Effect of Leaf Extract of *Psidium guajava* (Guava) on the Hematological Indices of Albino Rats

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ABSTRACT

This study was to determine the effect of aqueous and methanolic extracts of *Psidium guajava* leaves on the hematological indices of liver functions in albino rats and compare the effect of the extracts on body weight of the rats on weekly interval for four weeks. A total of eighty-four rats were divided into two experimental groups of thirty six rats per group. One group of rats was treated with aqueous extract while the other was treated with methanolic extract. Also, a common control group which was made of twelve rats. Each experimental group was made up of three treatment sub-group which consisted of different concentration levels of 200 mg/kg, 400 mg/kg and 800 mg/kg. A sub-group consisted of three replicates of four rats per replicate. Blood samples were collected from rats in each replicate of treatment and control groups and tested for various hematological parameters using standard methods.

The result of the study showed that a significant increase ($P < 0.05$) was observed from the result obtained from packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and hemoglobin (Hb). The body weight of the rats generally dropped as a result of oral administration of *P. guajava* extract. The findings from these studies tended to suggest that a moderate ingestion of the extracts was beneficial to the body. However, the beneficial effect extended to the fact that it can be used in the treatment of obesity, blood diseases and building the immune system due to its ability to lower the body weight and also increase all the hematological parameters.

(Keywords: haematological parameters, hematological indices, guava leaves)

INTRODUCTION

Psidium guajava is a plant belonging to the family *Myrtaceae*. It is grown in all parts of the world and cultivated for its leaf, fruit, bark, fleshy calyx root twigs or seeds. The plant has been found to thrive on a wide range of soil conditions and tolerate a pH range from 4.5 to 9.4, propagates easily, and bears fruit relatively quickly. It is also salt resistant (Morton, 1987).

Psidium guajava is commonly called Guava although it has several names in many part of the world. Such names include *Bayabas, Jambu burung, Lal peyara, advaba, madhuria, kalimbahin, malakabeng,* etc. However, in Nigeria, it is known as *gwaabaa* (Hausa), *woba* (Efik), *ugwoba* (Igbo), and *guata* (Yoruba) (Iwu, 1993).

Phytochemical analysis of the fruit shows that it contains vitamin A, iron, calcium and phosphorous (Iwu, 1993). Manganese with oxalic and malic acids, saponin, oleanolic acid, flavonoids, guaijavarine and quercetin (Arima and Danno, 2002). Whereas the fruit skin is rich in Ascorbic acid and carbonyl compound which gives it the strong odor. The chemical analysis of the leaves shows that it contains essential oil with different component which include malic acid, menthol, guayavolic acid, flavonoids, and quercetin. In addition, the leaves also contain fat, resin, mineral salt, tannin, chlorophyll, cellulose, etc. (Nadkarni and Nadkarni, 1999). Whereas the root analysis shows that it contains salt, carbohydrate, tannin, sterols, etc. (Iwu 1993).

Psidium guajava originated from Central America and Mexico, although today the plant is distributed to Africa and to the Pacific region. It is a low evergreen shrub with branches and downy twigs. It has thin smooth-copper colored bark

which peels off and show its greenish layer beneath. The leaves are green due to chlorophyll pigment in them, short-petioled, oval or oblong and irregular in outline. It has conspicuous parallel veins. It has petals which are easily shed and stamen with pale-yellow anthers. The fruit has strong, sweet, musky odor. It may be round, ovoid or pear shaped with sepals at the apex. The skin is fleshy, thick, yellowish, and flavorful. The central pulp is slightly darker and filled with hard yellowish seed. The seeds are chewable and are many although some are seedless (Morton, 1987; Baby and Mini, 2011).

Psidium guajava has been reported to be of great medicinal uses which are antibacterial, anti-candidal, anti-dysenteric, antiseptic, anti-anxiety, menstrual stimulant etc. (Abdelrahim *et al.,* 2002). These medicinal uses are elaborated below:

Anti-Cancer Activity: A study reveal that aqueous extract of *Psidium guajava* budding leaves has been shown to possess anti-prostate cancer in a cell (Baby and Mini, 2011). The control of high blood pressure has contributed to reduction in mortality from stroke and coronary heart disease.

