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# Anti-anemic potential of beetroot (*Beta Vulgaris*), pineapple (*Ananas comosus*) and papaya (*Carica papaya*) juice in phenylhydrazine treated Wistar rats.

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## ABSTRACT

Beetroot, pineapple and papaya are the most popular fruits and legumes with several therapeutical effects. This study aimed to assess the anti-anemic potential of beetroot (Beta Vulgaris), pineapple (Ananas comosus) and papaya (Carica papaya) juice in phenylhydrazine treated male Wistar rats. Twenty-five male rats were used. Anemic model was induced in 20 rats by daily injection with phenylhydrazine (40mg/Kg b.w) for two successive days. After one day, the rats were randomly divided into five subgroups as follows: Group A (negative healthy control), group B (positive control), group C (reference), group D and E receiving the beetroot, pineapple and papaya juice at 1mL and 2mL/Kg(b.w.) concentrations respectively. At the end of day 22 of experiment, the animals of all groups were sacrificed and blood samples were taken to determine the blood glucose, haemoglobin and complete blood count, immunological and iron status parameters. The results showed that the beetroot, pineapple and papaya juice significantly ($P<0.05$) affected the increase of the haemoglobin, complete blood count (except white blood cells and men corpuscle volume), immunological parameters (except platelet) and iron status biomarkers as compared to the reference group. Furthermore, several doses of beetroot, pineapple and papaya juice slightly ($p\leq 0.05$) increased the levels of blood glucose as compared to untreated group rats. The concentration that gives the best effect in increasing the haemoglobin level, haematological and immunological parameters and iron status biomarker is 2mL/Kg(b.w.) of natural fruit juice. The findings suggest that this fruit juice can be a medical alternative in the prevention and management of anemia in children and women.

**Keywords:** Haemoglobin, fruits, iron, Haematological, beetroot, anti-anemia.

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# INTRODUCTION

Anemia, which is characterized by a Haemoglobin level below 11.0 g/dL, is one of the most prevalent and potentially serious forms of nutrient deficiency anemias in the world. Also, it is a major public health problem in developing countries, affecting children, pregnant and lactating mothers[1,2,3]. The most important factors known to be associated with this condition are socio-economic status, poor diet, poor sanitation, various infections and infestations [4,5,6]. The World Health Organization (WHO) reported that an estimated 42% of children aged < 5 years are anemic worldwide. The burden is even higher in Africa, reaching 62.3%[7]. According to the most recent Demographic and Health Survey (DHS) and the Multiple Indicators Cluster Survey (MICS), the prevalence of anemia among children aged < 5 years in Cameroon is around 57%[8]. Anemia has a range of adverse consequences including: poor cognitive performance, poor growth of infants, preschool and school aged children, impairment of physical capacity and work performance of adolescents and adults, reduction in immune competence and increased morbidity from infections in all age groups[9].

Many authors reported that fruits and vegetables consumption promotes good health. Therefore, wide publicity and recommendation were given for their consumption because they have been implicated for their health promoting properties in most developed countries[10]. Furthermore, according to Ogbe et al.[11] various methods for treating anemia have been used which include iron. However, there are various drugs for the treatment of anemia which are not affordable to many poor people especially those living in rural area. Among the most important nutrient deficiencies can lead to nutritional anemia are iron, folic acid, vitamin B6, vitamin B12, vitamin C, protein and antioxidants.

Today, the development of science has led to the discovery and elaboration of exogenous natural and synthetic substances that can boost the metabolism of iron to limit the complications associated with anemia. While some of the substances are synthetic, a whole lot are of plants origin[12]. In rural areas of Cameroon, fresh fruits are consumed within short periods of seasonal availability after picked from wild trees or after harvesting. These fruits are carrying mainly from rural to urban areas or are mostly imported from neighbouring countries periods of abundance. These fruits are consumed in several forms: fresh, sliced[13] added to dishes and beverages or processed into natural juice[14].

