Comparative Haematopoietic Effect of Ascorbic Acid and Aqueous Extract of Psidium Guajava Leaves in Male Rats

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Abbreviations:
Hb: Haemoglobin, PCV: packed cell volume, RBC: red blood cell, WBC: white blood cell

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Abstract
The comparative haematopoietic potential of vitamin C and aqueous extract of P. guajava leaves (AEPGL) was investigated in rats. The study was carried out in Biochemistry Department, University of Calabar, Nigeria. Twenty one rats used in this study were randomly divided into three groups, with seven rats each. The rats in Group1 (i.e., control) received distilled water as placebo, while the remaining 2 experimental groups were respectively gavaged with 200 and 600mg/kg body weight of vitamin C and AEPGL, respectively, daily for 30 days. Phytochemical analysis of the extract indicated the presence of alkaloids, flavonoids, saponins, tannins, reducing compounds. The results showed that both vit C and AEPGL produced a significant increase (p<0.05) in PCV, Hb concentration, RBC and WBC counts, as well as neutrophils, lymphocytes and monocytes differential counts, compared respectively with the control. These results support the haematopoietic potentials of vitamin C and the extract, and that the haematopoietic potential of AEPGL is higher than that of vitamin C. The results of this study therefore give credence to the traditional use of P. guajava leaves in the treatment of anaemia.

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1. Introduction

In traditional medicine, the use of herbs in different preparations for the treatment of various ailments is greatly relied upon, especially by rural dwellers. There is a long history of traditional medicinal and agricultural uses of different herbs, in many countries. In traditional/folk medicine, some herbs have been considered as drugs and therefore generally safe and effective in the treatment of various diseased conditions (Treasure, 2000). On this account, some investigators are of the opinion that some herbal plants are usually oriented in the same general therapeutic direction and are complementary or synergistic, often non – specific but very rarely adverse (Treasure, 2000 Chevellier, 1996).

Psidium guajava is a large tropical evergreen shrub or small shade tree plant with long history of traditional and nutritional uses in many countries. It is known to contain several secondary metabolites, including alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes, and about 80 mg of vitamin C (ascorbic acid) per 100 g of guava (Begum et al., 2002; Suntornsuk et al., 2002). Some investigations examined antiamaebic, antibiotic, anti diabetic, antihyperglycemic, antimitogenic, antispasmodic, and sedative effects, as well as anticough and narcotic-like activities of the plant. However, most scientific evidence relates to the clinical efficacy of guava in treating gastrointestinal disorders (Lozoya et al., 2002; Goncalves et al., 2005). Literature reports reveal that traditional communities in India, Bolivia, Brazil, Egypt, China and Africa use different parts of this plant, particularly the leaves,
as anti-inflammatory, hemeostatic, antibiotic, antidiarrhoea, and anticholera agents (Olajide et al., 1999; Lozoya et al., 2002; Goncalves et al., 2005).

In the recent past, a good number of medicinal plants have been reported to be traditionally employed to alleviate anaemia. Some of these plants include Telfeira occidentalis, Combretum dolichopetalum, Alliuus asalloncicum, Bougainv spectabilis, Pterospermum ferbifugum, Sorghum bicolor, Jatropha curcas, Flacourtia flavenscens, Ageratum conyzoides and Bralliantasia nitens (Alada, 2000; Ajayi et al., 2000; Ogwumike, 2002; Owoyole et al., 2002; Agbor et al., 2005; Dina et al., 2006; Ita et al., 2007; Akah et al., 2009). Also, vitamin C has been reported to reverse the negative effect of gasoline and some heavy metals on haematopoietic potential of vitamin C.

Anaemia is known to constitute a serious health problem in many tropical countries because of the prevalence of different forms of parasitic infections, including malaria (Dacie and Lewis, 1994). In anaemia there is decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in females (Okochi et al., 2003). In the tropics, due to endemicity of malaria and other parasitic infections, between 10 to 20% of the population possess less than 10 g/dl of Hb in the blood (Diallo et al., 2008). The determination of haematological indices provides physiological information on a proper blood assessment. According to Okonkwo et al. (2004), accurate laboratory determination of blood parameters remain the only sensitive and reliable foundation for ethical and rational research, diagnosis, treatment and prevention of anaemia. This study therefore assessed and compared the haematopoietic potential of P. guajava leaf extract and ascorbic acid in rat model, considering the fact that P. guajava is known to be rich in ascorbic acid and that different parts of the plant have been reported to be useful in the management of various diseases

2. Objective of Research

To determine the comparative haematopoietic effect of Psidium guajava leaves and ascorbic acid in male rats.

3. Materials and Methods

3.1 Identification and preparation of Plant Materials

Fresh leaves of P. guajava were collected in July 2013 from local garden at the University of Calabar, Calabar, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden, and the Voucher specimen was deposited in the herbarium of the same University. The leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and then ground into fine powder using an electric dry mill (Moulinex). 200g of the ground powder was soaked in 1.0l of distilled water for 48 hours at room temperature. The mixture was filtered into 500ml conical flask with Watman filter paper (No.1). The filtrate was dried at a temperature of 30°C for 10 hours to produce a gel-like extract, which weighed 20.5g. Appropriate concentration of the extract was then subsequently made by dilution with distilled water into 200/mg/kg body weight and administered to the animals.