Antibacterial Activity: *Psidium guajava* contains a number of chemical constituents which are reported to possess antibacterial properties (Caceres *et al.,* 1993). Flavonoids extracted from the leaves were reported to have strong antibacterial action. Also, data have showed that aqueous and hydroalcoholic extracts of guava leaves present anti-microbial activity (Arima and Danno, 2002; Qadan *et al.,* 2005). *Psidium guajava* leaves have long been recognized for their antibacterial activity. They were shown to inhibit both Gram-positive and Gram-negative bacteria such as *S. aureus, Streptococcus mutans, Pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus spp., Shigella spp.* and *E. coli* (Cowen, 1999; Perez-Gutierrez *et al.,* 2008). The bark has also been shown to exhibit antibacterial effect. This effectiveness of guava as an antimicrobial has been confirmed (Abdelrahim *et al.,* 2002,).

Anti-Diarrheal Activity: In Nigeria, leaf infusion is taken for constipation and with red potash for dysentery. The ripe fruit is mildly laxative, and the unripe fruit is astringent and anti-diarrheic (Burkill, 1997). The young leaf also is used for dysentery and diarrhea. The methanol extract of the leaves was reported to inhibit the growth of *Salmonella, Shigella spp,* and *Escherichia coli*. This result confirmed its effectiveness as an antidiarrheal (Lin *et al.,* 2002).

Anti-Inflammatory Effect: The essential oil has been proven to have anti-inflammatory effect. It reduces oedema formation induced by carrageenan, granuloma formation induced by cotton pellets (Kavimani, *et al.,* 1997). Another study by Sen *et al.,* (1995) and Ojewale (2005) confirmed the anti-inflammatory activity and also showed antipyretic activity and potent anti-arthritic activity. Another study shows that the methanol extract of guava leaves was found to inhibit oedema induced by carrageenan and pain induced by acetic acid.

Antioxidant: Compounds in aqueous extracts of guava leaves have been reported to present antioxidant action and some of their pharmacological effects could be related to interaction in calcium channels (Morales *et al.,* 1994: Qjan and Nihorimbere, 2004).

Antispasmodic: The plant is aromatic antispasmodics; a decoction of the young leaves and shoots is prescribed in the West Indies for antispasmodic baths in India and Ghana (Ayensu, 1978). They are also recommended for swollen legs. Also, a decoction of the young leaves and shoots are used for spasm, fevers, worms and diabetes (Ticzon, 1997).

Diabetics; The leaf infusions are used in the Cape for diabetics. This study is confirmed by Ticzon, (1997) that the decoction of the young leaves and shoots are used for diabetics, fever etc. Also, water in which the fruit is soaked is good for thirst in diabetes (Conway, 2001).

Kidney Problems: A study shows that the young leaves and shoots are used for inflammation of the kidney and kidney problems (Ticzon, 1997). In India the leaf decoction is used for nephritis that is an inflammation of the kidney (Ayensu, 1978).

Oral care: A study shows that in South America the tender leaves are chewed for bleeding gums and hangovers, if chewed before drinking. Indians use a leaf decoction is for mouth sores, bleeding gums. A decoction is used as a gargle for sore mouth. Also, a decoction of the root-bark is used as a mouth wash for swollen gums and a decoction of the leaves makes efficacious gargle

for swollen gum and ulceration of the mouth (Nadkarni and Nadkarni, 1999).

Parturient: A study shows that a combined decoction of leaves and bark is given to expel the placenta after childbirth. It is also used to regulate menstrual periods or used as a douche for vaginal discharge and to tighten and tone of virginal walls after childbirth (Ticzon, 1997).

Vaginal Disorders: A study shows that a decoction of the leaves is used as a vaginal and uterine wash, especially in leucorrhea where it can be infused and applied as a douche (Conway, 2001). The leaves in decoction are recommended for uterine hemorrhage. The same decoction is used as a wash for vaginal and uterine problems and especially where an astringent remedy is needed (Ticzon, 1997).

Skin Use: A study shows that the leaves are a remedy for itches and the leaves in decoction is used as a wash for ulcers (Ticzon, 2001). A decoction of the bark and or leaves or flower infusion is used for wounds, ulcers and skin sores. In addition, the antioxidant activity will provide a caring environment for the skin (Masuda *et al.,* 2003). A decoction of the leaves is used in scurvy and for ulcers. (Nadkarni and Nadkarni, 1999). The leaves ground up with kaolin and water to a paste, are applied in Ghana to the body as ointment for measles. It is used for acne lesions (Qadan *et al.*, 2005).

However, most of the above-mentioned researchers conducted short-term research and suggested that further research work should be done on long-term bases so as to make better suggestion on the uses and effect of *Psidium guajava* extract.

