TITLE PAGE

EFFECT OF LEAF EXTRACTS OF *PSIDIUM GUAJAVA* (GUAVA) ON THE BIOCHEMICAL AND HAEMATOLOGICAL INDICES OF ALBINO RATS

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APPROVAL PAGE

I approve that this project was carried out under my supervision by Ofoegbu, Edmund Chinwendu, in the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka in partial fulfillment of the requirements leading to the award of Masters Degree in Physiology

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DEDICATION

This work is dedicated to the most gracious God for his extravagant grace and mercy towards me.

ACKNOWLEGEMENTS

With a heart full of joy and mouth full of thanks, I appreciate the Ever Sufficient God for his provision financially and otherwise throughout the period of this project. The lord has been gracious to me from the beginning till the end of this research. I give unreserved thanks to my supervisor, Prof, B. O. Mgbenka for his direction and patience throughout the supervision of the research. Words are grossly insufficient in expressing my immense gratitude to him. The formal Head of Zoology and Environmental Biology Department, Prof, J. E. Eyo should not be left unmentioned. I appreciate your moral support and the encouragement you gave me.

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ABSTRACT

This study was to investigate the effect of aqueous and methanolic extracts of *Psidium guajava* leaves on the biochemical and haematological indices of liver functions in rats, and compare the effect of the extracts on body weight of albino rats on weekly interval for four weeks. A total of eight four rats were divide into two experimental group of thirty six rats per group and one was treated with aqueous extract while the other was treated with methanolic extract also a common control group made of twelve rats. Each experimental group was made up of three treatment sub-group which consists of different concentration levels of 200 mg/kg, 400 mg/kg and 800 mg/kg. A sub-group consists of three replicate of four rats per replicate. Blood samples were collected from rats in each replicate of treatment and control groups and tested for various biochemical and haematological parameter using standard method. The result of the study showed that there was a significant increase ($P < 0.05$) in the concentration levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP). Whereas there was a significant decrease ($P > 0.05$) in most of the concentration levels of serum cholesterol compared to the control. However, 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 haemoglobin (Hb). The body weight of the rats generally dropped as a result of oral administration of *P. guajava* extract. Previous data tend to suggest that a moderate ingestion of the extracts is beneficial to the body although the increase in the liver enzymes (ALT, AST, ALP and ACP) tend to suggest a dysfunction in the physiology of the liver. However the beneficial effect extends 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 haematological parameter.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 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).

The plant can be seriously attacked by pest and disease. Such pests include citrus flat mite, bark-eating caterpillars, red-banded thrips, aphids etc, which attack different part of the plant whereas diseases of guava plant include mucor rot, alga spotting, stem-end rot, etc. (Morton, 1987). Phytochemical analysis of the fruit shows that it contains vitamin A, iron, calcium and phosphorous, manganese with oxalic and malic acids, saponin, oleanolic acid, flavonoids, guaijavarine and quercetin (Iwu, 1993; 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 contain 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). However, Okwu and Ekeke (2003) analysis of the twig shows that it contains essential elements which include

calcium, magnesium, phosphorous potassium and sodium. In addition, it contains the micro elements like zinc, iron, copper etc. (Arima and Danno, 2002).

1.2 Justification of the study

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 biochemical and haematological indices using an animal model in order to make an inference on the safety of its consumption by humans.

1.3 Objectives of the study

The objectives were to:

1 Compare the effects of the extracts on the body weight of the albino rats

2 Determine the effects of aqueous and methanolic extracts of *Psidium guajava* leaves on the biochemical indices of albino rats.

3 Determine the effects of aqueous and methanolic extracts of *Psidium guajava* leaves on the hematological indices on Albino rats.

1.4 Literature Review

1.4.1 Origin and morphology of *Psidium guajava*

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).

1.4.2 Uses of *Psidium guajava* leaves

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). A high fruit diet proved effective in improving systolic blood pressure and diet quality in adolescents with high blood pressure. The control of high blood pressure has contributed to reduction in mortality from stroke and coronary heart disease (Baby and Mini, 2011).

Antibacterial activity: *Psidium guajava* contains a number of chemical constituents which are reported to possess antibacterial (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 antidiarrhoel (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 decotion 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 leucorrhoea 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.

1.5 Biochemical Indices in a Man

According to Voet and Voet, (2004) Biochemists study the elements, compounds and chemical reaction controlled by enzymes and take place in all organisms. Biochemistry of blood gives an insight of what is happening within the body. Damaged tissues and cells release specific enzymes that can easily be detected with medical equipment as abnormal. These enzymes can be:

1.5.1 Acid phosphates (ACP): Acid Phosphatase (ACP) is an enzyme of the Hydrolase class of enzymes which acts in an acidic medium (pH = 4.9). It is widely distributed and found in high concentrations in the liver, red blood cells and the prostate. Whereas increased levels of the prostatic fraction are usually associated with prostatic carcinomas, the increased levels of the non prostatic fraction are normally associated with liver diseases, hyperparathyroidism, and Pagetôs disease (Sood, 2006).

1.5.2 Alanine aminotransferase (ALT): This is mostly found in liver although it is found in different tissues. Its increase in the liver is found in hepatitis, obstructive jaundice, cirrhosis and other hepatic disease. It can also result to myocardial infection (Sood, 2006).

1.5.3 Alkaline phosphatase (ALP): Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes that acts in an alkaline medium (pH = 10.0). It occurs in high concentrations in the liver, biliary tract epithelium and in the bones. However, normal levels have been found to be age dependent and usually increase during bone development. More often, increased levels are

always associated with liver and bone disease. Moderate increases are seen in Hodgkinôs disease and congestive hearts failure (Sood, 2006).

1.5.4 Aspartate aminotransferase (AST): Aspartate Transaminase (AST) is an enzyme found mainly in the heart muscle, liver cells, skeletal muscle and kidneys. Any injury to these tissues usually results in the release of the enzyme in the blood. Elevated levels are found in myocardial infection, Cardiac operations, Hepatitis, Cirrhosis, acute pancreatitis, acute renal disease, primary muscle disease. Decreased levels may be found in Pregnancy, Beri Beri and Diabetic Ketoacidosis (Sood, 2006).

