Volume 1 | Issue 2 | Jul - Dec 2023
ISSN: 2984-9896

# Anti-Anemic Effect of Breadfruit (*Artocarpus altilis*) Leaf Ethanolic Extract in Sprague Dawley Rats

**Jodeisah C. Cabaltican**
**Ernels Pascal D. Castillo**
**Graciela Antoni Maris B. Lauigan**
**Mark Ian C. Nortado**
**Kendryx A. Tangonan**
**Jennifer Lloren Luyun**
*College of Allied Health Sciences, Cagayan State University,Andrews Campus*
*Tuguegarao City, Cagayan, Philippines*

## ABTRACT

This study is about investigating the anti-anemic effects of various concentrations (25%, 50%, 75%, and 100%) of ethanolic extract from the leaves of breadfruit (*Artocarpus altilis*) on the hemoglobin concentration and red blood cell count on Sprague Dawley rats with carboplatin-induced anemia. It was done using randomized trial experimental research design using thirty (30) male Sprague Dawley randomly distributed into six groups (n=5) treated with varying concentrations (25%, 50%, 75%, and 100%), positive and negative controls. Based on the statistical analysis, the findings of this study showed that only Group 3 treated with 25% concentration did not reach the minimum threshold of hemoglobin concentration while all of the concentration yielded normal results in terms of red blood cell count. There was a significant difference in the anti-anemic effect among varying concentrations of the Breadfruit (*Artocarpus altilis*) leaf ethanolic extract in terms of hemoglobin concentration, that is, both 75% and 100% concentration yielded higher Hemoglobin concentration than 25%. Also, for red blood cell count where 25% concentration significantly differs from all the other concentrations, yielding lower RBC count than all other concentration levels. There was no significant difference in the anti-anemic effects of 75% and 100% concentrations when compared with positive control, in terms of hemoglobin concentration. Whereas negative control yields a significantly lower red blood count than the 50%, 75%, and 100% concentration levels of leaf extract.

***Keywords:*** *Anti-Anemia, Breadfruit (Artocarpus altilis), Ethanolic Extract, Sprague dawley rats*

## INTRODUCTION

Around the world, iron deficiency (ID.) is the most widely recognized reason for frailty, and it is especially common in ruined or impoverished nations such as African and Asian countries[1, 2]. The Centers of Disease Control and Prevention expressed Anemia to be influencing more than two billion individuals around the world, accounting for more than 30% of the world's population. It is more widespread in resource-poor nations, but it also affects many people in the developed world[3].

In Asian countries, this remains a major public health challenge, with a prevalence rate of 47% among non-pregnant and 52% among pregnant women in South and Southeast Asian (SSEA) countries varying according to geographic regions[4, 5]. In the Philippines, a considerable proportion of the population is malnourished in some form or another, including nutritional deficiencies. Iron deficiency (manifested as iron deficiency anemia) is the most common form of malnutrition in the Filipino population, especially children. The pervasiveness of lack of iron frailty or iron deficiency anemia in kids younger than five is still high, ranging from 20 to 50 percent depending on the location[6, 7]. Similarly, in a study conducted by de Jong N., around half of pregnant women in the Philippines are anemic. They considered a factor which may contribute to the infant mortality rate, one of the highest in Southeast Asia8.

Dietary iron insufficiency, Vitamin A deficiency, and Beta-thalassemia trait were the top three contributing factors worldwide. Compared to 27.0 percent (26.7–27.2) in 1990, the prevalence of anemia in 2019 was 22.8 percent (22.6–23.1). The number of instances of anemia has increased from 1.42 billion (1.41–1.43) billion in 1990 to 1.74 billion (1.72–1.76) billion in 2019. With a combined frequency of 39.7 percent (39.0–40.4) in the same year, the incidence was highest among children under five. Anemia instances were mild in 54.1 percent (53.8– 54.4) of cases, moderate in 42.5 percent (42.2–42.7), and severe in 3.4 percent (3.3–3.5). In 2019, anemia was responsible for 58.6 (40.14–81.1) million years of disability. Western Sub- Saharan Africa, South Asia, and Central Sub-Saharan Africa had the largest burdens[1].

Breadfruit (*Artocarpus altilis*), a member of the "breadfruit complex" belonging to the Mulberry Family Moraceae, is a tropical fruit characterized by prickly, round to oblong yellow- green fruit and alternating dark green leaves with either obovate to ovate lobs to clear pinnate dissected lobs. Breadfruit can be propagated easily from root shoots or cuttings, by air-layering branches, or from seeds. Although breadfruit has wide ecological adaptability, it grows best in warm and humid condition and hence is widely distributed in Africa, Central and South America, India, Southeast Asia, and other tropical regions of the world. So, with easy propagation and favorable condition in the country that supports its growth and cultivation, no wonder breadfruit thrives in nearly every Filipino backyard, one of the native but neglected fruit in the country[9].

