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Evaluating *Psidium guajava* (Guava) Leaf Extracts Ability to Improve Biochemical and Haematological Indices in Anaemic Rats

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ABSTRACT: *Psidium guajava* (Guava) leaf is used to cure a variety of illnesses, including cough, gastrointestinal pain, gastroenteritis, diarrhea and inflammatory conditions. This study aimed to determine the effect of aqueous extract of *Psidium guajava* leaf on biochemical and haematological parameters on phenylhydrazine-induced rat. Twenty-Five male Wistar rats, aged between 8 – 10 weeks, were divided into five groups of five rats per group. Group A served as "Normal control", Group B was induced but not treated, thus served as "Negative control", Group C was treated with Ferrous (50 mg/kg body weight) while Groups D and E received 300 mg/kg and 600 mg/kg body weight of leaf extract of *P. guajava* daily, respectively, throughout the duration of the experiment. Anemia was induced via oral administration of 20 mg/kg body weight of phenylhydrazine daily for fourteen consecutive days. Graded doses of the extracts were given by oral gavage once a day for 14 days. The antianaemic effects of *P. guajava* demonstrated significant increase ($P < 0.05$) in the hemoglobin (HGB), packed cell volume (PCV) and red blood cell (RBC) count of the extract-treated groups compared to the anemic control group. There was a better increase in the HGB levels of 600 mg/kg (13.50±0.11) compared to anaemic control group (10.13±0.22). The PCV increased more in 600 mg/kg *P. guajava* (41.00±0.40) compared to anaemic group (26.25±0.85). A significant ($P < 0.05$) increase was observed in the RBC count of 600 mg/kg *P. guajava* (5.12±0.05) compared to (3.52±0.23). The outcomes suggest promising therapeutic efficacy of *P. guajava* extract and ferrous treatments in mitigating anemia-induced hematological alterations.

Keywords: *Psidium guajava* (Guava leaf), HB, Anaemia, Rat.

Introduction

Anaemia is a common hematological condition characterized by a deficiency in the number or quality of red blood cells or the hemoglobin concentration, which impairs the ability of blood to carry sufficient oxygen to tissues and organs. It is a major global health concern that can leads to a variety of complications, including fatigue, weakness and impaired oxygen transport (WHO, 2020). The condition may arise due to several factors such as nutritional deficiencies, chronic diseases, blood loss or genetic disorder (Kassebaum, 2016). Among the various types of anaemia, iron deficiency anaemia and anaemia due to blood loss are most prevalent. Despite the availability of conventional treatments, including iron supplementation, erythropoiesis-stimulating agents, and blood transfusions here is a growing interest in exploring natural alternatives due to their bioactive compounds, affordability, safer and fewer side effects compared to synthetic drugs. (Sani *et al.*, 2020).

Psidium guajava (guava) is a tropical fruit-bearing plant widely known for its nutritional and medicinal properties. Guava is rich in vitamins, minerals, flavonoids and antioxidants (Sahoo *et al.*, 2013). The leaves of *P. guajava* have been shown to possess a range of bioactive compounds, such as flavonoids, tannins and phenolic acids that may exhibit therapeutic effects against several health conditions (Chaudhary *et al.*, 2020). Traditional medicine often utilizes guava leaf extracts to treat various ailments, including gastrointestinal disorders, diabetes and hypertension, while recent studies have also indicated potential hematological benefits (Ravichandran *et al.*, 2019).

Guava leaves contain significant amounts of antioxidants, tannins, flavonoids, and other phytochemicals that may play a role in enhancing hematopoiesis (the production of blood cells) and improving the overall health of the blood. Preliminary studies have suggested that guava leaf extracts could influence various biochemical and hematological indices, such as hemoglobin levels, red blood cell count, and platelet count, in animal models of anaemia. (Chaudhary *et al.,* 2020).