1.5.5 Serum cholesterol: It is the Lipid seen in brain tissues, blood and bile. It is needed for the formation of steroids and cellular membranes and it is common with arteriosclerotic vascular disease. The cholesterol is metabolized by the liver and moved in the blood by lipoproteins. An increased level of serum cholesterol are found in hypothyroidism, cirrhosis, nephritic syndrome, hyperlipidaemia, where as decreased level are found in malnutrition, malabsorption, anemia and liver disease (Sood, 2006).

1.6 Haematological Indices of Man

Haematology originated from Greek *ôhalmaô* and *ôlogos'* which means the science that studies blood. Blood is very different from other body tissue. The blood is made up of a solid and a liquid portion. The liquid portion is called the plasma and contains blood cells which are white blood cells (leucocyte), red blood cells (erythrocyte) and platelet (thrombocyte). The solid portion is comprised of cells which are the basic unit of life. All living organisms are made of one or many cells. Unicellular organisms e.g. bacteria are made of one cell where as multicellular organism like plant and animals are made of more than one cell. Cells have nucleus and organelles. Most cells share common characteristics but in multicellular organism each cell

performs a specialized function. The blood cells are very specialized and it is the only tissue made of cells that do not stick together. In animals, the blood is made up of fluid of variable composition. The Erythrocytes (red blood cell ï RBC), leucocytes (white blood cell ï WBC) and thrombocytes (platelets) are suspended in plasma. By using anticoagulant, the formed elements can be separated from the plasma. The fluid that remains after separation of the cloth is called serum. Serum = plasma ï fibrinogen. Hematological techniques is concerned with the cellular formed elements of blood, their concentration and number, the relative distribution of various types of cells and the structural or biochemical abnormalities that promote disease (Sood, 2006). The blood acts as a transport medium. It is contained in blood vascular system and in vertebrates. It is circular by means of contraction of the heart. Blood cells float separately throughout the body inside vessels called arteries and veins. Blood transports oxygen and food to other tissue whereas it transports carbondioxide and Nitrogenous waste from tissues to excretory organs for disposal. Furthermore, it carries hormones and acts as a defense system by producing anti-bodies (Sood, 2006). Abnormalities in any of the blood component lead to infection or disorders. Disorders involve anemia, polycythemia, leukemia, bleeding disorders, hypoalbuminaemia (albumin deficiency of gamma-globulin).

Medically, the number of each blood component present in a liter of blood varies with age and sex and whenever this range values are affected either by increase or decrease, it leads to one abnormality or disorder.

Moreover some blood disorders are inherited while others are caused by blood poison. Also, some drugs can cause blood abnormalities as a side effect (Diggs, 1985).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Identification and Collection of Plant Material

Fresh leaves of *Psidium guajava* were collected from the Botanical garden of the Plant Science and Biotechnology Department in University of Nigeria Nsukka, Enugu State. Sample of the plant specimen was identified and authenticated in Plant Science and Biotechnology Department. The specimen were deposited at the herbarium and preserved.

2.2 Preparation of the Extract

The method of extraction followed that of Uboh *et al.,* (2010). The leaves were air-dried for 2 weeks and then ground into fine powder using an electric dry mill (Moulinex). A total of 200 g of the ground powder was soaked in 1 liter each of distilled water and methanol for 48 hours at room temperature. The mixtures were differently filtered into 500 ml conical flask with Watman filter paper (No.1). The filtrate was dried at a temperature of 30ºC for 10 hours to produce a gel-like extract, which weighed 20.5 g. Appropriate concentration of the extracts were then subsequently made by dilution with distilled water.

2.3 Procurement, Management of Experimental animals

Eighty four male albino rats were purchased from the laboratory animal unit of the Department of Zoology and Environmental Biology in University of Nigeria, Nsukka, Enugu State. The rats were acclimatized in the experimental animal house for one week before the commencement of the experiment. They were kept in stainless wire-rat cages equipped with drinkers and feacal collecting trays in a clean experimental animal house. The rats were fed with broiler chicks mash. The faecal droppings were removed daily. The rats were weighed with Mettler, electronic balance PC 2000 during treatment. All the rats in both treatment and control

groups were allowed free access to food and water, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. They were separated into seven groups of 12 rats per group and the rats were differentially marked for identification.

2.4 Determination of LD_{50}

The method of determination followed that of Lorke (1983). A test was conducted using three graded doses of aqueous and methanolic extracts (10, 100 and 1000 mg/kg) of *Psidium guajava.* Each dose served as a group with six albino rats. Three rats for each of the extracts. There was no death recorded. After 24 hours, another three higher doses of (1500, 3000, and 5000) mg/kg of body weight were used as group with six albino rats for both the extracts. There was no lethality recorded in all the dose levels of *Psidium guajava* extracts administered orally to rats. The observable sign on the administration of the highest dose of 5000 mg/kg of both extracts were restlessness, panting and reddish eye but no death was recorded hence LD_{50} was not determined. Therefore a lower dose of 200 mg/kg, 400 mg/kg and 800 mg/kg were used.

2.5 Experimental Design

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 replicate 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 groupsô i.e (0.2 ml, 0.4 ml, 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	TREATMENT TWO	TREATMENT THREE
		200 mg/kg	400 mg/kg	800 mg/kg
NUMBER OF RATS	12	12	12	12

Table 2: Experimental design of rats treated with methanolic extract

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

2.6. Collection of Blood Sample and Preparation of Serum Samples for Analysis

The method adopted for the collection of blood and serum preparation followed that of Hoff (2000). Blood samples were collected from the animal with a Pasteur pipette with the aid of capillary inserted at the corner of the eye ball. Part of the whole blood collected was introduced into anticoagulant blood sample containers. The blood samples were then taken for blood tests analysis.

Similarly, some whole blood were collected from the animals in the different groups through ocular puncture and introduced into clean 5 ml centrifuge tubes. The blood sample was centrifuged at a speed of 3000 revolutions per minute (rpm) for 5 minutes. The supernatants (sera) were collected and used for the various biochemical analyses.