Moreover, Breadfruit (*Artocarpus altilis*) offers a wide array of nutrients, minerals, and vitamins. Breadfruit is low in fat, low-calorie foods and is rich in dietary fiber, potassium, phosphorous, magnesium, copper, iron, calcium, vitamin C, thiamin, riboflavin, niacin, pantothenic acid, B6, and folate10. According to USDA Nutrient Database, 2015, seeded breadfruit is high in carbohydrates and a good source of energy. It also contains a lot of iron, which is necessary for a number of aspects of the body, such as red blood cells and hemoglobin synthesis. In a study by Ajiboye, B. et al., diabetic rats fed with *Artocarpus altilis* fruit based- diet showed a significant increase in all the hematological parameters11. Numerous research findings highlight that breadfruit has anti-anemic effects that alleviate hematopoiesis of blood cells, as shown in the study by Adepeju et.al.[1, 2].

Aside from its fruit which is usually served as a delicious dish in some provinces in the country, other parts, including the leaves, roots, and stems are also used medicinally and serve other health purposes. Artocarpus extracts and metabolites from leaves, stem, fruit, and bark contain numerous beneficial biologically active compounds, and these compounds are used in various biological activities, including antibacterial, antitubercular, antiviral, antifungal, antiplatelet, antiarthritic, tyrosinase inhibitory, and cytotoxicity[1, 3]. Also, Breadfruit (*Artocarpus altilis*) contain some antioxidant properties that were also found in its close relative Jackfruit (*Artocarpus heterophyllus*) which were traditionally added in diets to combat anemia. This further spark interest among researchers to further investigate Breadfruit (*Artocarpus altilis*) anti-anemic activities.

And since the best treatment and management for anemia includes a diet plan rich in iron or iron supplementation, the researchers considered the use of Breadfruit (*Artocarpus altilis*) in the study. Other research studies utilized the fruit, roots, and stem of the breadfruit; however, in this study, the researchers mainly focused on the use of its leaves to determine the anti-anemic effect of Breadfruit (*Artocarpus altilis*) leaf ethanolic extract[15].

Coincidentally, Breadfruit (*Artocarpus altilis*) thrives in regions of the world where high prevalence rates of anemia were also reported, including the Philippines.

### *Objectives of the Study*

Thus, due to its year round-availability, wide distribution, high nutrient profile, and considering that little information on the anti-anemic effect of breadfruit has not been well established, the researchers aimed to evaluate the anti-anemic effect of ethanolic leaf extract of Breadfruit (*Artocarpus altilis*) in anemic induced male Sprague Dawley rats. Long ignored, Breadfruit (*Artocarpus altilis*) a hardly known underutilized backyard tree may well be on its way to becoming a source of iron supplementation, an extensively grown crop in plantations, and a common sight on Filipino dinner tables. Generally, this study aims to determine the anti-anemic effects of breadfruit (*Artocarpus altilis*) leaf ethanolic extract in Sprague Dawley rats.

Specifically, this study aims to:
Determine the anti-anemic effects of the varying concentrations (25%, 50%, 75%, 100%) of the breadfruit (*Artocarpus altilis*) leaf ethanolic extract in Sprague Dawley rats in terms of: Hemoglobin concentration Red blood cell count

## MATERIALS AND METHODS

### *Research Design*

An experimental research design, particularly a complete randomized trial, was used in this study to determine the anti-anemic effects of the varying concentrations (25%, 50%, 75%, 100%) of the breadfruit (*Artocarpus altilis*) leaf ethanolic extract in Sprague-Dawley rats in terms of hemoglobin concentration and red blood cell count.

### *Collection of medicinal plant*

The Breadfruit (*Artocarpus altilis*) leaves were collected from Abulug, Cagayan.

### *Identification of plant materials*

The collected plant was authenticated at the Cagayan Valley Department of Agriculture through the Bureau of Plant Industry at Carig, Tuguegarao City, Cagayan for the confirmation of the plant taxonomy.

### *Animal Model*

The experiment was carried out using thirty (30) 10-week-old male Sprague Dawley rats weighing approximately one hundred thirty (130) grams purchased from an authorized shop. Male rats were utilized as they have greater and more stable hematologic parameters than female rats14 to provide less variability in the results. The research animal model was acclimatized for seven (7) days in a shoe box type cage with bedding included in accordance with OECD test guideline 423 and housed at a standard rat housing and protocol at the Philippine Institute of Traditional Alternative Health Care Region (PITAHC) prior to the conduct of the experiment. The rats were given free access to food and water, and maintained room temperature of 25 ± 5OC with humidity of 50-70% and natural 12-hour day-night cycle following the International Animal Care and Use Committee (IACUC) Standard Procedure[1].

***Preparation and Extraction of Plant Material***

In this procedure, researchers utilized the maceration procedure in extracting the leaf which was conducted at Cagayan State University - Andrews Campus. The collected breadfruit (*Artocarpus altilis)* leaves were removed from the stem, cleaned, and air-dried at room temperature for 4 days and oven dried at 60OC for 2 days. The dried pieces were finely ground by means of a mechanical process. 1kg of powdered leaves was macerated on 2 liters of 95% ethanol for 72 hours at room temperature. The extracts were filtered and concentrated using a rotary evaporator at 40OC. A total amount of 163.6g of the leaf ethanolic extract was obtained.
16.25g of leaf ethanolic extract were dissolved in 65ml of distilled water to produce a 25% concentration of the extract. 32.5g of leaf ethanolic extract were dissolved in 65ml of distilled water to produce a 50% concentration of the extract.