Beetroot (*Beta Vulgaris*) is one of the most important vegetables consumed worldwide. Scientifically, beetroot is recognized to increase exercise stamina and running performance[15]. It is also utilized in management of hypertension and anemia[16,17]. Due to its red color, traditionally, beetroot is claimed to be useful in haematological disorders, its juice has obvious nutritional, medicinal and health benefits. Its also considered as rich supply of vitamins and minerals such as iron, magnesium and excellent source of folate[18].

Pineapple (*Ananas comosus*) has long been one of the most popular of the non citrus tropical and subtropical fruits, largely because of its attractive flavor and refreshing sugar acid balance[19]. It is cultivated from a crown cutting of the fruit, possibly flowering in 20-24 months and fruiting in the following 6 months. Phytochemical screening of the pineapple reveal the presence of saponin, glycoside, flavonoid and tannins and vitamins such as B1, B2, B3, B5, B6, B9 and C. Minerals such as calcium, magnesium, phosphorous, potassium, sodium and zinc have also been identified in the fruit[20]. The fruit juice aids digestion[21].

Papaya, in the household, consumers ordinarily sprinkle lime juice on pawpaw before consumption because of the flavour impact it has on the pawpaw. Carica papaya is a woody herb, growing up to 10-12 feet and is relatively short lived. Numerous substances of health value including carbohydrates, fibers, vitamins, A, B, B2, B3, B6, C, E and K and minerals such as iron, zinc, magnesium, calcium, copper and selenium have been identified in Carica papaya fruits[22].

Juice blending is one of the best methods to improve the nutritional quality of the juice. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used[23]. This work is therefore designed to assess the anti-anemic potential of beetroot (*Beta Vulgaris*), pineapple (*Ananas comosus*) and papaya (*Carica papaya*) juice in phenylhydrazine treated Wistar rats with the aim of making useful recommendations on their consumption to prevent anemia.

## MATERIALS AND METHOD

### Collection and identification of samples

The fully matured, ripe, freshly and harvested beetroot, pineapple and papaya were purchased from the local market of Yaounde and then identified at the National Herbarium by comparison with the material of Daniel Dang 89 using the specimen collection N°18648/SRF/CAM for the pineapple, with the material of Betti Jean de Lagarde 243 using the specimen collection N°66220HNC for the papaya and finally with the material of Daniel Dang N°351 using the specimen collection N°25664/SRF/CAM.

### Preparation of juice sample

The preparation of juice sample were done according the protocol of Ashurst [24].

- Pineapples were decrowned, washed with clean tap water and rinsed twice in chlorinated water, then rinsed with distilled water and then peeled out, removed feves and sliced crushed in a grinder, then juice extract by using hydraulic press and the extracted juice was again filtered by using a four layer muslin cloth to remove remaining pomace.
- Beetroots were washed with clean tap water and rinsed twice in chlorinated water, then rinsed with distilled water and then peeled out and sliced, crushed in a grinder, then juice extract by using hydraulic press and the extracted juice was again filtered by using a four layer muslin cloth to remove remaining pomace.
- Papayas were washed with clean tap water and rinsed twice in chlorinated water, then rinsed with distilled water and then peeled out, seeded and sliced, crushed in a grinder, then juice extract by using hydraulic press and the extracted juice was again filtered by using a four layer muslin cloth to remove remaining pomace.

Beetroot, pineapple and papaya juices were extracted separately with addition of distilled water 1:1 (v/w). After that, the juice of beetroot, pineapple and papaya has been blended in ratio 17:17:66 respectively[25]. The juice was divided into 10 mL aliquots in 15 mL Falcon tubes and stored at 4±1°C until used. Fresh preparation of the juice was done every 3 days.

**Analytical methods**

Moisture, total protein, lipids, crude fibers, carbohydrates, soluble sugars, ash and pH were determined in juice according to the methods described by official methods of analysis[26], also minerals content including, iron, calcium and magnesium, were estimated using atomic absorption spectrometer, then vitamin C was determined by compleximetreic method according to A. O. A. C[26], finally carotenoids were determined by spectrophotometric method according to Rodriguez-Amaya and Kimura [27].