2.7 Biochemical Analysis

2.7.1. Assay for acid phosphatase (ACP)

This was assayed according to Fishman and Lerner (1953). The principle of this assay involves the formation of phosphate and phenol from the reaction:

Phenyl phosphate + $H_2O \rightarrow$ phosphate + phenol

Phenol is condensed with 4-aminoantipyrine and oxidized with alkaline fermicyanide to give a red complex whose intensity is equal to the acid phosphatase activity.

Methodically, different test tubes were labeled test, control standard and Reagent Blank. A total of 1 ml each of solution 1 and 3 (appendix 1) were pipetted into test tube labeled test and control respectively, mixed and warmed for about 10 minutes at 37^o C in a water bath. 0.1 ml of sample

(serum) was added to the test tube labeled test. The content was properly mixed and incubated at 37^{o}C for 60 minutes.

Similarly, 1.1ml of solution 1 was added to test tubes labeled standard and reagent blank, and 1.0 ml of working phenol standard was added to test tube labeled standard. Consequently 0.8 ml of NaOH solution, 1.2 ml of solution 5 and 1.0 ml each of solution 6 and 7 were added to all the test tubes and the contents thoroughly mixed. The absorbance of the various test tubes was measured at 500 nm on a spectrophotometer (Spectro 21D Pec medicals).

2.7.2. Assay for alanine aminotransferase (ALT)

A Randox commercial enzyme kit of Reitman and Frankel (1957) was used. This assay was based on the principle that pyruvate is formed from

$$\alpha - Oxoglutarate + L - alanine \rightarrow L - glutamate + pyruvate.$$

ALT was measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4-denitrophenyl hydrazine.

Procedure: 0.5 ml of the ALT substrate phosphate buffer was pipetted into test tube labeled B (sample blank) and T (sample test) respectively. Furthermore, 0.1 ml serum sample was added to sample test tube (T) only and properly mixed, then incubated for exactly 30 minutes in a water bath at 37^{o}C.

After incubation, 0.5 ml of 2, 4 dinitrophenyl hydrazine was added to both test tubes B and T immediately. The entire content was mixed thoroughly and allowed to stand for 20 minutes in a water bath at 25^{o}C. Then 5.0 ml of NaOH solution was added to both test tubes B and T and mixed evenly. The absorbance of the sample was taken at wavelength of 546 nm after 5 minutes.

2.7.3. Assay for alkaline phosphatase (ALP)

A Randox commercial enzyme kit of Fishman and Lerner (1953) was used for the assay. It is based on the principle that phosphate and P-nitrophenol are formed from the reaction:

P-nitrophenylphosphate + H_2O Ÿ phosphate + P-nitrophenol

0.02 ml of the sample was pipetted into test tube and 1.0 ml of the reagent was also added to the sample and mixed thoroughly. The absorbance of the content was measured at 405 nm on a spectrophotometer (Spectro 21D Pec Medicals).

2.7.4. Assay for aspartate aminotrasferase (AST)

A Randox commercial enzyme kit according to the method of Reitman and Frankel (1957) was used for the assay. It is based on the principle that oxaloacetate is formed from the reaction: Ŭ-oxoglutarate + L-aspartate Ÿ L-glutamate + oxaloacetate.

Glutamic-oxaloacetic acid transaminase (AST) activity was measured by checking the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine .

A total of 0.5 ml of the AST substrate phosphate buffer was pipetted into each of the test tubes marked reagent blank (B) and sample (T) test. More so, 0.5 ml of the serum sample was added to the sample (T) test tube only and mixed thoroughly; 0.1 ml of distilled water was added only to the reagent blank (B) test tube. The entire media were mixed and incubated for 30 minutes in a water bath at 37^{o}C. After incubation, 0.5 ml of 2, 4-dinitrophenyl-hydrazine was added to the two test tubes reagent blank and sample test, mixed properly and allowed to stand for 20 minutes at 25^{o}C.

Finally, 5.0 ml of NaOH solution was added to the two (B and T) test tube and mixed. The absorbance of sample was read at wavelength of 546 nm against the reagent blank after 5 minutes.

2.7.5. Assay for serum cholesterol

A Randox commercial enzyme kit method of Rosechlau *et al.* (1974) was used. The total serum cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen perioxide and 4-aminoantipyrine in the presence of phenol and peroxides:

$$\text{Cholesterolester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{cholestene ï 3 ï one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4\text{ -Aminoantipyrine} \xrightarrow{\text{Peroxides}} \text{quinoneimine} + 4H_2O$$

Methodically three different test tubes labeled blank, standard and sample test were used. 10 ml of distilled water was pipetted into a test tube labeled blank, and 10 ml of cholesterol standard was pipetted into another test tube labeled standard. 10 ml of the serum sample was pipetted into another test tube labeled sample test. A total of 1000 εL of the reagent solution was added to all the three test tubes and mixed thoroughly. The experimental test tubes were incubated for 5 minutes at 37ِگ. The absorbance was measured after 60 minutes at a wavelength of 500 nm.

2.8. Haematological Analysis

2.8.1. Haemoglobin estimation

The method used in the haemoglobin estimation followed that of Sood (2006). The method is based on the principle of making an acid haematin solution of blood under experimentation in the graduated tube and then comparing it with the sealed comparison tubes containing the standard acid haematin.

A graduated text tube rinsed with water and methylated spirit was thoroughly dried up before being used. Then normal HCl solution was filled into the graduated tube up to 2 gms mark using a dropper. A micropipette was then filled up with blood sample by sucking up to 20 cm. the small amount of blood adhering to the outside of the micropipette was wiped off using sterilized cotton wool. The blood in the micropipette was added to the normal HCl solution in the graduated tube. The pipette was introduced carefully into the tube. The acid haematin solution was thoroughly stirred using a glass rod and then allowed to stand for 10 minutes. Later, the acid haematin solution was gradually diluted by adding 10ml of distilled water in drops. The addition of each drop of distilled water to the solution was stirred and its colour matched with that of the standard sealed tubes. The addition of distilled water continued until the colour of the acid haematin solution fades away as compared to that of the standard comparison tubes. The reading was taking just before the colour faded away.

