**Potentials of ethanol leaf extract of *C. aconitifolius* in Phenylhydrazine-induced Anemic rats**

**Abstract**

Anemia is a common health challenge caused by a decrease in hemoglobin concentration, packed cell volume and red blood cells. Some medicinal plants have antianaemic properties and can be used as a remedy to treat anemia. This study investigates the anti-anemic properties of ethanol extract of *Cnidoscolus aconitifolius* (EECA) leaves in phenylhydrazine-induced anemic rats. Acute toxicity (LD50) was done using Lorke’s method. Twenty-five (25) male Wistar albino rats were randomly divided into five groups of five rats each and used for the antianemic studies. Group A, B and C served as the normal control, anemic untreated (negative control) and anemic group treated with 1ml/kg emzoron (positive control) respectively. Groups D and E were treated with 100mg/kg and 200mg/kg of ethanol extract of *C. aconitifolius* respectively by oral gavage once a day for 14 days. Anemia was induced by intraperitoneal injection of 20 mg/kg phenylhydrazine for four consecutive days in groups B to E. At the end of 14 days treatment, biochemical analysis were done using standard diagnostic methods and haematological parameters were analysed using an automated haematology analyzer (Mindray-BC-5300). The result of the LD50 study showed that the extract may not be very toxic. The extract was able to restore the impaired biochemical parameters caused by phenylhydrazine to normal after 14 days of treatment. The antianemic effects of EECA was 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 after 14 days of treatment compared to the anemic untreated control group. There was a better increase in the hemoglobin levels of 100 mg/kg EECA (13.40±0.35) compared to 200 mg/kg EECA (12.50±0.45). Also, the packed cell volume increased more in 100 mg/kg EECA (40.97±0.87) compared to 200 mg/kg EECA (38.73±1.63). A better increase was observed in the red blood cell count of 100 mg/kg EECA (5.83±0.35) compared to 200 mg/kg EECA (5.55±0.44). The EECA was well tolerated by the animals as was seen from the results of the biochemical parameters. The extract also improved the haematological parameters of the animals. The animals that were treated with 100 mg/kg bodyweight yielded a far much better result by totally restoring the haematological parameters of the phenylhydrazine-induced anemic rats to normal without any observable alterations in the biochemical parameters investigated.

**Keywords**: Anemia, ethanol extract, Biochemical, Hemoglobin, Packed cell volume, Red blood cells.

**INTRODUCTION**

The appearance of anemia is as a result of the reduction of the number of erythrocytes below the normal range (Barcellini and Fattizzo, 2015). The reduction of the quality or size of erythrocytes can lead to the onset of anemia. Anemia is a devastating health problem affecting people living in both the developed and developing world. Globally, it affects about 1.74 billion (22.8%) of the world’s population (Gardner, 2020). The findings of a Nationally conducted systematic review and meta-analysis indicated 23% of school children were anemic (Tezera et al., 2018). Anemia occurs at all ages, however; reproductive-age women, preschool, and school-age children are affected more (McLean et al., 2009). Various diseases manifest both in children and adults such as malnutrition, pregnancy, and malaria that could lead to anemia.

The occurrence of drug-induced hemolytic anemia is very rare, and is therefore estimated at 1 per million people (Barbaryan *et al*., 2013). Phenylhydrazine (PHZ) is one drug with a toxic effect on red blood cells that could be useful for the treatment of polycythemia vera and fever. The negative effect of PHZ on red blood cells limits its medicinal use, and its (PHZ’s) activation of reactive oxygen species production has been linked to oxidative stress (Berger, 2007). Oxidative stress has been shown to be involved in the aging and apoptosis of erythrocytes, thus inducing hemolysis (Fibach and Rachmilewitz, 2008; Fujii *et al*., 2021).

Anti-anemic synthetic drugs are mostly not affordable, even when one can afford the drugs, sometimes the side effects are not tolerable. Thus, owing to the difficulties experienced in the management and control of anemia due to poverty, ignorance, and lack of accessible healthcare in most parts of Africa, there is need to study and utilize indigenous medicinal plants with anti-anemic properties that will help to manage this condition, especially in Nigeria where there is high prevalence of anemia among children and pregnant women (Ezeigwe *et al*., 2022). Plant and plant products over the years have been utilized as a source of medicine. Herbal medicines are assumed as greater importance in health care (Gerard and Bryan, 2015; WHO, 2017) in many developing countries.