**Animals**

Twenty-five (25) males rats (150-200g) were provided by the animal house of the Laboratory of Biochemistry, Pharmacology and Phytochemistry of Faculty of Medicine and Biomedical Science (FMBS) of University of Yaounde 1. The rats were acclimated for 7 days before used. During the experiments, the temperature of the animal house was adjusted at 23 ± 2°C under a 12 h dark/light cycle with free access to food and distilled water daily. Animals were housed in separate stainless steel cages and raised in a well ventilated room.

**Diet**

A standard diet consisted of 20% milk and fish meal, 55% wheat flour and corn starch, 10% soya oil, 4% salt and bone meal, 1% poly vitamins, 2% cellulose and 8% distilled water.

**Experimental design**

The local committee approved the experimental design and the protocol conforms to the guidelines of the European Community. Hemolytic anemia were induced to all groups except negative healthy control group. Daily intraperitoneal injection with phenyl hydrazine (40 mg/kg b.w.) for two consecutive days. The anemia was confirmed when the red blood cells and the Haemoglobin reduced by almost 30%[28]. Then, the animals were divided into five sub groups, including negative healthy control as follow:

- Group A (negative healthy control): non anemic rats, given distilled water for 14 days.
- Group B (positive control) : anemic rats, given distilled water for 14 days after anemia.
- Group C (reference) anemic rats, given Vitafer (syrup of iron and folic acid with vitamin B12) for 14 days after anemia at dose of 1ml/kg b.w.
- Group D (test 1) anemic rats, given beetroot, pineapple and papaya juice at dose of 1ml/kg b.w. for 14 days after anemia.
- Group E (test 2) anemic rats, given beetroot, pineapple and papaya juice at dose at dose of 2ml/kg b.w. for 14 days after anemia.

After 22 days, the rats were fasting overnight before sacrificing under ethyl ether anesthesia. Blood samples were taken from the rats' heart to determine blood glucose, Haematological, immunological and iron status parameters.

**Analysis**

**Blood glucose**

The concentration of glucose was determined after enzymatic oxidation in the presence of glucose oxidase, the hydrogen peroxide formed reacted under catalysis of peroxidase with phenol and 4-aminophenazone forming a red violet quinine imine dye as indicator[29].

**Haematological and immunological analysis**

The following blood parameter were analyzed: haemoglobin, red blood cells (RBC), white blood cells (WBC) haematocrit (%HCT), blood platelets count, haemoglobin content, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes, neutrophils, eosinophils, basophils, monocytes, lymphocytes were determined by using an automated haematology analyzer (Beckman Coulter)[30].

**Iron status parameters**

Serum ferritin and transferrin were determined in plasma through by immune turbidimetric assay methods using commercial kit (Biodiagnostics, United Kingdom). Serum iron were assess by colorimetric enzymatic ferene method using commercial kit (SG Mitalia, Italia). The procedures were conducted as stated in the manufacturer's instructions[2].

**Statistical Analysis**

The obtained data were expressed in mean ± standard deviation (SD). The data were analyzed using one-way ANOVA, followed by Tukey post-hoc test, using IBM/SPSS program 20. A value of $p \leq 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

The results obtained in the study are summarized in table 1-5, figure 1 and comprising chemical composition of beetroot, pineapple and papaya fruit juice, anti-nutrient contents of fruit juice, haematological parameters in Wistar rats, immune cells profile in Wistar rats and iron status parameters in Wistar rats and blood glucose of Wistar rats.

**Chemical Composition of beetroot, pineapple and papaya juice**

The results of chemical composition (proteins, lipids, ashes carbohydrates, minerals, vitamin C and carotenoids) of natural fruit juice in the present study were shown in table 1.