2.8.2. Blood haematocrit/packed cell volume (PCV)

The packed cell volume or haematocrit is the volume of red blood cells expressed as a percentage of the whole blood volume. Its determination followed the method of Sood (2006). With the aid of a needle and syringe, a capillary tube was filled three-quarter ($\frac{3}{4}$) full and the

open end of the tube sealed with sealing wax (plastocine). The filled capillary tubes were placed in a micro-haematocrit centrifuge with the sealed side facing outside. The content was centrifuged at 3500 rpm (revolution per minute) for 5 minutes. The packed cell height (red cell column) as well as the total height comprising of the height of the red cell and plasma were measured in millimeters using a ruler. The haematocrit was calculated using the formula below:

$$\text{Hct\%} = \frac{Height\ of\ red\ cells\ (mm)}{Height\ of\ red\ cells\ plasma\ (mm)} \times \frac{100}{1}$$ (Sood, 2006).

2.8.3. White blood cell count:

The method used to determined white blood cell count followed that of Sood (2006). A pipette was filled with capillary blood till 0.5 marks and the tip of the pipette cleaned. More so the white blood cell diluting fluid was drawn till the 1 mark of the pipette. The fluid and blood were mixed gently avoiding bubbling and the counting chamber was placed on top of it at the right place. The fluid-blood mixture was carefully transferred into the counting chamber (charging the chamber) using a fine bore Pasteur pipette. Caution was taken during charging of chamber to avoid overflow. At the end of the charging process, the cells were allowed to settle at the bottom of the chamber for 2 minutes. The under part of the chamber was cleaned and placed on the stage of the microscope. The white blood cells were immediately counted out in the four larger corner squares under the 10x power objective so as to avoid the drying up of fluid. The calculation of the number of cells per cubic millimeter of blood was as follows:

$$\frac{Cells\ count \times Blood\ Dilution \times Chamber\ depth}{Area\ of\ chamber\ counted}$$

$$= \frac{Number\ of\ cells\ counted \times 20 \times 10 \times (Depth\ Factor)}{4}$$ (Sood, 2006)

= number of cells counted x 50.

2.8.4. Red blood cell count

The method for the red blood cell count was according to Sood (2006). Blood was drawn to the 0.5 mark of the pipette. The tip of the pipette was wiped clean before the diluting fluid was drawn to the 101 mark. The mixture was mixed by shaking for 3 minutes. The chamber was charged and the red blood cells were counted under 40x objective of the microscope in the 80 smallest squares and the calculation was made using the formular:

$$\text{RBC Count} = \frac{No\ of\ Counted \times Dilution\ factor \times Depth\ factor}{Area\ Counted}$$ (Sood, 2006).

2.9. Statistical Analysis

The statistical analysis was conducted using GENSTAT. The mean values of the data were subjected to analysis of variance (ANOVA), whereas means of treatment groups were separated using DUNCAN multiple range test. Level of significance was set at 0.05.

CHAPTER THREE

RESULTS

3.1 Effect of the Extracts *Psidium guajava* on the Biochemical Indices of Albino Rats

Table 3 showed the result of the effects of the overall administration of aqueous and methanolic extracts on the biochemical 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. However, the effect of the different dose levels of the extracts on the biochemical parameters showed some weekly variations in Table 3 below.

TABLE 3. Effects of *Psidium guajava* on the biochemical indices of albino rats

TREATMENT (mg/kg)	ALT (U/L)	AST (U/L)	CHOL (mg/dL	ACP (U/L)	ALP (U/L)
Aqueous					
200	17.08±1.99[b]	14.48±5.43[b]	167.4±5.76[e]	2.983±0.95[d]	48.83±6.47[c]
400	16.92±6.99[c]	16.25±5.60[c]	158.3±12.2[b]	2.743±0.87[c]	56.08±4.48[d]
800	19.92±5.81[d]	21.18±9.82[f]	166.0±12.7[d]	2.917±0.53[d]	59.08±8.83[f]
Methanol					
200	26.42±9.25[e]	16.01±10.6[b]	167.7±12.4[e]	2.767±0.86[c]	45.08±6.73[b]
400	30.66±8.14[f]	18.87±7.24[d]	153.3±14.1[a]	2.387±1.05[b]	50.83±6.39[c]
800	32.91±9.19[g]	19.19±6.93[e]	157.3±8.53[b]	3.003±1.04[d]	58.00±6.99[e]
CONTROL	16.42±7.07[a]	13.41±6.19[a]	161.8±12.9[c]	2.216±2.56[a]	24.17±2.44[a]

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

3.1.1 Weekly effect of *P.guajava* on alanine aminotransferase, ALT (U/L) level.

Table 4 showed the weekly effect of concentration on ALT. it was observed that in the first week of treatment the concentration levels of methanol extract increased significantly ($P < 0.05$) when compared with the control. In the second week, aqueous extract of 200 and 400 mg/kg produced comparable effect to the control. In the third week, aqueous extract of 400 mg/kg body weight of the treatment group decreased significantly whereas aqueous extract of 200 mg/kg produced comparable effect to the control. All the methanol extract produced a significantly increased ($P < 0.05$) compared to the control in the same third week. It was also observed that in the fourth week of treatment, aqueous extract of 800mg/kg produced comparable effect to that of the control. At the same week four, aqueous extract of 200 mg/kg increased significantly ($p < 0.05$) and produced comparable effect to aqueous extract of 400 and 800 mg/kg respectively. Considering the effect of time, there was a significant increase ($P < 0.05$) at all the concentration levels in the fourth week.

TABLE 4. Results of the effect of *Psidium guajava* on alanine aminotransferase, ALT (U/L) level.

