

Effects of Methanol Extract of *Parkia biglobosa* Stem Bark on the Liver and Kidney Functions of Albino Rats

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Abstract: Extract of *Parkia biglobosa* stem bark is used in treating of malaria, diarrhoea, pains and host of other diseases in Nigerian traditional medicine (NTM). The aim of this research was to determine the beneficial properties of *Parkia biglobosa* as drug and its effect on the vital organs (such as liver and kidney). To achieve this, parameters such as ALP, ALT, AST, catalase, urea, PCV, RBC count, MDA and creatinine concentrations in the blood were evaluated as well as the phytochemical screening. The results revealed that the extract did not induce significant changes in some of the hepatic and hematological parameters determined (such as ALT, MDA, AST, catalase and RBC). A decrease in alkaline phosphatase activity and PCV concentrations were also recorded. These results suggest that the hepatic integrity of the rats were preserved as the parameters evaluated did not show alteration in concentrations and activity of hepatic markers. On the other hand, urea and creatinine levels increased significantly indicating possibility of kidney damage. The phytochemical screening revealed the presence of tannins, alkaloids, plant-protein, flavonoids, saponins, terpenes, glycosides and reducing sugars in the methanol extract. This study has shown the functional characteristics and effects of the methanol extracts of the stem bark of *P. biglobosa* on liver and kidney function of albino rats in short time treatment with the extract as the liver remained Intact whereas the kidney reflected changes.

Key words: *Parkia biglobosa* • Stem bark • Hepatic • Hematological • Phytochemical • Liver and Kidney Functions

INTRODUCTION

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have beneficial effect on long-term health when consumed by humans and can be used to effectively treat human diseases. At least 12,000 of such chemicals have been isolated so far; a number estimated to be less than 10% of the total [1]. Most of the claims of efficacy of the extracts derived from *P. biglobosa* stem bark have been scientifically established, little information on their effects on body organs (kidney and liver) are however, available.

P. biglobosa have been used in Nigeria and other West African rural communities to treat variety of diseases [2]. The efficacy of the various preparations of *P. biglobosa* is widely acclaimed by the Hausa communities of northern Nigeria for the treatment of such diseases as malaria, diabetes mellitus and pains. The stem

barks is boiled in water and taken as a decoction for the treatment of malaria, inflammatory diseases and infections to diarrhoea [3]. The bark soaked in ethanol is also used in some communities for antidiarrhoeal properties and as an effective anti- snake venoms that protects against neurotoxic, haemotoxic and cytotoxic effects of poisonous snakes [4]. Also, the leaves, fruits and seeds of *P. biglobosa* have also been used to manage various diseases [5].

The bark is also used with lemon for wounds and ulcers. In cote d' Ivoire and Nigeria, bark infusion is used as a tonic for diarrhea [6]. Bark is used as a mouthwash, vapour inhalant for toothache, or for ear complaints. It is macerated in baths for leprosy and used for bronchitis, pneumonia, skin infections, sores, ulcers, bilharzia, washes for fever, malaria, diarrhoea, violent colic and vomiting, sterility, venereal diseases, guinea worm, oedema and rickets and as a poison antidote. The leaves are also used for burns and toothache as well as for sore



Fig. 1: Bark, flower pod, leaves, seeds of *Parkia biglobosa*
Source: [10].

eyes in Gambia [7]. Recently, the attention of researchers has been drawn to the great potentials in *Parkia biglobosa* as a source of an antibacterial agent. [8], have all reported the presence of plant secondary metabolites which are known to exhibit antibacterial activity against a wide range of organisms. This paper presented the report of the efforts made to identify the compounds in the stem bark of *Parkia biglobosa* responsible for their bioactivity.

The origin of *Parkia biglobosa* is traced to the West African sub-region where it was first encountered by the Scottish surgeon, Mungo Park as he explored the Niger basin between 1795–1799. He went ahead to describe this tree in his writing “Travels in the interior districts of Africa. Robert Brown described the genus *Parkia* in 1826. He named it after Mungo Park, who made 2 remarkable journeys of exploration into the interior of West Africa in 1795-1797 and 1805. *Parkia biglobosa* is a multipurpose fodder tree that belongs to the family *Mimosaceae*.

Parkia biglobosa occurs in a belt between 5°N and 15°N, from the Atlantic coast in Senegal to southern Sudan and northern Uganda. The belt is widest in West Africa (maximum 800 km) and narrows to the east. It was probably introduced to São Tomé and Príncipe. Trial plantations have been established in Tanzania and African locust bean was introduced to the Caribbean region over 200 years ago, probably as a consequence of the slave trade and later possibly to Guyana. The use of the fermented beans of African locust bean dates back many centuries and was already described in the 14th century.

