it is the body's first line of defense during infections, an inflammatory disorder, and stress. The increase in free radicals and stress caused by PHZ may have led to the elevation of neutrophils. It could be deduced that the extracts, which have been reported to act as an antioxidant due to their contents (flavonoids, polyphenols, vitamin C and E) (12) may have cleared the free radicals created by PHZ thereby leading to the reduction of the neutrophils after treatment with the extracts.

The results showed an increase in the eosinophil and basophil counts after phenylhydrazine-induced anemia. This may be due to the inflammation caused by PHZ. It has been reported that inflammations and allergic reactions can lead to an increase in eosinophil and basophil counts (29,30). Basophil and eosinophil decreased values in the treated groups show that the plant does not contain harmful substances that can cause a further allergic reaction and could have helped to decrease the toxic effects of PHZ. Eosinophils and basophils are types of WBC, so the decrease in WBC may have led to the decrease in eosinophil and basophil counts.

Phenylhydrazine was reported by Unami et al (31) to increase MCV, MCH, and MCHC. This study showed an increase in MCV in the phenylhydrazine-induced anemic groups, though the increase in MCH and MCHC was not observed in all the induced groups. These increases may have resulted from the presence of free plasma Hb caused by the PHZ (24) by creating oxidative stress. At the end of the study, there was a reduction in the concentration of MCH and MCHC in the extract-treated groups, which may have resulted from the antioxidant components of the leaf extracts.

Conclusion

The findings from this study suggest that aqueous extract of *F. capensis* leaf is effective in the treatment and management of anemia. However, when used in combination with the aqueous extract of *C. aconitifolius*, an improved result on the haematological parameters of anemia was observed. From this, it can be inferred that the combination of the aqueous extract of *F. capensis* and *C. aconitifolius* offers a better remedy to anemic conditions and its complications.

Significant Statement

This study authenticated the anti-anemic property of aqueous extracts of *Ficus capensis* with its combination with aqueous extract of *Cnidoscolus aconitifolius* in phenylhydrazine-induced anemic rats. It is the first research that investigated the use of these two extracts as a combination in the treatment of anemia.

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Authors' contributions

OCE, CFE, and ELI designed the research. OCE, CFE, and ELI were involved in data curation. OCE and NHI analyzed the data. OCE and CFE wrote the paper. OCE, CHO, and CMO edited the paper. All authors read and approved the final paper.

Conflict of interests

Authors hereby declare no conflict of interest.

Ethical considerations

All authors hereby declare that "Principles of laboratory animal care" were followed. All experimental protocols were examined and approved by the Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka, Nigeria in line with the recommendations for Animal Care and Use in Research, Education and Testing (ACURET). The ethical approval code is NAU/AREC/0010A.

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