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	8.666 ± 0.57^{b1}	11.66 ± 0.56^{a2}	19.66 ± 0.57^{b3}	28.33 ± 0.55^{c4}
400	9.667 ± 0.58^{c1}	13.67 ± 0.57^{b2}	16.67 ± 0.56^{a3}	27.66 ± 0.56^{bc4}
800	11.66 ± 0.57^{d1}	18.65 ± 0.57^{c2}	22.66 ± 0.57^{c3}	26.66 ± 0.55^{ab4}
Methanol				
200	16.00 ± 0.00^{e1}	20.33 ± 0.58^{d2}	30.67 ± 0.58^{d3}	38.67 ± 0.57^{d4}
400	20.66 ± 0.58^{g1}	25.67 ± 0.56^{e2}	36.66 ± 056^{e3}	39.66 ± 0.58^{e4}
800	19.66 ± 0.56^{f1}	30.66 ± 0.57^{f2}	38.66 ± 0.57^{f3}	42.65 ± 0.57^{f4}
Control	7.667 ± 0.56^{a1}	12.66 ± 0.58^{ab2}	20.00 ± 0.00^{b3}	25.67 ± 0.56^{a4}

**Means within the same column followed by different letters are significantly different ($P < 0.05$), mean values in a row with different figures are significantly different ($P < 0.05$).*

3.1.2 Weekly effect of *P. guajava* on aspartate aminotransferase, AST (U/L)

Table 5 showed the weekly effect of concentration on AST. It showed that in the first week, there was a significant increase ($P < 0.05$) in all the dose levels compared to the control. However aqueous and methanol extract of 400 mg/kg produced comparable effect. In the second week, there was a significant increase in all the dose levels compared to the control. However methanol dose level of 200 mg/kg was significantly low ($P > 0.05$) whereas methanol and aqueous extract of 400 and 800 mg/kg respectively produced comparable effect. In the third week, aqueous extract of 200 mg/kg was significantly low compared to that of the control whereas aqueous extract of 400 mg/kg produced comparable effect to the control. Furthermore there was a significant increase ($P < 0.05$) in all the dose levels of the treatment group in the fourth week compared to the control. However methanol extract of 200 and 400 mg/kg produced comparable effect. Considering the effect of time on the different extract doses, it was observed that there was a significant increase ($P < 0.05$) in the fourth week at all the concentration dose levels of the extract.

TABLE 5: Results of the effect of *Psidium guajava* on aspartate aminotransferase, AST (U/L)

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	9.333 ± 0.57^{c1}	10.33 ± 0.58^{c2}	15.93 ± 0.11^{a3}	22.33 ± 0.56^{b4}
400	10.73 ± 0.46^{d1}	12.67 ± 0.58^{d2}	16.93 ± 0.12^{b3}	24.67 ± 0.58^{c4}
800	11.96 ± 0.06^{e1}	14.07 ± 0.12^{e2}	22.84 ± 0.28^{e3}	35.86 ± 0.23^{f4}
Methanol				
200	8.333 ± 0.57^{b2}	8.067 ± 0.05^{a1}	20.66 ± 0.57^{d3}	27.00 ± 0.00^{d4}
400	10.33 ± 0.29^{d1}	14.33 ± 0.57^{e2}	22.83 ± 0.28^{e3}	27.96 ± 0.05^{d4}
800	12.66 ± 0.58^{f1}	15.13 ± 0.12^{f2}	18.96 ± 0.05^{c3}	30.00 ± 0.00^{e4}
Control	5.933 ± 0.12^{a1}	9.833 ± 0.28^{b2}	16.90 ± 0.17^{b3}	21.00 ± 1.73^{a4}

**Means within the same column followed by different letters are significantly different ($P < 0.05$), mean values in a row with different figures are significantly different ($P < 0.05$).*

3.1.3 Weekly effect of *P. guajava* on alkaline phosphatase (ALP) (U/L) level.

Table 6 showed the result of the weekly effect of concentration levels on ALP. It was observed that there was a significant increase ($P < 0.05$) at all the dose levels of the extracts in the first week of the treatment while aqueous and methanol extract of 200 and 400 mg/kg respectively produced comparable effects. In the second week there was a significant increase ($P < 0.05$) in all dose levels compared to the control whereas aqueous and methanol extract of 400 mg/kg produced comparable effect. At the third and fourth week, there was a significant increase ($P < 0.05$) in all the dose levels compared to that of the control. However aqueous and methanol extract of 200 mg/kg and 400 mg/kg produced comparable effect in the fourth week. Furthermore, consideration on the effect of time revealed that all the concentration levels of the extract produced significant increase ($P < 0.05$) in the fourth week.

TABLE 6: Results of the effect of *Psidium guajava* on alkaline phosphatase (ALP) (U/L) level

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	41.66±0.57^{c1}	44.66±0.56^{c2}	51.33±0.57^{c3}	57.66±0.54^{c4}
400	53.67±0.55^{f2}	51.33±1.15^{d1}	56.66±0.58^{e3}	62.68±0.56^{d4}
800	48.66±0.58^{e1}	53.66±0.57^{e2}	63.64±0.56^{g3}	67.34±0.58^{f4}
Methanol				
200	36.64±0.57^{b1}	42.66±0.55^{b2}	46.68±0.54^{b3}	54.33±1.15^{b4}
400	41.66±0.56^{c1}	50.33±0.54^{d2}	52.66±0.57^{d3}	58.66±0.58^{c4}
800	47.33±0.55^{d1}	58.66±0.58^{f2}	60.33±0.57^{f3}	65.64±0.57^{e4}
Control	21.66±0.58^{a1}	24.67±0.57^{a2}	22.66±0.58^{a1}	27.68±0.57^{a3}

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

3.1.4 Weekly effect of *P. guajava* on acid phosphatase (U/L) level.

In Table 7 the result of the weekly effect of concentration on ACP showed that there was a significant increase ($P < 0.05$) at all the dose levels of the treatment group from the first week to the fourth week when compared to that of the control. However aqueous extract of (200 and 400) mg/kg body weight of the treatment group produced comparable effect in the first week. Also, aqueous and methanol extract of 400 mg/kg produced comparable effect in the second week while aqueous and methanol extract of 200 mg/kg were not significantly different in the fourth week. Considering the effect of time, all the concentration levels of the extract produced a significant increase ($P < 0.05$) at the fourth week.

TABLE 7: Results of the effect of *Psidium guajava* on acid phosphatase (U/L) level.