African locust bean is a multipurpose tree that is as highly valued as Shea butter tree. Fermented seeds (‘soumbala’, ‘dawadawa’, ‘netetu’) serve primarily as a condiment for seasoning sauces and soups. Roasted

seeds are used as a coffee substitute known as ‘Sudan coffee’ or ‘café nègre’. The mealy pulp from the fruits is eaten or is mixed with water to make a sweet and refreshing drink rich in carbohydrates. Boiled pods are used to dye pottery black; the ash is applied as a mordant. The bark is rich in tannins and may be used for tanning hides, but the resulting leather is often of moderate quality especially with regard to colour, which is often reddish, uneven and darkens when exposed to light. The leaves are sometimes eaten as a vegetable, usually after boiling and then mixed with other foods such as cereal flour. Young flower buds are added to mixed salads. In West Africa the bark, roots, leaves, flowers, fruits and seeds are commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is the most important for medicinal uses, followed by the leaves [9]. Medicinal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension and disorders of the respiratory system, digestive system and skin. In veterinary medicine, a root decoction is used to treat coccidiosis in poultry. Green pods are crushed and added to rivers to kill fish. The nutritional value of the fish is not adversely affected so long as they are cooked or dried. The fruit pulp is used as an ingredient of feed for pigs and dogs. The seeds are added to poultry feed after treatment to remove their anti-nutritional properties. The leaves are a useful, but not very palatable fodder.

Aim of the Study: The aim of the present study was to extract and analyse the various phytochemicals in *P. biglobosa* and check their effects on organ functions of albino rats.

Objectives of the Study: To achieve the above aim, the following were done:

- Qualitative determination of phytochemicals
- Determination of ALT activity
- Determination of ALP activity
- Determination of AST activity
- Determination of catalase activity
- Determination of urea concentration
- Determination of malondialdehyde concentration
- Determination of packed cell volume
- Determination of red blood cell concentration
- Determination of creatinine concentration

MATERIAL AND METHODS

Materials

Plant Material: The stem bark of *P.biglobosa* was collected in the month of November, 2012 at the University of Nigeria. The authentication was done by Pharm. Ezea of Pharmacy Department, University of Nigeria, Nsukka. The plant material was cleaned and dried under shade to avoid destruction of active compounds. The dried material was ground with an electric grinder into powder. This was stored in an air-tight container ready for extraction.

Animal Material: The animals used in this work were wister albino rats of both sex of about 12-18 weeks. They were obtained from an animal house at Nsukka. The animals were allowed to acclimatize in the environmental conditions for one week, with 12 hours light/dark cycle maintained on a regular vital feed and water.

Instruments/Equipment:

Beakers	Pyrex
Centrifuge	PAC, Pacific
Conical flask	Pyrex
Dissecting set	Gold cross
Filter paper	Whatman
Glass funnels	Kimax
Laboratory mill	Thomas-Wiley (model 4)
Measuring cylinder	Pyrex
Micropipette	Perfect
Mortar	Lifescan (J and J)
pH meter	Ecoscan
Pipette	Technico
Refrigerator	Thermocool
Separating funnel	Pyrex
Spatula	Pyrex
Syringe	Lifescan

Test tube	Pyrex
Thermometer	Zeal
Water bath	Gallenkamp, London
Weighing balance	Metler HAS
Microheamatocrit reader	London
Triple beam balance (MB 2610)	London
Digital photo colorimeter	German
Havard trip balance 2kg-5lb capacity.	Harvard
Chemicals and Reagents:	
Absolute methanol	BDH, England
Acetone	Sigma, London
Aluminium chloride	BDH, England
Ascorbic acid	May & Baker
Bismuth carbonate	BDH, England
Chloroform	Sigma, London
Carbon tetrachloride	Cartivalue
Disodium hydrogen phosphate	Merck
Distilled water	STC, UNN
Ethyl acetate	BDH, England
Fehling's solutions A and B	BDH, England
Ferric chloride	Merck
Ferrous sulphate	BDH, England
Glacial acetic acid	Sigma, London
Hydrochloric acid	BDH, England
Iodine crystals	Merck
Lead acetate	BDH, England
Millon's reagent	BDH, England
Mercuric chloride	Sigma, London
Naphthylene diamine dihydrochloride	BDH, England
Olive oil	Goya, Nigeria
Picric acid	Lab Tech Chemicals, London
Potassium dihydrogen sulphate	BDH, England
Potassium iodide	East Angelis
Potassium sulphate	May & Baker, England
Potassium hydroxide	May & Baker, England
Potassium chloride	Merck
Phosphoric acid	Sigma, London
Saponin	Lab tech Chemicals, London
Sodium tungstate	BDH, England
Sulphuric acid	BDH, England
Tannic acid	Sigma, London
Trichloroacetic acid	BDH, England
Tween 80	Sigma, London