**Table 1: Chemical composition of beetroot, pineapple and papaya juice**

| Parameters | Fruit juice |
|---|---|
| pH | 4.79±0.00 |
| **Proximate chemical composition (g/100g)** | |
| Moisture | 92.25±0.34 |
| Protein | 1.24±0.45 |
| Lipid | 0.26±0.01 |
| Ash | 0.20±0.01 |
| Crude fiber | 0.02±0.02 |
| Soluble sugars | 1.19±0.03 |
| Total sugars | 6.03±0.01 |
| **Minerals content (mg/100g)** | |
| Iron | 1.29±0.05 |
| Ca | 6.03±0.05 |
| Mg | 7.87±0.01 |
| **Bioactive compounds (mg/100mL)** | |
| Vit C | 50.85±0.01 |
| Carotenoids | 0.01±0.00 |

Data are expressed as mean ± standard deviation of triplicate experiments

The chemical composition of beetroot, pineapple and papaya juice showed that it was an acidic (pH = 4.79), poor in protein (1.24%), lipids (0.26%). Total sugars and solubles sugars content was 6.03% and 1.19% respectively. Crude fiber was 0.02%. The mineral composition of natural fruit juice was obtained in table 1. The calcium, iron and magnesium content was 6.03%, 1.29% and 7.87% respectively. The fruit juice was rich in vitamin C (50.85%). Carotenoid content was 0.01%. These results are similar to those obtained by Bhavya[31] with some changes due to the variety of the genotypes and the matrix of fruits. Indeed, the study is exhibited that the beverage developed from blend of beetroot, pineapple and orange had a pH of 4.2-5.00%, total sugars of 8.65-10.13%, protein of 0.89-1.12% and lipids of 0.21-0.26% for the juices. The acidic pH could be due to the presence of the pineapple drink in the mixture. It was reported in the literature that pineapple juice had a pH of 3.5 while papaya juice had a pH of 4.5 and beetroot pH of 5[31]. The low pH inhibits the growth and proliferation of microorganism. An acidic pH preserves the beverage against possible microbiological spoilage, for good preservation[32]. In addition, Champagne and Phillippy[33] showed that an acidic pH favoured the absorption of cations by limiting the action of several anti-nutrients such as phytic acid.

Beetroot (Beta Vulgaris), pineapple (Ananas comosus) and papaya (Carica papaya) juice exhibited a iron, calcium and magnesium concentrations being 1.29, 6.03 and 7.87 mg/100mL respectively. However, the mineral content of the juice was higher compared to 100% beetroot juice and 100% beetroot-pineapple juice (v/v) which was 0.175 to 0.214mg/100mL, 0.136 to 0.182 mg/100mL and 0.87 to 1.98 mg/100mL for iron, calcium and magnesium respectively[34]. Iron is a very crucial microelement which is related to anemia prevention and treatment. It plays a major role in cell mediated immunity, in the control of haematopoiesis during infections and in respiratory exchange; it also enters in the composition of haemoglobin[35]. Calcium is known to be a macronutrient necessary for the development of teeth, bones and the release of hormones. Magnesium is an active component of several enzymes systems in which thymine pyrophosphate is a cofactor. Oxidative phosphorylation is greatly reduced in the absence of magnesium. Mg is also an essential activator for the phosphate-transferring enzymes myokinase, kinase, and creatine kinase [36].

Vitamin C and carotenoids obtained in this study were 50.85 and 0.01 mg/100mL. The vitamin C content were higher than that of mandarin and corossol beverages. However, the vitamin C of the juice is lower than that of orange (125.40mg/100ml), lemon (87.90mg/100mL) nectars[37]. Vitamin C is an extremely important nutrient for the body. It helps make red blood cells and maintain healthy red blood cells. Vitamin C plays important role in intestinal absorption of iron, especially in low levels of serum iron[1], protects cells against oxidative stress and is involved in the absorption of iron. The carotenoid content was significantly lower compared to the mango nectar (2.1mg/100mL)[38]. Also, carotenoids significantly increased iron uptake from ferrous fumarate and NaFe-EDTA and were capable of partially overcoming the inhibition produced by tannic acid[39]. In the same line, the results of Hamidah et al.[40] stated that iron plays a role in the formation and maturation of erythrocytes in the process vitamin C serves as a trigger of the iron.