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	1.900 ± 0.10^{e1}	2.284 ± 0.02^{c2}	3.683 ± 0.03^{g3}	4.066 ± 0.06^{e4}
400	1.933 ± 0.06^{e1}	1.966 ± 0.05^{b2}	3.184 ± 0.02^{e3}	3.890 ± 0.01^{d4}
800	2.282 ± 0.03^{f1}	2.693 ± 0.01^{e2}	3.000 ± 0.00^{d3}	3.693 ± 0.02^{b4}
Methanol				
200	1.783 ± 0.02^{d1}	2.366 ± 0.05^{d2}	2.884 ± 0.03^{c3}	4.033 ± 0.05^{e4}
400	1.084 ± 0.03^{b1}	1.900 ± 0.00^{b2}	2.783 ± 0.02^{b3}	3.794 ± 0.01^{c4}
800	1.483 ± 0.02^{c1}	2.860 ± 0.06^{f2}	3.483 ± 0.03^{f3}	4.183 ± 0.03^{f4}
Control	0.593 ± 0.01^{a1}	0.897 ± 0.01^{a1}	1.594 ± 0.01^{a2}	2.783 ± 0.02^{a3}

**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 ($P < 0.05$).*

3.1.5 Weekly effect of *P. guajava* on serum cholesterol level

In Table 8, the result of the weekly effect of concentration on cholesterol showed that there was a significant increase ($P < 0.05$) in all the dose levels of the treatment group in the first week with exception to methanol extract of 400 mg/kg which was significantly low ($P > 0.05$) when compared to that of the control. In the second week, methanol extract of (400 and 800) mg/kg were significantly low ($P > 0.05$) compared to that of the control while aqueous extract of (200 and 800) mg/kg produced comparable effect. However other dose levels were significantly different compared to the control. At the third week, dose level of aqueous extract of 200 mg/kg were significantly high ($P < 0.05$) while aqueous and methanol extract of 800 mg/kg produced comparable effect. However other dose levels were significantly low ($P > 0.05$). At the fourth week aqueous extract of 800 mg/kg was significantly high ($P < 0.05$) while other dose levels were significantly low ($P > 0.05$) compared to that of the control. With effect to time, it was observed that dose levels of aqueous 400 mg/kg, methanol 400 and 800 mg/kg produced significant increase ($P < 0.05$) at the first week, aqueous 200 mg/kg in the third week while aqueous 800 mg/kg and methanol 400 mg/kg at the fourth week of treatment.

TABLE 8: Results of the effect of *Psidium guajava* on serum cholesterol level

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	159.3 ± 1.15^{c1}	165.6 ± 0.58^{e2}	173.3 ± 1.15^{f4}	171.4 ± 1.16^{d3}
400	174.7 ± 0.57^{f4}	160.7 ± 0.57^{d3}	155.6 ± 0.57^{c2}	142.0 ± 1.73^{a1}
800	171.3 ± 1.15^{e3}	164.6 ± 0.58^{e2}	147.3 ± 1.15^{a1}	180.6 ± 1.15^{g4}
Methanol				
200	181.3 ± 1.14^{g4}	176.7 ± 0.55^{f3}	161.3 ± 1.16^{d2}	151.7 ± 1.53^{b1}
400	139.4 ± 1.15^{a1}	146.6 ± 0.56^{a2}	151.0 ± 1.73^{b3}	175.3 ± 0.58^{e4}
800	169.3 ± 1.14^{d4}	153.3 ± 0.55^{b2}	147.0 ± 1.15^{a1}	159.3 ± 1.15^{c3}
Control	144.6 ± 0.57^{b1}	159.3 ± 1.15^{c2}	164.0 ± 1.73^{e3}	179.3 ± 1.15^{f4}

**Mean values within the same column followed by different letters are significantly different ($p < 0.05$), whereas mean value in a row with different figures are significantly different ($P < 0.05$).*

3.2 Effect of *Psidium guajava* Extracts on Haematological Indices of Albino Rats.

Table 9 showed the results of the overall effects of the administration of the aqueous and methanolic extracts on some haematological indices of albino rats. It was observed that there was a significant increase ($P < 0.05$) at all the dose levels of the treatment group on the haematological indices of the albino rats when compared to that of the control. However the effect of the different dose levels of the extract on the haematological parameters showed some differences.

TABLE 9: Effects of *P.guajava* on the Haematological parameters

TREATMENT(mg/kg)	PCV (%)	RBC($10^6 mm^3$)	WBC($10^6 m$)	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 ($P < 0.05$).*

3.2.1 Weekly effect of *P. guajava* on packed cell volume (PCV)(%)

Table 10 showed the weekly effect of different concentration on PCV. It was observed that in week one, the PCV of methanol extract of (800 and 400) mg/kg body weight of the treatment group increased significantly ($P < 0.05$) when compared to that of the control and the other concentration levels of the extract. In the second week, the PCV of aqueous extract of 800 mg/kg produced comparable effects to that of the control while PCV of methanol extract of 400 mg/kg body weight of the treatment group was significantly low ($P > 0.05$). At the third week of treatment, the PCV of methanol extract of 400 mg/kg increased significantly and produced comparable effect to those of methanol and aqueous extracts of 200 and 400 mg/kg respectively. No significant difference ($P > 0.05$) was observed between the PCV of these groups (methanol 400, 200 and aqueous 200) mg/kg and the control. Furthermore, the PCV of the control group was the least in the third week. In fourth week, the PCV of methanol extract of 200 mg/kg produced significant increase ($P < 0.05$) followed by methanol extract of 800 mg/kg. There was no significant difference ($P > 0.05$) in the PCV of methanol 400, aqueous (800 and 200) mg/kg and the control. With respect to time, PCV of all treatment groups increased significantly ($P < 0.05$) in the third week with the exception of methanol 200 mg/kg that produced significant increase ($P < 0.05$) in the fourth week.