Methods

Preparation of Methanol Extract of *P.Biglobosa* Stem

Bark: Pulverized leaf sample of *P.biglobosa* (500g) was weighed out into a beaker; using Harvard trip. balance of 2Kg- 5lb (5lb was converted to 200g). Using measuring cylinder, 1000ml of methanol was measured and added into the bottle and was allowed to stand for 48h. After 48h, it was filtered using whatman filter paper into a conical flask (500ml×3). The filtrate was poured into a disc plate to enable evaporation at room temperature for 5 days, leaving the extract of the *P.biglobosa* leaf in the bowl. Using spatula the extract was collected and stored in a sample collection container at room temperature.

Determination of Percentage Yield:

$$\% \text{ YIELD} = \frac{\text{Weight of extract}}{\text{Weight of pulverized stem bark}} \times 100$$

Determination of Phytochemicals

Reagents Preparation: The phytochemical was carried out based on the procedures outlined by [11].

Preparation of Reagents for Phytochemical Analysis

5% (w/v) Ferric Chloride Solution: 5.0 g of ferric chloride was dissolved in 100 ml of distilled water.

Ammonium Solution: 187.5 ml of the stock concentrated ammonium solution was diluted in 31.25 ml of distilled water and then made up to 500 ml with distilled water.

45% (v/v) Ethanol: 45 ml of absolute ethanol was mixed with 55 ml of distilled water.

Aluminium Chloride Solution: 0.5 g of aluminium chloride was dissolved in 100 ml of distilled water.

Dilute Sulphuric Acid: 10.9 ml of concentrated sulphuric acid was mixed with 5.0 ml of distilled water and made up to 100 ml.

Lead Sub-Acetate Solution: 45 ml of 15% lead acetate (i.e. 15.0 g of lead acetate in 100 ml of distilled water) was dissolved in 20 ml of absolute ethanol and made up to 100 ml with distilled water.

Wagner's Reagent: 2.0 g of iodine crystals and 3.0 g of potassium iodide were dissolved in a 40 ml of distilled water and then made up to 100 ml (with distilled water).

Mayer's Reagent: 13.5 g of mercuric chloride was dissolved in 50 ml of distilled water. Also, 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. The two solutions were mixed and the volume made up to 100 ml with distilled water.

Dragendorff's Reagent: 0.85 g of bismuth carbonate was dissolved in 100 ml of glacial acetic acid and 40ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water. Both solutions were mixed to give a stock solution.

Molisch Reagent: 1.0 g of α -naphthol was dissolved in 100ml of absolute ethanol.

2% (v/v) Hydrochloric Acid: 2.0 ml of concentrated hydrochloric acid was diluted with some distilled water and made up to 100 ml.

1% (w/v) Picric Acid: 1.0 g of picric acid was dissolved in 100 ml of distilled water.

Qualitative Phytochemical Analysis of *P. Biglobosa*:

The phytochemical analysis of the plant was carried out on both fresh and dried samples according to the method of [11] in order to identify its active constituents.

Test For Alkaloids: 0.2 g of the sample was boiled with 5 ml of 2% HCl on a steam bath. The mixture was filtered and 1ml portion of the filtrate was treated with 2 drops of the following reagents.

Dragendorff's Reagent: An orange precipitate indicates the presence of alkaloids.

Mayer's reagent: A creamy-white precipitate indicates the presence of alkaloids.

Wagner's Reagent: A reddish-brown precipitate indicates the presence of alkaloids.

Picric Acid (1%): A yellow precipitate indicates the presence of alkaloids.

Test For Flavonoids: 0.2 g of the sample was heated with 10 ml ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrate was used for the following tests.

Ammonium Test: 4 ml of the filtrate was shaken with 1 ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicates the presence of flavonoids.

Aluminium Chloride Test: 4 ml of the filtrate was shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow colouration that indicates the presence of flavonoids.

Test For Glycosides: About 2.0 g of the sample was mixed with 30 ml of distilled water and heated in a water bath for 5 minutes. The mixture was filtered and the filtrate used for the following tests. 0.3 ml of Fehling's solutions A and B was added to 5 ml of the filtrate until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides.

About 15 ml of dilute sulphuric acid was used instead of distilled water; the above process was repeated and filtered. 0.3 ml of Fehling's solutions A and B was added to 5 ml of the filtrate until it turned alkaline (tested with litmus paper) and heated on a water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides.