3.2 Phytochemical screening

The aqueous extract of P. guajava leaves was screened for the presence / absence of alkaloids, flavonoids, polyphenols, saponins, tannins and reducing compounds according to the methods described by Trease and Evans (2002).

3.3 Handling and treatment of Animal

Twenty one adult male albino rats weighing between 150-250g obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar Nigeria, were used for the study. The rats were divided into three groups with seven rats each, as follows:

Group I (control group receiving distilled water as placebo),

Group II (test group receiving vitamin C),

Group III (test group receiving aqueous extract of P. guajava leaves).

The rats were acclimatized in the experimental animal house for one week before the commencement of the experiment. The animals, housed in stainless steel cages under standard conditions; ambient temperature 28.0 ± 2.0°C and humidity 46%, with a 12 hr light/dark cycle, were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water ad libitum, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

The animals in test groups II and III received 200 and 600mg/kg body weight oral daily doses of vitamin C and aqueous extract of P. guajava leaves, respectively, using orogastric tubes and syringes. This lasted for a period of 30 days and the experiments were conducted between the hours of 09.00 am and 10.00am daily. Rats in the control groups I were administered, by oral gavage, with 5ml of distilled water (placebo).
Synthetic vitamin C was obtained from the Sigma Chemicals, Poole England and used for the study. A stock solution of vitamin C was prepared by dissolving 20g of vitamin C powder in 500ml of distilled water out of which a dose of 200mg/kg body weight was administered to animals in 5ml of vehicle daily for 30 days. All the animal experiments were carried out in accordance with the guidelines of the Institution’s Animal Ethical Committee.

3.4 Collection and analysis of blood
All the animals were anaesthetized with chloroform vapour, twenty-four (24) hours after last day of extract and vitamin C administration, and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of EDTA sequestrene sample bottles. The whole blood samples were analysed for haematological indices within 24 hours of collection. The haematocrit or packed cell volume was determined according to the haematocrit method, while the haemoglobin concentrations were determined by cyanomethaemoglobin method, described by Alexander and Griffiths (1993). The red blood cell, white blood cell and the differential white blood cell counts were estimated by the visual method described by Dacie and Lewis (1991).

3.5 Statistical Analyses
The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student’s t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using SPSS windows. A significant change was considered acceptable at p < 0.05.

4. Results
The results of the study on the comparative effect of ascorbic acid and aqueous extract of P. guajava leaves on some haematological indices in male rats are presented in Tables 1 and 2, as well as Figure 1. While the result of the phytochemical screening of the leaf extract is presented in Table 3. The mean values of the packed cell volume, haemoglobin concentration, red and white blood cells counts as well as the neutrophil, leucocyte and monocyte differential counts obtained for rats treated with ascorbic acid and the leaf extract were observed to be significantly higher (p < 0.05), compared respectively with the control values (Tables 1 and 2). However, the respective comparative percentage increase in these haematological indices of the group of rats treated with the leaf extract was noted to be significantly higher (p < 0.05), compared to the values obtained for the rats treated with ascorbic acid (Fig. 1). The results of this study showed that although both ascorbic acid and aqueous extract of P. guajava leaves possess haematopoietic potentials, the leaf extract’s potential is higher than that of the ascorbic acid in rat model.

The phytochemical screening of the leaf extract showed the presence of alkaloids, flavonoids, saponins, tannins and reducing compounds (Table 3). However, the relative proportion of the alkaloids, flavonoids, polyphenols and reducing compounds detected in the leaf extract was observed to be comparatively higher than the relative proportion of saponins and tannins.

5. Discussion
In this study, aqueous extract of P. guajava leaves is reported to cause a significant increase in packed cell volume, haemoglobin concentration, as well as red and white blood cells counts. These results agree with the report by Agbaje (Agbaje et al., 2009) that Syzygium aromaticum (L.) merr significantly increased RBC, Hb and PCV. This indicated that the some chemical constituents of the leaf extract might have interacted with haematopoietic tissues and stimulated the production of RBC. Correlating the results of this study, S. aromaticum has been reported by Agbaje, (2008) to contain flavonoids, which are free radical scavengers. These components possibly competed with Hb in RBC for oxygen, causing in hypoxic condition, which might have then stimulated the synthesis and production of RBC. Also, the metabolic products of the extract’s constituents might have directly stimulated the kidney to facilitate the formation and secretion of erythropoietin, which is the humoral regulator of RBC production (Kobzick and Schoen, 1994; Sanchez-Elsner et al., 2004; Imoru et al., 2006). It is therefore very likely that P. guajava leaf extract contains erythropoietin-like principle(s), which is responsible for the high RBC and PCV values recorded in this study.