TABLE 10: Results of the effect of *Psidium guajava* on packed cell volume (PCV)(%)

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	20.67 ± 1.15^{a1}	25.67 ± 0.58^{b2}	27.67 ± 0.57^{b3}	24.66 ± 0.57^{a2}
400	24.33 ± 0.58^{b1}	25.00 ± 0.00^{b1}	30.33 ± 0.58^{c2}	25.67 ± 0.56^{b1}
800	24.33 ± 1.15^{b1}	27.67 ± 0.56^{c2}	28.67 ± 0.57^{b2}	23.66 ± 0.58^{a1}
Methanol				
200	27.67 ± 0.58^{d2}	24.67 ± 0.58^{b1}	31.33 ± 0.56^{c3}	34.33 ± 0.57^{d4}
400	30.67 ± 0.56^{e3}	22.33 ± 0.57^{a1}	31.33 ± 0.57^{c3}	23.66 ± 0.58^{a2}
800	30.33 ± 0.58^{e4}	24.67 ± 0.57^{b1}	28.33 ± 0.56^{b3}	26.67 ± 0.56^{c2}
Control	25.33 ± 0.57^{c2}	27.67 ± 058^{c3}	24.66 ± 0.58^{a1}	24.00 ± 0.00^{a1}

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

3.2.2 Weekly effect of *P. guajava* on red blood cell (RBC) ($10^6 mm^3$) level

Table 11 showed the result of the weekly effect on different concentration of RBC. It was observed that in the first and second week, all dose levels of the extract increased significantly ($P < 0.05$) when compared to the control while aqueous and methanol extracts of 200 and 400 mg/kg produced comparable effect in the second week. In the third week, methanol extract of (200, 400 and 800) mg/kg were significantly similar and produced comparable effects to the control. Methanol extract of 800 mg/kg produced comparable effect to aqueous extract of 400 mg/kg whereas aqueous and methanol extracts of 200 mg/kg and 800 mg/kg also produced comparable effect. At the fourth week, RBC of aqueous extract of 200 mg/kg was significantly similar ($P > 0.05$) to the control while aqueous and methanol extracts of 400 mg/kg and 200 mg/kg respectively produced comparable effects. However there was a significant increase ($P < 0.05$) in the other dose levels of both aqueous and methanol extracts. The effect of time on consumption of the extract revealed that aqueous 400 and methanol 200 mg/kg significantly elevated ($P < 0.05$) RBC in the second week of treatment whereas other dose level increased in the last week.

TABLE 11: Results of the effect of *Psidium guajava* on red blood cell (RBC) (10^6mm^3) level

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	3.183 ± 0.03^{f1}	3.483 ± 0.03^{c2}	3.950 ± 0.09^{d3}	4.167 ± 0.05^{a4}
400	3.667 ± 0.05^{e3}	4.183 ± 0.03^{e4}	3.083 ± 0.03^{b1}	3.483 ± 0.03^{b2}
800	4.000 ± 0.00^{g3}	3.683 ± 0.02^{d1}	3.983 ± 0.02^{d2}	4.100 ± 0.10^{d4}
Methanol				
200	2.967 ± 0.06^{d1}	4.467 ± 0.02^{f4}	3.783 ± 0.06^{c3}	3.483 ± 0.02^{b2}
400	2.783 ± 0.03^{c1}	3.483 ± 0.03^{c3}	3.003 ± 0.05^{a2}	4.000 ± 0.00^{c4}
800	2.283 ± 0.03^{b1}	2.783 ± 0.03^{b2}	3.067 ± 0.03^{b3}	4.283 ± 0.03^{e4}
Control	2.000 ± 0.00^{a1}	2.697 ± 0.06^{a2}	3.000 ± 0.00^{a3}	3.000 ± 0.00^{a3}

**Means value within the same column followed by different letters are significantly different ($p < 0.05$) whereas mean values within the same row followed by different figures are significantly different.*

3.2.3 Weekly effect of *P. guajava* on white blood cell (WBC) (10^3mm^3) level.

Table 12 showed the result of the weekly effect of the different concentration levels of the extract on WBC. It was observed that in the first and second week, the WBC of all dose levels of the extract increased significantly ($P < 0.05$) when compared to that of the control. In the third week, aqueous and methanol extracts of 400 mg/kg and 200 mg/kg produced comparable effect. WBC of aqueous extract of 200 mg/kg produced comparable effect ($P > 0.05$) to that of the control whereas other dose levels of the extract increased significantly ($P < 0.05$). It was also observed that the WBC of aqueous extract of 200 mg/kg and 400 mg/kg had decreased significantly ($P < 0.05$) in the fourth week when compared to that of the control. However there was a significant increase ($P < 0.05$) in all the other dose levels in the same week. Considering the effect of time on different extract administration, it was observed that WBC of methanol 200 mg/kg produced significant increase ($P < 0.05$) at the fourth week, while aqueous (200 and 800) mg/kg and methanol 400 mg/kg in the third week whereas aqueous 400 mg/kg in the second week. However some of the extract dose level produced comparable effect.

TABLE 12: Result of the effect of *Psidium guajava* on white blood cell (WBC) (10^3mm^3) level.

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	4517 ± 28.9^{c12}	5017 ± 28.9^{c3}	4883 ± 28.8^{a2}	4450 ± 0.00^{a1}
400	4802 ± 29.0^{e1}	6102 ± 2.89^{f3}	4983 ± 29.0^{b2}	4883 ± 28.6^{b1}
800	5017 ± 28.9^{f1}	5210 ± 17.3^{e2}	6183 ± 28.8^{e4}	5696 ± 5.77^{f3}
Methanol				
200	4617 ± 28.8^{d1}	4817 ± 28.8^{b12}	4997 ± 5.77^{b2}	5400 ± 0.00^{d3}
400	4416 ± 29.0^{b1}	5117 ± 28.9^{d2}	6116 ± 28.9^{d4}	5783 ± 28.8^{g3}
800	5283 ± 28.8^{g1}	6216 ± 28.7^{g4}	5783 ± 28.7^{c3}	5616 ± 28.9^{e4}
Control	4300 ± 0.00^{a1}	4696 ± 5.77^{a2}	4883 ± 28.7^{a3}	5083 ± 28.9^{c4}

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

3.2.4 Weekly effect of *P. guajava* on Haemoglobin (Hb) (g/DL) serum level

Table 13 showed the weekly effect of different dose levels of aqueous and methanolic extracts on Hb. It was observed that there was a significant increase ($P < 0.05$) in all the dose levels of the treatment group in the first week when compared to the control. It was also observed that in the second week, aqueous extract of 400 mg/kg and 200 mg/kg respectively produced comparable effects to methanol extract of 800 mg/kg while the other dose levels recorded a significant increase ($P < 0.05$). In the third week, methanol extract of 200 and 400 mg/kg produced comparable effects while aqueous extracts of 200 mg/kg and methanol extract of 800 mg/kg also produced comparable effects. However there was a significant increase in all the dose levels of the treatment group in the same week. It was observed that in the fourth week, there was a significant increase ($P < 0.05$) in all dose levels compared to the control, whereas aqueous and methanol extracts of 800 mg/kg and 200 mg/kg produced comparable effects. However with effect to time, dose levels of all aqueous extract produced significant increase ($P < 0.05$) in the third week, methanol 200 mg/kg in the fourth week while methanol 400 and 800 mg/kg in the first week.