This study aimed to evaluate the effects of *P. guajava* leaf extracts in improving the biochemical and haematological indices in anaemic Wistar rats. By assessing parameters like blood hemoglobin levels, red and white blood cell counts, liver and kidney function markers, and other biochemical markers, this research seeks to support the possible application of guava leaf extracts in the treatment of anemia. Gaining insight into how guava leaf extracts affect these indicators may pave the way for the creation of complementary or other options for anemia, especially in environments with low resources.

Materials and methods

The experimental protocol used in this study was approved by the Ethics Committee of Michael Okpara University of Agriculture, Umudike, and conforms with the guide for the care and use of animals in research and teaching of Michael Okpara University of Agriculture, Umudike (Abia State, Nigeria)

Animals: Eight-weeks-old Male Wistar rats, weighing between 160 and 200 g, were obtained from the Laboratory Animal Unit, College of Natural and Applied Sciences, Zoology and Environmental Biology Department, Michael Okpara University of Agriculture, Umudike. They were fed on commercial growers mash (Guinea feeds®) and water *ad libitum*, and placed in a controlled environment in the Animal House at the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, for 2 weeks for acclimatization.

Plant collection and identification: Fresh leaves of *P. guajava* were obtained from Amaoba Community, Ikwuano LGA in Abia State Nigeria, in the month of March 2023 and were identified at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, by a plant taxonomist, Dr. F. E. Mukah.

Preparation of the extracts: Two hundred and fifty grams (250 g) of the leaves were air dried at room temperature for 2 weeks and subsequently pulverized using a grinding machine at the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike. Thereafter 200 g of the powdered sample was boiled for thirty minutes and was thereafter filtered first with clean handkerchief and then with filter paper. The filterate was then evaporated in a water bath. The paste obtained was stored then stored at 4 °C in a refrigerator for laboratory analyses.

Acute toxicity: For the acute toxicity evaluation, the modified Lorke's method used by Orieke *et al*. (2019) was adopted with little modification. For each extract, two stages were involved in the experiment. In the first stage, 9 Wistar rats were assigned to 3 groups (A, B and C) of 3 rats each and were treated with 10, 100 and 1000 mg/kg of the extract, respectively. Animals were thereafter monitored for the manifestations of toxicity signs, behavioural changes and death within 24 hours. Time of onset, intensity and duration of these symptoms, if any were recorded. With zero mortality recorded, the study proceeded to the second phase which also involved the use of 9 rats assigned to 3 groups (A – C). Single treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg, respectively. The animals were again monitored for toxicity signs, behavioural changes and death within 24 hours. When no mortality was observed at the end of the period, the highest dose used (5000 mg/kg) was repeated on another set of 3 rats to serve as a confirmatory test and was observed within 24 hours and a further one week.

Acute toxicity values calculated using Lorke's formula stated as:

$$LD_{50} = \sqrt{A \; x \; B}$$

A = Maximum dose that produced no mortality, B = Minimum dose that produced mortality

Experimental design for the anaemia study and administration of extract: A preventive study model was adopted to evaluate the haemoprotective activity of the extract. Twenty-Five rats, assigned to 5 groups of five rats each, were treated as shown below. Induction of anaemia was via oral administration of 20 mg/kg body weight of phenylhydrazine daily for 4 days. The design is summarized below:

Group 1: Positive control
Group 2: 20 mg/kg phenylhydrazine only (Negative control)
Group 3: Fesolate 50 mg/kg + phenylhydrazine (20 mg/kg)
Group 4: Extract 300 mg/kg + phenylhydrazine (20 mg/kg)
Group 5: Extract 600 mg/kg + phenylhydrazine (20 mg/kg)

Blood sample collection: Under chloroform anaesthesia, 2 rats from each group were dissected on days 3, 5, and 8. They were first weighed and then anaesthetized by placing them in a closed jar containing cotton wool soaked with 5 ml of chloroform anaesthesia. They were then dissected using surgical scissors to expose the heart; two blood samples were immediately withdrawn from the vena cava of each rat via cardiac puncture. The first sample was collected into a vial containing disodium salt of ethylene diamine tetra acetic acid (EDTA) anticoagulant. The second blood sample was collected into a plain sample vial without any anticoagulant.