There is an increasing dependence on medicinal plants by a large number of the population in developing countries as a good alternative for the prevention and treatment of various illnesses including anemia. Medicinal plants have been used over the years in rural communities to treat infections and diseases, and have had undoubtedly good results hence; there has been a level of reliance on plants as a whole. The relevance attached to the use of medicinal plants is derived from their affordability, minimal side effects, and accessibility compared to modern medicines (Ezebuiro *et al.,* 2020).

*Cnidoscolus aconitifolius*, known as tree spinach (English), efo iyana ipaja, or efo Jerusalem (Yoruba) is commonly found growing in the Western part of Nigeria. Different ethnic groups over the years have used *C. aconitifolius* as a vegetable (Silalahi, 2019) and in traditional medicine. This plant is easy to find in the surrounding environment such as yards, roadsides, and cultivated land (Irsyam *et al*., 2020). The leaf of *C. aconitifolius* has a beautiful structure with dense branching pattern which makes it often used as decoration and living fence. Research has shown that *C. aconitifolius* is rich in natural antioxidant (Kuti and Konoru, 2004), which scavenges free radicals and the edible part of the plant which tastes like spinach when cooked is the leaf. It serves as important nutritional source of protein, vitamin, minerals (calcium, iron and phosphorus), some of which are factors necessary for erythropoiesis (Akachukwu *et al.,* 2014). Reports have also shown that *Cnidoscolus aconitifolius* is used to boost blood parameters. According to Iwelewa *et al.* (2005), the plant is believed to have a blood-boosting effect, and so is commonly taken by pregnant women and young children who are anemic. This study, therefore, aims at adding more scientific information on the antianemic potentials of crude ethanol extract of *C. aconitifolius* in phenylhydrazine-induced anemic rats.

**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 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 waterbath at 500C. 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.

***Test Animals***

A total of 38 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 fromVital 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 (LD50) evaluation***

The median lethal dose (LD50) for each of the extracts were determined using Lorke’s method (1983). Thirteen (13) male rats were used for the determination of the median lethal dose of the ethanol extract. 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 extracts.

***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 ethanol extract of *C. aconitifolius* leaf**

**Group E: Anemia + 200 mg/kg bw. of ethanol extract of *C. aconitifolius* leaf**

***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 Weight***

The weight 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 extract has an effect on the body weight 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.

***Haematological Analysis***

Haematological parameters were determined using automated haematology analyzer (Mindray-BC-5300). The haematological parameters that were analysed 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 (BCO3-), Total Calcium (Tcal) and Ionized Calcium (ncal).

**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 (Trinder, 1969 and Tietze et al., 1990). Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula (Friedwald et al., 1972). 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 (1978). The reaction depends on the formation of complex between malondialehyde and thiobarbituric acid (TBA). Serum volume of 0.4ml was collected into the test tubes; 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituric acid and then mixed thoroughly.

The reaction mixture was then placed in 100oC 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:

Optical density x extinction co-efficient

Time amount of sample

Where the extinction coefficient value is 1.56 x 10-5M-1CM-1

The unit is expressed as mol/MDA/mg of protein.

***Data Analysis***

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (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.

**RESULTS**

**Results**

**Acute Toxicity (LD50) Test**

The result of the acute toxicity study revealed the safety of the extract for consumption. It showed that the ethanol leaf extract of *C. aconitifolius* was not toxic. There were two acute toxicity testing phases. The low doses of administration (10, 100 and 1000mg/kg body weight) of the extract showed no visible signs of toxicity in the experimental animals within 24 hours of administration (Table 1). At the second phase, doses were increased to 1600, 2900 and 5000 mg/kg body weight. Even at these high administered doses, no death of the experimental rats was recorded within 24 hours of administration. Though, at the dosage of 2900mg/kg body weight, a slight weakness was observed while the rat administered 5000mg/kg body weight was weak.

