Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(Research Article)

# Anti-diarrheal effects of ethanol leaf extract of *Eleusine Indica* in Castor Oil induced diarrhea using mice model

Chima Ernest Orji [1], Nkemakonam Chinecherem Madu [1], Izunna Somadina Okwelogu [2], Josephat Chekwube Obasi [3], Chinenye Henrietta Nedum [4], Cyril Onyeka Ogbue [4], Victoria Nonyelum Olli [5] and Jude Nnaemeka. Okoyeh [6,*]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe, University, P.M.B. 5025 Awka, 420110 Anambra State, Nigeria.*
[2] *Department of Medical Laboratory Science, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Nigeria.*
[3] *Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, P.M.B. 5025 Awka, 420110 Anambra State, Nigeria.*
[4] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe, University, P.M.B. 5025 Awka, 420110 Anambra State, Nigeria.*
[5] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus, Anambra State, Nigeria.*
[6] *Department of Medical Laboratory Science, School of Nursing and Health Sciences, Neumann University, One Neumann Drive, Aston, PA, 19014. USA.*

Magna Scientia Advanced Biology and Pharmacy, 2024, 12(02), 011–022

Publication history: Received on 15 May 2024; revised on 29 June 2024; accepted on 02 July 2024

Article DOI: https://doi.org/10.30574/msabp.2024.12.2.0031

## Abstract

Ancient civilizations employed medicinal herbs to treat ailments. Medicinal herbs are essential for treating chronic disorders. Different medicinal plants can treat comparable ailments, depending on the country. In many rural communities in developing countries, particularly in Africa, therapeutic medicines and remedies derived from indigenous plants are almost invariably the only readily available and cost-effective alternatives to traditional diarrhea medicines. The study aims to evaluate the anti-diarrheal activity of the *Eleusine indica* ethanol extract in mice. The study used fresh *Eleusine indica* leaves. The extracts were administered at concentrations of 250, 500, and 1000 mg/kg for 28 days. On the 29th, the rats were induced with diarrhea, and blood samples were collected through the retro-orbital plexus before being sacrificed. The serum from the collected blood was used to run hematological and electrolyte tests. The liver and kidney function tests were analyzed using standard methods. The determination of antidiarrheal activity was done using the following models: Castor oil (CO) induced diarrhea, CO induced enteropooling activity, and CO induced gastrointestinal motility. The 80% ethanol extract produced significant ($p<0.001$) antidiarrheal activity in all three models tested. The hematological, renal, hepatic, and electrolyte parameters of extract-treated mice were not significantly ($p > 0.05$) different from those of the control group. There was no statistically significant difference ($p > 0.05$) in the chloride, sodium, potassium, calcium, and bicarbonate levels of the extract-treated groups when compared to the control group. The findings of the studies demonstrated the antidiarrheal activities of *Eleusine indica* leaves, which could be a therapeutic option against diarrhea.

**Keywords:** *Eleusine indica;* Diarrhea; Ethanol; Extract; Leaves

* Corresponding author: Jude N. Okoyeh

Copyright © 2024 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

## 1. Introduction

Diarrhea is a prevalent illness that can range in intensity and cause. Diarrheal diseases are the second most common cause of death in children under the age of 5 globally, resulting in almost 1.7 billion cases and 525,000 deaths annually [1]. The majority of deaths caused by diarrhea occur in low- and middle-income countries (LMICs), particularly in sub-Saharan Africa and South Asia, accounting for roughly 90% of these deaths[2] . Diarrhea is a significant public health issue affecting children in Nigeria, with a prevalence rate of 18.8% - one of the highest in sub-Saharan Africa and exceeding the regional norm of 16%. Each year, diarrhea is responsible for more than 16% of child fatalities in Nigeria, causing around 150,000 deaths primarily among children under the age of five [3]. The epidemiology of childhood diarrhea in Nigeria shows that the incidence is highest in the first two years of life and declines as children grow older [4]. Factors associated with the high prevalence of diarrhea include poor water quality, lack of access to improved sanitation, and unhygienic practices in food preparation and disposal of excreta [5].There is also a regional discrepancy, with a higher prevalence of diarrheal disease in the northern parts of the country compared to the south.

The assessment of diarrhea is contingent upon its duration, intensity, and the existence of specific accompanying symptoms. The presence of blood in the stool, regardless of how often it occurs or its quality, is a clear indication of acute diarrheal diseases or dysentery [6].A diarrheal condition is commonly categorized as acute, chronic, or persistent. Acute diarrhea, the most prevalent form of diarrhea disorders, typically begins suddenly, is caused by infections, and typically resolves within a span of 14 days. Acute diarrhea is characterized by the sudden occurrence of three or more loose or watery bowel movements per day, lasting for a maximum of 14 days[7]. On the other hand, chronic or persistent diarrhea is diagnosed when the episode lasts longer than 14 days. Chronic diarrhea primarily arises from congenital abnormalities in the body's digestive and absorptive processes and persists for a minimum of 14 days.

The main difference between acute and chronic diarrhea is duration. Chronic diarrhea is beyond 4 weeks, while acute is less 2 weeks [8]. Acute diarrhea caused by viruses or bacteria is usually mild and self-limiting. If persistent, chronic diarrhea may suggest major medical issues and require additional evaluation and treatment. In some cases, it might be self-limiting [9]. Chronic diarrhea can be osmotic, secretory, or inflammatory, requiring different diagnosis and therapy. Different types of diarrhea exhibit various pathophysiological changes in electrolyte and water transport. These changes include increased luminal osmolarity, increased electrolyte secretion, decreased electrolyte absorption, and accelerated intestinal motility, resulting in decreased transit time [10] .These modifications result in the buildup of fluid in the digestive system, which triggers a process known as enteropooling. Various research have employed plant extracts and isolated chemicals to address or avert diarrhea[10],[11].