Moreover, a beneficial effect on the differential white blood cell counts, particularly the neutrophils, is also reported for P. guajava leaves extract. This finding suggests that the extracts probably contain some agents that can stimulate the production of leucocytes. This is in line with the fact that the presence of some leucocytes production stimulating agents have been reported for some commonly prescribed medicinal plants (AL-Mamary, 2002; Imoru et al., 2006). The
Table 1: Effect of ascorbic acid and aqueous extract of *P. guajava* leaves on some haematological indices in male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>PCV (%)</th>
<th>Hb (g/dl)</th>
<th>RBC (x 10^6 cells mm^-3)</th>
<th>WBC (x10^3 cells mm^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>40.56 ± 1.52</td>
<td>11.96 ± 3.32</td>
<td>1.25 ± 0.06</td>
<td>4.42 ± 3.42</td>
</tr>
<tr>
<td>II</td>
<td>48.72 ± 2.34*</td>
<td>15.73 ± 1.05*</td>
<td>1.48 ± 0.08*</td>
<td>5.23 ± 2.88*</td>
</tr>
<tr>
<td>III</td>
<td>53.85± 1.86*+</td>
<td>18.98 ± 2.20*+</td>
<td>1.61 ± 0.07*+</td>
<td>6.10 ± 1.56*+</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD, n = 7. *P < 0.05 compared with group I, +P < 0.05 compared with group II.

Table 2: Effect of ascorbic acid and aqueous extract of *P. guajava* leaves on differential white blood cell counts in male rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Eosinophils (%)</th>
<th>Monocytes (%)</th>
<th>Basophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>66.74 ± 3.24</td>
<td>32.76 ± 1.86</td>
<td>0.56 ± 0.20</td>
<td>2.88 ± 0.60</td>
<td>0.54 ± 0.21</td>
</tr>
<tr>
<td>II</td>
<td>84.68 ± 3.01*</td>
<td>40.65 ± 2.44*</td>
<td>1.35 ± 0.36*</td>
<td>4.32 ± 1.08*</td>
<td>1.30 ± 0.34*</td>
</tr>
<tr>
<td>III</td>
<td>93.86 ± 2.56*+</td>
<td>48.83 ± 3.32*+</td>
<td>1.28 ± 0.56*</td>
<td>5.02 ± 1.76*+</td>
<td>1.42 ± 0.26*+</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD, n = 7. *P < 0.05 compared with group I, +P < 0.05 compared with group II.

Table 3: Phytochemical profile of aqueous extract of *P. guajava* leaves.

<table>
<thead>
<tr>
<th>Phytochemical components</th>
<th>Relative proportion present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>++</td>
</tr>
<tr>
<td>Reducing Compounds</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys: ++ = Highly present, + = Present.

Figure 1. Effect of ascorbic acid (Vit C) and aqueous extract of *P. guajava* leaves (AEFGL) on the comparative percentage increase in haematological indices in male rats.
results also showed that *P. guajava* leaf extract caused a significant increase in the circulating neutrophils during differential count. Neutrophil is known to be the main type of WBC that attack and destroy bacteria, viruses and other injurious agents in the body. Hence, WBC is well known to be responsible for defending the body against infection and tissue damage. The results of this present study suggest that *P. guajava* leaf extract may be considered to contain potent immunostimulants, thereby justifying their use in the treatment of some immune-related diseases in herbal medicine. From the results of this present study, it was observed that the extract enhanced haematopoietic activity in rats, giving an indication that the extract possesses a beneficial effect in the management of anaemic conditions. Anaemia is a clinical condition whereby the haematological indices, such as haemoglobin concentration and total red blood cell counts, fall below the normal range for a given age, sex and population (Ibu et al., 1999). It is generally known that any chemical agent with the potential of boosting the level of haematological indices, without any serious negative effect on other tissues, may be considered for the management of anaemia, either in folk or orthodox medicine. The results of this study indicated that the extract of *P. guajava* leaves may possibly serve as an acceptable blood booster in an anaemic condition or prophylactic purpose. Although the specific mechanism(s) through which the extract facilitated the increase in these haematological indices was not ascertained in this study, an indication assumed to be a direct effect of the extract on the haematopoietic systems may be suspected. It is possible that the extract contains such constituent(s) that can interact and stimulate the formation and secretion of erythropoietin, haematopoietic growth factors/committed stem cells. Specifically, stimulations of haematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000).

Moreover, the haematopoietic potential of the leaf extract of *P. guajava* may also be related to its antioxidant activity. The phytochemical screening showed that the extract contains flavonoids which are powerful antioxidant polyphenolic compounds. Torell et al. (1986) and Faure et al. (1990) have shown that flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membranes. Also, results have shown that flavonoids from *Helichrysum* genus inhibit the formation of superoxide ions and hydroxyl radicals, which are two strong peroxidation agents (Facino et al., 1990). This antioxidant activity may protect both the haematopoietic committed stem and the formed blood cells from the attack of the reactive free radicals in the body. However, the study on the specific mechanism(s) associated with the positive effect of the extract on haematological indices in male rats is in progress. From the results of this study, it may be concluded that the extract from *P. guajava* leaves possesses some haematopoietic properties that could be possibly utilized in haematinic therapy.

**Author’s Contribution and Competing Interests**

No competing interest

**References**