**Table 1: Acute toxicity studies of ethanol leaf extract of *C. aconitifolius***

|  |  |  |  |
| --- | --- | --- | --- |
| **Phase** | **Dose mg/kg** | **Death recorded in rats** | **Observations** |
| **First** | **10** |  | **none** |
|  | **100** |  | **none** |
|  | **1000** |  | **none** |
| **Second** | **1600** |  | **none** |
|  | **2900** |  | **Slightly weak** |
|  | **5000** |  | **weak** |

Number of rats per phase = 3 and 1 respectively. Number of deaths per group = 0

**Bodyweight**

The weights of the rats were ascertained before, during treatment and before sacrifice (Table 2). There were significant increase (*p<*0.05) in the weight of the rats across all the groups when compared to their weights before induction. The increase in their weight may not be as a result of the administered extract since there were no noticeable increase in weight when compared to other groups that were not treated with the extract, but their weight could be normal increase in weight due to their feeding.

**Table 2: Bodyweight of phenylhydrazine-induced anemic rats treated with ethanol leaf extract of *C. aconitifolius*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups** | **Weight (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 crude ethanol extract of *C. aconitifolius* | 126.40±2.58 | 135.80±2.52 | 147.40±3.66 | 156.80±8.24\* |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 127.60±1.78 | 130.20±2.85 | 144.20±4.68 | 158.20±2.35\* |

Table is expressed as mean ± SEM; *p<*0.05 significant difference compared to day 0.

**Random Blood Glucose Concentration**

There were no observable changes in the glucose levels when compared with the normal control and anemic groups as revealed in Table 3. As such, all the groups random blood glucose concentration were within normal throughout the period of the experiment.

**Table 3:** Random blood glucose concentrations ofphenylhydrazine-induced anemic rats treated with ethanol leaf extract of *C. aconitifolius*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 crude ethanol extract of *C. aconitifolius* | 94.40±9.33 | 90.80±7.09 | 110.4±5.11 | 101.00±7.17 |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 89.60±6.11 | 86.80±7.09 | 110.4±5.11 | 101.00±7.17 |

Table is expressed as mean ± SEM; *p<*0.05 significant difference compared to anemic untreated

**Haematological Analysis**

Haematological parameters (Tables 4 to 16) were used to ascertain the Haematological effect of ethanol leaf extract of *C. aconitifolius* on phenylhydrazine-induced anemic rats.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.50b | 8.30±1.55 |
| Anemia + Standard drug (Emzoron) | 13.17±0.27 | 10.60±0.60b | 14.10±0.10c |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 12.87±0.43 | 9.53±0.27b | 13.40±0.35c |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 12.87±0.12 | 9.37±0.55b | 12.50±0.45c |

Table is expressed as mean ± SEM; bSignificant decrease with respect to day 0; cSignificant increase with respect to day 5.

Table 4 revealed haemoglobin concentration of which the treatment groups respectively showed significant decrease and increase when compared to day 0 and day 5. Induction of anemia caused a significant (p<0.05) reduction in the haemoglobin concentrtation of the rats. Treatment with the ethanol extract of *C. aconitifolius* (p<0.05) significanytly restored the haemoglobin concentration to normal.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±1.77b | 23.73±2.87bd |
| Anemia + Standard drug (Emzoron) | 40.47±0.99 | 33.43±2.97b | 43.87±0.74c |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 38.03±1.08 | 26.83±1.41b | 40.97±0.87c |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 46.67±1.56 | 27.83±2.38b | 38.73±1.63c |

Table is expressed as mean ± SEM; bSignificant decrease with respect to day 0; cSignificant increase with respect to day 5; dSignificant decrease with respect to day 5.

Table 5 revealed packed cell volume of the treatment groups showed significant decrease and increase when compared to day 0 and day 5. Induction of anemia caused a significant (*p*<0.05) reduction in the packed cell volume of the rats. Treatment with the ethanol extract of *C. aconitifolius* (p<0.05) significantly restored the packed cell volume to normal.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.25b | 3.54±0.38b |
| Anemia + Standard drug (Emzoron) | 8.37±0.36 | 6.27±0.64b | 5.86±0.15b |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 8.13±0.20 | 5.20±0.27b | 5.83±0.35b |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 7.94±0.16 | 4.98±0.20b | 5.55±0.44b |

Table is expressed as mean ± SEM; bSignificant decrease with respect to day 0.

