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Pharmacobiochemical Studies of The effect of Ethylacetate Fraction of Cnidoscolus aconitifolius Leaves in Phenylhydrazine-Induced Anemic Rats.

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Abstract
Background: *Cnidoscolus aconitifolius Euphorbiaceae leaves have been used separately in traditional medical practice to treat different ailments, of which anemia is one. This study aims to evaluate anti-anaemic property of ethylacetate fraction of Cnidoscolus aconitifolius leaves in phenylhydrazine-induced anemic rats.*
Methods: *Twenty-five rats weighing 120–150g were randomized into five groups (A–E) of five rats each. Group B-D were induced with anemia. Groups A and B served as the normal control and anemic control respectively, while Groups C, D, and E were treated with a standard drug (Emzoron), 100mg/kg body weight ethylacetate fraction of ethanol extract of* C. aconitifolius*, and 200mg/kg body weight ethylacetate fraction of ethanol extract of* C. aconitifolius*, respectively. The hematological and biochemical analysis was carried out using standard diagnostic techniques.*
Results: *Following the induction of phenylhydrazine, the rats became anemic. The results showed that there were significant increases (*$p<0.05$*) in the Hb, PCV, RBC, MCV, MCH and the platelet values and decreases in the MCHC and WBC values after the 14th day treatment. It was also observed that there was significant decrease (*$p<0.05$*) in the values obtained for liver function parameters (ALP, AST, ALT, total bilirubin, and direct bilirubin levels), urea, creatinine, Electrolytes (K^+, Na^+,Cl^-,BCO_3^-,T^{cal} and n^{cal}), Total Cholesterol (TCHOL), Low-density Lipoprotein (LDL-C), Triglycerides (TRIG), Very Low-density Lipoprotein (VLDL-C), Lactate dehydrogenase and Malondialdehyde (MDA) and a significant increase (*$p<0.05$*) in obtained values for High-density Lipoprotein (HDL) in all the extract-treated groups compared with the anemic-untreated. The values obtained for most of these biochemical parameters in the extract-treated groups were in the range of normal control showing that the extract did not, in any way, alter the biochemical parameters.*
Conclusion: *Ethylacetate fraction of ethanol extract of* Cnidoscolus aconitifolius *was found to ameliorate the effects of phenylhydrazine-induced anemia on haematological parameters and may promote liver function parameters, maintain normal serum electrolyte level and kidney function indices, stimulate reduction of "bad cholesterols" and increase "good cholesterol" and reduce lipid peroxidation.*
Keywords: Cnidoscolus aconitifolius*, anemia, phenylhydrazine, hematological, parameters, peroxidation.*

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I. Introduction

Anemia, sometimes called erythrocytopenia, is a medical condition in which the capacity of the blood to transport oxygen is insufficient, either because of too few red blood cells, or because of too little hemoglobin[1,2]. It can result in impaired cognitive development, fatigue, paleness and in severe cases, increased risk of mortality[3]. Anaemia is a public health problem that affects populations in both rich and poor countries[4]. Globally, anaemia affects 1.62 billion people (95%), which corresponds to 24.8% of the population (95%)[5]. The highest prevalence is in preschool-age children (47.4%, 95%), and the lowest prevalence is in men (12.7%, 95%)[6]. The World Health Organization estimates that the prevalence of anaemia in pregnancy varies from 53.8% to 90.2% in developing countries and 8.3% to 23% in developed countries[7]. However, the population group with the greatest number of individuals affected is non-pregnant women (468.4 million, 95%). Prevalence of anemia among women of reproductive age (% of women ages 15-49) in Nigeria was 49.80 as of 2016. Prevalence of anemia among children (% of children under 5) in Nigeria was 68.30 as of 2016[8]. Although the

primary cause is iron deficiency, this can also be as a result of poor nutrition, high prevalence of blood parasites such as plasmodium, trypanosomes and helminths infestation[9].

The occurrence of anemia is due to the various red cell defects such as production defect (aplastic anemia), maturation defect (megaloblastic anemia), defects in hemoglobin synthesis (iron deficiency anaemia), genetic defects of hemoglobin maturation (thalassaemia) or due to the synthesis of abnormal hemoglobin (haemoglobinopathies, sickle cell anaemia and thalassaemia) and physical loss of red cells (hemolytic anemias)[10]. The different types of anemia have conventional treatments. Ferrous sulfate is used in the treatment of iron-deficiency anemia[11]. The cost of medical care for anemia is high and as a result the rural populations in various parts of the world do not have access to high quality drugs for the treatment of anaemia, so they rely on plants and herbal products for the treatment of anaemia[9,12].

Medicinal plants have been found to be useful in the formulation and production of modern drugs which can aid in the treatment of various blood deficiencies[13] including anemia especially in developing countries. In Nigeria, the medicinal properties of plants are empirically appreciated. Nigeria is blessed with numerous indigenous edible plants that are utilized locally, and one of these herbal resources is *Cnidoscolus aconitifolius*. Extracts of *Cnidoscolus aconitifolius* (CA) have been reported to possess medicinal properties ranging from hepatoprotective, anti-anemic, anti-diabetic, anti-bacterial, and anti-cardiovascular[14,15,16,17].

