



Bioactive Principles Present in Aqueous Extracts of Oil Bean (*Pentacletra macrophylla* Benth) and their Effect in the Haematological and Biomedical Profile of Male Albino Wistar Rats

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ABSTRACT

The bioactive principles present in oil bean (*Pentaclethra macrophyllabenth*) and their effects in the haematological and biochemical profile of male albino wistar rats were studied using 22 male albino wistar rats weighing 180–200g. The study is aimed at ascertaining the impact of prolonged administration of *Pentaclethra macrophyllabenth* on the haematological and biochemical profile of albino wistar rats. The 22 male albino rats were acclimatized in the animal house for 14 days. After this, they were divided into three groups (A, B, and C) respectively. Group A was the control group (6 rats), Group B (8 rats) on low dose of the extracts, while Group C (8 rats) on high dose of the extract. Group B and C received the extract for 28 days while Group A received normal rat feed and water ad libitum. Blood samples were collected from the animals by cardiac puncture and placed in E.D.T.A (Ethylene diamine tetracetic acid) and in plain tubes for the analysis. Results showed that *Pentaclethra macrophyllabenth* increased the haemoglobin concentration, packed cell volume, platelet count, and WBC count. It also caused elevation of the liver function profiles in the test rats due to prolonged administration. It could be deduced from this research that the extract of *Pentaclethra macrophyllabenth* contains some bioactive principles capable of altering the biochemical and haematological profile of rats.

INTRODUCTION

The African oil bean (*Pentaclethra macrophyllabenth*) is a tropical tree crop. The plant is mostly found in the forest of eastern, western, and central Africa. Grows approximately 6 meters in girth, and 21 meters in height, (Keay et al 1969). It belongs to leguminous family and subfamily of mimosoidae, (Keay 1969). Okafor (1982) recognized *Pentaclethra macrophyllabenth* as a food tree species for outlying farms in the forest zone. Though there are several staple foods such as maize, rice, beans, etc., yet there are still great needs to supplement these food products with certain unpopular food which are equally packed with loads of essential vitamins needed for healthy functioning of the body; one of such is the African oil bean seed. The African oil bean seed is an excellent source of energy, protein, amino acids, phosphorous, magnesium, iron, vitamins, calcium, manganese, and copper (Achinewhu, 1980). It is an excellent source of phytonutrients such as tannins, alkaloids, flavonoids, sterols, glycosides, and saponins (Ikhuoria et al, 2006). Notwithstanding, the high nutritional contents of the African oil bean seeds studies revealed that the fermentation process which they undergo before consumption usually eradicates most of these minerals and vitamins such as phosphorous (Enejiugha et al, 2005).

The economical and medical application of oil bean seed include; due to its anti-inflammatory properties, both the bark and the seeds can be used for producing local ointment for treating itches, insect bites, cuts, and wounds, the bark for African oil bean tree exhibits anti-helminthic properties, thus can be used for preparing herbal remedies for treating and destroying parasitic worms. African oil bean seeds contain saponins, and alkaloids that possess antibacterial properties, thus can be used for fighting *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Bacillus subtilis* (Cugnani et al, 1985). The African oil bean seeds are an excellent source of oil, thus can be used commercially for producing soaps, candles, and cooking oil. The edible seeds are enclosed in brownish shells which can be used for decorations and for making beads, traditional dancing costumes, dresses, rosaries, hand bangles, bags and necklaces. The woods serve as fire woods, and can also be used for generating charcoals. It can also be used as a source of dietary estrogen (Phytoestrogen). Dietary estrogen can be employed in nutritional supplements and pharmaceutical preparations and vitamins supplements to provide better nutrition and aid in controlling obesity (Okorie et al, 2006).

Various use of African oil bean in human life prompted our curiosity to embark on this study to see if the oil bean seed can be used to control the haemopoietic and biochemical profile of albino wistar rats, to find out if the oil bean seed has any toxic effects on the liver enzymes and to know if the bioactive principles present can be used to reduce high haemoglobin concentration as in polycythemia or

improve low haemoglobin concentration as in anaemic rats.

MATERIALS AND METHODS

ANIMALS:

The 22 male albino wistar rats were randomly selected and were kept in animal house in wire mesh cages under standard conditions (temperature 25-30°C; 12hr light and 12hr cycles) with free access to food and water ad libitum. They were stabilized for two weeks before being used for the experiment.

EXPERIMENTAL DESIGNS:

Twenty-two male albino wistar rats weighing 160-180g were randomly selected and divided into three groups namely groups A, B, and C. Group A (control) consists of 6 rats. Group B and C are the test group receiving low dose (Grp B) and high dose (Grp C) of aqueous extract of oil bean seed. They were fed for 28 days with the extracts and water ad libitum, while the control rats were fed with rat feed and water ad libitum.