48.75g of leaf ethanolic extract were dissolved in 65ml of distilled water to produce a 75% concentration of the extract. 65g of leaf ethanolic extract were dissolved in 65ml of distilled water to produce a 100% concentration of the extract. The concentrations were stored at $4^{0}$C.

***Induction of anemia***

Anemia was introduced by a single intravenous injection of carboplatin with a dosage of 60 mg/kg at physiologic saline based on the protocol utilized in Woo S. et al.[16].

***Experimental protocol***

Six groups of five male rats were randomly selected and formed. All the groups were anemic and were treated by either Ferrous sulfate or the breadfruit (*Artocarpus altilis*) leaf ethanolic extract (25%, 50%, 75%, 100%) from Day 2 to Day 15 except for Group 1, which served as the negative control group.

Group 1 or the negative control group consisting of anemic rats that were not given any treatment. Group 2 or the positive control group consisting of anemic rats treated with Ferrous sulfate with a dose of 4.0 mg/kg once a day through oral gastric gavage from D2 to D1517. Group 3 or the Anemic rats were treated with 1mL/100g 25% breadfruit (*Artocarpus altilis*) leaf ethanolic extract through oral gastric gavage from D2 to D15. Group 4 or the Anemic rats were treated with 1mL/100g 50% breadfruit (*Artocarpus altilis*) leaf ethanolic extract through oral gastric gavage from D2 to D15. Group 5, Anemic rats treated with 1mL/100g 75% breadfruit (*Artocarpus altilis*) leaf ethanolic extract through oral gastric gavage from D2 to D15. And lastly, Group 6 or the Anemic rats were treated with 1mL/100g 100% breadfruit (*Artocarpus altilis*) leaf ethanolic extract through oral gastric gavage from D2 to D15[18].

***Collection and Analysis of Blood Sample***

Roughly 0.5 mL of blood was obtained on EDTA microtainer tubes at days 0 (D0; before induction of anemia) 1 (D1), 3 (D3), 7 (D7), 11 (D11), and 15 (D15)19 20 through tail snipping. Blood samples were analyzed using an automated hematology analyzer for hemoglobin concentration and red blood cell count. The following reference values were used: hemoglobin concentration = 10.4-16.5 g/dL and red blood cell count = 3.6-13 $x10^{12}/L$[21].

***Statistical Treatment***

To determine the anti-anemic effects ofthe varying concentrations (25%, 50%, 75%, 100%) of the breadfruit (*Artocarpus altilis*) leaf ethanolic extract in Sprague Dawley rats in terms of hemoglobin concentration and red blood cell count, a measure of central tendency specifically the mean was used in this study.
One-way ANOVA was used to determine whether there is a significant difference in the anti-anemic effects among the varying concentrations of the breadfruit (*Artocarpus altilis*) leaf ethanolic extracts. The same statistical tool was also used to determine if there is a significant difference in the anti-anemic effects of breadfruit (*Artocarpus altilis*) leaf ethanolic extracts compared to the positive (Ferrous sulfate) and negative (distilled water) controls.

***Experimental Layout***

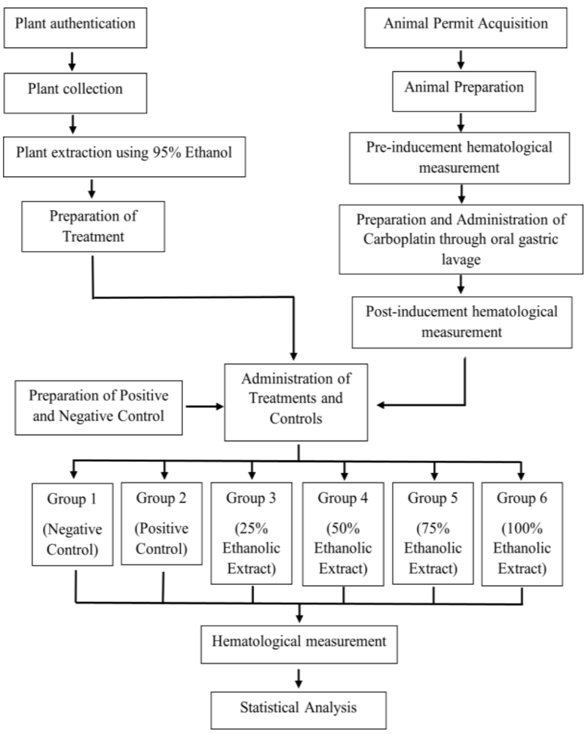


**Figure 1.** The Analysis Framework of the Anti-Anemic Effect of Breadfruit (*Artocarpus altilis*) Leaf Ethanolic Extract in Sprague Dawley Rats

Figure 1 shows the procedures done during the course of the study. The collection of the leaves was done in Abulug, Cagayan. The ethanol extraction of the leaves was done in Cagayan State University - Andrews Campus. The induction of anemia was done at the Philippine Institute of Traditional and Alternative Health Care (PITAHC) laboratory located at Carig, Tuguegarao City.
The first two groups were the positive and negative control wherein the positive control received ferrous sulfate with a dose of 4mg/kg and the negative control received distilled water. Groups three to six received breadfruit (Artocarpus altilis) leaf ethanolic extract with varying concentrations (25%, 50%, 75%, and 100% respectively) at a dose of 1mL/100g. The administration of treatments was done in PITAHC. The collection and analyses of blood samples was done in six sets: Day 0 (before induction of anemia), Day 1, Day 3, Day 7, Day 11, and Day 15.
The data were gathered at Waggie Tail Veterinary Clinic located at Pengue-Ruyu, Tuguegarao City and statistical analysis was done in Cagayan State University - Andrews Campus.