Cnidoscolus aconitifolius is an evergreen, perennial shrub of the Family Euphorbiaceae commonly found in the tropics. Popularly called "chaya tree spinach" in Mexico, where it was first cultivated as a household leafy green vegetable. In Nigeria, it is commonly known as "Iyana Ipaja" (Yoruba), "obarandu" (Igbo) or "hospital too far" (Niger Delta) due to its perceived health benefits. It has been demonstrated to contain phenols, saponins, cardiac glycosides and Phlobatannin[18]. *C. aconitifolius* leaves have been shown to have a high content of ascorbate, β-carotene, protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin and rich in vitamin A, vitamin B2, vitamin B1, vitamin B9, vitamin C, vitamin D, vitamin E and vitamin K, vitamin B3, vitamin B6, vitamin B12, and vitamin E[19]. The present study was aimed at determining the ameliorative effects of ethylacetate fraction of ethanol extract of *Cnidoscolus aconitifolius* on the hematological and biochemical parameters, in phenylhydrazine-induced anemic rats.

II. Methods

Sample Collection and Identification

The leaves of *C. aconitifolius* were collected from Adazi-Ani, Anaocha Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAU/168.

Preparation of Ethanol Extract of C. aconitifolius Leaf

The leaves were properly washed and air dried at room temperature for two weeks. The dried leaves were pulverized into powder using corona manual grinding machine. Exactly 1.5 kg of the pulverized leaf powder of *C. aconitifolius* was soaked in 6 litres of 70% ethanol for 24 hrs for ethanol extraction. The ethanol mixture was sieved using muslin cloth and filtered using Whatman no 1 filter paper. The filtrate was concentrated using water bath at 50^0C. The biological yield of the extract after extraction was 173.6g. The ethanol extract was stoppered in universal bottles and preserved in the refrigerator for use.

Fractionation of C. aconitifolius Leaf Extract

The crude ethanol leaf extract (173.6g) was fractionated by the method of Wu *et al*.[20]. The method involved successive extraction by increasing polarity with n-hexane, chloroform, ethyl acetate, n-butanol and water. Twenty-five grams (25g) of the ethanol extract was dissolved in 250ml of Methanol/Water (MeOH/H2O) (1:1) mixture and shaken with n-hexane (2x250ml). Combined extract was left to dry on the bench to yield n-hexane fraction. Methanol (MeOH) was further fractionated by successive solvent extraction with chloroform (1x250ml), ethyl acetate (2 x 200ml) and n-butanol (1x250ml). Each fraction was left to evaporate to dryness on the bench to yield n-hexane fraction (3.23g), chloroform fraction (9.75g), ethyl acetate fraction (5.63g), n-butanol fraction (2.50g) and water fraction (3.89g).

Test Animals

A total of 25 male Wistar albino rats weighing between 120–150g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages under standard environmental conditions (27°C±3°C, 12-hour light/dark cycle) in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment for one week before use. The animals were fed Vital grower's

mash pellets purchased from Vital Feed Distributor at Awka, Anambra state and fed *ad libitum*. At the end of the one-week acclimatization period, the animals were weighed, grouped and labeled.

Acute toxicity (LD_{50}) evaluation

The median lethal dose (LD_{50}) for each of the extracts were determined using Lorke's method[21]. Thirteen (13) male rats were used for the determination of the median lethal dose. The thirteen (13) rats were randomized into six groups; three rats each for the first phase which was given 10, 100 and 1000mg/kg bw and one rat each for the second phase which was given 1600, 2900 and 5000mg/kg bw. The animals were monitored for changes in behaviour and mortality within 2 hrs, 24 hrs and 14 days after a single administration of the fraction.

Study design for Antianemic Properties

A total of twenty-five (25) male Wistar rats were randomized into 5 groups of 5 rats each. After the induction of anemia with phenylhydrazine, the animals were treated for 14 days after which blood was collected by cardiac puncture under ketamine anesthesia and used for haematological and biochemical analysis. They were grouped as follows:

Group A: Normal Control
Group B: Negative Control (Anemic untreated)
Group C: Positive Control (Std Drug-Emzoron)
Group D: Anemia + 100 mg/kg bw. of ethylacetate fraction of *C. aconitifolius* leaves
Group E: Anemia + 200mg/kg bw. of ethylacetate fraction of *C. aconitifolius* leaves

Induction of Anemia

Anemia was induced intraperitoneally in the rats using 20mg/kg b.w. of phenylhydrazine for four consecutive days. The animals were confirmed to be anemic on the 5th day before the commencement of treatment. Blood was collected by *retro orbital sinus* for hematological analysis before and after the induction of anemia to monitor the animals for the symptoms of anemia before the commencement of treatment.

Determination of Bodyweight

The bodyweight of the experimental subjects was checked using an electronic weighing scale. The weight of the rats were monitored before, during, and after the experiment to know whether the chloroform fraction has an effect on the bodyweight of the experimental rats.

Random Blood Glucose Concentration

The blood glucose levels of the rats were checked before the induction of anemia, during, and after treatment using One Touch Glucometer (Life Scan, USA) and test strips based on the method of Trinder 1972.

Hematological Analysis

Hematological parameters were determined using automated hematology analyzer (Mindray-BC-5300). The hematological parameters that were analyzed include Haemoglobin (HGB), Packed Cell Volume (PCV), Red Blood Cells (RBC), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBC), Neutrophils (NEUT), Lymphocytes (LYMPH), Monocytes (MON), Eosinophils (EOS), Basophils (BAS).

Liver Function Test

Serum biochemical indices routinely estimated for liver functions were analysed. They include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Kidney Function Test

Urea and creatinine were analysed using Randox test kits. The procedures were carried out according to the manufacturer's instructions.