Induction of anemia caused a significant reduction (p<0.05) in red blood cell count of the groups induced (Table 6). Treatment with graded doses of ethanol extract of *C. aconitifolius* did not cause a noticeable increase in the red blood cell count.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **PLT (109/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±65.03b | 488.70±168.90b |
| Anemia + Standard drug (Emzoron) | 713.00±4.62 | 756.30±10.17 | 572.00±115.9 |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 742.30±25.31 | 940.30±57.03 | 706.00±50.24d |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 774.30±33.24 | 825.70±7.31 | 773.30±97.30 |

bSignificant decrease with respect to day 0; dSignificant decrease with respect to day 5.

Induction of anemia caused a non-significant (p<0.05) increase in the platelet count of the groups treated with the standard drug and the graded doses of the extract while it caused a significant decrease (p<0.05) in the anemia untreated group (Table 7). Treatment with the standard drug and the extracts did not cause any observable change in the platelet count.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±2.80a | 66.83±1.10ad |
| Anemia + Standard drug (Emzoron) | 51.17±1.10 | 53.47±1.18 | 75.00±2.69ac |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 50.00±1.49 | 51.67±0.12 | 70.80±5.17ac |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 52.77±4.72 | 55.70±2.59 | 70.47±4.87ac |

aSignificant increase with respect to day 0; cSignificant increase with respect to day 5; dSignificant decrease with respect to day 5.

The mean corpuscular volume of phenylhydrazine-induced anemic rats did not reveal any significant difference (Table 8). However, treatment with the extracts caused a significant increase (p<0.05) in the mean corpuscular volume of the groups treated with the standard drugs and the extracts.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.90a | 23.40±0.06ad |
| Anemia + Standard drug (Emzoron) | 16.53±0.52 | 17.10±1.51 | 24.10±0.45ac |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 15.70±0.72 | 18.43±0.52a | 23.23±1.85ac |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 16.07±0.67 | 18.83±0.33a | 22.73±1.71ac |

aSignificant increase with respect to day 0; cSignificant increase with respect to day 5; dSignificant decrease with respect to day 5.

The mean corpuscular haemoglobin of phenylhydrazine-induced anemic rats did not reveal any significant difference (Table 9). However, treatment with the extracts caused a significant increase (*p*<0.05) in the mean corpuscular haemoglobin of the groups treated with the standard drugs and the extracts.

**Table 10:** Mean Corpuscular Hemoglobin Concentration (MCHC) of phenylhydrazine-induced anemic rats treated with ethanol leaf 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±0.26ac |
| Anemic Untreated | 27.70±0.75 | 28.50±0.10 | 35.10±0.56ac |
| Anemia + Standard drug (Emzoron) | 28.10±0.38 | 31.97±1.18a | 32.17±0.67a |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 28.40±0.55 | 35.63±0.97a | 33.10±0.26a |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 29.10±0.71 | 33.93±1.06a | 32.27±0.32 |

aSignificant increase with respect to day 0; cSignificant increase with respect to day 5.

Induction of anemia using phenylhydrazine caused a significant increase (*p*<0.05) in the mean corpuscular haemoglobin concentration (MCHC) of the groups treated with the standard drug and the extracts (Table 10). However, treatment with the standard drug and the graded doses of the extract did not cause any significant difference (*p*>0.05) in the mean corpuscular haemoglobin concentration of the groups treated with the standard drugs and the extract groups.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **WBC (109/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±5.94a | 32.28±4.82ac |
| Anemia + Standard drug (Emzoron) | 7.73±0.73 | 31.65±4.75a | 14.96±2.31ad |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 9.00±0.97 | 41.36±4.66a | 13.30±4.12ad |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 9.20±2.26 | 42.56±5.62a | 14.66±5.93ad |

aSignificant increase with respect to day 0; cSignificant increase with respect to day 5; dSignificant decrease with respect to day 5.

Induction of anemia with phenylhydrazine caused a significant increase (p<0.05) in the white blood cells of all the induced groups compared to the uninduced (Table 11). Treatment with the extract caused a significant decrease (p<0.05) in the white blood cells of all the treated groups.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±1.59bd |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 29.30±2.25 | 33.37±2.80 | 12.57±0.75bd |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 28.07±0.29 | 31.60±0.72 | 22.07±4.62bd |

bSignificant decrease with respect to day 0; dSignificant decrease with respect to day 5.