Medicinal plants offer a hopeful opportunity for finding novel medications to treat diarrhea. They include both non-pharmacological and pharmacological approaches [12];[13].These therapeutic therapies, derived from indigenous plants, consistently outperform conventional medicines in the underdeveloped countries The presence of tannins, alkaloids, saponins, flavonoids, steroids, and/or terpenoids in many plants has been identified to contribute to their anti-diarrhea properties. These medicines are easily accessible and economical for managing diarrhea in rural populations [14]. This sparked the study on the anti-diarrheal effect of *Eleusine indica* ethanol leaf extract on castor oil-induced diarrhea in a mouse model. The findings of this study will help to confirm the local use of this plant in the treatment of both acute and chronic diarrhea.

## 2. Materials and methods

### 2.1. Apparatus and Equipment

The following laboratory apparatus and equipment were used in the course of this study: glass column, flasks, beakers, test tubes, measuring cylinders, animal cages, dissecting kits, funnel, cotton wool, desiccators, mortar and pestle, scissors, syringes (1ml, 2ml, and 5ml), Petri dish, cannula, hand gloves, Eppendorf tubes, sample bottles, and laboratory record book. Other equipment were: rotary evaporator, analytical weighing balance (Metler H30, Switzerland), Water bath (Techmel & Techmel, Texas, USA), Acurex Chemistry Analyzer (SR NO: 7047,England), National Blender (Japan), digital vernier caliper (Battenfeld Technologies Inc., USA), electric hot plate (DB-1A), Hematology auto-analyzer (Sysmex Kx-21N, United States) and Ion selective electrode detector (ISE 4000, France).

### 2.2. Reagents and drug

The following chemicals and drug were used in the course of the study: ethanol (JHD, Guangdong Guanghua Schi-Tech. Ltd., China), formaldehyde 40% w/v, castor oil, carrageenan, tween 80, formalin solution, chloroform (Sigma Aldrich, St. Louis, MO, USA), and loperamide (Imodium®).

### 2.3. Animals

Health albino mice of either sex, average weight (18–30 g), were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. They were housed in clean metal cages, supplied with clean drinking water, and fed with commercial pelleted feed (Guniea Feed®, Nigeria) in the animals' house, School of Pharmacy, Agulu. The National Institute of Health Guidelines for care and use of laboratory animals (Pub No. 85-23, revised 1985) guided the handling of the animals.

### 2.4. Collection and authentication of plant materials

Fresh leaves of *E. indica* were collected from the botanical garden of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The plant was authenticated by a taxonomist at the Department of Botany, while voucher number *PCG/474/P/052* was issued at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu campus.

**Figure 1** Photograph of *Eleusine indica* collected from the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

### 2.5. Extraction of plant material

The dried leaves of E. indica were ground into a fine powder using an electric blender and stored in a sealed amber bottle to maintain cleanliness and prevent air exposure. Next, 750 grams of the powdered leaf material underwent a cold maceration process using 80% ethanol. The mixture was continuously stirred for a duration of 48 hours. The liquid that passed through the filter was collected and made more concentrated by removing the water using a heated bath at a temperature of 400 °C. Prior to utilization, the extract was stored in a refrigerator. The extract's percentage yield was determined using a procedure outlined by Zhang et al.[15]:

$$\%\ \text{yield} = \frac{\text{mass of dry extract}}{1000\ g} \times \frac{100}{1}$$

### 2.6. Acute Toxicity Test

The acute toxicity LD50 test was performed in two phases on 13 mice weighing an average of 30g using the modified Lorke's method[16]. In the first stage, the animals were divided into three groups of three mice each (n = 3), and the extract was administered at three different dose levels (10, 100, and 1000 mg/kg) based on body weight. The animals were watched for 24 hours. Because there were no deaths in the first phase, extracts at concentrations of 2000, 3000, 4000, and 5000 mg/kg were administered for four groups of one animal each. Animals were inspected for a further 24 hours. The number of deaths was recorded for each group, and the LD50 was computed as follows:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$; where: $D_0$ = highest dose that gave no mortality; $D_{100}$ = Lowest dose that produced mortality.

### 2.7. Phytochemical screening

*2.7.1. Qualitative phytochemical screening*

The plant's raw extracts were examined for the existence of :alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, phenol, reducing sugars, and glycosides using established techniques and procedure written by Trease and Evans [17].

## 2.8. Sub-acute toxicity study

This test was carried out similar to Agbor et al.[18]. A total of 20 mice of either sex (18-25 g) were randomly assigned to four groups. The control group (1) had 5 animals and received 10 ml/kg of distilled water, while groups (2-4) had 5 rats each and received 250, 500, and 1000 mg/kg of the extract, respectively. After collecting blood samples to determine baseline hematological and biochemical parameters, the animals were dosed once daily for 28 days. On the 29th day, blood samples were taken from the retro-orbital plexus into EDTA and plain tubes for hematological and biochemical analysis.

## 2.9. Liver, Kidney, Electrolytes and Hematological Tests

The serum concentrations of liver and kidney function enzymes: alanine transaminase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) ,urea and creatinine were determined spectrometrically with commercially available randox reagents kit (Randox Laboratories,UK) similar to Aba et al.,,[19].The serum electrolytes: Sodium ($Na^+$), potassium ($K^+$), chloride ($Cl^-$), and bicarbonate($HCO_3^-$) were analyzed similar to Ernest et al., [20] while hematological parameters(PCV, HB, RBC, WBC and Platelets) were determine using hematological auto-analyzer similar to Amaza et al., [21].

## 2.10. Anti-diarrhea study and experimental design

The experimental animals were randomly divided into three groups (negative control, positive control, and three test groups), with five animals in each group (n = 5). The negative control received vehicle (10 ml/kg, distilled water), and the positive control received Loperamide (2 mg/kg). The test groups (groups 3, 4, and 5) received different doses of the extract (250, 500, and 1000 mg/kg, respectively) orally, which was determined based on the acute oral toxicity test.

