



**EFFECT OF METHANOLIC EXTRACT OF PSIDIUM GUAJAVA  
LEAVES EXTRACT ON PHENYLHYDRAZINE INDUCED ANAEMIA  
IN ADULT FEMALE WISTAR RAT.**

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#### ABSTRACT

**Aim:** This study was carried out to determine the potential of methanolic leaf extract of *Psidium guajava* on phenylhydrazine induced anaemia in adult female wistar rat. **Material and Method:** Thirty adult female rats weighing between 100g and 110g were assigned into six groups of five rats each. Group A served as a healthy Control group. Group B, C and D were induced with anaemia using phenylhydrazine intraperitoneally for four days with a dose concentration of 10mg/kg body weight; this was followed by the oral

administration of methanolic extract of *psidium guajava* with a dose concentration of (100mg/kg, 300mg/kg and 500mg/kg body weight) respectively for 14days. Group E was administered with 100mg/kg body weight of the extract only while F received 10g of phenylhydrazine with 500mg/kg body weight of extract simultaneously. **Result:** The results obtained showed that administration of phenyl hydrazine caused a significant decrease in packed cell volume as well as haemoglobin when compared with the control group ( $P < 0.05$ ). However, on treatment with the methanolic extract of *Psidium guajava*, the level of PCV and

haemoglobin were significantly increased ( $P < 0.05$ ). **Conclusion:** This observation probably implies that methanolic extract of *Psidium guajava* could be of benefit in the treatment of anaemia related cases.

**KEYWORDS:** *Psidium guajava*, phenyl hydrazine, anaemia, wistar rat.

## INTRODUCTION

Anaemia is the most prevalent ailment among children and pregnant women in the developing countries. It is associated with low blood in the system. as a decrease in the amount of red blood cells (RBCs) or the amount of hemoglobin in the blood.<sup>[1]</sup> The prevalence of anaemia particularly in Nigeria rural areas is high. This is mainly due to malnutrition.<sup>[2,3]</sup> Anaemia can result in decrease resistance to infection, fatigue low productivity, poor academic performance, impaired cognitive development, impaired psychomotor development and increased risk of maternal mortality.<sup>[4]</sup>

Plants play a very critical role in the health care needs for the treatment of anaemia, and to enhance the immunological response against disease conditions. Plant leaves extracts are essentially curative and can boost the humoral and cell mediated immunity against viruses, bacteria, fungi, and protozoa.<sup>[5]</sup>

*Psidium guajava* is of important benefit in the folk medicine. It belongs to the family myrtaceae. It is commonly known as Guava in English. Guava is well known for its food and nutritional values throughout the world.<sup>[6]</sup> Guava is very rich in tannins, Phenols, Saponins, triterpenes, essential oils, Carotenoids, lectins, fiber and fatty acids. It also contains appreciable source of pectin that is a dietary fibre. *Psidium guajava* is rich in antioxidants, lutein and lycopene.<sup>[7]</sup> The leaves of guava are rich in flavonoids and quercetin. The flavonoids have shown antibacterial activity, while quercetin contributes to antidiarrheal effect of guava. It is capable of relaxing intestinal smooth muscles and prevent bowel contractions. Also, flavonoids show antispasmodic activity.<sup>[8]</sup> Different parts of guava have been used for treating stomach and diarrhea in many developing countries. Leaves extract of Guava are used to treat respiratory and gastrointestinal disorders. It is also used as cough sedative, anti diarrheic as well as in the management of hypertension.

In recent times, anaemia is the most common cause of mortality in children and pregnant women in Nigeria. The increase in the incidence of anemic condition in Nigeria is due to

poor dieting or nutritional pattern. In the continuous search for solution to the problem of anaemia mainly among children and pregnant women in Nigeria, suggestions have been expressed of the need to enhance the nutritive quality of our local food through better processing and enrichment. Guava is one of such plants worthy of attention. It is widely consumed in Nigeria especially for the majority of the people in the Eastern part of Nigeria. There have been some scientific reports on the use of herbs in the treatment of anaemia.<sup>[3]</sup> These studies had clearly elucidated the facts that really some plants herbs are effective against anaemia. Anaemia if not properly managed will continue to increase mortality rate. However, little information is known on the relevance of *Psidium guajava* in the treatment of anaemia.

This present study was carried out using phenylhydrazine to induce anaemia in female wistar rats to determine the effective potential of the leaf extracts of *psidium guajava* in rat.

## **MATERIALS AND METHODS**

### **Plant Material**

The leaves of *Psidium guajava* was collected from a local garden in Okofia Anambra State, Nigeria between 8<sup>th</sup> and 10<sup>th</sup> November, 2013. The leaves were identified and authenticated by Sir G.O. Ogbuozole of Botany department, Nnamdi Azikwe University Awka, Nigeria.