Induction of anemia with phenylhydrazine caused a significant increase (*p*<0.05) in the neutrophil of all the induced groups compared to the uninduced (Table 11). Treatment with the extract caused a significant decrease (*p*<0.05) in the neutrophil of all the treated groups.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±1.80b | 69.10±1.08c |
| Anemia + Standard drug (Emzoron) | 65.33±2.72 | 52.53±2.13b | 82.13±1.63ac |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 67.17±1.52 | 57.03±2.98b | 87.13±0.64ac |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 64.80±2.35 | 52.57±1.35b | 84.20±2.71ac |

aSignificant increase with respect to day 0; bSignificant decrease with respect to day 0 cSignificant increase with respect to day 5.

Induction of anemia with phenylhydrazine caused a significant decrease (p<0.05) in the lymphocyte count of all the induced groups compared to the uninduced (Table 11). Treatment with the extract caused a significant increase (p<0.05) in the lymphocyte of all the treated groups.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.03bd |
| Anemic Untreated | 0.43±0.03 | 1.47±0.03 | 0.03±0.03bd |
| Anemia + Standard drug (Emzoron) | 0.37±0.03 | 1.40±0.06 | 0.00±0.00bd |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 0.43±0.03 | 1.50±0.00 | 0.17±0.03d |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 0.37±0.07 | 1.40±0.00 | 0.20±0.00d |

bSignificant decrease with respect to day 0; dSignificant decrease with respect to day 5.

Induction of anemia with phenylhydrazine caused a significant increase (p<0.05) in the monocyte count of all the induced groups compared to the uninduced (Table 11). Treatment with the extract caused a significant decrease (p<0.05) in the monocyte count of all the treated groups.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.03bd |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 0.17±0.03 | 0.20±0.00 | 0.13±0.09 |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 0.13±0.03 | 0.13±0.03 | 0.17±0.03 |

bSignificant decrease with respect to day 0; dSignificant decrease with respect to day 5.

Induction of anemia with phenylhydrazine did not cause a significant increase (*p*>0.05) in the eosinophil count of all the induced groups compared to the uninduced (Table 15). Treatment with the extract did not cause a significant difference in the eosinophil count of all the treated groups.

**Table 16:** Basophils (BAS) of phenylhydrazine-induced anemic rats treated with ethanol leaf extract of *C. aconitifolius*.

|  |  |  |  |
| --- | --- | --- | --- |
| **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±0.00bd |
| Anemic Untreated | 0.10±0.00 | 0.33±0.03 | 0.00±0.00bd |
| Anemia + Standard drug (Emzoron) | 0.17±0.03 | 0.37±0.03 | 0.00±0.00bd |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 0.23±0.09 | 0.40±0.00 | 0.00±0.00bd |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 0.13±0.03 | 0.27±0.03 | 0.00±0.00bd |

bSignificant decrease with respect to day 0; dSignificant decrease with respect to day 5.

Induction of anemia with phenylhydrazine caused a significant increase (p<0.05) in the basophil count of all the induced groups compared to the uninduced (Table 16). Treatment with the extract caused a significant decrease (*p*<0.05) in the basophil count of all the treated groups.

**Biochemical Analysis**

**Effect of Extract on Liver Function Parameters**

Induction of anemia consecutively for four days using phenylhydrazine caused a significant increase (p<0.05) in the liver function parameters (aspartate transaminase, alkaline phosphatase, alanine transaminase, total bilirubin and direct bilirubin) compared to the normal control group which was left uninduced (Table 17). Treatment with the ethanol extract of *C. aconitifolius* significantly (p<0.05) reduced the liver function parameters compared to the anemia untreated group which remained high.

**Table 17:** Effect of treatment with ethanol leaf extract of *C. aconitifolius* 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±1.15e | 62.50±9.97e | 30.00±2.08e | 2.10±0.32e | 0.85±0.17e |
| Anemia + Standard drug (Emzoron) | 21.67±1.33h | 42.73±14.10h | 16.67±2.60h | 1.48±0.16h | 0.34±0.12h |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 20.67±3.28h | 35.33±5.52h | 17.33±5.04h | 1.36±0.14h | 0.31±0.02h |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 19.00±2.08h | 37.50±2.51h | 15.00±3.06h | 1.67±0.21 | 0.28±0.02h |

eSignificant increase with respect to normal control; hSignificant decrease with respect to anemic untreated.