## 2.11. Determination of anti-diarrheal activity

### *2.11.1. Castor oil induced diarrhea*

This study used the method as described by Ezekwesili et al.[22]. Five groups of Swiss albino mice (n = 5 each) were used. Diarrhea was induced orally in each mouse using castor oil (1 ml/kg b.wt.). Groups 4, 5, and 6 received three different doses of plant extract (low-250, medium-500, and high-1000 mg/kg body weight) orally after one hour of castor oil treatment. Group 1 received normal saline orally (10 ml/kg) and served as the control, while group 2 served as the standard and received Loperamide (2 mg/kg body weight, respectively). The actual dose of loperamide administered to the mice was determined using Nair and Jacob [23] equation. Individual metal cages lined with white, nonabsorbent paper housed the animals. Fecal output was assessed by collecting the fecal material for 8 hours after drug administration, drying it for 2 hours, and then weighing it. The percentage fecal output (% FOP) was calculated as follows:

$$\% \text{ FOP} = \frac{\text{Ft x 100}}{Fc}$$

Where
Ft = mean fecal weight of each test group,
Fc = fecal weight of control group.

$$\% \text{ Inhibition of defecation} = \frac{\text{Mo} - \text{M X 100}}{Mo}$$

Where;
Mo: mean defecation of control;
M: mean defecation of test sample/standard drug.

### *2.11.2. Castor oil induced enteropooling model*

The method described by Robert et al.[24]was used to determine the effect of extract on intraluminal fluid accumulation. For this experiment, the albino mice were grouped and treated with castor oil as described under the grouping and dosing sections. One hour after treatment, the mice were sacrificed by cervical dislocation. The abdomen of each mouse was opened, and the whole length of the intestine, from the pylorus to the caecum, was ligated, dissected, and carefully removed.

The small intestines were weighed, and the intestinal contents were collected by milking into a graduated tube to measure the volume. The empty intestines were reweighed, and the difference between the two weights was calculated. The percentage reduction in intestinal secretion and the weight of intestinal contents were determined by using the following formulas:

$$\textbf{\% of inhibition by using MVSIC} = \frac{\text{MVICC}-\text{MVICT}}{\text{MVICC}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

where MVSIC: mean volume of the small intestinal content; MVICC: mean volume of the intestinal content of the control group; MVICT: mean volume of the intestinal content of the test groups.

$$\textbf{\% of inhibition by using MWSIC} = \frac{\text{MWICC}-\text{MWICT}}{\text{MWICC}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

where MWSIC: mean weight of the small intestinal content; MWICC: mean weight of the intestinal content of the control; MWICT: mean weight of the intestinal content of the test groups.

*2.11.3. Gastrointestinal motility test (Charcoal meal)*

This test assessed the effect of the leaves of E. indica on normal gastrointestinal transit. The gastrointestinal motility test was carried out according to Onyejekwe et al.[25]. Five groups of albino mice (n = 3 each) were used for the experiment. The animals were fasted for 24 hours before the experiment, with free access to water. Group 1 received normal saline (10 ml/kg body weight.) and served as a control vehicle; group 2 was administered 3 mg/kg body weight of the standard drug loperamide; and groups 3, 4, and 5 were treated with low, medium, and high doses (in mg/kg body weight.) of the extract orally. After 5 minutes, 1 ml of charcoal meal (5% charcoal suspension in 2% aqueous tragacanth) was administered orally to each animal. The mice were sacrificed by cervical dislocation. The abdomen and the entire length of the intestine were removed and placed lengthwise on white paper, and the intestinal distance moved by the charcoal meal from pylorus to caecum was measured after one hour. The peristaltic index and percentage of inhibition were calculated using the following formula, as written by Than et al.[26].

$$\text{Peristalsis index} = \frac{\text{Distance travelled by charcoal mean}}{\text{Length of small intestine}} \times \frac{100}{1}$$

$$\text{\% inhibition} = \frac{\text{Dc}-\text{Dt}}{\text{Dc}} \times \frac{100}{1}$$

where, Dc: Mean distance travelled by the control; Dt: Mean distance travelled by the test group.

### 2.12. Statistical Analysis

The data recorded from the study were analyzed using the statistical package for social sciences (SPSS-27). The data was subjected to one-way analyses of variance (ANOVA), followed by a post hoc turkey's test. P-values in the in the range ($p<0.05$) were considered to be statistically significant. Results were presented as the mean ± standard error of the mean (SEM) of sample replicates.

## 3. Results

### 3.1. Results of Phytochemical Analysis

The ethanol extract of ***E. indica*** contained various plant phytochemicals such as: saponins, carbohydrates, alkaloids, flavonoids, tannis, cardiacglycosides, and reducing sugar. Flavonoids, saponins, alkaloids, and tannis, as shown in Table1.

**Table 1** Phytochemicals in *E. indica*

| | Phytochemical | Crude extract |
|---|---|---|
| 1 | Saponins | + + + |
| 2 | Tannins | ++ |
| 3 | Carbohydrates | - |
| 4 | Reducing Sugars | + |
| 5 | Flavonoids | ++ |
| 7 | Alkaloids | +++ |
| 8 | Terpeniods | ++ |
| 9 | Glycosides | +++ |
| 10 | Steroids | ++ |
| 11 | Fats and oils | ++ |
| 12 | Proteins | + |
| 13 | Acidic compounds | - |

**Key:** - = Not Present; + = Present in small concentration;++ = Present in moderately high concentration +++ = Present in high concentration

### 3.2. Castor oil induced diarrhea

All control animals developed diarrhea after receiving castor oil for one hour. Significant (*P<0.05; **P<0.01; ***P<0.001) inhibition of diarrheal effects was produced by the ingestion of different doses of ethanol leaf extract of ***E. indica*** across the treatment groups. 1000 mg/kg gave the highest activity inhibition of 93.74%. This inhibition displayed was higher than those of the standard antidiarrheal drug loperamide (2 mg/kg), 87.60%. A significant inhibition was also achieved by 500 mg/kg (76.11%), while 250 mg/kg (44.37%) showed a moderate antidiarrheal effect (Table 2).