Blood tests: Approximately 5 ml of blood samples was collected in both EDTA and plain sample vials. Blood samples collected were used in the determination of haematological indices and liver enzymes.

(i) Estimation of haematological indices: Haematological parameters such as haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) and number of platelets were assessed by the standard haematological measurements using an automatic haematological assay analyser (Beckman Coulter, USA).

(ii) Determination of liver enzymes: Blood serum enzyme levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP) were determined using standard laboratory procedure at the department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike Laboratory.Treatments were via the oral route and lasted for 14 days. Body weights were determined at the beginning and end of treatment.

Statistical analysis: The results were expressed as the mean ± standard error of mean (SEM) which were calculated from duplicate laboratory test values. Differences among each group were investigated using one-way analysis of variance (ANOVA) with post hoc test for least significant difference (LSD) assuming equal variances using the IBM SPSS statistic software version 22 (SPSS: Statistical Package for Social Sciences). Significant difference was accepted at $p < 0.05$.

Results

Acute toxicity: Administration of aqueous extract of *P. guajava* to rat even at the highest dose of 5,000 mg/kg did not produce any death in the treated groups. No signs of acute toxicity were also observed

Hematological and biochemical analyses: Table 1 shows the effect of the extract on hematological parameters such as PCV, HBC, red blood cell count (RBC),TLC, neutrophil count, lymphocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Mean corpuscular hemoglobin concentration (MCHC). Significant ($p < 0.05$) increase was found in the MCV count of the group given the highest concentration of the extract. While others showed no significant difference when compared with the control.

The effect of the extract on some serum enzymes (AST, ALT, ALP) and some biochemical parameters such as total bilirubin, conjugate bilirubin, and creatinine are presented in Table 2. There were significant increases ($p < 0.05$) in the activities of ALT, AST and ALP when compared with the control group.

Table 1: The effect of leaf extract of *P. guajava* on some haematological parameters

Parameters	Control	Anemic Untreated	Standard Drug (Ferrous)	300 mg *P. guajava*	600 mg *P. guajava*
PCV (%)	44.75±0.63^{a}	26.25±0.85^{d}	38.00±1.08^{b}	40.25±0.85^{c}	41.00±0.40^{c}
HBC (g/dl)	16.25±0.10^{a}	10.13±0.22^{d}	12.47±0.18^{b}	13.43±0.11^{c}	13.50±0.22^{c}
RBC (×106)	7.09±0.09^{a}	3.52±0.23^{d}	6.39±0.99^{b}	5.07±0.13^{c}	5.12±0.05^{c}
Neutrophil count (%)	8.66±0.14^{c}	11.43±0.35^{a}	10.06±0.11^{b}	10.16±0.11^{b}	10.31±0.14^{b}
MCV (fl)	63.09±0.28^{c}	75.01±2.36^{a}	70.56±0.60^{b}	73.49±0.43^{ab}	76.16±0.37^{a}
MCH (pg)	22.91±0.15^{b}	28.99±126^{a}	23.20±0.55^{b}	27.98±0.65^{a}	28.07±0.35^{a}
MCHC (g/dl)	36.32±0.30^{b}	38.62±0.48^{a}	32.87±0.50^{c}	38.08±0.84^{a}	36.86±0.65^{ab}

Different superscripts across a row indicate significant difference in means at $p < 0.05$

Table 2: The effect of leaf extract of *P. guajava* on some biochemical parameters