Electrolyte Concentration

The serum electrolyte concentration was analysed using AFT-300 electrolyte analyzer. The whole blood sample of the Wistar rat was centrifuged at 4000 rpm for 10 mins. The serum was separated and used for the analysis. The probe of the electrolyte analyser aspirates the serum of the Wistar rat which passes through the electrodes, aspiration pump and the electronic circuits which measure and process the electromotive force to

give the test ion concentration. The electrolytes that were analyzed include Potassium ion (K^+), Sodium ion (Na^+), Chloride ion (Cl^-), Bicarbonate ion (BCO_3^-), Total Calcium (T^{cal}) and Ionized Calcium (n^{cal}).

Lipid Profile

The lipid profile (Total Cholesterol, Triglycerides, High-Density Lipoprotein-cholesterol, Low-Density Lipoprotein-cholesterol and Very Low-Density Lipoprotein-cholesterol) were determined using Randox test kits[22,23]. Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula[24]. The procedure used was according to the manufacturer's instructions provided in the manual.

Lactate Dehydrogenase

Serum lactate dehydrogenase enzyme was determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Lipid Peroxidation

Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) assay method of Buege and Aust[25]. The reaction depends on the formation of complex between malondialehyde and theobarbituric acid (TBA).0.4ml of serum was collected into the test tubes; 1.6mlof 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituricacid and then mixed thoroughly.

The reaction mixture was then placed in 100^{o}C boiling water for 15 minutes, allowed to cool and centrifuged at 3000 rpm for 10 minutes.The supernatant was collected and the optical density recorded at 532nm against reagent blank containing distilled water.

The lipid peroxidation activity was calculated using the formula:

$$\frac{\text{Optical density}}{\text{Time}} \times \frac{\text{extinction co-efficient}}{\text{amount of sample}}$$

Where the extinction coefficient value is $1.56 \times 10^{-5} M^{-1} CM^{-1}$
The unit is expressed as umol/MDA/mg of protein.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

III. Results

Results of the Acute Toxicity (LD_{50}) Test

The result of acute toxicity (LD_{50}) study revealed that the ethylacetate fraction of the ethanol extract may not be toxic following Lorke's method. No death was recorded in the first and second phase of the administration. Although the rat that was given the highest dose (5000 mg/kg) showed some symptoms of toxicity as recorded in table 1, the LD_{50} value is said to be above 5000mg/kg which entails that the fraction may not be very toxic as the toxicity level did not result to death.

Table 1: Acute toxicity studies of ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Phase	Dose mg/kg	Death recorded in rats	Observations
First	10	0/3	None
	100	0/3	None
	1000	0/3	None
Second	1600	0/1	None
	2900	0/1	Slightly weak
	5000	0/1	Weak

Result of Bodyweight

The weights of the rats were recorded on day 0 (before the induction of anemia), day 5 (after four days of consecutive induction of anemia), day 12 (after seven days treatment) and day 19 (after 14 days of treatment) (Table 2). The induction of anemia did not show any significant difference in the weight of the rats when the test groups were compared with the control groups. Although, as treatment progressed, a significant ($p<0.05$) increase in weight was observed in both the test and control groups on days 12 and 19 compared to days 0 and

5. The weight of the rats increased normally in the course of treatment. The gain in weight cannot be attributed to the treatment regimen, as can be seen from the results comparing the anemic-untreated with the normal rats and the test groups.

Table 2: Bodyweight of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Bodyweight (g)			
	Before induction Day 0	**After 4th day of ind. of anemia Day 5**	**After 7th day of treatment Day 12**	**After 14th day of treatment Day 19**
Normal Control	133.20±2.54	143.60±3.33	150.00±4.83	161.20±4.68
Anemic Untreated	126.00±3.11	133.00±2.89	156.50±7.10	162.80±4.55
Anemia + Standard drug (Emzoron)	125.60±5.14	129.80±3.98	144.80±4.77	155.00±4.11
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	134.60±5.16	143.60±5.03	150.00±5.34	161.00±5.38
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	126.80±4.34	133.00±2.85	141.00±4.70	149.60±3.39

Effect on Random Blood Glucose Levels

The random blood glucose levels were observed to determine the effect of treatment with aqueous extract of *C. aconitifolius* on random blood glucose levels. The random glucose levels of the rats were recorded on day 0 (before the induction of anemia), day 5 (after 4 days of consecutive induction of anemia), day 12 (after 7 days treatment) and day 19 (after 14 days of treatment) (Table 3). The rats maintained normal blood glucose levels before, during, and after treatment.

Table 3: Random blood glucose concentrations of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Glucose Level (g/dl)			
	Before induction Day 0	**After 4th day of ind. of anemia Day 5**	**After 7th day of treatment Day 12**	**After 14th day of treatment Day 19**
Normal Control	94.80±11.20	81.40±4.03	102.00±2.98	107.80±11.00
Anemic Untreated	100.40±8.77	82.40±5.26	104.0±6.36	99.50±2.78
Anemia + Standard drug (Emzoron)	96.00±6.45	78.60±3.01	114.60±7.81	105.60±5.91
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	92.60±7.88	87.00±2.17	101.20±1.66	100.0±3.29
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	108.60±4.83	82.00±6.01	96.60±6.50	103.40±6.01

Result of Haematological Analysis

Effect on Haemoglobin (HGB) Concentration

The group induced and treated with 200 mg/kg ethylacetate fraction had the highest haemoglobin concentration (14.50 g/dl ± 0.31) after treatment (14 days of treatment) while the Anemic untreated group (negative control group) had the least haemoglobin concentration (8.30±1.55) after treatment. The Hgb concentration of some experimental groups (Anemia + Standard drug, Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius* & Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*) increased significantly (p<0.05) above the anemic untreated while the haemoglobin concentration of the normal control group increased but not significantly.