PREPARATION OF AQUEOUS Oil bean seed;

The oil bean seeds were bought from local market around the university. The specie was identified and authenticated by a taxonomist in biological science before being used. 200g of the oil bean seed were boiled in a liter of distilled water 1hr 30mins. It was filtered, and allowed to cool at room temperature. The hard shells were broken and the seeds removed, washed, cut, and blended using mortar and a pestle (Samuelson et al, 1992). They were soaked in water overnight in 500ml of distilled water and filtered the following day using Whatman No.1 filter paper. A part of the filtrate 300ml was double diluted to be used (1.0ml) for group B on low dose while the remaining filtrate was used undiluted (neat) in the volume of 1.0ml for group C rats on high dose of the aqueous extract of oil bean seed.

PHYTOCHEMICAL ANALYSIS OF OIL BEAN SEEDS:

The oil bean seeds were screened for the presence or absence of various metabolites using standard phytochemical screening procedures as described by Harbourne (1973) and Trease et al, 1996. The extracts were tested for calcium, reducing sugar, flavonoids, tannins, glycosides, alkaloids, saponins, acidic compounds, resins, fats and oil, carbohydrates, and steroids.

TOXICITY STUDIES:

The toxicity study (LD_{50}) of the extract in albino wistar rat was determined using Lorke's method. The procedure of determining the lethal dose is by increasing the

concentration of the extracts administered into the rats (per body weight) in each group of eight (8) rats for five days. The doses used were 1000mg/kg, 2000mg/kg, 2,500mg/kg, 3000mg/kg, 4000mg/kg. The mortality rate was determined after 18hrs and analyzed graphically.

BIOCHEMICAL TEST:

The biochemical test was carried out by the method described by Baker et al, 1998 for determining the various parameters in the liver functions.

STATISTICAL ANALYSIS:

The results got in the study were represented as mean and standard deviation (Mean \pm S.D) and analysis of variance was done using the student-t-test to determine the level of significance.

RESULTS:

TABLE 1: The phytochemical analysis of *Pentacletra macrophyllabenth*

CONSTITUENTS OF EXTRACT OF <i>P.macrophyllata</i>							
	Alkaloids	Carbohydrate	Calcium	Sugar	Flavenoids	Tanins and Saponins	Resins, Terpenoids, acidic compound
Degree of constitution	+	++	++	+	++	+++	-

- Negative
- + Present in small concentrations
- ++ Present in moderate high concentration
- +++ Present in very high concentration

TABLE 2: Haematological profile of albino rats before the administration of extracts and 28 days after the administration of aqueous extract of *Pentacletra macrophyllabenth*

Groups	Hbg/dl \pm S.D	PCV % \pm S.D	WBC/mm ³ \pm S.D	Platelet count $\times 10^9/l$ \pm S.D
Control rats Grp A, n=6(Extract free)	12.40 \pm 6.39	37.30 \pm 1.5	4,300 \pm 26	165 \pm 39
Before Extract Grp B, n=8 (Low dose)	12.08 \pm 3.05	36.2 \pm 1.42	4,280 \pm 14	164 \pm 21
Grp C, n=8 (High dose)	12.24 \pm 4.18	36.6 \pm 2.52	4,340 \pm 40	170 \pm 32
28 days after extract administration Group B, n=8 (Low dose)	14.15 \pm 3.15	43.2 \pm 5.02	4,475 \pm 47	172 \pm 12
Group C, n=8 (High dose)	14.50 \pm 3.52	43.1 \pm 6.45	4,463 \pm 38	175 \pm 20
P. Value	P<0.05	P<0.05	P<0.05	P<0.05

There is an increase in Hb, PCV, and platelet count in the test rats treated with aqueous extract of *P.macrophyllabenth* compared to their corresponding controls.

TABLE 3: Shows the Liver function indices in albino wistar rats on aqueous extract of *P.macrophyllabenth* (oil bean).

Groups	Total Bilirubin Mg/dl (x ± S.D)	Conjugated Bilirubin mg/dl (x ± S.D)	Alkaline Phosphate iu/l (x ± S.D)	Aspartate Transaminase iu/l (S.D)	Alanine Transaminase iu/l (x ± S.D)
Control rats n=6 (Extract free)	0.32 ± 0.2	0.2 ± 0.1	54 ± 1.5	6.2 ± 0.9	4.7 ± 0.5
Before Extract Group B n=8	0.31 ± 0.1	0.25 ± 0.1	52 ± 0.8	6.0 ± 0.4	5.2 ± 0.3
Group C n=8	0.43 ± 0.2	0.34 ± 0.1	54 ± 1.0	6.2 ± 0.7	6.0 ± 0.8
28 days after extract Administration Low dose Group B n=8	1.65 ± 0.35	2.46 ± 0.83	162 ± 3.5	34.9 ± 7.0	32.6 ± 4.0
Group C n=8	1.68 ± 0.43	2.35 ± 0.74	159 ± 0.73	35.2 ± 1.6	30.2 ± 3.6
High dose Group B n=8	2.04 ± 0.81	4.05 ± 1.262	303 ± 25	63.3 ± 5.8	59.2 ± 1.8
Group C n=8	3.08 ± 0.64	4.25 ± 1.08	302 ± 22	74.2 ± 4.5	68.5 ± 1.6
P value	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

There was a significant increase in the liver function indices due to administration of aqueous extract of *P.macrophyllabenth* to the test rats compared with their corresponding control.