Due to the medicinal uses of *P. guajava* and its potency in alternative medicine, it is therefore necessary to investigate the physiological effects of this plant material on hematological indices using an animal model in order to make an inference on the safety of its consumption by human.

This study therefore tried:

1 To compare the effects of the extracts on the body weight of the albino rats

2 To determine the effects of aqueous and methanolic extracts of *Psidium guajava* leaves on the hematological indices of albino rats.

METHODS

As shown below, a total of 84 albino rats were used. The experiment was divided into two major groups as shown in Table 1 and Table 2 (aqueous extract and methanolic extract group). The first major group (aqueous extract) as shown in Table 1 was made up of 36 albino rats which were further divided into three treatment groups (A, B and C) comprised of 12 rats per group. The treatment groups (A, B, and C) were exposed to three different aqueous extract concentration levels namely 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively. Each treatment group contained three replicates comprising of four rats per replicate which were differentially marked for easy identification.

Similarly, the second major group (methanolic extract group) was made up of a total of thirty-six (36) rats which were divided into three treatment groups (A, B, and C) comprised of twelve albino rats per group.

The treatment groups (A, B, and C) were administered three different methanolic extract concentration levels namely 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight of the extracts, respectively. Each treatment group contained three replicates of four rats per replicates which were differentially marked for easy identification.

Both aqueous and methanolic treatment group had a common control group comprised of twelve rats. The control group was administered only distilled water in the same proportion as the treatment groups (i.e., 0.2 ml, 0.4 ml, and 0.8 ml for 200 mg/kg, 400 mg/kg, and 800 mg/kg) per kg, respectively. The portal of administration was orally by injection. The experiment lasted for four weeks but on weekly basis, one rat was sampled from each replicate in both aqueous and methanolic groups and control group.

Table 1: Experimental Design of Rats Treated with Aqueous Extract.

TREATMENT	CONTROL GROUP	TREATMENT ONE 200 mg/kg	TREATMENT TWO 400 mg/kg	TREATMENT THREE 800 mg/kg
NUMBER OF RATS	12	12	12	12

Table 2: Experimental Design of Rats Treated with Methanolic Extract.

TREATMENT	TREATMENT ONE 200 mg/kg	TREATMENT TWO 400 mg/kg	TREATMENT THREE 800 mg/kg
NUMBER OF RATS	12	12	12

RESULTS

Research Question 1: Effect of *Psidium guajava* extracts on the hematological indices of albino rats.

Table 3: Effects of *P. guajava* on the Hematological Parameters.

TREATMENT(mg/kg)	PCV (%)	RBC(10^6mm^3)	WBC(10^6m)	Hb(g/dL)
Aqueous				
200	24.67±2.74^a	3.704±0.42^c	3967±163^a	14.06±1.67^c
400	26.08±2.23^b	3.604±0.41^c	4443±192^b	15.59±1.54^d
800	26.08±2.31^b	3.941±0.17^d	5527±473^d	15.93±1.30^d
Methanol				
200	29.50±3.85^d	3.680±0.56^c	4986±374^c	12.88±1.34^b
400	27.00±4.24^c	3.318±0.49^b	5333±667^d	14.44±1.69^c
800	27.50±2.23^c	3.104±0.76^b	5725±351^e	16.22±1.04^e
Control	24.92±1.78^a	2.774±0.53^a	4740±302^d	9.500±1.20^a

**Means within the same column followed by different letters are significantly different (P < 0.05).*

Table 3 showed the result of the effects of the overall administration of aqueous and methanolic extracts on the haematological indices of the rats. It was observed that there was a significant increase (P < 0.05) in all the parameters when compared to that of the control.

Research Question 2: Weekly effect of *P. guajava* on body weight of albino rats.

Table 4: Results of the effect of *Psidium guajava* on body weight of albino rats.

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	164.3±1.15^{e1}	201.6±2.88^{f3}	170.4±0.58^{b2}	239.3±1.15^{f4}
400	128.6±0.57^{a1}	147.6±0.57^{b2}	167.3±0.54^{a3}	214.0±3.46^{c4}
800	132.3±0.56^{b1}	158.7±0.58^{c2}	167.6±0.57^{a3}	210.7±0.58^{b4}
Methanol				
200	130.0±0.00^{a1}	203.3±2.89^{f3}	197.7±0.56^{e2}	224.6±0.54^{d4}
400	150.3±0.57^{d2}	135.6±0.57^{a1}	189.3±0.58^{d3}	202.6±0.57^{a4}
800	140.6±1.15^{c1}	170.6±1.15^{d2}	184.7±0.55^{c3}	202.7±0.57^{a4}
Control	170.6±1.15^{f1}	189.4±0.57^{e2}	201.6±2.88^{f3}	234.6±0.58^{e4}