**Table 2** Antidiarrheal effects of ethanol leave extract of *E. indica* on castor oil induced diarrheal model in mice

| Treatment/ Dose | Onset of diarrhea (Min) | No of wet feces | Total number of feces | Average weight of wet feces (gm) | Average weight of total feces (gm) | % Inhibition of diarrhea | % Weight of wet feces | % Weight of total feces |
|---|---|---|---|---|---|---|---|---|
| Distilled water 10ml/kg | 65.77± 1.22 | 8.79±0.99 | 8.79±0.99 | 0.68±0.64 | 0.68±0.46 | | | |
| Loperamide 2mg/kg | 167.89±0.79 | 1.09± 0.32 | 5.89±0.12 | 0.10±0.05 | 0.17±0.05 | 87.60*** | 14.71 *** | 25.00*** |
| *E.indica* 250mg/kg | 98.99±0.45 | 4.89 ±0.18 | 7.56±0.19 | 0.15±0.21 | 0.31±0.09 | 44.37* | 22.09* | 45.59* |
| *E.indica* 500mg/kg | 162.78±1.69 | 2.10±0.79 | 5.33±0.41 | 0.12±0.48 | 0.20±0.67 | 76.11** | 17.64** | 29.41** |
| *E.indica* 1000mg/kg | 197.08±0.65 | 0.55±0.49 | 1.66±0.28 | 0.09±0.52 | 0.12±0.32 | 93.74*** | 13.24*** | 17.65*** |

All the values are expressed as mean ±SEM n=5 in each group. Data analyzed by ONE WAY ANOVA followed by Tuckey post hoc test.: *P<0.05; **P<0.01; ***P<0.001 significantly different from control.

### 3.3. Effects of ethanol leave extract of *E. indica* on gastrointestinal fluid accumulation in mice

**Table 3** Effects of ethanol leave extract of *E. indica* on gastrointestinal fluid accumulation in mice

| Treatment/Dose | Volume of intestinal contents (ml) | % Inhibition | Weight of intestinal contents (gm) | % Inhibition |
|---|---|---|---|---|
| Distilled water 10ml/kg | 0.96 ± 0.32 | | 1.94 ± 0.65 | |
| Loperamide 2mg/kg | 0.22 ± 0.17 | 77.08*** | 0.41 ± 0.05 | 78.87*** |
| *E.indica* 250mg/kg | 0.42 ± 0.33 | 56.25* | 0.63 ± 0.09 | 67.53* |
| *E.indica* 500mg/kg | 0.27 ± 0.05 | 71.88** | 0.45 ± 0.08 | 76.80** |
| *E.indica* 1000mg/kg | 0.15 ± 0.49 | 84.38*** | 0.21 ± 0.07 | 89.18*** |

All the values are expressed as mean ±SEM n=5 in each group. Data analyzed by ONE WAY ANOVA followed by Tuckey post hoc test.: *P<0.05; **P<0.01; ***P<0.001 significantly different from control.

In Table 3, the extract showed a significant reduction in both the average volume and weight of intestinal contents (AVIC and AWIC) at all tested doses as compared to the control. The percentage inhibition of volume of intestinal contents was found to be 67.53% ($p < 0.05$), 76.80% ($p < 0.01$), and 89.18% ($p < 0.001$) at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively.

### 3.4. Effect of ethanol leave extract of *E.indica* on castor oil induced intestinal transit in mice

As shown in Table 4, the extract significantly inhibited charcoal meal's intestinal transit at all tested doses. The data revealed that the percentage reduction of gastrointestinal transit of charcoal was 53.37% ($p < 0.05$), 73.99% ($p < 0.01$), and 84.26% ($p <0.001$) at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively. The overall result was dose-dependent.

**Table 4** Effect of ethanol leave extract of *E.indica* on castor oil induced intestinal transit in mice

| Treatment/Dose | Length of small intestine (cm) | Distance moved by the charcoal meal (cm) | Peristaltic index (%) | % Inhibition |
|---|---|---|---|---|
| Distilled water 10ml/kg | 56.87 ± 0.22 | 51.31 ± 0.12 | 90.22 ± 1.47 | |
| Loperamide 2mg/kg | 59.15 ± 1.44 | 10.52 ± 0.28 | 17.79 ± 0.76*** | 80.28*** |
| *E.indica* 250mg/kg | 58.66 ± 0.32 | 24.68 ± 0.68 | 42.07± 0.54* | 53.37* |
| *E.indica* 500mg/kg | 56.98 ± 0.40 | 15.65 ± 1.50 | 23.47± 0.41** | 73.99** |
| *E.indica* 1000mg/kg | 59.43 ±0.77 | 8.44 ± 1.85 | 14.20 ± 1.26*** | 84.26*** |

All the values are expressed as mean ±SEM n=5 in each group. Data analyzed by ONE WAY ANOVA followed by Tuckey post hoc test.: *P<0.05; **P<0.01; ***P<0.001 significantly different from control.

### 3.5. Effects of *E. Indica* extract on hematological parameters

Table 5 presents the hematological parameters of the test groups and the control group. The leaf extract of *E. indica* studied at all treatment dose levels (250 mg/kg, 500 mg/kg, and 1000 mg/kg) had a good hematological tolerance for 28 days of the study. The hematological parameters of the extract-treated rats were not significantly different from those of the control group.

**Table 5** Effect of *E. indica* extract on hematological parameters of the treated and untreated rats for 28days

| Duration | Treatment | PCV (%) | RBC ($10^6$/ ul) | Hemoglobin (g/dl) | PLAT ($10^3$/ul) | PCT (%) | WBC ($10^3$/µl) |
|---|---|---|---|---|---|---|---|
| Baseline | Control | 45.91±0.54 | 6.64 ±0.69 | 14.98 ±1.12 | 893.18±0.20 | 0.96±0.05 | 13.86±0.84 |
| | 250 mg/kg | 46.07±0.86 | 6.49 ±0.41 | 15.36±0.54 | 892.07±0.66 | 0.96±0.09 | 13.63±0.77 |
| | 500 mg/kg | 45.99±2.21 | 6.75±0.33 | 15.03 ±0.38 | 889.11±0.78 | 0.96±0.08 | 13.98±2.50 |

| | 1000mg/kg | 46.23±0.39 | 6.51±0.27 | 15.11 ±0.87 | 887.74±1.49 | 0.96±0.07 | 14.12±0.56 |
|---|---|---|---|---|---|---|---|
| Day 29 | Control | 45.83±0.43 | 6.55±0.28 | 14.92±0.35 | 886.99±1.22 | 0.96±0.03 | 13.88±0.18 |
| | 250 mg/kg | 46.22±0.27 | 6.49±0.76 | 15.16±0.24 | 851.06±0.17 | 0.96±0.04 | 13.94±0.37 |
| | 500 mg/kg | 45.87±0.63 | 6.54±0.21 | 15.34±0.19 | 836.68±0.44 | 0.95±0.02 | 14.02±0.55 |
| | 1000mg/kg | 46.38±0.81 | 6.53±0.25 | 15.24±0.42 | 853.06±0.37 | 0.95±0.06 | 14.09±0.49 |

Values are expressed as Mean ± SEM for (n =5/group). Values across treatments groups are not significantly different from each other at p<0.05 (Analyzed by ANOVA followed by Tukey's, test).