Test for Proteins: About 5 ml of distilled water was added to 0.1 g of the sample. The mixture was left to stand for 3 hours and then filtered. 2 ml portion of the filtrate was added to 0.1 ml of Millon's reagent. The mixture was shaken and kept for observation. A yellow precipitate indicates the presence of proteins.

Test For Carbohydrates: About 0.1 g of the sample was shaken vigorously with water and filtered. To the aqueous filtrate was added few drops of Molisch reagent followed by vigorous shaking again. Then, 1 ml of concentrated sulphuric acid was carefully added down the side of the test tube to form a layer below the aqueous solution. A brown ring at the interface indicates the presence of carbohydrates.

Test For Reducing Sugars: A quantity, 0.1 g of the sample was shaken vigorously with 5 ml of distilled water and filtered. To the filtrate was added equal volumes of Fehling's solutions A and B and shaken vigorously. A brick-red precipitate indicates the presence of reducing sugars.

Test For Saponins: About 0.1 g of the sample was boiled with 5 ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests.

Emulsion Test: A quantity, 1 ml of the filtrate was added to two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

Frothing Test: A quantity, 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

Test for Tannins: A quantity, 2 g of the sample was boiled with 5 ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions.

Lead Sub Acetate Solution: To 1 ml of the filtrate was added 3 drops of lead sub acetate solution. A gelatinous precipitate indicates the presence of tannins.

Bromine Water: To 1 ml of the filtrate was added 0.5 ml of bromine water and then observed for a pale brown precipitate.

Ferric Chloride Solution: A quantity, 1 ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

Test for Oils: A quantity, 0.1 g of the sample was pressed between filter papers and the papers observed. Translucency of the filter paper indicates the presence of oils.

Test for Resins

Precipitate Test: A quantity, 0.2 g of the sample was extracted with 15 ml of 96% ethanol. The alcoholic extract was poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Colour Test: A quantity, 0.12 g of the sample was extracted with chloroform and concentrated to dryness. The residue was redissolved in 3 ml of acetone and 3 ml of concentrated HCl added. and heated in a water bath for 30 minutes. A pink colour which changes to magenta indicates the presence of resins.

Test for Terpenoids and Steroids: About 9 ml of ethanol was added to 1 g of the sample and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath and 5 ml of hot water was added. The mixture was allowed to stand for 1 hour and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml

of the chloroform extract in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface shows the presence of steroids.

Another 0.5 ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicates the presence of terpenoids.

Animal Studies: The animals used in this work were Wistar albino rats of about 12-18 weeks. The animals were acclimatized for one week under standard environmental condition, with 12 hours light/dark cycle maintained on a regular vital feed and water.

The sixteen rats were weighed and grouped into four (3) cages: A and B and Control according to their body weight. Each cage contained four rats per cage. Each rat in each cage was marked with picric acid to differentiate them. The rats received methanol extract of *P.biglobosa* stem-bark extract orally for a period of 14 days except the control that received only feed and water. The rats in cage-A received 200mg/kg of the extract, cage-B received 400mg/kg of the extract and cage C -control received only feed and water. The dose was administered to the rats according to their body weight as shown below.

$$\text{VOL. AMOUNT} = \frac{\text{mg/Kg b.w}}{1000} \times \frac{\text{Wt. of animal in grams}}{\text{Conc. in mg/ml of extract}}$$

STOCK CONC = 1g = 20ml of normal saline

1000 mg = X

X = 50mg/ml.

b.w = body weight

Preparation of Normal Saline: 0.9% normal saline was prepared by dissolving 0.9g NaCl in 100ml of distilled water.

Preparation of Drugs: 1g of the extract was dissolved in 20ml of 1% normal saline and used for just 2 days to avoid fungi and bacteria attack.

Haematological Studies of *P.biglobosa* Extract on Rats Determination of Packed Cell Volume

Principle: When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

Method: Using microhaematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until they are approximately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with plastic seal and placed in the medial grooves of the centrifuge, head exactly opposite each other, with the sealed end away from the centre of the centrifuge. All tubes were spun for five minutes at 1000 rpm. The tubes were removed as soon as the centrifuge had stopped spinning.

Calculation: PCV was obtained for each tube using microhaematocrit-reader by measuring the height of the RBC column and expressing this as a ratio of the height of the total blood column.

$$\text{PCV (\%)} = \frac{\text{Height of cell column}}{\text{Height of total blood column}} \times 100$$

Determination of Red Blood Cell Counts (RBC)

Principle: When whole blood is diluted with an isotonic fluid, it prevents lysis and facilitates counting of the red cells. Some isotonic solutions in use include Hayem's solution, Gower's solution or 0.85% NaCl solutions.