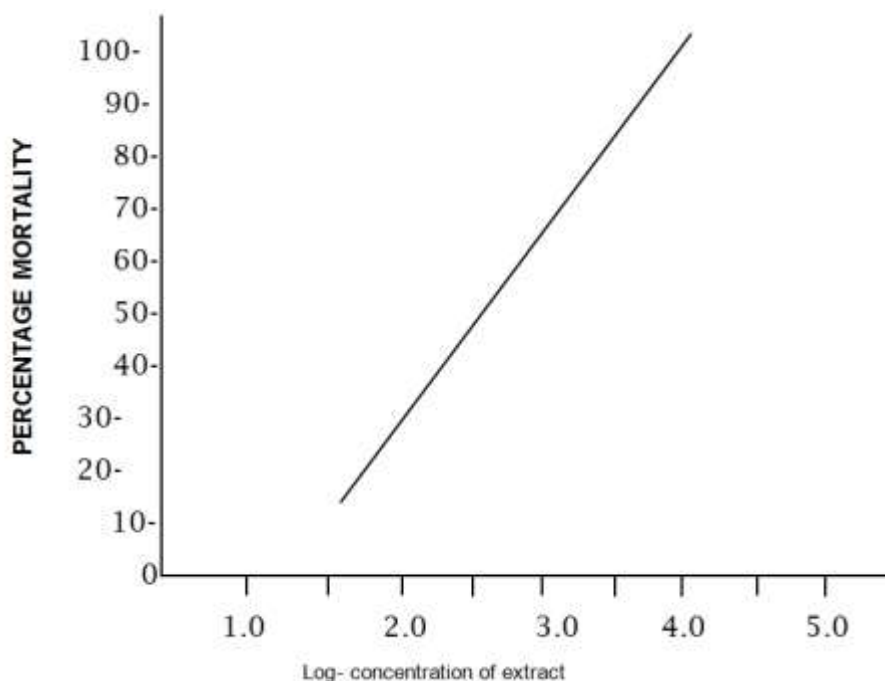


FIGURE 1: Shows the lethal dose (LD₅₀) of *P.macrophyllabenth* (oil bean).

The lethality dose of rats on extract of *P.Macrophylla* is 2,6000mg/kg

DISCUSSION

The bioactive principles present in aqueous extracts of *Pentaclethra macrophylla* (oil bean), and its effects in the haematological and biochemical profile of male albino wistar rats has been studied. The low and high dose of aqueous extract of *Pentaclethra macrophylla* has an effect on the haematological and biochemical indices of the test rats. The significant increase on the haemoglobin concentration from 12.08 ± 3.05 (Grp C) to 14.15 ± 3.15 (Grp B) and 14.50 ± 3.52 (Grp C) though within normal range (12.40-18.0g/100ml), indicates oil bean seeds contain haemopoietic bioactive principles and it can be administered in cases of decrease in blood level (anaemia). The rich mineral composition of oil bean seed makes it a good low cost of protein and amino acids. The increase intake of the food, increases the haemoglobin value. The extract is also rich in iron. These substances are essential for erythropoiesis to occur (Sembulingham, 2010). An increase in erythropoietin leads to an increase in packed cell volume in conditions characterized by low PCV values such as found in anaemia (Nutritional deficiency anaemia), pregnancy, and cirrhosis of the liver. There was an increase in white cell count and platelet count (Table 2) in the test rats (Grp B and C), though within the normal range of WBC and platelet count. This increase indicates that the extract possesses the bioactive principles which boost immunity, as an increase in white blood cell count increases the mechanism of the body. The principle responsible for boosting the defense mechanism is protein.

On the effect of the bioactive principles on the liver function of the test rats, it was discovered that it also affected the total bilirubin and conjugated bilirubin positively. From the result of phytochemical analysis conducted (Table 1) on the extract, it indicated the presence of sodium, calcium, and water. These substances increase the quantity of bile and in turn increase the amount of bilirubin produced, since bilirubin is one of the bile pigments (Sembulingham, 2010). The bioactive principles present in the extract could help boost liver function by increasing the bile flow. The abnormal increase in the alkaline phosphatase, aspartate transaminase and alanine transaminase in the test rats (Table 3) could be indicative that the extract could cause liver problems when taken in high dose for a long time. It was observed that the test groups especially the high dose groups were considerably less active than the control animals indicating bodily weakness due to the effect of the extract.

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