Parameters	Control	Anemic Untreated	Standard Drug (Ferrous)	300 mg *P. guajava*	600 mg *P. guajava*
AST (mg/dL)	35.50±2.10^{d}	110.75±4.52^{a}	84.25±2.29^{b}	73.50±1.32^{c}	77.50±3.01^{bc}
ALT (mg/dL)	27.25±1.11^{c}	80.50±2.40^{a}	62.25±2.93^{b}	57.25±2.14^{b}	56.25±2.66^{b}
ALP (mg/dL)	84.50±1.55^{c}	148.7±2.50^{a}	113.25±3.57^{b}	110.00±4.30^{b}	106.75±1.38^{b}
Bilirubin (mg/dL)	0.61±0.04^{c}	2.032±0.18^{a}	1.17±0.05^{b}	1.24±0.08^{b}	1.09±0.02^{b}
T/Protein (mg/dL)	6.11±0.08^{a}	4.22±0.08^{d}	5.32±0.11^{b}	4.75±0.09^{c}	5.28±0.12^{b}
Albumin (mg/dL)	3.26±0.07^{a}	2.06±0.03^{c}	2.58±0.14^{b}	2.62±0.04^{b}	2.80±0.11^{b}
Globulin	2.86±0.04^{a}	2.16±0.05^{c}	2.74±0.11^{a}	2.13±0.09^{c}	2.48±0.08^{b}

Different superscripts across a row indicate significant difference in means at $p < 0$.

Discussion

Depending on the species, plants typically contain different chemical compositions (referred to as phytochemicals). Many plants are recognized to provide both medicinal and economic benefits. Those with medicinal significance are frequently utilized as herbal remedies to maintain and restore good health (Uboh *et al.,* 2010). It is well recognized that eating a range of regional herbs and vegetables improves human health in many ways, including preventing and curing illnesses, as plants have long been a practical and natural source of therapeutic substances.

The anti-anemic effects of aqueous leaf extracts of *P. guajava* in PHZ-induced anemia in Wister rats were investigated in this study. It was observed that the administration of phenylhydrazine-significantly decreased the levels of packed cell volume and haemoglobin value. However, upon treatment with aqueous extract of *P. guajava* for two weeks, the PCV and Hb values reversed significantly.

Similarly, when compared to the control, the levels of red blood cell and neutrophil counts were significantly elevated at the doses of 300 mg/kg and 600 mg/kg body weight (Behera *et al.*, 2012). The antianaemic effect of *P. guajava* may be linked to the presence of phytochemicals, vitamins and minerals constituents, which are well-known hemopoietic factors that directly affect bone marrow blood cell production.

The findings of this study suggest that *P. guajava* leaf extract could be a suitable blood booster for preventative or anemic conditions. This activity is thought to be a direct effect of the extract on the hematopoietic systems, even if the precise mechanism or mechanisms by which the extract assisted the increase in these hematological indices were not determined in this investigation. One or more of the constituents in the extract may interact to promote the production and release of erythropoietin, hematopoietic growth factors and committed stem cells. In particular, it has been observed that erythropoietin and hematopoietic growth factor stimulations speed up production of new blood cells (Aruna *et al.,* 2013).

From the results of this investigation, the aqueous extract of *P. guajava* leaves enhances hepatocellular function. In general, the integrity of tissues following exposure to a pharmacological agent or agents can be indirectly accessed through analyses of the activities of some basic liver function enzymes in the plasma or serum. These enzymes are usually liver markers, whose plasma concentrations above the homeostatic limits could be associated with various forms of disorders which affect the functional integrity of the liver tissues. The aqueous extract of *P. guajava* leaves had a non-significant effect on the liver function enzymes in this study. The current study's findings thus demonstrated that the functional integrity of the liver tissues may not be harmed by the use of *P. guajava* leaf aqueous extract as a liver tonic in various regions of the world.

Conclusion

In conclusion, the results of the present study indicate that aqueous extract of *P. guajava* leaves was discovered to be nontoxic at an acute level, reversing PHZ-induced haemolytic anaemia and suggested to possess hepatoprotective ability, the aqueous extract of the plant leaves may be used in the management of anaemia related cases until otherwise established.

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Nil

Conflict of interest

There are no conflicts of interest.

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