Table 4: Haemoglobin (HGB) concentration of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	HGB (g/dl)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	12.43±0.50	12.13±0.19	11.70±1.40
Anemic Untreated	12.20±0.34	8.73±0.50[b]	8.30±1.55
Anemia + Standard drug (Emzoron)	13.17±0.27	10.60±0.60[b]	14.10±0.10[c]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	13.20±0.56	10.13±0.78[b]	13.17±1.07[c]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	12.63±0.49	8.80±1.56[b]	14.50±0.31[c]

[b]Significant decrease with respect to day 0; [c]Significant increase with respect to day 5.

Effect on Packed Cell Volume (PCV)

The Normal control group had the highest PCV (45.83±2.13), then, the group induced and treated with 200 mg/kg ethylacetate fraction of *C. aconitifolius* experimental group (44.67±0.45) after 14 days treatment while the anemic untreated (negative control group) had the least PCV (23.73±3.42) level after treatment (table 5). The PCV level of the experimental groups increased significantly (p<0.05) above the anemic untreated.

Table 5: Packed Cell Volume (PCV) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* leaves.

Groups	PCV (%)		
	Before induction Day 0	After 4th day of induction of anemia Day 5	After 14th day of treatment Day 19
Normal Control	46.87±1.72	46.73±2.66	45.83±2.13
Anemic Untreated	37.43±0.46	30.57±1.77[b]	23.73±2.87[bd]
Anemia + Standard drug (Emzoron)	40.47±0.99	33.43±2.97[b]	43.87±0.74[c]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	43.13±1.47	30.33±3.78[b]	40.77±3.58[c]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	45.70±3.43	35.87±2.68[b]	44.67±0.45[c]

[b]Significant decrease with respect to day 0; [c]Significant increase with respect to day 5; [d]Significant decrease with respect to day 5.

Effect on Red Blood Cell (RBC) Count

The negative control group (Anemic Untreated) had the lowest RBC (3.54±0.38) after 14 days treatment while the normal control group had the highest RBC (6.38±0.74) count after treatment (table 6). The RBC count of all experimental groups and normal control group showed a significant (p<0.05) increase compared to the anemic untreated after 14 days treatment.

Table 6: Red Blood Cells (RBC) count of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* leaves.

Groups	RBC (g/dl)		
	Before induction Day 0	After 4th day of induction of anemia Day 5	After 14th day of treatment Day 19
Normal Control	7.91±0.06	7.85±0.32	6.38±0.74
Anemic Untreated	8.26±0.18	3.15±0.25[b]	3.54±0.38[b]
Anemia + Standard drug (Emzoron)	8.37±0.36	6.27±0.64[b]	5.86±0.15[b]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	7.61±0.37	4.86±0.80[b]	5.42±0.38[b]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	7.52±0.26	3.99±1.01[b]	6.14±0.17[c]

[b]Significant decrease with respect to day 0; [c]Significant increase with respect to day 5.

Effect on Platelets (PLT) count

The Platelet count of all experimental group and normal control group increased above the anemic untreated after 14 days treatment but not significant (p>0.05) as can be seen in table 7. Normal control group had the highest platelet count (780.70±81.77).

Table 7: Platelets (PLT) count of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* leaves.

Groups	PLT (10^9/L)		
	Before induction Day 0	After 4th day of induction of anemia Day 5	After 14th day of treatment Day 19
Normal Control	798.70±36.29	794.00±52.62	780.70±81.77
Anemic Untreated	751.30±81.88	445.70±65.03[b]	488.70±168.90[b]
Anemia + Standard drug (Emzoron)	713.00±4.62	756.30±10.17	572.00±115.9
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	705.00±96.67	813.00±114.50	696.00±106.40
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	783.00±37.98	867.00±111.22	763.00±82.95

[b]Significant decrease with respect to day 0.

Effect on Mean Corpuscular Volume (MCV)

The group induced and treated with 100 mg/kg ethylacetate fraction of *C. aconitifolius* had the highest MCV (75.06±3.23). The MCV of experimental groups shows a decrease below the anemic untreated after 4 days of induction of anemia. However, they increased significantly after 14 days treatment except the normal control group (table 8).

Table 8: Mean Corpuscular Volume (MCV) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* leaves.

Groups	MCV (fL)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	57.40±1.23	59.50±1.30	55.77±0.55
Anemic Untreated	52.67±1.09	97.47±2.80	66.83±1.10[ad]
Anemia + Standard drug (Emzoron)	51.17±1.10	53.47±1.18	75.00±2.69[ac]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	51.57±6.48	63.77±5.03	75.06±3.23[a]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	51.73±1.95	73.60±19.90	72.83±1.73[a]

[a]Significant increase with respect to day 0; [c]Significant increase with respect to day 5; [d]Significant decrease with respect to day 5.

Effect on Mean Corpuscular Hemoglobin (MCH)

The group induced and treated with 100 mg/kg ethylacetate fraction of *C. aconitifolius* had the highest MCH (24.33±0.96). The MCH of normal control group decreased below the anemic untreated while the MCH of the rest of the experimental group increased significantly (p<0.05) above the anemic untreated value after 14 days treatment (table 9).