Vitamin C can reduce $Fe^{3+}$ to $Fe^{2+}$ in the gut making it easy to absorb iron. It was reported that the conversion of iron in the form of ferric to ferrous occurs in the stomach with the help of HCl. In patients with iron deficiency anemia the production of stomach acid decreases so it is difficult to convert iron in the form of ferri ($Fe^{3+}$) to ferro ($Fe^{2+}$). The presence of vitamin C SH (sulfidril) and amino acid sulfur can increase iron absorption because it can reduce iron in the form of ferric to ferro. Vitamin C can increase iron absorption an from food through the formation of ferro ascorbate complexes.

The estimated value of the anti-nutrients content are indicated in table 2.

**Table 2: Anti-nutrients content of beetroot pineapple and papaya juice (mg/100g DM).**

| Parameters | Fruit juice |
|---|---|
| Tannins | 0.84±0.31 |
| Oxalates | 1.54±0.12 |
| Phytates | 0.05±0.01 |
| Saponins | 0.21±0.03 |

Data are expressed as mean ± standard deviation of triplicate experiments. DM : Dry matter.

The anti-nutrients content were 0.84, 1.54, 0.05 and 0.21% for tannins, oxalates, phytates and saponins respectively. The findings in this study were lower than those obtained by Nwozol et al.[41] on levels of anti-nutritional factors in some wild edible fruits of Northern Nigeria. The anti-nutrients content of our juice revealed that it is safe to drink. Indeed, the anti-nutrients content are lower than the safe dose for tannins (150-200mg/day), for phytates (2000-2600 mg/day) and for oxalates (200-500mg/day)[42]. The presence of anti-nutritional factors can interferes with the absorption of divalent minerals by forming insoluble salt with them which is responsible for the formation of kidney stones[43].

**Effect of beetroot, pineapple and papaya juice on the haematological and immunological parameters in Wistar rats.**

Haemoglobin, complete blood cells and immune cells profile in Wistar rats are illustrated in table 3 and table 4. Haemoglobin concentrations varied from 9.25g/dL (group B) to 14.2g/dL (group A). There was a significant difference ($P<0.05$) between the different groups analysed. The 7.75±0.01 and 7.08±0.01 values of RBC of rats administrated with 1mL (group D) and 2mL (group E) of juice was significantly higher ($P<0.05$) than the value of 6.60±0.10 observed in the reference (group C) and 4.75±0.05 in the positive control group (group B). The 8.93 ±0.01 value of rats administrated with 2mL (group E) of juice was significantly lower ($P<0.05$) than the value of 11.2 ±0.01 (group B) and higher than the value of 6.20 ±0.10 obtained in the reference group (group C).

**Table 3: Effect of fruit juice on the levels of haematological parameters.**

| Parameters | Groups | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| HB (g/dL) | 14.2±0.01$^{d}$ | 9.25±0.02$^{a}$ | 11.6±0.1$^{b}$ | 12±0.00$^{bc}$ | 12.5±0.5$^{c}$ |
| RBC (*10$^{6}$/µL) | 7.10±0.10$^{c}$ | 4.75±0.05$^{a}$ | 6.60±0.10$^{b}$ | 7.08±0.01$^{c}$ | 7.75±0.01$^{d}$ |
| WBC (*10$^{3}$/µL) | 9.60±0.10$^{d}$ | 11.2±0.01$^{e}$ | 6.20±0.10$^{a}$ | 8.75±0.01$^{b}$ | 8.93±0.01$^{c}$ |
| %HCT | 50.60±0.10$^{e}$ | 33.75±0.05$^{a}$ | 37.20±0.20$^{b}$ | 41.80±0.10$^{c}$ | 45.00±1.00$^{d}$ |
| MCV(fL) | 83.60±0.30$^{a}$ | 118.50±0.50$^{e}$ | 114.80±0.10$^{d}$ | 101.00±1.00$^{c}$ | 97.75±0.01$^{b}$ |
| MCH (pg) | 30.60±0.10$^{c}$ | 25.50±0.20$^{a}$ | 25.20±0.10$^{a}$ | 26.00±1.00$^{a}$ | 28.00±1.00$^{b}$ |
| MCHC (g/dL) | 33.80±0.10$^{e}$ | 21.25±0.01$^{a}$ | 29.40±0.10$^{b}$ | 31.60±0.10$^{c}$ | 32.06±0.01$^{d}$ |