### 3.6. Effect of *E. indica* extract on Biochemical indices (liver and Kidney markers)

From results of parameters in Table 6, there was no statistical significant difference (p>0.05) in ALT, AST, ALP, urea and creatinine levels of extract treated groups when compared to control group.

**Table 6** Effect of *E. indica* extract on Biochemical indices (liver and Kidney markers)

| Time | Treatment | **AST(U/L)** | **ALT(U/L)** | **ALP(IU/L)** | **UREA (MG/DL)** | **CREATININE (MG/DL)** |
|---|---|---|---|---|---|---|
| Baseline | Control | 24.32±0.36 | 46.22±0.40 | 66.49 ±0.53 | 25.06±0.48 | 1.17±0.12 |
| | 250 mg/kg | 24.72±1.64 | 44.76±0.41 | 65.58±0.47 | 24.49±0.31 | 1.19±0.27 |
| | 500 mg/kg | 24.99±0.44 | 44.61±0.69 | 65.41 ±0.13 | 25.16±1.22 | 1.19±0.11 |
| | 1000mg/kg | 25.14±0.59 | 46.00±0.18 | 66.76 ±0.29 | 24.47±1.49 | 1.14±0.48 |
| Day 29 | Control | 24.69±0.33 | 45.34±0.53 | 66.96±2.36 | 24.94±0.44 | 1.15±0.32 |
| | 250 mg/kg | 25.15±0.25 | 45.95±0.38 | 66.53±1.19 | 24.93±0.68 | 1.17±0.28 |
| | 500 mg/kg | 25.26±0.71 | 45.91±0.26 | 66.08±0.77 | 24.85±0.90 | 1.15±0.17 |
| | 1000mg/kg | 25.44±0.30 | 45.92±1.15 | 66.62±0.26 | 24.95±0.49 | 1.16±1.26 |

Values are expressed as Mean ± SEM for (n =5/group). Values across treatments groups are not significantly different from each other at p<0.05 (Analyzed by ANOVA followed by Tukey's, test).

### 3.7. Effects of leaves extract on Serum electrolyte levels

There was no statistical significant difference (p>0.05) in chloride, sodium, potassium, and bicarbonate levels of extract treated groups when compared to control group (Table 7).

**Table 7** Effects of leaves extract of *E. indica* on Serum electrolyte levels

| **Time** | **Treatment** | **Sodium (mEq/L)** | **Potassium (mEq/L)** | **Chloride (mEq/L)** | **$HCO_3$ (mEq/L)** | **Calcium (mEq/L)** |
|---|---|---|---|---|---|---|
| Baseline | Control | 143.44±0.12 | 4.28±0.36 | 102.78±0.18 | 29.00±0.43 | 8.14±0.14 |
| | 250 mg/kg | 141.78±0.58 | 4.14±0.33 | 102.02±1.44 | 28.93±0.22 | 8.44±0.28 |
| | 500 mg/kg | 142.01±0.28 | 4.28±1.39 | 102.89 ±0.66 | 28.97±0.49 | 8.21±1.00 |
| | 1000mg/kg | 143.11±0.13 | 4.30±0.54 | 103.97 ±1.16 | 28.39±0.41 | 8.11±0.32 |
| Day 29 | Control | 142.35±0.44 | 4.32±0.28 | 102.81±0.76 | 28.67±0.26 | 8.15±1.11 |
| | 250 mg/kg | 143.16±0.18 | 4.35±0.55 | 103.63±0.39 | 28.45±0.23 | 8.09±1.41 |
| | 500 mg/kg | 143.44±0.37 | 4.29±0.20 | 103.77±0.47 | 28.65±0.45 | 8.12±0.40 |
| | 1000mg/kg | 142.84±0.76 | 4.32±0.41 | 103.82±0.65 | 28.96±0.86 | 8.31±1.06 |

Values are expressed as Mean ± SEM for (n =5/group). Values across treatments groups are not significantly different from each other at p<0.05 (Analyzed by ANOVA followed by Tukey's, test).

## 4. Discussion

There is growing evidence on the effects of phytochemicals from medicinal plants in controlling diarrhea. These plants' phytochemical analysis has identified several active compounds with anti-diarrheal properties, especially, in sub-Saharan Africa [27]. Flavonoids are believed to inhibit intestinal motility and hydro-electrolytic secretion, which are altered in diarrhea [28].For instance, Tannins, found in most anti-diarrheal herbs, have astringent and anti-inflammatory properties [29]. Moreover, they reduce intestinal motility and hydro-electrolyte secretion and can act as antimicrobial agents, inhibiting the growth of pathogens associated with diarrheal diseases like E. coli [30].Our result in Table 1 has shown that *E. indica* contains all of these phytochemicals.

The third model in this study, enteropooling, was designed to assess the secondary components of diarrhea. In this model, the 80% ethanol extract significantly reduced the average volume and weight of intestinal contents at all tested doses when compared to the control. The in vivo anti-diarrheal index (ADI) is a measure of the cube root of the combined effects of three parameters, such as purging frequency in the number of wet stools, delay in the onset of diarrheal stools, and intestinal motility [31]. Generally, a higher ADI value indicates a measure of how effective an extract is in treating diarrhea [32],[33]. ADI was increased with dose, suggesting the dose-dependent nature of this parameter. In this study, the 80% ethanol extract exhibited the highest in vivo ADI value at corresponding doses, confirming its superior antidiarrheal properties.