Table 9: Mean Corpuscular Hemoglobin (MCH) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	MCH (pg)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	16.40±1.29	15.50±0.66	18.33±0.20
Anemic Untreated	15.96±0.84	27.80±0.90[a]	23.40±0.06[ad]
Anemia + Standard drug (Emzoron)	16.53±0.52	17.10±1.51	24.10±0.45[ac]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	15.07±0.42	21.57±2.27[a]	24.33±0.96[a]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	15.73±0.54	23.13±2.40[a]	23.67±0.20[a]

[a]Significant increase with respect to day 0; [c]Significant increase with respect to day 5; [d]Significant decrease with respect to day 5.

Effect on Mean Corpuscular Hemoglobin Concentration (MCHC)

Negative control (Anemic Untreated) group had the highest MCHC count (35.10±0.56). The MCHC of all the experimental group decreased below the anemic untreated after 14 days treatment but only the normal control group and the group treated with the standard drug were significant (table 10).

Table 10: Mean Corpuscular Hemoglobin Concentration (MCHC) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius.*

Groups	MCHC (g/dl)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	26.23±2.33	26.13±1.57	32.90±0.26[ac]
Anemic Untreated	27.70±0.75	28.50±0.10	35.10±0.56[ac]
Anemia + Standard drug (Emzoron)	28.10±0.38	31.97±1.18[a]	32.17±0.67[a]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	30.03±1.34	33.73±1.62[a]	32.40±0.21
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	29.10±0.78	34.03±4.75[a]	32.50±0.59

[a]Significant increase with respect to day 0; [c]Significant increase with respect to day 5.

Effect on White Blood Cell (WBC) Count

The white blood cell count of all experimental group increased significantly (p<0.05) after 4 days induction of anemia and decreased significantly below the anemic untreated after 14 days treatment except for the normal control group (table 11). Negative control (Anemic Untreated) group had the highest white blood cell count (32.28±4.82).

Table 11: White Blood Cells (WBC) count of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	WBC (10^9/L)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	11.17±0.92	11.43±1.86	14.45±0.93
Anemic Untreated	9.14±1.85	21.51±5.94[a]	32.28±4.82[ac]
Anemia + Standard drug (Emzoron)	7.73±0.73	31.65±4.75[a]	14.96±2.31[ad]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	7.49±0.39	33.74±13.60[a]	15.40±2.05[ad]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	10.18±1.67	44.57±13.97[a]	11.21±1.80[d]

[a]Significant increase with respect to day 0; [c]Significant increase with respect to day 5; [d]Significant decrease with respect to day 5.

Effect on Neutrophils (NEUT) count

All the experimental groups increased after 4 days of induction of anemia except the normal control. The groups induced and treated with Emzoron, 100 mg/kg and 200mg/kg ethylacetate fraction of *C. aconitifolius* shows a significant decrease (p<0.05) with respect to the anemic untreated 14days treatment (table 12).

Table 12: Neutrophils (NEUT) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Neut (%)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	27.77±0.35	27.53±1.67	24.83±6.67
Anemic Untreated	29.53±0.66	32.13±1.08	30.70±0.96
Anemia + Standard drug (Emzoron)	27.40±1.04	30.57±1.08	17.80±1.59[bd]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	27.23±1.44	31.83±1.94	17.37±2.98[bd]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	26.40±1.12	32.13±2.05	20.20±4.11[d]

[b]Significant decrease with respect to day 0; [d]Significant decrease with respect to day 5.

Effect on Lymphocytes (LYMPH) count

All the experimental groups decreased significantly (p<0.05) after 4 days of induction of anemia except the normal control. The groups induced and treated with Emzoron (standard drug), 100 mg/kg and 200mg/kg ethylacetate fraction of *C. aconitifolius* shows a significant (p<0.05) increase with respect to the anemic untreated 14 days treatment (table 13).

Table 13: Lymphocytes (LYMPH) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Lymph (%)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	70.10±0.25	70.70±0.80	74.67±6.87
Anemic Untreated	66.60±1.57	52.97±1.80[b]	69.10±1.08[c]
Anemia + Standard drug (Emzoron)	65.33±2.72	52.53±2.13[b]	82.13±1.63[ac]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	67.97±1.41	59.23±1.83[b]	82.47±3.03[ac]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	70.37±1.16	57.03±5.55[b]	79.47±4.11[c]

[a]Significant increase with respect to day 0; [b]Significant decrease with respect to day 0 [c]Significant increase with respect to day 5.

Effect on Monocytes (MON) count

The experimental groups increased significantly (p<0.05) after 4 days of induction of anemia except the normal control. The groups induced and treated with Emzoron (standard drug), 100 mg/kg and 200mg/kg ethylacetate fraction of *C. aconitifolius* shows a significant decrease (p<0.05) with respect to the anemic untreated after 14 days treatment (table 14).

Table 14: Monocytes (MON) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Mon (%)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	0.33±0.03	0.37±0.33	0.03±0.03[bd]
Anemic Untreated	0.43±0.03	1.47±0.03	0.03±0.03[bd]
Anemia + Standard drug (Emzoron)	0.37±0.03	1.40±0.06	0.00±0.00[bd]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	0.43±0.03	1.43±0.03	0.17±0.12[d]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	0.37±0.03	1.43±0.12	0.10±0.10[d]

[b]Significant decrease with respect to day 0; [d]Significant decrease with respect to day 5.