**Effect of Extract on Kidney Function Parameters**

Induction of anemia consecutively for four days using phenylhydrazine caused a noticeable increase in the kidney function parameters (creatinine and urea) compared to the normal control group which was left uninduced (Table 18). Treatment with the ethanol extract of *C. aconitifolius* significantly (*p*<0.05) reduced the creatinine concentration compared to the anemia untreated group which remained high.

**Table 18:** Effect of treatment with ethanol leaf extract of *C. aconitifolius* 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±1.18e | 2.10±0.40 |
| Anemia + Standard drug (Emzoron) | 10.70±0.45h | 1.57±0.20 |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 9.670±0.29h | 1.70±0.06 |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 11.23±1.43h | 1.60±0.17 |

eSignificant increase with respect to normal control; hSignificant decrease with respect to anemic untreated.

**Effect of Extract on Electrolyte Levels**

Induction of anemia did not cause much effect in the serum electrolyte (Potassium, Sodium, Chloride ion, Bicarbonate ion, Total calcium and Ionized calcium) concentrations (Table 19). However, treatment with the ethanol extract of *C. aconitifolius* caused a significant (*p*<0.05) increase in the chloride ion of the treated groups compared to the anemic untreated. Also, an increase was observed in the sodium ion concentration of the group treated with 200mg/kg bodyweight of the extract compared with the normal and untreated control groups.

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Groups** | **K+ (mmol/L)** | **Na+ (mmol/L)** | **Cl- (mmol/L)** | **BCO3- (mmol/L)** | **Tcal (mmol/L)** | **ncal (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±0.52fh | 130.66±1.33 | 106.7±0.67eg | 21.33±0.88 | 1.07±0.18 | 0.53±0.09 |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 6.07±0.24 | 132.0±0.58 | 109.3±0.33eg | 21.67±0.33 | 1.07±0.07 | 0.53±0.03 |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 6.63±0.90 | 133.67±1.33e | 110.30±0.33eg | 22.00±1.53e | 0.93±0.09fh | 0.43±0.03fh |

eSignificant increase with respect to normal control; fSignificant decrease with respect to normal control; gSignificant increase with respect to anemic untreated; hSignificant decrease with respect to anemic untreated.

**Effect of Extract on Lipid Profile**

Induction of anemia consecutively for four days using phenylhydrazine caused a noticeable increase in the lipid profile parameters (Total cholesterol, High-Density-Lipoprotein cholesterol, Triglycerides, Low-Density-Lipoprotein cholesterol and Very low-Density Lipoprotein) compared to the normal control group which was left uninduced (Table 20). Treatment with the ethanol extract of *C. aconitifolius* significantly (*p*<0.05) reduced the total cholesterol concentration compared to the anemia untreated group which remained high.

**Table 20:** Effect of treatment with ethanol leaf extract of *C. aconitifolius* 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±3.76e | 35.33±4.70 | 38.67±3.84e | 95.27±8.73e | 9.07±0.93e |
| Anemia + Standard drug (Emzoron) | 113.60±3.18h | 42.00±5.03 | 32.67±2.03 | 65.13±8.23h | 6.53±0.41h |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 103.67±10.17h | 40.00±4.04 | 34.00±4.73 | 56.87±7.01h | 6.80±0.95h |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 95.33±14.88h | 40.33±8.09 | 36.33±4.18h | 47.27±6.68 | 7.27±0.84 |

eSignificant increase with respect to normal control; hSignificant decrease with respect to anemic untreated.

**Effect of Extract on Lactate Dehydrogenase Activity**

Induction of anemia consecutively for four days using phenylhydrazine caused a significant (*p*<0.05) increase in the lactate dehydrogenase activity compared to the normal control group which was left uninduced (Table 21). Treatment with the ethanol extract of *C. aconitifolius* significantly (*p*<0.05) reduced the lactate dehydrogenase activity compared to the anemia untreated group which remained high.

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

|  |  |
| --- | --- |
| **Groups** | **LDH (U/L)** |
| Normal Control | 294.67±12.41 |
| Anemic Untreated | 427.00±24.02e |
| Anemia + Standard drug (Emzoron) | 249.30±22.28h |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 319.70±24.31h |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 297.70±11.29h |

eSignificant increase with respect to normal control; hSignificant decrease with respect to anemic untreated.