The hematopoietic system is more sensitive to the effects of toxic compounds [34]. Therefore, assessing hematological parameters is essential in establishing the effect of plant extracts on the animal's blood system [35]. In this study, the investigated hematological parameters did not show significant variations compared to the control. Furthermore, this investigation is relevant to risk assessment, as changes in hematological parameters significantly predict human toxicity when interpreted from animal studies [36]. The hematological analysis revealed that E. indica did not significantly alter the measured parameters. A normal hematological profile observed in *E. indica*-treated groups when compared to the control group further justified the nontoxic nature of *E. indica* [37].

Serum AST, ALT, and ALP values are used to determine liver disease and damage. ALT increases with any liver injury [38], and hepatocyte injury, especially membrane breakage, releases ALT and AST from the cytosol [39]. There was no significant differences ($p > 0.05$) in AST, ALT, and ALP levels between extract-treated and control groups. This suggests that the extract did not impair liver function. These liver enzyme markers were not affected much, suggesting the extract is not hepatotoxic. Significant increases in serum ALT, AST, and ALP indicate hepatotoxicity [40]. The values presented were not significantly different from the control group ($p > 0.05$).

In cases of renal failure in humans, there is typically a proportional increase in serum urea and creatinine levels as renal function gradually declines[41]. According to Gounden et al. [42] ,creatinine levels rise only when there is significant damage to functional nephrons. Moreover, no statistically significant difference (nsp > 0.05) was reported in urea and creatinine levels with regard to extract-treated and control groups. The extract of E. indica has an $LD_{50}$ above 5000 mg/kg, indicating safe acute exposure. The study found that the extract did not affect renal function. Therefore, lack of significant changes in these renal markers suggests that *E. indica* is not nephrotoxic.

Our findings on effects of *E. indica* on serum electrolytes was to ensure safety on users of the plants. This test is essential since diarrhea is associated with electrolyte imbalance. Any substance or herbal remedy that depletes electrolyte imbalance might be detrimental to the patient. According to Shah et al.[43], electrolyte imbalances, such as hyponatremia and hypokalemia, are common in individuals with diarrhea and dehydration, and these imbalances can contribute to mortality in severe cases. Therefore, maintaining electrolyte balance is crucial during episodes of diarrhea to prevent complications associated with dehydration and electrolyte disturbances[44]. Abnormal levels of serum electrolytes, such as potassium, sodium, bicarbonate, and chloride, may suggest tubular dysfunction [45]. This is because a healthy kidney is responsible for maintaining the proper balance of electrolytes in the body. The levels of sodium, chloride, and potassium in all of the groups treated with *E. indica* in this study did not differ from the normal control group, which stayed within the relevant safe range.

## 5. Conclusion

The results of this study demonstrated that *E. indica* extract has encouraging anti-diarrheal properties. The dual inhibitory effects of the 80% ethanol extract fractions on castor oil-induced gastrointestinal motility and fluid secretion were responsible for their overall antidiarrheal activities. The overall antidiarrheal effect can be attributed to the presence of bioactive secondary metabolites, which can vary in polarity from non-polar to polar. These metabolites

include flavonoids, tannins, terpenoids, and steroids. Their antidiarrheal activities can be induced either individually or in combination. Moreover, other tests conducted to determine the effects of *E. indica* on blood, liver, kidney, and electrolytes show that the plant does not affect these parameters. These findings provide scientific support for the folkloric repute of *E. indica* as a treatment for diarrhea.

## Compliance with ethical standards

### *Acknowledgments*

The authors duly acknowledge the utilization of facilities located at the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

### *Disclosure of conflict of interest*

No competing interest is disclosed by any of the writers.

### *Statement of ethical approval*

The study received ethical approval of the Nnamdi Azikiwe University-Animal Research Ethics Committee (NAU-AREC) with approval number: ***NAU/AREC/2023/00073.*** The animals were monitored by members of the animal ethics committee during the course of the study to ensure that they were humanely sacrificed and the remains were properly disposed of.

## References

[1] R. M. Hartman *et al.*, Risk Factors for Mortality Among Children Younger Than Age 5 Years With Severe Diarrhea in Low- and Middle-income Countries: Findings From the World Health Organization-coordinated Global Rotavirus and Pediatric Diarrhea Surveillance Networks, *Clin. Infect. Dis. An Off. Publ. Infect. Dis. Soc. Am.*, vol. 76, no. 3, p. e1047, Feb. 2023, doi: 10.1093/CID/CIAC561.

[2] K. L. Kotloff, J. A. Platts-Mills, D. Nasrin, A. Roose, W. C. Blackwelder, and M. M. Levine, Global burden of diarrheal diseases among children in developing countries: Incidence, etiology, and insights from new molecular diagnostic techniques, *Vaccine*, vol. 35, no. 49 Pt A, pp. 6783–6789, Dec. 2017, doi: 10.1016/J.vaccine.2017.07.036.

[3] M. D. Dairo, T. F. Ibrahim, and A. T. Salawu, Prevalence and determinants of diarrhoea among infants in selected primary health centres in Kaduna north local government area, Nigeria, *Pan Afr. Med. J.*, vol. 28, Oct. 2017, doi: 10.11604/PAMJ.2017.28.109.8152.

[4] H. Hussein, Prevalence of Diarrhea and Associated Risk Factors in Children Under Five Years of Age in Northern Nigeria: A Secondary Data Analysis of Nigeria Demographic and Health Survey 2013, 2017.

[5] A. Kehinde Peter and U. Umar, Combating diarrhoea in Nigeria: the way forward, *J. Microbiol. Exp.*, vol. Volume 6, no. Issue 4, Jul. 2018, doi: 10.15406/JMEN.2018.06.00213.

[6] M. A. da Cruz Gouveia, M. T. C. Lins, and G. A. P. da Silva, Acute diarrhea with blood: diagnosis and drug treatment, *J. Pediatr. (Rio. J).*, vol. 96, no. Suppl 1, p. 20, Mar. 2020, doi: 10.1016/J.JPED.2019.08.006.

[7] J. A. Rudolph and P. A. Rufo, Diarrhea, *Encycl. Infant Early Child. Dev. Three-Volume Set*, vol. 1, pp. V1-394-V1-401, Nov. 2022, doi: 10.1016/B978-012370877-9.00342-X.