Effect on Eosinophils (EOS) count

The groups induced and treated with Emzoron (standard drug), 100 mg/kg and 200mg/kg ethylacetate fraction of *C. aconitifolius* shows a significant (p<0.05) decrease with respect to the anemic untreated after 14 days treatment (table 15).

Table 15: Eosinophils (EOS) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Eos (%)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	1.13±0.03	1.10±0.06	0.27±0.17
Anemic Untreated	0.23±0.07	0.13±0.03	0.17±0.09
Anemia + Standard drug (Emzoron)	0.23±0.03	0.17±0.03	0.07±0.03[bd]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	1.03±0.07	0.20±0.06	0.00±0.00[b]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	0.97±0.12	0.20±0.00	0.23±0.13[b]

[b]Significant decrease with respect to day 0; [d]Significant decrease with respect to day 5.

Effect on Basophyls (BAS) count

The experimental groups increased after 4days of induction of anemia except the normal control. The groups induced and treated with Emzoron (standard drug), 100 mg/kg and 200mg/kg ethylacetate fraction of *C. aconitifolius* shows a significant (p<0.05) decrease with respect to the anemic untreated after 14days treatment (table 16).

Table 16: Basophyls (BAS) of phenylhydrazine-induced anemic rats treated with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves.

Groups	Bas (%)		
	Before induction Day 0	**After 4th day of induction of anemia Day 5**	**After 14th day of treatment Day 19**
Normal Control	0.10±0.00	0.10±0.00	0.00±0.00[bd]
Anemic Untreated	0.10±0.00	0.33±0.03	0.00±0.00[bd]
Anemia + Standard drug (Emzoron)	0.17±0.03	0.37±0.03	0.00±0.00[bd]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	0.27±0.07	0.33±0.03	0.00±0.00[bd]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	0.17±0.03	0.30±0.06	0.00±0.00[bd]

[b]Significant decrease with respect to day 0; [d]Significant decrease with respect to day 5.

Result of Biochemical Analysis

Effect on Liver Function Parameters

The result of the effect of administration of aqueous extract of *C. aconitifolius* to the experimental subjects on liver function parameters is represented in table 17. The induction of anemia with phenylhydrazine caused an increase in the liver function parameters (ALP, AST, ALT, total bilirubin, and direct bilirubin levels). Treatment with the ethylacetate extract of *C. aconitifolius* significantly (p<0.05) reduced the liver function parameters in all the treatment groups compared to the anemic-untreated.

Table 17: Effect of treatment with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves on liver function parameters of phenylhydrazine-induced anemic rats.

Groups	AST (U/L)	ALP (U/L)	ALT (U/L)	T. BIL (mg/dl)	D. BIL (mg/dl)
Normal Control	17.67±0.88	30.57±3.58	10.33±1.20	1.14±0.16	0.22±0.04
Anemic Untreated	29.00±1.15^{e}	62.50±9.97^{e}	30.00±2.08^{e}	2.10±0.32^{e}	0.85±0.17^{e}
Anemia + Standard drug (Emzoron)	21.67±1.33^{h}	42.73±14.10^{h}	16.67±2.60^{h}	1.48±0.16^{h}	0.34±0.12^{h}
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	20.33±2.19^{h}	39.63±4.52^{h}	13.67±3.84^{h}	1.53±0.13	0.32±0.12^{h}
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	18.33±0.33^{h}	32.30±5.43^{h}	17.00±0.58^{h}	1.52±0.14	0.39±0.12^{h}

^{e}Significant increase with respect to normal control; ^{h}Significant decrease with respect to anemic untreated.

Effect on Kidney Function Parameters

The result of the effect of administration of ethylacetate fraction of *C. aconitifolius* to the experimental subjects on kidney function parameters is represented in table 18. The induction of anemia with phenylhydrazine caused a significant (p<0.05) increase in the urea and creatinine levels in all the rats induced except the normal control group which was not induced. Treatment with the ethylacetate extract of *C. aconitifolius* reduced the urea and creatinine levels in all the treatment groups compared to the anemic-untreated with only urea being significantly higher (p<0.05). However, a better reduction in urea and creatinine levels were recorded in the groups that were treated with 100 mg/kg ethylacetate fraction of *C. aconitifolius* compared to the groups that were administered 200 mg/kg ethylacetate fraction of *C. aconitifolius* and the standard drug.

Table 18: Effect of treatment with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves on kidney parameters of phenylhydrazine-induced anemic rats.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Normal Control	9.27±1.28	1.73±0.03
Anemic Untreated	15.17±1.18^{e}	2.10±0.40
Anemia + Standard drug (Emzoron)	10.70±0.45^{h}	1.57±0.20
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	9.50±0.21^{h}	1.53±0.18
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	9.77±0.59^{h}	1.87±0.28

^{e}Significant increase with respect to normal control; ^{h}Significant decrease with respect to anemic untreated.

Effect on Electrolyte Levels

Induction of anemia increased the potassium ion (K^{+}), sodium ion (Na^{+}), chloride ion (Cl^{-}), bicarbonate ion (BCO_3^{-}), total calcium (T^{cal}) and ionized calcium (n^{cal}) in all the groups except the normal control that was not induced as shown in table 19. A reduction was observed in the electrolyte levels of the test groups compared to the anemic-untreated group.

Table 19: Effect of treatment with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves on electrolyte levels of phenylhydrazine-induced anemic rats.