Method: Using the Thoma (manual counting) method, anticoagulated blood was drawn up to the 0.5ml mark in the RBC count pipette and diluted to a 101 mark with RBC diluting fluid (1: 200 dilution). Dilution was repeated with the replicate tube. Counting chamber was cleaned; both pipettes were shaken three times; counting chamber filled (first expelling the first 4 drops of the mixture), allowing approximately three minutes for the RBCs to settle. Red cells were counted using the counting steps as follows:

The filled counting chamber was carefully placed on the microscope stage.

Using low power (x10 objective) the large centre square was placed in the middle of the field of vision and the entire large square was carefully examined for even distribution of RBCs.

The high-dry objective was carefully changed, moving the counting chamber so that the small upper left corner square (this square is further sub-divided into 16 even smaller squares) is completely in the field of vision.

All the RBCs were counted in the squares, also counting the cells on the two of the margins but excluding those lying on the other two sides.

Calculation:

The RBCs (in mm^3) = cells counted \times correction for volume \times correction for dilution
 = RBCs counted in 5 small squares $\times 200 \times 1.0/0.2$ (or 50)
 = number of RBCs counted in five squares $\times 10^4$

Analysis of P.biglobosa Extract on Rats Liver Markers Determination of Alanine Aminotransferase (ALT)

Principle: ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540 nm.

Method: The blank and sample test tubes were set up in duplicates. 0.1 ml of serum was pipetted into the sample tubes. To these were added 0.5 ml buffer solution containing phosphate buffer, L-alanine and α -oxoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37°C ml and pH 7.4. 0.5 ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to both tubes while 0.1 ml of sample was added to sample blank tube. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25°C . About 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 540 nm.

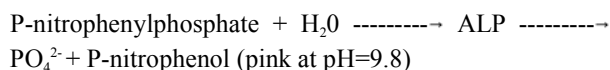
Determination of Aspartate Aminotransferase (AST)

Principle: AST or SGOT is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 546 nm.

Method: The blank and sample test tubes were set up in duplicates. About 0.1ml of serum was pipetted into the sample tubes. 0.5 ml of Reagent 1 was pipette into both sample and blank tubes. The mixtures were thoroughly mixed and incubated for exactly 30 minutes at 37°C ml and pH 7.4. 0.5 ml of Reagent 2 containing 2, 4-dinitrophenylhydrazine was added into all the test tubes followed by 0.1 ml of sample into the blank tubes. The tubes were mixed thoroughly and incubated for exactly 20 minutes at 25°C . 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minutes at 546 nm.

Determination of Alkaline Phosphatase (ALP)

Principle: The principle is based on the reaction involving serum alkaline phosphatase and a colorless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns pink that can be determined spectrophotometrically.



Method: The blank and sample test tubes were set up in duplicates. 0.05 ml of sample was pipetted into the sample test tubes. 0.05 ml of distilled water was pipetted into the blank tube. 3.0 ml of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at 405 nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one minute intervals.

Calculation: Alkaline phosphatase was calculated as follows:

$$\text{Activity of ALP (in U/L)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 3300$$

RESULTS

Qualitative Phytochemical Analysis: The phytochemical test is used to analyze the bio-active compounds (phytochemicals) present in the plant extract and the degree of occurrence. The more the plus (+) signs, the more level of occurrence. The minus (-) sign represents no occurrence.

Table 1: Phytochemistry table

Chemical compounds	Methanol extract
Alkaloids	+++
Flavonoids	+++
Glycosides	+++
Acidic compounds	—
Saponins	+++
Tannins	+++
Reducing sugar	++
Plant proteins	+++
Fats and oil	—
Resins	—
Carbohydrate	+++
Steroids	+
Terpenoids	+