[8] D. G. Feleke, E. S. Chanie, F. T. Admasu, S. Bahir, A. T. Amare, and H. K. Abate, Two-week prevalence of acute diarrhea and associated factors among under five years' children in Simada Woreda, South Gondar Zone, Northwest Ethiopia, 2021: a multi-central community based cross-sectional study, *Pan Afr. Med. J.*, vol. 42, May 2022, doi: 10.11604/PAMJ.2022.42.12.32599.

[9] R. G. Afzalpurkar, L. R. Schiller, K. H. Little, W. C. Santangelo, and J. S. Fordtran, The self-limited nature of chronic idiopathic diarrhea, *N. Engl. J. Med.*, vol. 327, no. 26, pp. 1849–1852, Dec. 1992, doi: 10.1056/NEJM199212243272605.

[10] S. E. Bahekar and R. S. Kale, Antidiarrheal activity of ethanolic extract of Manihot esculenta Crantz leaves in Wistar rats, *J. Ayurveda Integr. Med.*, vol. 6, no. 1, p. 35, Jan. 2015, doi: 10.4103/0975-9476.146542.

[11] M. K. Rahman, M. A. U. Chowdhury, M. T. Islam, M. A. Chowdhury, M. E. Uddin, and C. D. Sumi, Evaluation of Antidiarrheal Activity of Methanolic Extract of Maranta arundinacea Linn. Leaves, *Adv. Pharmacol. Sci.*, vol. 2015, 2015, doi: 10.1155/2015/257057.

[12] D. J. Cangemi and B. E. Lacy, Management of irritable bowel syndrome with diarrhea: a review of nonpharmacological and pharmacological interventions, *Therap. Adv. Gastroenterol.*, vol. 12, 2019, doi: 10.1177/1756284819878950.

[13] A. Ketenci and M. Zure, Pharmacological and non-pharmacological treatment approaches to chronic lumbar back pain, *Turkish J. Phys. Med. Rehabil.*, vol. 67, no. 1, p. 1, 2021, doi: 10.5606/TFTRD.2021.8216.

[14] G. N. Teke, J. R. Kuiate, V. Kueté, R. B. Teponno, L. A. Tapondjou, and G. Vilarem, Antidiarrheal activity of extracts and compound from Trilepisium madagascariense stem bark, *Indian J. Pharmacol.*, vol. 42, no. 3, p. 157, May 2010, doi: 10.4103/0253-7613.66839.

[15] Y. Zhang, X. Chen, C. Gueydan, and J. Han, Plasma membrane changes during programmed cell deaths., *Cell Res.*, vol. 28, no. 1, pp. 9–21, 2018, doi: 10.1038/cr.2017.133.

[16] D. Lorke, A new approach to practical acute toxicity testing, *Arch. Toxicol.*, vol. 54, no. 4, pp. 275–287, Dec. 1983, doi: 10.1007/BF01234480.

[17] W. C. Evans, *Pharmacognosy*, 16th ed. Saunders Elsevier, 2009.

[18] G. A. Agbor, F. Longo, E. A. Makong, and P. A. Tarkang, Evaluation of the antidiarrheal and antioxidant properties of Justicia hypocrateriformis, *Pharm. Biol.*, vol. 52, no. 9, pp. 1128–1133, 2014, doi: 10.3109/13880209.2013.879189.

[19] P. E. Aba, K. O. Ugwueze, S. O. Onoja, C. O. Okorie-Kanu, and A. O. Anaga, Hepatoprotective and antioxidant properties of the methanol leaf extract of Diaphananthe bidens in acetaminophen-induced hepatotoxicity in rats, *Trop. J. Pharm. Res.*, vol. 18, no. 11, pp. 2371–2377, 2019, doi: 10.4314/tjpr.v18i11.20.

[20] O. C. Ernest *et al.*, Toxicological effects of two major types of potash used as food additives in Nigeria: Biochemical, hematological, and histopathological analysis of major organs in Wistar rats, *Int. J. Sci. Res. Arch.*, vol. 11, no. 2, pp. 1904–1915, Apr. 2024, doi: 10.30574/IJSRA.2024.11.2.0678.

[21] I. B. Amaza, A. Maidala, and Isidahomen, Haematological and serum biochemical indices of growing rabbits fed graded levels of Yam peel meal as replacement for maize 1 1 2 Nigerian Society for Animal Production Nigerian Journal of Animal Production ©, *Abstr. Nig. J. Anim. Prod*, vol. 2020, no. 4, pp. 167–175, 2020.

[22] J. O. Ezekwesili, U. U. Nkemdilim, and C. U. Okeke, Mechanism of antidiarrhoeal effect of ethanolic extract of Psidium guajava leaves, vol. 22, no. 2, 2010, Accessed: May 11, 2024. [Online]. Available: http://www.klobex.org/bkrAlsoavailableonlineathttp://www.bioline.org.br/bkBKR2010184/22203

[23] A. Nair and S. Jacob, A simple practice guide for dose conversion between animals and human, *J. Basic Clin. Pharm.*, vol. 7, no. 2, p. 27, 2016, doi: 10.4103/0976-0105.177703.

[24] A. Robert, J. E. Nezamis, C. Lancaster, A. J. Hanchar, and M. S. Klepper, Enteropooling assay: A test for diarrhea produced by prostaglandins, *Prostaglandins*, vol. 11, no. 5, pp. 809–828, May 1976, doi: 10.1016/0090-6980(76)90189-1.

[25] V. N. Onyejekwe, O. . Afieroho, and K. . Abo, Anti-Diarrheal Evaluation of the Aqueous Ethanol Extract of Jateorhiza macrantha (Hook F.) Exell Mendonça (Menispermaceae) Leaves, *Saudi J. Biomed. Res.*, vol. 05, no. 02, pp. 25–29, 2020, doi: 10.36348/sjbr.2020.v05i02.004.

[26] A. Than, H. J. Kulkarni, W. Hmone, and S. J. Tha, Anti-diarrhoeal Efficacy of Some Burmese Indigenous Drug Formulations in Experimental Diarrhoeal Test Models, *Int. J. Crude Drug Res.*, vol. 27, no. 4, pp. 195–200, 1989, doi: 10.3109/13880208909116903.