***Ethical Consideration***

In the treatment and improvement of human and animal illnesses, laboratory or model animals were utilized. In the light of scientific progress, the selection of the most appropriate test method was given for a purpose in line with existing test guidelines, laws, and protocols.
The testing laboratory and researchers should consider all existing test guidelines, laws, and protocols protecting the welfare of animals, including:

Animal Welfare Act of 1998 (RA 8485)

Department of Agriculture AO No. 40. Rules and Regulations on the Conduct of Scientific Procedures Using Animals.

Philippine Association of Laboratory Animal Sciences (PALAS) Code of Practice for the Care and Use of Laboratory Animals in the Philippines.

All procedures were be done according to OECD Test Guideline initial consideration including:
OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24. Doses of test compounds that are known to produce severe pain and discomfort as a result of their corrosive or irritating properties need not be administered22.
OECD (2000) Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety

Evaluation Environmental Health and Safety Monograph Series on Testing and Assessment No 19. Animals that are clearly in agony or displaying indications of severe and long-term misery shall be removed and compassionately killed and are treated in the same way as animals who died during the test. This separate Guidance Document outlines the criteria for deciding whether or not to kill stricken or extremely sick animals, as well as how to recognize when an animal's death is predicted or imminent[23].

The researchers should likewise stick and shun any type of logical offense like manufacture, misrepresentation, or copyright infringement in proposing, performing, surveying research, or in detailed research results. Such unfortunate behavior should be submitted purposefully, intentionally, or in negligence of acknowledged practices as characterized by the National Scientific Foundation (2001)[24].

## RESULTS AND DISCUSSION

This chapter represents the results and discussion of all data collected after accomplishing the different method of experimentation that evaluates the effectiveness of breadfruit (*Artocarpus altilis*) leaf ethanolic extract as anti-anemia on anemically induce sprague dawley rats after treatment of the extract and Ferrous sulfate.
Anemia was induced in Sprague Dawley rats with a single dose of carboplatin (60 mg/kg) administered intravenously to the rats via the tail vein. Hematological responses of animal models including hemoglobin concentrations and red blood cell count were measured before and after induction of carboplatin, and after the introduction of if fruit-leaf sample extract collected from breadfruit (*Artocarpus altilis*) to determine potential anti-anemic effects of the varying concentrations (25%, 50%, 75%, 100%) for up to 15 days.
A total of six groups of five male rats were randomly selected and formed. All the groups were anemically induced and treated either with Ferrous sulfate or ethanolic leaf extract collected from breadfruit (*Artocarpus altilis*) leaf with varying concentrations from Day 2 to Day 15 except for Group 1, which served as the negative control group.

A After carboplatin administration, the RBC counts and hemoglobin concentrations showed a biphasic decline before their nadir at around 8 days (day 1), which was expected from the reduction in reticulocyte counts. The following table shows the varying pattern of expression in hemoglobin (table 1.1) and RBC counts (table 1.2) before and after the administration of different concentrations of breadfruit (*Artocarpus altilis*) leaf ethanolic extract and positive and negative control. Animal models treated with positive (Ferrous sulfate) and negative (distilled water) controls showed significant differences in the anti- anemic effects of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts.

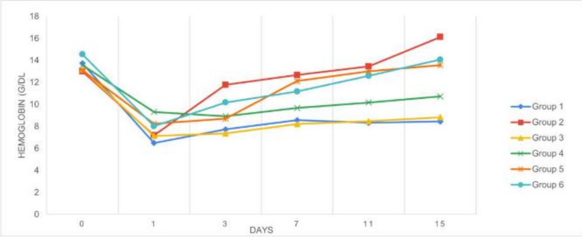


**Figure 2.1** Hemoglobin concentration of Sprague dawley rats before and after the administration of various concentrations of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts, positive control and negative control

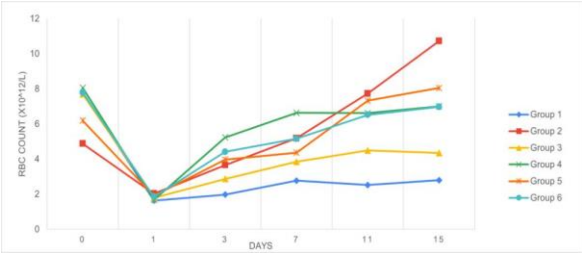


**Figure 2.2** RBC count of Sprague dawley rats before and after the administration of various concentrations of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts, positive control and negative control.

Figures 2.1 and 2.2 show the line graph on the estimated mean concentration of blood hemoglobin and the RBC count collected from the animal models in multiple days. The increasing concentration of *Artocarpus altilis* leaf ethanolic extracts correlates highly with the effectiveness of the extract. As evident in the table, the significant pattern of expression in the anti-anemic effects was shown due to treatment with varying concentrations (25%, 50%, 75%, 100%) of the breadfruit (Artocarpus altilis) leaf ethanolic extracts.