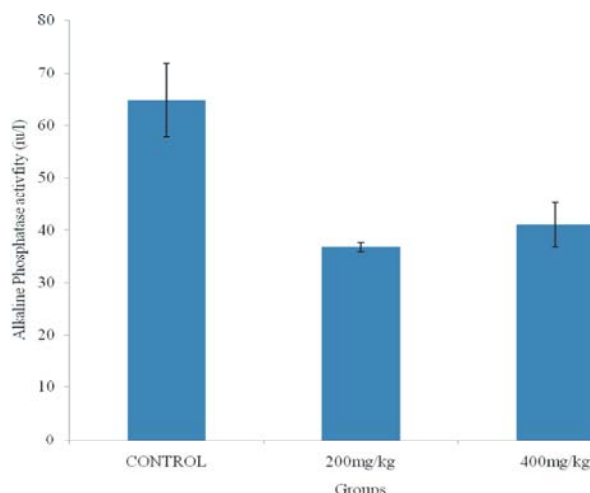


Fig. 2: Alkaline Phosphatase Activity in the rats treated with *P. biglobosa*

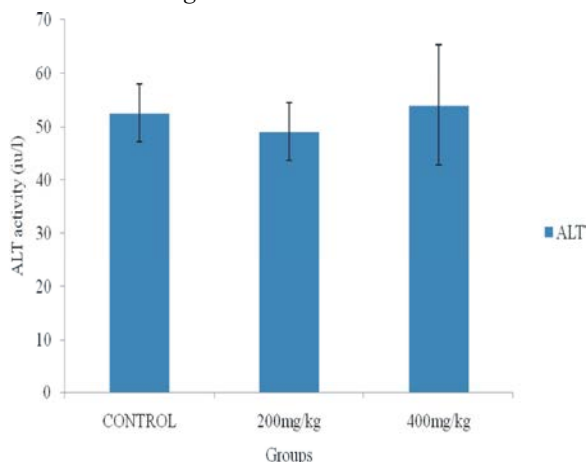


Fig. 3: Alanine Amino Transferase Graph activity in rats treated with *P. biglobosa*

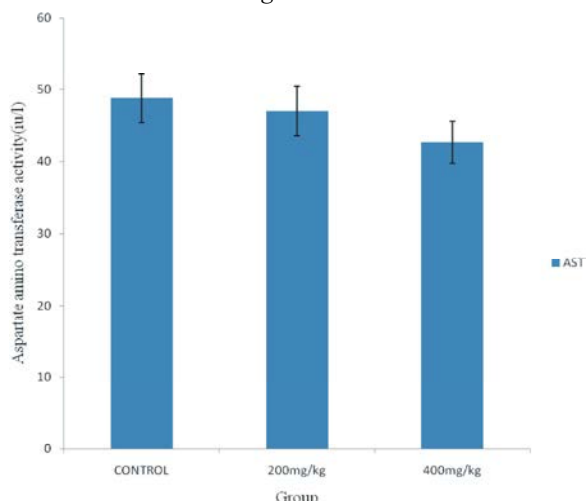


Fig. 4 : Aspartate amino transferase activity in rats treated with *P. biglobosa*

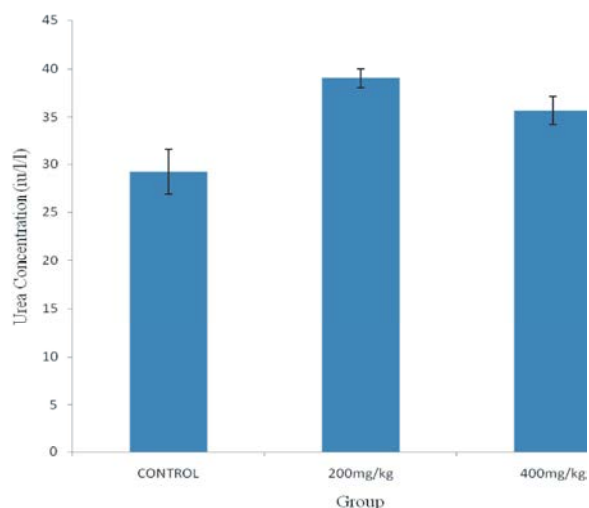


Fig. 5: Urea concentration in blood serum of rats treated with *P. biglobosa*

Determination of Alkaline Phosphatase (ALP) in Rats:

The result in Figure 2 shows that at $P>0.05$, there was significant decrease in the level of Alkaline phosphatase with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Alanine Amino Transferase (ALT) in rats:

The result in Figure 3 shows that at $P>0.05$, there was no significant difference in the level of Alanine Amino Transferase with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Aspartate Transaminase (AST):

The result in Figure 4 shows that at $P>0.05$, there was no significant difference in the level of Aspartate amino transferase with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Urea in Rats:

The result in Figure 5 shows that at $P>0.05$, there was significant increase in the level of Urea with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Creatinine in Rats:

The result in Figure 6 shows that at $P>0.05$, there was a significant increase in the level of serum Creatinine with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Malondialdehyde (MDA) in Rats:

The result in Figure 7 shows that at $P>0.05$, there was no significant difference in the level of serum malondialdehyde with respect to the control at extract concentrations of 200mg/kg and 400mg/kg.