[27] M. T. A. Plaatjie *et al.*, A scoping review on efficacy and safety of medicinal plants used for the treatment of diarrhea in sub-Saharan Africa, *Trop. Med. Heal. 2024 521*, vol. 52, no. 1, pp. 1–19, Jan. 2024, doi: 10.1186/S41182-023-00569-X.

[28] E. A. Palombo, Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function, *Phyther. Res.*, vol. 20, no. 9, pp. 717–724, Sep. 2006, doi: 10.1002/PTR.1907.

[29] I. Ky, A. Le Floch, L. Zeng, L. Pechamat, M. Jourdes, and P. L. Teissedre, Tannins, *Encycl. Food Heal.*, pp. 247–255, Jan. 2016, doi: 10.1016/B978-0-12-384947-2.00683-8.

[30] M. Piero, E. Ajwang, G. Omwenga, and C. Muruthi, In Vivo antidiarrheal effect and phytochemical characterization of Plectranthus barbatus Andrews, *J. Adv. Biotechnol. Exp. Ther.*, vol. 7, no. 1, p. 239, 2024, doi: 10.5455/jabet.2024.d20.

[31] Z. D. Kifle, B. B. Kidanu, T. Y. Tadesse, T. F. Belachew, and S. A. Atnafie, Evaluation of In Vivo Antidiarrheal Activity of Solvent Fractions of Hagenia abyssinica (Rosaceae) in Swiss Albino Mice, *Evid. Based. Complement. Alternat. Med.*, vol. 2021, 2021, doi: 10.1155/2021/8828331.

[32] F. P. Ching, E. K. I. Omogbai, R. I. Ozolua, and S. O. Okpo, Antidiarrhoeal activities of aqueous extract of Stereospermum kunthianum (Cham, Sandrine Petit) stem bark in rodents, *African J. Biotechnol.*, vol. 7, no. 9, pp. 1220–1225, 2008, Accessed: May 11, 2024. [Online]. Available: https://www.ajol.info/index.php/ajb/article/view/58652

[33] M. Molla, N. Gemeda, and S. M. Abay, Investigating Potential Modes of Actions of Mimusops kummel Fruit Extract and Solvent Fractions for Their Antidiarrheal Activities in Mice, *Evid. Based. Complement. Alternat. Med.*, vol. 2017, 2017, doi: 10.1155/2017/4103410.

[34] T. V. Dougnon *et al.*, Toxicological Characterization of Ten Medicinal Plants of the Beninese Flora Used in the Traditional Treatment of Diarrheal Diseases, *Evid. Based. Complement. Alternat. Med.*, vol. 2021, 2021, doi: 10.1155/2021/6676904.

[35] G. S. Maina, N. J. Muriithi, M. J. Kiambi, and B. M. Mwangi, Determination of Hematological Effects of Methanolic Leaf Extract of Vernonia lasiopus in Normal Mice, *J. Blood Lymph*, vol. 05, no. 03, 2015, doi: 10.4172/2165-7831.1000139.

[36] W. . Arika and D. . Nyamai, Hematological Markers of In Vivo Toxicity, *J. Hematol. Thromboembolic Dis.*, vol. 04, no. 02, 2016, doi: 10.4172/2329-8790.1000236.

[37] N. D. Hall, H. Zhang, J. P. Mower, J. S. McElroy, and L. R. Goertzen, The Mitochondrial Genome of Eleusine indica and Characterization of Gene Content within Poaceae, *Genome Biol. Evol.*, vol. 12, no. 1, p. 3684, Dec. 2020, doi: 10.1093/GBE/EVZ229.

[38] H. K. Björnsson, S. Olafsson, O. M. Bergmann, and E. S. Björnsson, A prospective study on the causes of notably raised alanine aminotransferase (ALT), *Scand. J. Gastroenterol.*, vol. 51, no. 5, pp. 594–600, May 2016, doi: 10.3109/00365521.2015.1121516.

[39] R. Chinnappan *et al.*, Low-Cost Point-of-Care Monitoring of ALT and AST Is Promising for Faster Decision Making and Diagnosis of Acute Liver Injury, *Diagnostics 2023, Vol. 13, Page 2967*, vol. 13, no. 18, p. 2967, Sep. 2023, doi: 10.3390/DIAGNOSTICS13182967.

[40] A. S. Lee, J. Persoff, and S. M. Lange, Liver Function Tests, *Mayo Clin. Med. Man.*, pp. 373–387, Jul. 2023, doi: 10.1201/b14283-48.

[41] E. M. Brookes and D. A. Power, Elevated serum urea-to-creatinine ratio is associated with adverse inpatient clinical outcomes in non-end stage chronic kidney disease, *Sci. Rep.*, vol. 12, no. 1, p. 20827, Dec. 2022, doi: 10.1038/S41598-022-25254-7.

[42] V. Gounden, H. Bhatt, and I. Jialal, Renal Function Tests, *StatPearls*, Jul. 2022, Accessed: Jan. 09, 2023. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK507821/

[43] G. S. Shah, B. K. Das, S. Kumar, M. K. Singh, and G. P. Bhandari, Acid base and electrolyte disturbance in diarrhoea., *Kathmandu Univ. Med. J. (KUMJ)*, vol. 5, no. 1, pp. 60–62, 2007.

[44] S. Vepraskas, H. Toth, and M. Weisgerber, Acid–Base and Electrolyte Disturbances, *Nelson Pediatr. Symptom-Based Diagnosis*, pp. 831-850.e1, Jan. 2018, doi: 10.1016/B978-0-323-39956-2.00046-7.

[45] O. C. Ezeigwe, F. A. Nzekwe, O. F. Nworji, C. F. Ezennaya, E. L. Iloanya, and K. K. Asogwa, Effect of Aqueous Extract of F. capensis Leaves and Its Combination with C. aconitifolius Leaves on Essential Biochemical Parameters of Phenylhydrazine-Induced Anemic Rats, *J. Exp. Pharmacol.*, vol. 12, pp. 191–201, 2020, doi: 10.2147/JEP.S254003.