**Mean value within the same column followed by different letters are significantly different (P < 0.05). *Mean value within the same row with different figures are significantly different (P < 0.05).*

Table 4 showed the result of the weekly effects of the concentration on body weight of the albino rat. It was observed that there was a significant decrease ($P > 0.05$) in all the dose level at the first week when compared to that of the control. In the second week, aqueous extract of 200 mg/kg and methanol extract of 200 mg/kg were significantly high ($P < 0.05$) whereas other concentration levels in the same week were significantly low ($P > 0.05$) compared to that of the control. In the third week, aqueous and methanol extracts of 400 and 800 mg/kg produced comparable effects, although there was a significant decrease ($P > 0.05$) in all the other dose levels compared to the control. At the fourth week, it was observed that aqueous extract of 200 mg/kg was significantly high ($P < 0.05$) compared to the control.

Methanol extracts of 400 and 800 mg/kg produced comparable effects while other dose levels of the extracts were significantly low ($P > 0.05$) compared to the control. However considering the effect with time, the body weight of the rats produced significant increase ($P < 0.05$) in the fourth group in all the dose levels of the extract.

DISCUSSION AND CONCLUSION

It can be noted that plants generally have different chemical composition depending upon the species. A good number of plants generally have been known to be of economical and medicinal value. Some herbs have been considered as drugs and therefore generally safe and effective. Most herbs have been associated with broad actions on a number of physiological systems in concert unlike the pharmaceutical drugs which are usually designed to elicit a specific effect. The ones that are of medicinal value are majorly used as herbal remedy for the restoration and maintenance of good health. Some researchers on medicinal plants 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 rare adverse (Uboh *et al.,* 2010).

The determination of hematological indices provides physiological information on a proper blood assessment. According to Okonkwo *et al.,* (2010) accurate laboratory determination of blood parameters remains the only sensitive and reliable foundation for ethical and rational research, diagnosis, treatment, and prevention of diseases. The result also showed that the oral administration of both aqueous and methanol extract of *Psidium guajava* significantly increased the hematological parameters. This observation is in agreement with Uboh (2010), who stated that red blood cell, hematocrit, and hemoglobin concentration increased significantly ($P < 0.05$) on administration of aqueous extract in both male and female rats. He stated that the use of the aqueous extract of *P. guajava* leaves as liver tonic in some part of the world may not interfere with the functional integrity of the liver tissues and therefore indicate that the extract of *P. guajava* leaves may possibly serve as an acceptable blood booster in an anemic condition or prophylactic purpose. Although the specific mechanism(s) through which the extract facilitated the increase in these hematological indices were not known. It is therefore possible that the extract contains such constituent(s) that can interact and stimulate the formation and secretion of erythropoietin; hematopoietic growth factors/committed stem cells. Specifically, the stimulations of hematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray *et al.,* 2010; Uboh *et al.,* 2010)). This agreed with the result of Aberu *et al.* (2006), which showed that there was a significant increase ($P < 0.05$) in blood constituent from blood incubated with guava extract. This result therefore suggest that aqueous guava extract could present antioxidant action and, or alters the membrane structures involved in ion transport into cells thus decreasing the radiolabeling of blood constituent with technetium-99m.

The animals dropped in weight compared to the control group as a result of the administration of both extracts. This agreed with the result of *Hisae et al.,* (2012) in which there was a decrease in body weight of rats treated with guava leaf extract. The significant decrease ($P > 0.05$) in body weight of the experimental animals was also in agreement with the research of Ezekwesili *et al.* (2010) and Uboh *et al.* (2010). The loss of body weight in the treatment group administered with the extracts may be as a result of loss of appetite observed. This might have led to decrease in food intake or lesions in the intestine leading to malabsorption. The decrease in body weight may also be attributed to dietary palatability problem due to daily administration of *Psidium guajava* (Rabo, 1998).

In conclusion, the present study suggests that moderate and short-term ingestion of *Psidium*

guajava leaf extracts as traditional medicine can be of great advantage. The extract has more beneficial effects on the hematological than on the biochemical components of the body. The beneficial effect extends to the fact that it can serve as a blood booster and as well as anti-obesity because of its effect on blood cells and body weight, respectively.

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