To further determine the anti-anemic effects of varying concentrations (25%, 50%, 75%, 100%) of the breadfruit (Artocarpus altilis) leaf ethanolic extracts in sprague dawley rats in terms of Hemoglobin concentration and Red Blood Cell count, the mean ± SD was utilized.

**Table 1.** Anti-anemic Effects of the Varying Concentrations (25%, 50%, 75%, 100%) of the Breadfruit (*Artocarpus altilis*) Leaf Ethanolic Extract in Sprague Dawley Rats in Terms of Hemoglobin Concentration and Red Blood Cell Count.

| Treatment | Hemoglobin Concentration | Red Blood Cell Count ($x10^{12}/L$) |
|---|---|---|
| 25% concentration | 8.834 ± 1.0584 | 4.346 ± 1.0171 |
| 50% concentration | 10.7333 ± 2.1986 | 7.0033 ± 0.3591 |
| 75% concentration | 13.575 ± 1.1177 | 8.0475 ± 0.2924 |
| 100% concentration | 14.075 ± 2.0304 | 6.97 ± 1.1465 |
| Negative Control | 8.4533 ± 2.2124 | 2.8 ± 0.2718 |
| Positive Control | 16.125 ± 1.4592 | 10.7225 ± 1.3621 |

*Reference values: Hemoglobin concentration = 10.4-16.5 g/dL; Red blood cell count = 3.6 - 3 x1012/L

Table 1 shows the mean and standard deviation dimensions of the hemoglobin concentration and red blood cell count of the Sprague Dawley rats applied with corresponding concentrations of breadfruit (*Artocarpus altilis*) leaf ethanolic extract.

At 25% concentration, the hemoglobin concentration of the rats receiving the treatment did not reach the minimum threshold of normal hemoglobin concentration on average. The other three levels of concentration were in the range with the standard threshold of hemoglobin concentration. Phytochemical screening revealed the presence of tannins and polyphenols, flavonoids, leucoanthocyanins, steroids, and triterpenes. The considered secondary metabolites were phenolic compounds, vitamin C, monoterpenes, and organic acids25. The presence of secondary metabolites as well as Vitamin C in the ethanolic extract can be associated with the increase in hemoglobin concentration26. Increasing the amount of concentration also increases the amount of secondary metabolites present in the solution. This was noted in the study conducted by Goorani, S. et al., wherein there was a remarkable increase between the various groups27. A concentration-dependent increase in hemoglobin concentration was also showed in the study of Gnangoran28.

The standard deviation shows that the hemoglobin concentration of the Sprague Dawley rats treated with 25% concentration of breadfruit (*Artocarpus altilis*) leaf ethanolic extract yields the least dispersed measurements. On the other hand, hemoglobin concentration of the Sprague Dawley rats treated with 50% concentration yields the most dispersed measurements.

All treatments have yielded normal results in terms of red blood cell count. The standard deviation shows that the red blood cell counts of the Sprague Dawley rats treated with 75% concentration of Breadfruit (*Artocarpus altilis*) leaf ethanolic extract yields the least dispersed measurements. On the other hand, red blood cell counts of the Sprague Dawley rats treated with 100% concentration yields the most dispersed measurements. Breadfruit (*Artocarpus altilis*) is a good source of Vitamin C26 which can increase the absorption of iron fourfold that is used in the formation of erythrocytes29 which is reflected in the table. Red blood cell count also increases with the increase of the concentration of ethanol extract. However, 75% concentration yielded higher red blood cell count than the 100% concentration. This was contradicted in the study of Goorani, S. et al.27 where the 100% concentration still yielded higher red blood cell count than the other concentrations. Same evidence were deemed provided by Gnangoran28, Sènou20 and Rabeh et al.30

To determine whether there is a significant difference in the anti-anemic effects among the varying concentrations of the Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts, One-Way Analysis of Variance (ANOVA) was used.

**Table 2.1.** Test of significant difference in the anti-anemic effects among the varying concentrations of the breadfruit (Artocarpus altilis) leaf ethanolic extracts in terms of hemoglobin concentration.

| **Source of Variation** | **df** | **SS** | **MS** | **FV** | **PV** |
|---|---|---|---|---|---|
| Between Groups | 3 | 80.4948 | 26.8316 | 10.64 | 0.0011 |
| Within Groups | 12 | 30.2632 | 2.5219 | | |
| **Total** | **15** | **110.758** | **7.3839** | | |

According to Table 2.1, at 5% level of significance, there is sufficient evidence to say that at least one concentration of breadfruit (*Artocarpus altilis*) leaf ethanolic extracts differs significantly from the other concentrations in terms of hemoglobin concentration. This is evident on the p-value, which is less than the significance level of 0.05. To further determine what specific concentration differed from the other, Tukey's Honest Significant Difference (HSD) post-hoc test was used.

At 5% level of significance, there is enough evidence to say that there is a statistically significant difference between the 75% and 25% levels of concentration of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts in terms of hemoglobin concentration. Furthermore, 75% concentration yields a significantly higher hemoglobin concentration than the 25% concentration.