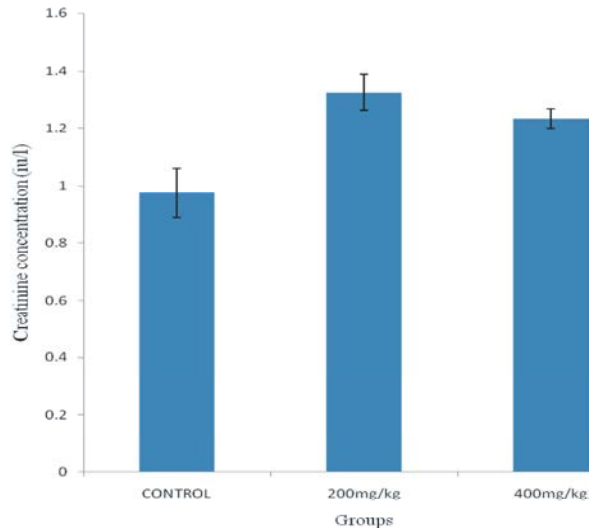


Fig. 6: Creatinine concentration in rats treated with *P. biglobosa*

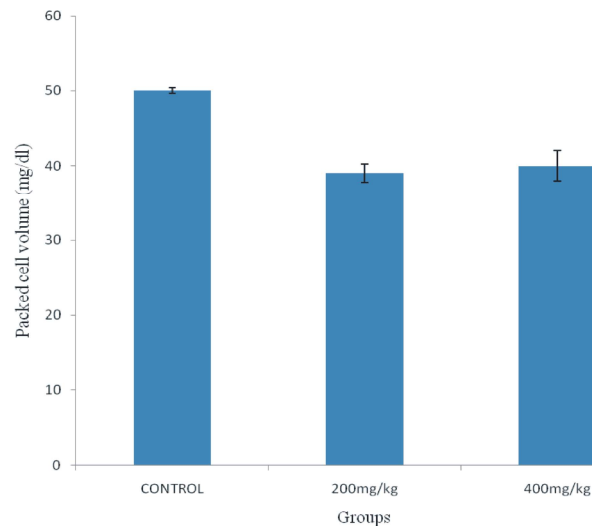


Fig. 9: Packed Cell Volume Test Graph

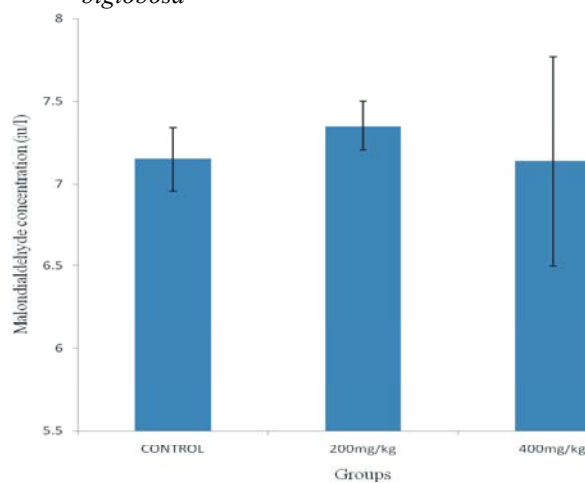


Fig. 7: Malondialdehyde concentration in rats treated with *P. biglobosa*

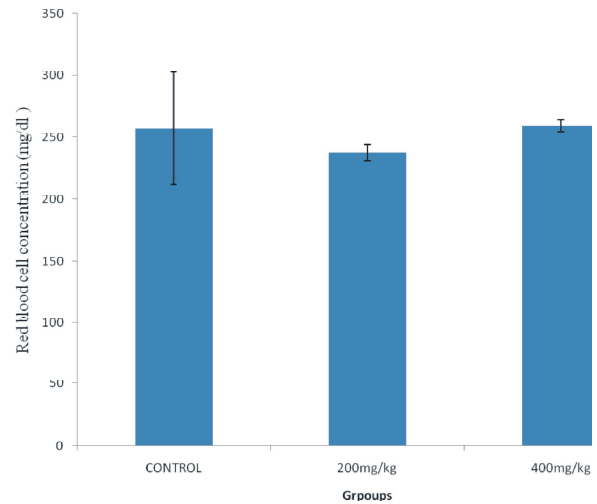


Fig. 10: Red Blood Count Graph concentration in rats treated with *P. biglobosa*

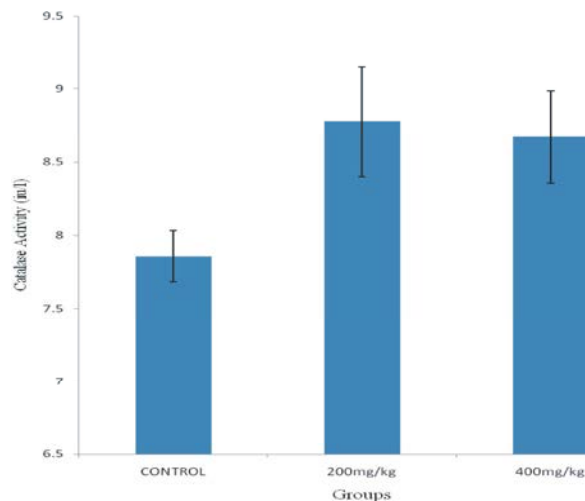


Fig. 8: Catalase activity in rats treated with *P. biglobosa*

Determination of Catalase Activity in Rats: The result in Figure 8 shows that at $P > 0.05$, there was no significant difference in the level of catalase with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Packed Cell Volume in Rats: The result in Figure 9 shows that at $P > 0.05$, there was no significant difference in the level of Packed cell volume with respect to the control at concentrations 200mg/kg and 400mg/kg.

Determination of Red Blood Cells in Rats: The result in Figure 10 shows that at $P > 0.05$, there was no significant difference in the level of Red blood cells with respect to the control at concentrations 200mg/kg and 400mg/kg.

DISCUSSION

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures disease preventive properties. Plants contain secondary metabolites (phytochemicals), which are present in various concentrations. These phytochemicals include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others. The active properties of many drugs found in plants are secondary metabolites [12]. Therefore, basic phytochemical investigation of *P. biglobosa* extracts for their major phytoconstituents is also vital. The hematological, liver, kidney and phytochemical analysis of *P. biglobosa* were investigated to determine any functional effect that may arise as a result of a short time animal exposure to the extracts within 24h period. The results of the study showed decrease in serum alkaline phosphatase level with respect to control and this was synonymous with the result of [5], who investigated the toxicity of the stem bark of *P. biglobosa* and found alkaline phosphatase level to be lower than that of the control at different doses of the extract administered. A rise in alkaline phosphatase level is usually a characteristics found in cholestatic liver disease [13]. As such, the significant reduction in ALP levels by the methanol extract of *P. biglobosa* shows that no possible cholestasis occurred at the dose levels tested [13]. There was also significant decrease in the plasma PCV of the rats at the end of the study. This could be as a result of feeding factor. On the other hand, there was an increase in serum creatinine and urea concentration relative to the control. However, the result was in contrast with the result of [5] that recorded no change in levels of urea and creatinine. It is worthy of note that at 400mg/kg dose, two out of four rats died making 400mg/kg the LD₅₀ (lethal dose-dose that killed 50% of the animals) and this also contrasted work of [5], who at 5000mg/kg dose recorded no casualty. The serum concentration of AST, ALT, MDA, catalase as well as the concentration of RBC did not show any significant difference with the concentration of same parameters in the control.