Groups	K^{+} (mmol/L)	Na^{+} (mmol/L)	Cl^{-} (mmol/L)	BCO_3^{-} (mmol/L)	T^{cal} (mmol/L)	n^{cal} (mmol/L)
Normal Control	8.20±1.40	127.33±1.86	97.00±0.00	18.67±1.20	1.27±0.03	0.60±0.00
Anemic Untreated	8.20±1.52	130.00±3.61	96.67±2.03	20.67±1.20	1.27±0.07	0.63±0.03
Anemia + Standard drug (Emzoron)	5.47±0.52^{fh}	130.66±1.33	106.7±0.67^{eg}	21.33±0.88	1.07±0.18	0.53±0.09
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	5.17±0.23^{fh}	136.00±2.08^{e}	101.3±0.88^{egj}	22.33±0.67^{e}	1.43±0.20^{i}	0.70±0.12^{i}
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	6.23±0.37	135.30±3.53^{e}	102.00±2.08^{egj}	21.33±1.45	1.37±0.03^{i}	0.70±0.00

[e]Significant increase with respect to normal control; [f]Significant decrease with respect to normal control; [g]Significant increase with respect to anemic untreated; [h]Significant decrease with respect to anemic untreated; [i]Significant increase with respect to standard drug; [j]Significant decrease with respect to standard drug.

Effect on Lipid Profile

The induction of anemia significantly (p<0.05) increased the TCHOL, (LDL-C, TRIG and VLDL while it significantly (p<0.05) decreased HDL-C in all the groups except the normal control group (table 20). The groups treated with the ethylacetate fraction of *C. aconitifolius* and standard drug showed a significant (p<0.05) decrease in the TCHOL, LDL, TRIG and VLDL levels compared with the anemic-untreated group. There was a significant (p<0.05) increase in the HDL-C of all the test groups except anemic untreated.

Table 20: Effect of treatment with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves on lipid profile of phenylhydrazine-induced anemic rats.

Groups	TCHOL (mg/dl)	HDL-C (mg/dl)	TRIG (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Normal Control	92.67±5.46	45.33±2.85	27.33±1.45	41.87±5.88	5.47±0.29
Anemic Untreated	139.67±3.76[e]	35.33±4.70	38.67±3.84[e]	95.27±8.73[e]	9.07±0.93[e]
Anemia + Standard drug (Emzoron)	113.60±3.18[h]	42.00±5.03	32.67±2.03	65.13±8.23[h]	6.53±0.41[h]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	108.70±9.87[h]	45.67±2.60	35.33±5.46	55.93±11.78[h]	7.07±1.09
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	120.70±3.18[e]	41.67±8.29	32.33±2.03	72.53±10.97[e]	6.47±0.41[h]

[e]Significant increase with respect to normal control; [h]Significant decrease with respect to anemic untreated.

Effect on Lactate Dehydrogenase Activity

Induction of anemia caused a significant (p<0.05) increase in the LDH level of all the groups except the normal control which was not induced as shown in table 21. Administration of 100 and 200mg/kg bodyweight of ethylacetate fraction of *C. aconitifolius* significantly (p<0.05) decreased the LDH level in all the test groups compared with the anemic-untreated group.

Table 21: Effect of treatment with ethylacetate fraction of crude ethanol extract of *C. aconitifolius* leaves on lactate dehydrogenase (LDH) activity of phenylhydrazine-induced anemic rats.

Groups	LDH (U/L)
Normal Control	294.67±12.41
Anemic Untreated	427.00±24.02[e]
Anemia + Standard drug (Emzoron)	249.30±22.28[h]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	330.67±9.53[h]
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	336.00±18.88[h]

[e]Significant increase with respect to normal control; [h]Significant decrease with respect to anemic untreated.

Effect of Extract on Lipid Peroxidation (Malondialdehyde)

Induction of anemia caused a significant (p<0.05) increase in the MDA level of all the groups except the normal control which was not induced as shown in table 22. Administration of 100 and 200mg/kg bodyweight of ethylacetate fraction of *C. aconitifolius* significantly (p<0.05) decreased the MDA level in all the test groups compared with the anemic-untreated group.

Table 22: Effect of treatment with crude ethanol extract of *C. aconitifolius* on malondialdehyde (MDA) concentration of phenylhydrazine-induced anemic rats.

Groups	MDA (µmol/L) x 10^{-9}
Normal Control	3.15±0.97
Anemic Untreated	4.51±0.26
Anemia + Standard drug (Emzoron)	2.44±0.58[h]
Anemia + 100 mg/kg ethylacetate fraction of *C. aconitifolius*	2.72±0.47
Anemia + 200 mg/kg ethylacetate fraction of *C. aconitifolius*	3.54±0.70

[h]Significant decrease with respect to anemic untreated.

IV. Discussion

Among all human diseases, anemia sometimes called erythrocytopenia, remains the most common blood disorder, affecting about a third of the global population[4]. Anemia is induced by phenylhydrazine which resulted to a fall in the PCV, RBC, Hb and MCV values obtained in the rats[12]. The results of the acute toxicity test showed that no death was recorded but at 5000 mg/kg, it was observed that the rat was weak, signifying that the ethylacetate fraction can be toxic at higher doses. Also, there is no significant difference in the weight gained by the experimental rats in all the test groups compared to that of the normal control group. This

indicates that the administration of ethylacetate fraction of *C. aconitifolius* have no significant effect on bodyweight. The blood glucose levels of the Wistar rats showed no significant difference ($p>0.05$) after the administration of ethylacetate fraction of ethanol extract of *C. aconitifolius*.