Data are expressed as mean ± standard deviation of triplicate determinations; means within the same line with different superscripts significantly different at p<0.05; A: Negative healthy control ; B: positive control ; C: reference group ; D: anemic rats treated with 1mL/Kg BW of fruit juice ; E: anemic rats treated with 2mL/Kg BW of fruit juice ; HB : Haemoglobin; RBC : Red Blood Cell ; WBC : White Blood Cell ; HCT : Haematocrit ; MCV : Mean Corpuscular Volume ; MCHC : Mean corpuscular Haemoglobin concentration ; MCH : Mean Corpuscular Haemoglobin.

**Table 4: Effect of fruit juice on the levels of immunological parameters.**

| Parameters | Groups | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| LYMP | 32.20±0.10$^{e}$ | 18.25±0.01$^{a}$ | 20.60±0.10$^{b}$ | 25.00±1.00$^{c}$ | 26.25±0.01$^{d}$ |
| MONO% | 5.20±0.10$^{e}$ | 2.50±0.1$^{a}$ | 3.17±0.01$^{b}$ | 4.20±0.10$^{c}$ | 4.80±0.10$^{d}$ |
| NEUT % | 56.60±0.10$^{e}$ | 18.50±0.10$^{a}$ | 20.60±0.10$^{b}$ | 49.60±0.10$^{c}$ | 52.25±0.01$^{d}$ |
| BASO% | 0.29±0.01$^{c}$ | 0.15±0.01$^{a}$ | 0.21±0.01$^{b}$ | 0.34±0.01$^{d}$ | 0.31±0.01$^{c}$ |
| Platelets(*10$^{3}$/µL) | 314.60±0.20$^{a}$ | 626.75±0.02$^{e}$ | 592.20±0.20$^{d}$ | 525.60±0.30$^{c}$ | 417.25±0.05$^{b}$ |
| EO% | 0.20±0.01$^{a}$ | 0.75±0.01$^{e}$ | 0.63±0.01$^{d}$ | 0.54±0.01$^{c}$ | 0.45±0.01$^{b}$ |

Data are expressed as mean ± standard deviation of triplicate determinations; means within the same line with different superscripts significantly different at p<0.05. A: Negative healthy control ; B: positive control; C: reference group ; D: anemic rats treated with 1mL/Kg b.w. of fruit juice ; E: anemic rats treated with 2mL/Kg BW of fruit juice; LYM: Lymphocytes; MONO: monocytes; NEUT: Neutrophils; BASO: Basophils; EO: Eosinophils.

Induction of anemia in rats resulted in a significant decrease at the 5% threshold in red blood cells, HTCs, MCH, MCHC, lymphocytes, monocytes, neutrophils and basophils. However, there was an increase in white blood cells, MCV, platelets and eosinophils as shown in Table 3 and 4. There was a significant increase (P <0.05) in the haematological parameters (%HCT, MCH, MCHC) in treatment group (group D and E) when compared with the negative control group (group A) and negative control (group B). Table 4 showed that there was a significant increased (P< 0.05) in lymphocytes, monocytes, neutrophils, basophils, eosinophils count in treatment group D and E when compared with the negative control group (group B) and reference group