Furthermore, the phytochemical analysis gave hint on the potentials of the stem bark of *P. biglobosa* and the possible explanation to results of the parameters above. The result showed an appreciable amount of flavonoid in the methanol extract of *P. biglobosa* and this corresponds with the result of [14], who recorded the same high concentration of flavonoids. A number of investigators have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including

arginine and glutamic acids possess hypoglycemic effects in various experimental animals model [15]. However, this hypothesis stipulates that plant which contain terpenoid and/or flavonoids possess hypoglycemic activities in diabetic and normal mammal. Therefore the hypoglycemic activity of the methanol extract of stem bark of *P. biglobosa* is confirmed due to the terpenoid present, which appears to be involved in the stimulation of the β -cells of the pancreas and the subsequent secretion of preformed insulin. One or more of the other chemical constituents of the plant especially flavonoid is also likely to have played a crucial role in the hypoglycemic action of the plant extract [14]. Saponin, plant protein and tannins were also present in appreciable amount. Study conducted by [16], showed that a large intake of tannins may cause kidney and liver damage.

CONCLUSION

This study has shown the effects of methanol extract of stem bark of *Parkia biglobosa* on not only hepatic and renal functions, but also on the general well being of the body. It can be said that methanol extract of stem bark of *P. biglobosa* due to presence of flavonoids and no change in malondialdehyde levels could possess good anti-oxidant properties. The extract seemed to be liver friendly as none of the liver markers were altered. However, renal function seem to have been implicated which reflected in rise in urea and creatinine concentrations.

Recommendation: At the 400mg/kg dose, two out of four rats died (which supposes an LD₅₀), however toxicological study was not carried out in this study to find the cause of the deaths. On this ground, We recommend that major toxicological studies should be done on the methanol extract of stem bark of *P. biglobosa*. Apart from the casualties recorded, methanol stem bark extract of *P. biglobosa* seem to be a good raw material for drug making as there were appreciable amounts of favourable phytochemicals present and the hepatic markers of the rats were preserved at the end of the study.

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