The haemoglobin concentration of the anaemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* were significantly ($p<0.05$) increased. The red blood cells supply oxygen to the cells and tissues of the body using hemoglobin[26]. Since haemoglobin is composed of an iron-containing substance called haem[27], an Increase in the haemoglobin concentration might be due to the iron and vitamin C content of extract of *C. aconitifolius*. Significant ($p<0.05$) increases in PCV of the anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius* was observed after treatment and this indicates that there is a production of red blood cell in the bone marrow[28]. PCV is the proportion of blood volume that is occupied by red blood cells[28].

The main function of the red blood cells (RBCs) which contain haemoglobin is to transport oxygen from the lungs to the tissues[29] and was significantly increased ($p<0.05$) in the anemic rats treated with ethylacetate fraction of ethanol extract of *C. aconitifolius*. This indicates that ethylacetate fraction of ethanol extract of *C. aconitifolius* have the ability to induce the release of erythropoietin, a glycoprotein hormone that functions as a cytokine for erythrocyte precursors in bone marrow. Medicinal plants are capable of ameliorating the majority of ailments which can be as a result of its phytochemical, mineral, vitamin and antioxidant composition[30,31,32,33].

MCV defines the size of the red blood cells, MCH quantifies the amount of hemoglobin per red blood cell and MCHC is the ratio of the MCH to the MCV[34]. When there is a malfunction in hemoglobin synthesis, it results in low MCV. In the current study, there is an increase in the MCV and MCH value of the experimental rats compared to the normal control and anemic untreated after 14 days treatment indicating that while the values MCHC went back to the normal values after the 14 days treatment. The decrease in MCHC could be as a result of suppression of hematopoiesis in the rats.

The increase in total WBCs count (> 11,000/μL) above normal range is known as Leukocytosis[35], which occurs in response to conditions such as anemia. It was observed in the group of anemic untreated rats. WBCs are part of the immune response. White blood cells (WBCs) of the group of anemic rats treated with the ethylacetate fraction of ethanol extract of *C. aconitifolius* decreased significantly ($p<0.05$). The decrease observed could be as a result of suppression of the production of WBCs in the bone marrow. Significant increase ($p<0.05$) in lymphocytes count in the anemic rats must have also resulted from stress-induced lymphocytosis and neutrophilia.

Liver is an organ of the body which is involved in many metabolic functions thus, it is prone to injuries induced from these activities[36,27]. The serum liver enzymes (AST, ALT & ALP) including bilirubin are parameters used to the presence of liver disease or potential harm to the liver. Usually, when the liver is injured, these enzymes which are not normally found in the serum rise and gets released into the serum[38,39]. This analysis shows that there was a significant increase ($p<0.05$) in the levels of ALP, AST, and ALT in the anemic-untreated group compared to the values of the normal control group. But the groups treated with different doses (100 & 200mg/kg) of ethylacetate fraction of ethanol extract of *C. aconitifolius* were observed to have a significant ($p<0.05$) reduction in the levels of these enzymes. This is indicative of the safety restorative potential of this leaf extract in the correct doses to the liver.

The rise of urea nitrogen and creatinine in the blood indicates impairment of kidney function[40]. In this assay, the significant decrease ($p<0.05$) in the values of urea and creatinine (mg/dl) with respect to the anemic control indicates no nephrotoxicity. The levels of these markers in the groups treated with 100 mg/kg & 200mg/kg of ethylacetate fraction of ethanol extract of *C. aconitifolius* were within range of the normal control group as seen in Table 17. Lipid profile is a panel of blood tests used to find abnormalities in lipids, such as cholesterol and triglycerides[41]. The effect of the ethylacetate fraction of ethanol extract of *C. aconitifolius* was examined on the lipid profile of the test groups. All groups showed significant decrease ($p<0.05$) in TCHOL, LDL-C, TRIG and VLDL-C, and a significant increase ($p<0.05$) in HDL-C levels with respect to the anemic-untreated group. This indicates that the ethylacetate fraction of ethanol extract of *C. aconitifolius* is effective. HDL is known to be the good cholesterol in the body because it absorbs cholesterol and carries it back to the liver to be expelled, preventing the arteries from being blocked by excess cholesterol thus, reducing the risk of cardiovascular diseases[12].

Lipid peroxidation is the oxidative degradation of lipid and its end-product malondialdehyde (MDA), is one of the important biomarkers for oxidative stress[42,43]. A significant increase ($p<0.05$) in MDA level was observed in the anemic-untreated group compared with the normal control. However, other groups treated with a standard drug and ethylacetate fraction of ethanol extract of *C. aconitifolius* (100mg/kg and 200mg/kg shows a significant decrease ($p<0.05$) in the level of MDA with respect to the anemic-untreated. This is indicative that the administration of ethylacetate fraction of ethanol extract of *C. aconitifolius* did not induce lipid peroxidation.

V. Conclusion

The results revealed that ethylacetate fraction of *C. aconitifolius* leaf is safe for therapeutic purposes and could help to combat anaemic disorders implicated with the induction of phenylhydrazine, mitigate the release of free radicals and as well protect the integrity of the liver. This could be as a result of active phytochemicals, minerals and vitamins detected in high quantities in the leaves of *C. aconitifolius.*

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Ethics Approval

The study was carried out in strict compliance with the guidelines for the Institutional Animal Care and Use Committee (IACUC) of Nnamdi Azikiwe University, Awka, Nigeria in line with the recommendations of Animal Care and Use in Research, Education and Testing (ACURET).

Author's Contribution

Conflict of interest

There is no conflict of interest in this manuscript.

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