**Effect of Extract on Lipid Peroxidation (Malondialdehyde)**

Induction of anemia consecutively for four days using phenylhydrazine caused a significant (*p*<0.05) increase in the malondialdehyde concentration compared to the normal control group which was left uninduced (Table 22). Treatment with the ethanol extract of *C. aconitifolius* at a dose of 100mg/kg significantly (*p*<0.05) reduced the malondialdehyde concentration compared to the anemia untreated group which remained high.

**Table 22:** Effect of treatment with ethanol leaf 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±0.58h |
| Anemia + 100 mg/kg crude ethanol extract of *C. aconitifolius* | 2.26±0.21h |
| Anemia + 200 mg/kg crude ethanol extract of *C. aconitifolius* | 2.78±0.45 |

hSignificant decrease for anemic untreated.

**DISCUSSION**

Anemia is one of the major conditions leading to mortality and morbidity worldwide and particularly in developing countries. Anemia is characterized by a decrease in erythrocyte count, circulating hemoglobin and packed cell volume. Children and adults are affected with the prevalence in pregnant women and children. In this present research, the anti-anemic property of ethanol extract of *C. aconitifolius* was determined. Phenylhydrazine caused a slight decrease in the bodyweight of the rats after induction of anemia. Loss of bodyweight is one of the clinical signs and symptoms of anemia which can be due to loss of appetite. The normal control rats ate normally during the experiment which led to a noticeable increase in their bodyweight thereby promoting weight gain. The decrease in bodyweight in anemic rats could be because of reduction in the activities of disaccharidases that catalyze the final step of carbohydrate digestion (Vieira *et al*., 2000). The bodyweight of the anemic rats treated with ethanol extract of *C. aconitifolius* increased significantly (*p*<0.05) in days 7 and 14 of the treatment compared to the normal control group.

The results obtained from the acute toxicity (LD50) study revealed that the extract may not be toxic. According to Lorke’s method, administration of 5000mg/kg of an extract which did not cause any death at such a high dose in an experimental subject is an indication that the extract may not be very toxic (Lorke, 1983). The fasting blood glucose concentration remained normal in all the groups before, during and after the experiment. This reveals that the administration of phenylhydrazine does not alter the glucose concentration of the rats. Also, it reveals that the graded doses of the extract do not in any way affect the glucose concentration of the experimental subjects. Administration of phenylhydrazine resulted in a significant decrease in hemoglobin, red blood cell counts and packed cell volume. Phenylhydrazine induced hemolytic anemia by decreasing the values of hemoglobin, erythrocyte count and hematocrit in experimental subjects. The decrease in hemoglobin level may be associated with hemolysis or alterations in heme biosynthesis because of `inhibition of iron binding with heme and decreased activity of enzymes involved in heme biosynthesis (Pandey *et al*., 2016).

The result of the study revealed that ethanol extract of *C. aconitifolius* significantly increased the hemoglobin concentration, erythrocyte count, and packed cell volume of the treated groups. In contrast to the anemic untreated rats, the values of the hematological parameters of the treated rats increased gradually over time until a complete restoration to normal levels was seen after day 14 of the treatment. This is an indication that the blood parameters are gradually regenerated by the body which will attest that phenylhydrazine administered consecutively for 4 days, does not interfere in the regeneration mechanism and does not permanently alter the body’s ability to regenerate blood parameters (Bleu et al., 2022). These results are in line with that of Ezeigwe *et al*. (2022) who observed an increase in the hemoglobin, packed cell volume and erythrocyte count of phenylhydrazine-induced rats treated with a combination of *F. capensis* and *C. aconitifolius*. The normal values of the blood parameters were also restored in the group of rats treated with emzoron (Blood tonic comprising of vitamins) which reveals that emzoron is effective in treating phenylhydrazine damage (Chinenye *et al*., 2019)

This may be because of the phytochemicals, minerals, and vitamin content of *C. aconitifolius* (Ezeigwe *et al*., 2020). The improvement in the haematological parameters may be because of the presence of tannins, alkaloids, flavonoids and phenolic compounds in the extract. Alkaloids and flavonoids are potent antioxidants that prevent and repair damage to red blood cells by free radicals or highly reactive oxygen species (Quattara-Soro *et al*., 2022). Most anti-anemic compounds are known to be free radical inhibitors, which reverse anemic conditions (Sheth *et al*., 2021). It’s been shown that alkaloids inhibit cyclic adenosine monophosphate phosphodiesterase, thus accumulating it. This singular effect stimulates protein phosphorylation and synthesis, which improves erythropoiesis (Katsayal and Lamai, 2011). These phytochemicals may have contributed to the anti-anemic activity of the extract by stimulating erythropoiesis in the bone marrow.