TABLE 13: Results of the effect of *Psidium guajava* on haemoglobin (Hb) (g/DL) serum level

TREATMENT(mg/kg)	WEEK1	WEEK2	WEEK3	WEEK4
Aqueous				
200	12.93 ± 0.12^{b2}	14.96 ± 0.06^{d3}	16.16 ± 0.28^{c4}	12.16 ± 0.28^{b1}
400	13.87 ± 0.11^{d1}	15.28 ± 0.03^{e2}	17.93 ± 0.11^{e3}	15.26 ± 0.23^{e2}
800	14.63 ± 0.12^{e1}	16.93 ± 0.11^{f2}	17.30 ± 0.17^{d3}	14.83 ± 0.28^{d1}
Methanol				
200	13.33 ± 0.29^{c3}	11.13 ± 0.12^{b1}	12.43 ± 0.12^{b2}	14.63 ± 0.11^{d4}
400	16.97 ± 0.05^{f4}	13.83 ± 0.28^{c2}	12.53 ± 0.06^{b1}	14.43 ± 0.12^{c3}
800	17.80 ± 0.00^{g3}	15.07 ± 0.05^{de1}	15.96 ± 0.05^{c2}	16.06 ± 0.05^{f2}
Control	8.033 ± 0.05^{a1}	8.833 ± 0.28^{a2}	10.13 ± 0.11^{a3}	11.00 ± 0.00^{a4}

**Means 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 ($P < 0.05$).*

3.3 Weekly Effect of *P. guajava* on Body Weight of Albino Rats

Table 14 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.

TABLE 14: Results of the effect of *Psidium guajava* on body weight of albino rat

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 ($P < 0.05$).*

CHAPTER FOUR

DISCUSSION

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).

Results from this study showed that the oral administration of both aqueous and methanol extracts of *Psidium guajava* increased the activities of the liver maker enzymes. The presence of the maker enzyme in the serum may give knowledge on organ dysfunction. Usually an elevation in the liver maker enzymes may indicate inflammation or damage to the cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes into the blood which may lead to increased liver enzymes into the blood (Wells *et al.,* 1986).

Acid phosphatase (ACP) is a hydrolase class of enzymes that acts in acidic medium and found in high concentration in the liver, red blood cell and the prostrate (Sood, 2006). Alkaline phosphatase (ALP) is an enzyme of the hydolase class which acts in alkaline medium and it is found in high concentration in the liver and biliary tract epithelium (Sood, 2006). Aspartate aminotransferase (AST) is an enzyme found mainly in heart muscle, liver cells, skeletal musles and kidneys. Alanine aminotransferase (ALT) is found in a variety of tissues but mainly seen in

the liver (Sood, 2006). Cholesterol is a lipid seen in the brain tissues, blood and bile. It is needed for the formation of steroids and cellular membranes and it is found in high concentration in arteriosclerotic vascular disease. Thus a significant increase which was observed in these liver maker enzymes activities following the administration of the extracts may be due to increased functional activity of the liver thereby resulting to new synthesis of complex molecules from simple molecules (Yakubu *et al.,* 2001; Orisakwe *et al.,* 2003). Hameid (2007) in his research suggested that increase in these liver maker enzymes may be due to release of enzymes from damaged hepatocytes. The elevation in these concentration levels showed the hepatotoxicity of the liver. These maker enzymes are liver specific thus an increase in its level is a clear indication of injury to the hepatocytes of the liver (Ellis *et al.,* 1978). The result also showed a significant increase ($P < 0.05$) in serum cholesterol level. An increased level of cholesterol may be as a result of deposit on the inner layer of blood vessels. The increased blood deposits tend to block blood vessels causing decrease in the flow of blood thereby leading to a disease condition called atherosclerosis.

However at some concentration levels, there was a significant decrease compared to the control thus indicating a decrease hepatotoxicity of the liver. This effect may be due to the presence of flavonoids in the extract which are said to be hepatoprotective (Trease and Evans, 1989). This observation agreed with the work of Sai *et al*. (1998) using green tea (*Camelia siemensis*) which attributed the hepatoprotection observed in their study to the presence of polyphenol. The significant decrease may be as a result of malnutrition, malabsorption, anemia or liver diseases (Sood, 2006). This also agreed with the result of *Hisae et al.,* (2002) who observed a significant decrease ($P > 0.05$) in ALT and AST of rats treated with guava leaves extract whereas the extract did not affect cholesterol in the liver.

In contrast however, Uboh *et al.* (2010) observed in his research that treatment of rats with aqueous extract of *P. guajava* leaves have no significant effect ($P > 0.05$) on the activities of the serum liver diagnostic enzymes ALT, AST and ALP as well as the concentrations of serum total proteins and albumins, compared respectively with the control. This observation indicated that the aqueous extract of *P. guajava* leaves did not exhibit any remarkable hepatotoxic effect in the animal model and his histopathological study also did not show any adverse alteration in the morphological architecture of the liver tissues in both sexes of the animal model.

The determination of haematological 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 haematological parameters. This observation is in agreement with Uboh (2010), who stated that red blood cell, haemotocrit and haemoglobin 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* leaf 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 radiolabelling 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 suggest 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 haematological 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 as a result of its effect on blood cells and body weight respectively.

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