At a significance level 5%, there is also enough evidence to say that there is a statistically significant difference between the 100% and 25% levels of concentration of Breadfruit (Artocarpus altilis) leaf ethanolic extracts in terms of hemoglobin concentration. Furthermore, 100% concentration yields a significantly higher hemoglobin concentration

**Table 2.1.1.** Multiple comparison of concentrations of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts in terms of hemoglobin concentration.

| Treatment i | Treatment j | Mean Difference (i-j) | Standard Error | Tukey p-value |
|---|---|---|---|---|
| 50% concentration | 25% concentration | 1.8993 | 1.1598 | 0.396 |
| 75% concentration | 25% concentration | 4.7410 | 1.0653 | 0.004* |
| 100% concentration | 25% concentration | 5.2410 | 1.0653 | 0.002* |
| 75% concentration | 50% concentration | 2.8417 | 1.2129 | 0.142 |
| 100% concentration | 50% concentration | 3.3417 | 1.2129 | 0.072 |
| 100% concentration | 75% concentration | 0.5000 | 1.1229 | 0.969 |

*mean difference is significant at 0.05 significance level

30 Rabeh NM, Kady KA, Elmasry HG, Abdelhafez BI. Effect Of Feeding Moringa Oleifera (Moringaceae) Leaves Extract On Rats With Induced Iron Deficiency Anemia. NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO. 2021:13276-87.

than the 25% concentration.We can therefore say with the evidence provided that the increasing concentration of *Artocarpus altilis* leaf ethanolic extracts correlates highly with the effectivity in terms of hemoglobin concentration.

**Table 2.2**. Test of significant difference in the anti-anemic effects among the varying concentrations of the breadfruit (*Artocarpus altilis*) leaf ethanolic extracts in terms of red blood cell count.

| Source of Variation | df | SS | MS | F Value | p-value |
|---|---|---|---|---|---|
| Between Groups | 3 | 34.3327 | 11.4442 | 15.98 | 0.0002 |
| Within Groups | 12 | 8.5961 | 0.7163 | | |
| **Total** | **15** | **42.9288** | **2.8619** | | |

According to Table 2.2, at 5% level of significance, there is sufficient evidence to say that at least one concentration of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts differs significantly from the other concentrations in terms of red blood cell count (x10^12/L). This is evident on the p-value, which is less than the significance level of 0.05. To further determine what specific concentration, differ from the other, Tukey's HSD post-hoc test was used.

**Table 2.2.1.** Multiple comparisons of concentrations of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts in terms of red blood cell count (x10^12/L).

| Treatment i | Treatment j | Mean Difference (i-j) | Standard Error | Tukey p-value |
|---|---|---|---|---|
| 50% concentration | 25% concentration | 2.6573 | 0.6181 | 0.005* |
| 75% concentration | 25% concentration | 3.7015 | 0.5678 | <0.0001* |
| 100% concentration | 25% concentration | 2.624 | 0.5678 | 0.003* |
| 75% concentration | 50% concentration | 1.0442 | 0.6464 | 0.407 |
| 100% concentration | 50% concentration | -0.0333 | 0.6464 | 1.00 |
| 100% concentration | 75% concentration | -1.0775 | 0.5985 | 0.32 |

*mean difference is significant at 0.05 significance level

At 5% level of significance, there is enough evidence to say that the 25% concentration level of Breadfruit (Artocarpus altilis) leaf ethanolic extracts significantly differs from all the other concentration levels in terms of red blood cell count. Moreover, the 25% concentration level of extract yields a significantly lower RBC count than all the other concentration levels.

**Table 3.1.** Test of significant difference in the anti-anemic effects of breadfruit (*Artocarpus altilis*) leaf ethanolic extracts compared to the positive (Ferrous sulfate) and negative (distilled water) controls in terms of hemoglobin concentration.

| Source of Variation | df | SS | MS | F Value | p-value |
|---|---|---|---|---|---|
| Between Groups | 5 | 187.8452 | 37.5691 | 13.75 | <0.0001 |
| Within Groups | 17 | 46.4398 | 2.7318 | | |
| **Total** | **22** | **234.2849** | **10.6493** | | |

Table 3.1 shows that at 5% level of significance, there is enough evidence to say that at least one concentration of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts differs significantly from the other concentrations or the control groups in terms of hemoglobin concentration. This is shown by the p-value being less than the significance level, 0.05. Tukey's HSD post-hoc test was used to further determine what specific concentration differ from the other.