### **Preparation of Plant Extract/Extraction**

The leaves of *psidium guajava* were washed thoroughly to remove dust and sand particles. It was then sundried for seven days. The dried leaves were ground into powder form with an electric blender. About 500grams of the pulverized dried leaves were cold macerated in 70% methanol for 48hours with intermittent shaking. Then it was filtered using Whatman filter paper and the rotary evaporator at 40°C. The extract was kept at 4°C in a refrigerator for further use.

### **Experiment Animals**

Thirty apparently healthy adult female wistar rats weighing between 100g to 110 were used for the study. They were kept in a clean plastic cage and housed in the experimental animal house of the department of physiology, college of Health Sciences Nnewi, Nnamdi Azikwe University. The wistar rats were acclimatized for a period of 14days, during which they were fed properly with commercially prepared growers mash made by Grand Cereals Ltd and

distilled water was provided *ad libitum*. The study was approved by the institutional Ethical Committee.

### **Experimental Design**

The animals were randomly assigned into six groups of five rats each. Each group was treated either with distilled wistar only or with single dose of variable doses of extract. These are classified as follows; Group A is the control that was only administered with the rat diet. Group B, C and D were phenylhydrazine induced received 100mg/kg, 300mg/kg and 50mg/kg body weight of extract respectively. Group E was administered with 100mg/kg body weight of the extract only. Group F was phenylhydrazine induced anaemia and simultaneously treated with 100mg/kg of extract.

### **Induction of Anaemia**

Anaemia was induced in group B, C and D using phenylhydrazine through intraperitoneally for four days with a dose concentration of 10mg/kg body weight. On the fifth day, 2mls of blood was collected from A,B,C and D for PCV and haemoglobin estimation to confirm anaemia.

### **Blood Collection**

After treatment with methanolic extract for 14days, all the animals (Group A to F) were weighed and anaesthetized in a glass jar containing cotton wool soaked in chloroform. Blood samples were collected by Cardiac Puncture using sterile needle and syringe. The blood samples were put into EDTA containers properly labeled for analysis within 24hours of collection.

### **Haematological Assay**

The haemoglobin level was measured by the cyanomethaemoglobin method.<sup>[9]</sup> The red blood cell count was determined by visual method<sup>10</sup>. Packed cell volume was assayed by using a micro haematocrit centrifuge while total white blood cell was measured by visual method.<sup>[9]</sup>

### **Statistical Analysis**

All results were expressed as mean  $\pm$  standard deviation. Data on the effect of methanolic extract of *Psidium guajava* was analyzed using one-way analysis of variance (ANOVA) followed by student's t-test.  $P < 0.05$  was considered as statistically significant.

**RESULTS****Table I: The final weight, packed cell value and haemoglobin values among different groups.**

GROUP	WEIGHT (G)	PCV %	Hb (g/dl)
A	125.98 ± 3.11	35.50 ± 3.11	11.75 ± 1.26
B	125.71 ± 0.01 <sup>a</sup>	35.50 ± 3.11	11.75 ± 0.96
C	125.72 ± 0.11	38.00 ± 4.30 <sup>a</sup>	12.80 ± 1.48
D	125.64 ± 0.22	37.67 ± 2.08 <sup>a</sup>	12.33 ± 0.55
E	125.69 ± 0.07	39.60 ± 2.41 <sup>a</sup>	13.20 ± 0.84 <sup>a</sup>
F	125.62 ± 0.02	40.00 ± 1.58 <sup>ab</sup>	13.40 ± 0.55 <sup>ab</sup>

a = P < 0.05 compared with Group A

b = P < 0.05 compared with Group B

**Table 2: the red blood cells platelets, and white blood cells, values of different groups administered with extract and control.**

GROUP	RBC	Platelet	WBC
A	5.36 ± 0.34	215.50 ± 77.93	14575 ± 3487
B	4.86 ± 0.86	174.33 ± 51.79	8025 ± 1711 <sup>a</sup>
C	5.70 ± 0.66	306.80 ± 45.36 <sup>b</sup>	12280 ± 4175
D	5.63 ± 0.47	236.67 ± 3.79 <sup>c</sup>	9866 ± 577
E	5.04 ± 0.54	239.40 ± 66.67	10140 ± 3158
F	5.86 ± 0.63	228.40 ± 62.20	6920 ± 2203 <sup>a,c</sup>

a = p < 0.05 compared with Group A

b = p < 0.05 compared with Group B

c = P < 0.05 compared with Group C

**DISCUSSION**

The exposure to phenylhydrazine may lead to damage to red blood cells, potentially resulting in anaemia.<sup>[11]</sup>

In this study, it was observed that administration of phenylhydrazine induced anaemia significantly decreased the level of packed cell volume and haemoglobin value. This is associated with the disruption of the red cell membrane by the phenylhydrazine. Phenylhydrazine is a strong oxidant agent, which is extensively used in industries, laboratory and therapeutic settings. Really, the ability of phenylhydrazine to cause removal of erythrocytes from circulation was the basis of its former use as a therapeutic agents for polycythemia vera a disorder in which there is increased red cell mass in the circulatory system.<sup>[12]</sup> The decrease

in the levels of PCV and Hb before treatment might be due to the disruption of RBC membrane.<sup>[13]</sup>