Some toxicity markers were investigated in the ethanol extract of *C. aconitifolius* by analysing some essential biochemical parameters. Liver function parameters used to detect the presence of potential harm or toxicity to the liver include the serum levels of the aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) enzymes, also, bilirubin concentrtations. Liver injury can cause a rise in the ALT. The release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage (Chikwendu *et al*., 2015). The anemic untreated rats showed a significant increase (p<0.05) in the serum liver enzymes compared to the uninduced normal control rats. Treatment with the ethanol extract of *C. aconitifolius* gradually restored the serum levels of the liver enzymes back to normal bringing their values closer to normal. These results suggest the safety of the leaf extract to the liver which could be by exhibiting protective and restorative potential in cases of liver damage or injury.

The levels of urea, creatinine, and electrolytes (K+, Na+, Cl-, BCO3-, Tcal, ncal) assayed for indicated no nephrotoxicity or tubular dysfunction as there was a noticeable significant decrease (p<0.05) in the values of these biomarkers as against that of the anemic untreated control. The levels of urea, creatinine, and electrolytes in the groups treated were within range of the normal control group. Elevated levels of blood urea and creatinine could be a sign of an underlying ill health affecting the kidneys. Serum levels of electrolytes such as potassium, sodium, bicarbonate, and chloride which are too high or too low are suggestive of tubular dysfunction (Hamidu *et al*., 2018).

Administration of phenylhydrazine significantly increases (p<0.05) the serum levels of total cholesterol (TCHOL), triglycerides (TRIG), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) and significantly reduced (p<0.05) the high-density lipoprotein cholesterol (HDL-C) in all the groups induced. Treatment with the graded doses of ethanol extract of C. aconitifolius restored the lipid serum levels to almost normal by reducing the concentration of sewrum TCHOL, TRIG, LDL-C, VLDL-C and increasing the HDL-C in all the treated groups. This is a good indication of the safety of the extract. HDL-C is known to be a good cholesterol, so it is less likely to end up in the arteries to form plaques, facilitating the prevention of cardiovascular risk factors (Hemalatha *et al*., 2019).

Lactate dehydrogenase (LDH) is an enzyme found in various tissues, including the heart, liver, kidneys, and muscles. Elevated values of LDH can lead to tissue damage, cardiac issues, liver diseases, kidney diseases, cancer and infections. Damages to tissues that have LDH in their cells release the enzyme into your bloodstream or other body fluids which is an indication of disease or injury. LDH is used to detect cell damage or cell death (Johnson-Davis, 2010). Phenylhydrazine significantly increased (p<0.05) the LDH activity observed in the anemic untreated group compared to the groups treated with the ethanol extract of *C. aconitifolius*. This is an indication that the extract has a restorative effect on the tissues from the anemic condition.

Malondialdehyde (MDA) is widely used as a biomarker for assessing oxidative stress caused by lipid peroxidation in biomedical fields and research. Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in the generation of free radicals causing cellular damage. Biomonitoring of MDA has been used in both in-vivo and in-vitro studies as a key biomarker for various disease patterns. Oxidative stress is the state of imbalance between the reactive oxygen species (ROS) and the ability of a biological system to detoxify readily the reactive intermediates (Zorawar *et al*., 2014). The results of the MDA analysis revealed that the extract has a restorative effect on the high MDA caused by the induction of anemia with phenylhydrazine thereby ameliorating the effect of oxidative stress in the tissues.

**Conclusion**

The findings of this study revealed that ethanol extract of *C. aconitifolius has* antianaemic potentials and can be easily accessed by anemic patients due to its availability and affordability. *C. aconitifolius* leaves improve haematological parameters, promote liver function parameters, maintain normal serum electrolyte level and kidney function indices, stimulate the reduction of "bad cholesterols", increase "good cholesterol" and reduce oxidative stress. The results of the hematological analysis proved that it boosts the erythrocyte count and positively enhances the hematological parameters without altering the biochemical functions of the experimental subjects.

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