**Table 3.1.1.** Multiple comparison of control groups and concentrations of Breadfruit (Art*ocarpus altilis*) leaf ethanolic extracts in terms of hemoglobin concentration.

| Treatment i | Treatment j | Mean Difference (i-j) | Standard Error | Tukey p-value |
|---|---|---|---|---|
| Positive Control | 25% concentration | 7.291 | 1.1087 | <0.0001* |
| Positive Control | 50% concentration | 5.3917 | 1.2623 | 0.006* |
| Positive Control | 75% concentration | 2.5500 | 1.1687 | 0.295 |
| Positive Control | 100% concentration | 2.05 | 1.1687 | 0.518 |
| Negative Control | 25% concentration | -0.3807 | 1.2070 | 1.000 |
| Negative Control | 50% concentration | -2.2800 | 1.3495 | 0.556 |
| Negative Control | 75% concentration | -5.1217 | 1.2623 | 0.009* |
| Negative Control | 100% concentration | -5.6217 | 1.2623 | 0.004* |

*mean difference is significant at 0.05 significance level

Table 3.1.1 shows the multiple comparison of the control group and the different concentration levels of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts in terms of hemoglobin concentration using the Tukey's HSD test. There is enough evidence, at 5% significance level, to say that the positive control yields a significantly higher hemoglobin concentration than the 25% and the 50% concentration levels of leaf extract. On the other hand, at 5% significance level, there is sufficient evidence to say that the negative control yields a significantly lower hemoglobin concentration than the 75% and the 100% concentration levels of leaf extract.

Table 3.2. Test of significant difference in the anti-anemic effects of breadfruit (*Artocarpus altilis*) leaf ethanolic extracts compared to the positive (Ferrous sulfate) and negative (distilled water) controls in terms of red blood cell count.

| Source of Variation | df | SS | MS | F Value | p-value |
|---|---|---|---|---|---|
| Between Groups | 5 | 145.8893 | 29.1779 | 34.66 | <0.0001 |
| Within Groups | 17 | 14.3097 | 0.8417 | | |
| **Total** | **22** | **160.1990** | **7.2818** | | |

Table 3.2 shows that the p-value is less than the level of significance, 0.05. Hence, at 5% level of significance, there is enough evidence to say that at least one concentration of Breadfruit (*Artocarpus altilis*) leaf ethanolic extracts differs significantly from the other concentrations or the control groups in terms of red blood cell count. Tukey's HSD post-hoc test was used to further determine what specific concentration differed from the other.

**Table 3.2.1.** Multiple comparison of control groups and concentrations of Breadfruit (Artocarpus altilis) leaf ethanolic extracts in terms of red blood cell count.

| Treatment i | Treatment j | Mean Difference (i-j) | Standard Error | Tukey p-value |
|---|---|---|---|---|
| Positive Control | 25% concentration | 6.3765 | 0.6155 | <0.0001* |
| Positive Control | 50% concentration | 3.7192 | 0.7007 | 0.001* |
| Positive Control | 75% concentration | 2.6750 | 0.6487 | 0.008* |
| Positive Control | 100% concentration | 3.7525 | 0.6487 | <0.0001* |
| Negative Control | 25% concentration | -1.5460 | 0.6700 | 0.244 |
| Negative Control | 50% concentration | -4.2033 | 0.7491 | <0.0001* |
| Negative Control | 75% concentration | -5.2475 | 0.7007 | <0.0001* |
| Negative Control | 100% concentration | -4.1700 | 0.7007 | <0.0001* |

*mean difference is significant at 0.05 significance level

Table 3.2.1 shows the multiple comparison of the control group and the different concentration levels of Breadfruit (Artocarpus altilis) leaf ethanolic extracts in terms of red blood cell count using the Tukey's HSD test. There is enough evidence, at 5% significance level, to say that the positive control yields a significantly higher red blood cell count than the Breadfruit (Artocarpus altilis) leaf ethanolic extract treatments.

On the other hand, at 5% significance level, there is sufficient evidence to say that the negative control yields a significantly lower red blood cell count than the 50%, 75% and the 100% concentration levels of leaf extract.

## CONCLUSION

In conclusion, the research findings indicate that Breadfruit (Artocarpus altilis) leaf ethanolic extract exhibits anti-anemic effects in Sprague Dawley rats. Specifically, the 50% and 75% concentrations of the extract showed a significant improvement in hemoglobin concentration, while all concentrations had a positive impact on red blood cell count. These results suggest the potential therapeutic value of Breadfruit leaf extract in combating anemia.

## RECOMMENDATIONS

Based on the findings and conclusions of our comprehensive data analysis, several recommendations can be made. Firstly, it is crucial to implement constant monitoring to assess the gradual impact of continued consumption of the ethanolic extract of the fruit sample. Secondly, it is worth noting that RBC indices and bleeding constituents, such as platelets, were not covered in this study, warranting further investigation. Moreover, future research should focus on examining more specific types of anemia, which were not within the scope of this study. Additionally, there is a need for extended testing and analysis to explore potential proliferative effects beyond the anti-anemia properties of the fruit. Furthermore, it is advisable to conduct a stability or shelf-life study on the plant extract product to ensure its long-term effectiveness. Consideration should also be given to alternative extraction methods apart from ethanol and the inclusion of additional hematological parameters in future testing protocols.

## REFERENCES

**Aka LO, Obidike RI, Igibokwe CO, Ezema WS. 2011.** The effect of feeding differently prepared breadfruit (Artocarpus altilis) on the hematology, serum biochemistry, live and relative organ weights in Albino Rats. Nigerian Veterinary Journal. 2011;30(1).