(group C) and increase was statistically significant (P<0.05) in treatment group D and E when compared with the healthy control group. Although platelet were significantly (P<0.05) enhanced in all the groups, the increased was significant positive control group ($626.75*10^3$µL) when compared with the reference group ($529.20*10^3$µL) and test groups ($525.60*10^3$µL and $417.25*10^3$µL) respectively for group D and group E. Phenyl hydrazine (PHZ) was used in this study to induced hemolytic anemia. According to[44, 45] PHZ is toxic for the body and cause lysis of RBC. Also, PHZ causes oxidative damage to red blood cells by increasing the formation of reactive oxygen species[46]. Induction of anemia in rats had caused a decrease in HB, complete blood cells and immunological parameters as shown in tables 3 and 4. The administration of natural fruit juice in anemic rats showed significant increases in HB, RBC, %HCT, MCH, MCHC, lymphocytes, monocytes, neutrophils, basophils and eosinophils in all test groups as sees in tables 3 and 4. The best results were found in group E (rats administrated with 2mL/Kg) and group D (rats administrated with 1mL/Kg) with significant changes compared to the reference group (group C) and positive control group (group B). But the concentration of all Haematological parameters of tested group remained low compared to the healthy group (group A). The decrease in the main blood parameters might be due to the hematolytic effect of phenylhydrazine[47] as a result of the oxidation of the oxyHaemoglobin and precipitation of Haemoglobin. The results obtained showed a significant increase in the number of WBC, MCV and platelet in negative control group (group B), therefore it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances (PHZ), which alter their normal physiological processes. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia[48]. The increase in haematological and immunological parameters in this study are similar to those of[49] who obtained the anti-anemic effect of some natural food supplements on rats and[50] who reported a significant increase in haematological parameters in rats treated for a fortnight with the aqueous extract of fluted pumpkin leaves. Many authors have reported that the use of beetroot-pineapple drink in the treatment of anemia might be beneficial to humans[35]. The combination of fruits and vegetables for the production of beverages could be a better alternative to synthetic products to prevent or combat iron deficiency[51]. Feeding with 1mL and 2mL of the beetroot, pineapple and papaya formulated drink resulted in an improvement in the values of all parameters that were affected. This drink has the ability to stimulate erythropoietin and restore red blood cell parameters to normal.

**Effect of beetroot, pineapple and papaya juice on the blood glucose**

The influence of beetroot, pineapple and papaya juice on blood glucose is shown in figure 1.

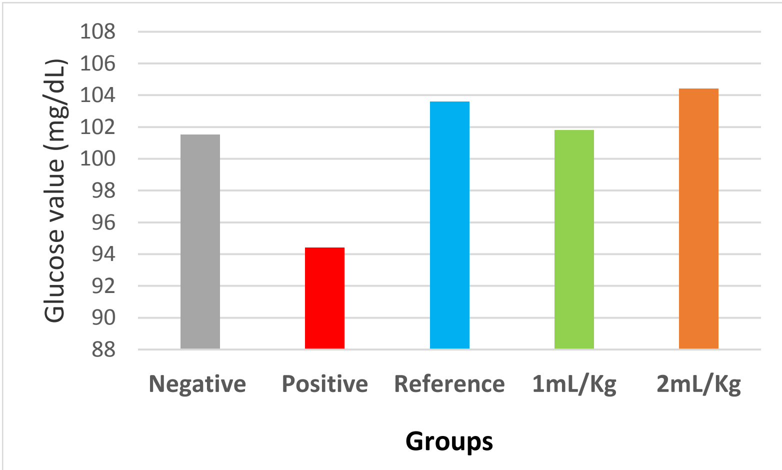


**Figure 1: Effect of beetroot, pineapple and papaya juice on blood glucose in male Wistar rats**

At the end of experiment, there was a significant difference (P<0.05) between the different groups tested. Blood glucose level in group E (administration of 2mL/Kg of juice) and reference group (group C) were higher compared to others groups. Blood glucose was low in anemic untreated group (group B) when compared to those values of others groups. Furthermore, these values (group D and E) were within the normal ranges consumption[52] which means that the glucose metabolism were not affected, showing the safety of beetroot, pineapple and papaya juice. These results are similar to those of[53] who found that treatment with shaddock fruit juice has beneficial effects on glucose tolerance and one reported by Hadijah et al.[54] who exhibited that mango, soursop and kasturi lime juice did not produce adverse effects in experimental rats. In contrast, the above mentioned, observations were different from the study of[55] who found that aqueous extract of *Telfairia occidentalis* (Pumpkin) leaf at doses of 2mL/day and 4mL/day given to albinos rats have decrease in glucose level of experimental rats when compared with that of the control. These differences may be due to the difference in the genotypes of fruits or green legumes. The glucose concentration is in concern when relates to the fruit juice consumption. This is due to the previous reports indicated the high intake of fruit beverages with the increase risk of diabetes[54]. However, many authors reported that increased consumption of fruits in adults is a good an effective strategy for decreasing energy consumption and for increasing and maintaining weight loss. In the same line, study by[56] suggested that the 100% fruit and vegetable juices were not associated with the increased risk of type 2 diabetes in Japanese women.