However, upon treatment with the methanolic extract of *psidium guajava* for two weeks, the anaemia state of the wistar rats were significantly reversed. Also, *psidium guajava* was able to prevent phenylhydrazine induced anaemia on treatment along with the administration of phenylhydrazine. This reversal was seen in the significant increase of PCV and Hb values. This is consistent with the work of Adeyemi *et al.*<sup>[14]</sup> In the same vein, Aruna *et al.*<sup>[15]</sup> reported antianaemic importance of the leave extract.

Similarly, the level of red blood cells, platelet and total WBC counts were significantly increased at the dose 250mg/kg and 500mg/kg body weight when compared with the control. This antianaemic effect of *psidium guajava* could be associated with the presence of phytochemicals vitamins and minerals constituents<sup>13</sup>. These constituents are well known haemopoietic factors that have direct influence on the production of blood cells in the bone marrow.

Although, there was slight increase in the red blood cells of all treated groups which was not significant. Statistically, on the other hand, the treatment with *psidium guajava* extract was able to significantly increase the values of the platelets in the group that received higher doses of the extract compared to group B which received a lower dose and control group A. similarly, it was observed that the level of WBC in treated groups significantly decreased when compared with the control group.

Interestingly, the weight of the treated rats with or without phenylhydrazine administration decreases significantly in relation to the weight of the control group. This decrease in weight seemed to be dose dependent. This shows that *psidium guajava* extract could be useful as a weight moderating agent. The mechanism of its action is however not clear even though it could probably be due to reduction in the food intake of the rats in which the inhabitation of feeding centre and stimulation of the satiety centre in the hypothalamus.

In conclusion, the methanolic extract of *psidium guajava* leaves reversed and prevented anaemia induced phenylhydrazine model of anaemia. Hence, this extract could be very useful in the management of anaemia related cases.

**REFERENCES**

1. Smith RE. "The clinical and economic burden of anemia." *The American Journal of Managed Care.*, 2010; 16: S59–66.
2. Bhaskaram P. Immunobiology of mild micronutrient deficiencies. *Br. J. Nutr.*, 2001; 85: S75-S80.
3. Ebuehi OAI and Mbara KC. Biochemistry studies of Iron fortified GArri Fed to Pheny Hyrdazine-induced anaemic rats. *American Journal of food technology*, 2011; 6(6): 472-482.
4. Bothwell TH and Macphail, A.P. The potential role of NaFeEDTA as an Iron fotificant. *Int. J. Vitam. Nutri Res.*, 2004; 74: 421- 434.
5. Aruna RK, Wazida T and Sinha MP. Effects of Psium guajava aqueous extracts on haematological profile and serum lipid variables of albino rats. *The Bioscan*, 2013; 8(2): 743-746.
6. Bera E, Bhattacharya S and Biswas M. Evaluation of acute anti-inflammatory activity of wistar albino rats. *Journal of Advanced Pharmacy Education and Research*, 2003; 3(1): 23-26.
7. Josep B and Priya MR. Review on nutritional, Medicinal and Pharmacoligical Properties of guava. *International journal of Pharma and Bio Sciences*, 2011; 2(1): 53-69.
8. Barbalho BM, Maiehado FMV, Goulart R, Brunati AC, Ottoboni AM, and Nicolau CC. Psidium Guajava. A plant of multipurpose medicinal applications. *Medicinal and aromatic plants*, 2012; 1(4): 104-110.
9. Cheesbrough, M (2000). *District laboratory Practice in Tropical countries Part 2.* Cambridge University Press. 299-309.
10. Baker FJ and Silvertan RE (1985). *Introduction to Medical Laboratory Technology.* Sixth Edition. Butterworths. 321-326.
11. Berger J. Phanylhydrazine haematotoxicity. *Journal of applied Biomedicine*, 2007; 5: 125-130.
12. Shelter MD and Hill HA. Reactions of hemoglobin with phenylhydrazine. A review of selected aspects. *Environmental Health Perspectives*, 1985; 64: 265-281.
13. Behera, S, Dixit, S., Bulhyya G., and Kar SK. Vitamin A status and hematological values in sickle cell disorder cases. *India J. med. SC.*, 2012; 66 (8):169-173.
14. Adeyemi OS, Akanji MA and Ekanem JI. Antianaemic properties of ethanolic extracts of *psidium guajava* in *Trypansome brucei* infected rats. *Res J. Pharmacol*, 2010; 4(3):74-77.

15. Aruna RK, Wazidu T. and Shina P. Effect of *Psidium Guajava* aqueous extract on haematological profile and serum lipid variables of albino rats J. life sciences, 2013; 8(2): 743-746.