**Agbangnan Dossa, C., Bothon, F., Kanfon, R., Medoatinsa, S., Bahou, G., Wotto, V., & Sohounhloue, D. 2018.** Nutritional Quality, Phenolic Compound Content and Radical Scavenging Potential of Artocarpus altilis of Benin. American Journal of Food Science and Technology. 2018; 6(4), 195–198. DOI: https://doi.org/10.12691/ajfst-6-4-9

**Bailey, R. L., West Jr., K. P., & Black, R. E. 2015.** The Epidemiology of Global Micronutrient Deficiencies. Annals of Nutrition and Metabolism, 66(Suppl. 2). 2015. https://doi.org/10.1159/000371618

**Bawa a'eed H, Webb M. 2016.** Nutritional and health effects of the consumption of breadfruit. 2016.

**Bregman DB, Morris D, Koch TA, He A, Goodnough LT. 2013.** Hepcidin levels predict nonresponsiveness to oral iron therapy in patients with iron deficiency anemia. American Journal of Hematology. 2013;88(2):97–101.

**Chambers, B. K. S. M. D. 2007.** Iron Deficiency Anemia. American Family Physician. March 1, 2007.

**de Jong, N., Romano, A. B. A., & Gibson, R. S. (2002).** Zinc and iron status during pregnancy of Filipino women. Asia Pacific Journal of Clinical Nutrition, 11(3), 186–193. https://doi.org/10.1046/j.1440-6047.2002.00289.x

**Detzel, P., & Wieser, S. (2015).** Food Fortification for Addressing Iron Deficiency in Filipino Children: Benefits and Cost-Effectiveness. Annals of Nutrition and Metabolism, 66(Suppl. 2), 35–42. https://doi.org/10.1159/000375144

**Ejidike BN, Ajileye O. 2007.** Nutrient composition of African breadfruit (Treculia africana) seed hull and its use in diets for the African giant land snail, Archachatina marginata. Pakistan Journal of Nutrition.;6(2):201-3.

**Encyclopaedia Britannica, Inc., & Petruzzello, M.** breadfruit | Description, History, Cultivation, & Uses.

**Gardner, W., & Kassebaum, N.** Global, Regional, and National Prevalence of Anemia and Its Causes in 204 Countries and Territories, 1990–2019. Current Developments in Nutrition, 4(Supplement_2). 2020. https://doi.org/10.1093/cdn/nzaa053_035

**Hashim, N. M.. 2022.** Prenylated flavones from Artocarpus altilis. Academia.Edu. 2017, February 15. Retrieved January 10, 2022.

**Jagtap UB, Bapat VA. 2010.** Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2010;129(2):142– 66.

**Kreissl A. 2009**: Malnutrition in the Philippines - perhaps a double burden? 2009.

**LawPhil Projects. 2000.** REPUBLIC ACT NO. 8976: Philippine Food Fortification Act of 2000. November 7, 2000.

**Lynch SR. 2003.** Fe/Physiology. In: Benjamin Caballero, editor. Encyclopedia of Food Sciences and Nutrition. 2nd edn. Oxford: Elsevier Science Ltd. 2003.

**Mbaeyi-Nwaoha, I. E., & Onwuka, C. P. 2014.** Comparative evaluation of antimicrobial properties and phytochemical composition of Artocarpus altilis leaves using ethanol, n-hexane and water. African Journal of Microbiology Research. 2014; 8(37), 3409–3421. https://doi.org/10.5897/ajmr2014.6930

**Milman N.** Anemia--still a major health problem in many parts of the world!

**Nguyen, Tadi. 2021.** Iron Supplementation. StatPearls. 2021.

**OECD (2000)** Guidance Document on Acute Oral Toxicity.

**OECD (2000)** Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation.

**Omobuwajo TO.** Overview of the status of breadfruit in Africa. Acta Horticulturae. 2007;(757):61–4.

**Pachuta Węgier L, Kubiak M, Liebert A, Clavel T, Montagne A, Stennevin A, et al. 2020.** Ferrous sulfate oral solution in young children with iron deficiency anemia: An open-label trial of efficacy, safety, and acceptability. Pediatrics International. 2020;62(7):820–7.

**Palanog, A. D. 2019.** Zinc and Iron Nutrition Status in the Philippines Population and Local Soils. Frontiers.

**Pradhan, C., Mohanty, M., Rout, A., Das, A.B., Satapathy, K.B., & Patra, H.K. (2013).** Phytoconstituent Screening and Comparative Assessment of Antimicrobial Potentiality of Artocarpus Altilis Fruit Extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 2013 Jan; 5(3).

**Ragone D. 2018.** Breadfruit— artocarpus altilis (parkinson) fosberg. Exotic Fruits. 2018.

**Ragone D. 2001.** Chromosome numbers and pollen stainability of three species of Pacific Island breadfruit (Artocarpus, Moraceae). American Journal of Botany. 2001 Apr;88(4):693-6.

**Ragone D.** Farm and forestry production and marketing profile for breadfruit (Artocarpus altilis).

**Ragone, D. 2006.** Artocarpus altilis (breadfruit). Species Profiles for Pacific Island Agroforestry, Version. 2006 Apr;2:1-7.

**Rohner, F., Bradley, A. W., Grant, J. A., Elizabeth, A. Y., Lebanan, M. A. O., Rayco-Solon, P., & Saniel, O. P.** Infant and Young Child Feeding Practices in Urban