**Effect of beetroot, pineapple and papaya juice on the iron status markers**

The data for iron, serum ferritin and transferrin in male Wistar rats are presented in table 5. Results revealed that induction of anemic rats' model by injection with PHZ to rats caused a significant ($P<0.05$) decline in iron, ferritin and transferrin concentrations compared to healthy control group. Supplementation with natural fruit juice showed very highly significant increase of iron, ferritin and transferrin in group E and D compared to negative and reference group. Moreover, the daily administration of beetroot, pineapple and papaya juice at 1mL and 2mL doses for 24 days could significantly ($P<0.05$) ameliorate the concentrations of Fe, ferritin and transferrin.

**Table 5: Effects of beetroot, ananas and papaya juice on the iron status markers**

| Parameters | Groups | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Iron µg/dL | 47.07±0.01$^{e}$ | 31.46±0.01$^{a}$ | 39.24±0.01$^{b}$ | 40.15±0.01$^{c}$ | 41.62±0.01$^{d}$ |
| Serum ferritin µg/L | 36.61±0.01$^{e}$ | 22.63±0.01$^{a}$ | 25.60±0.10$^{b}$ | 29.49±0.01$^{c}$ | 30.62±0.01$^{d}$ |
| Serum transferrin mg/dL | 295.00±1.00$^{e}$ | 193.37±0.01$^{a}$ | 207.50±0.10$^{b}$ | 213.00±1.00$^{c}$ | 233.12±0.01$^{d}$ |

Data are expressed as mean ± standard deviation of triplicate determinations; means within the same line with different superscripts significantly different at $p<0.05$. A: Negative healthy control ; B: positive control ; C : reference group ; D: anemic rats treated with 1mL/Kg BW of fruit juice ; E: anemic rats treated with 2mL/Kg b.w. of fruit juice.

Iron is an important element in the production of haemoglobin, the molecule that carries oxygen inside the red blood cells. It is an essential component of body system involved in the utilization of oxygen. Deficiencies in red blood cells lead to iron deficiency anemia. Serum ferritin concentration generally reflects the body's iron stores and is considered one of the most reliable indicators of iron status in patients[2]. About transferrin, the assessment of the plasma transferrin level is useful for the differential diagnosis of anemia and for monitoring its treatment. If the anemia is due to a defect in iron incorporation into erythrocytes, the transferrin level is normal or low but the protein is highly saturated with iron. The high blood iron content of group D and E compared to group B can be explained by the fact that beetroot, pineapple and papaya juice are rich in iron and vitamin C which facilitates the absorption of nonheme iron by improving the blood iron level. Indeed, many authors reported that beetroot has ameliorative effects on cyclophosphamide induced anemia in rats. Supplementation with diet rich in vegetables may increase consumption of antioxidants including carotenoids, ascorbate, tocopherol and phenolics which were found to inhibit the cellular damage induced by oxidative stress[11]. Similarly, results have been reported by previous researchers who revealed that beetroot and papaya are an excellent source of iron[11,40]. Therefore, beetroot, pineapple and papaya juice could potentially have hepatoprotective effect.

## CONCLUSION

This study suggest that beetroot, pineapple and papaya juice has high amount of nutritive chemical constituents that can be beneficial to children and women of reproductive age. Also, the study demonstrated that the administration of this natural fruit juice significantly hepatoprotective effect against hemolytic anemia induced in rats by phenyl hydrazine. This natural fruit juice also revealed amelioration in immunological and iron status parameters. Further, the findings can be used as baseline information for further scientific investigation for using this drink as an alternative medicine in the management of anemia and investigating in involving organs, antioxidants and other biological activities.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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