Effect of Aqueous Extract of *Parkiabiglobosa* Fruit Husk on Heamatological Parameters in Rats

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ABSTRACT

The toxic effect of the aqueous extract of Parkiabiglobosa fruit husk on haematological parameters is studied in albino rats. The oral acute toxicity is conducted using the standard method of Karber as modified by Aliu and Nwude. The prolonged toxicity is evaluated by estimating the haematological parameters such as red blood cells (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), white blood cells (WBC) count and the body weight. The results of the study show that the oral LD_{50} of the extract is 1120mg/kg b.w indicating that the extract is moderately toxic. The aqueous extract has no significant ($p>0.05$) effect on the RBC, HB and PCV but shows a significant ($p<0.05$) increase in WBC count on day 14-21. The body weight of the rats is significantly ($p<0.05$) increased by the administration of the extract which may imply that the extract is a taste receptor stimulant which stimulates appetite of the rats, thereby increasing feed consumption or the extract may have been responsible for depressing the satiety center leading to continuous consumption of feed resulting into increase body weight. In conclusion, the aqueous extract of Parkiabiglobosa fruit husk can be said to be safe but also with a significant ($p<0.05$) increase in total serum WBC count which may mean a possible stimulation of the immune system.

Keywords: *Aqueous extract, Parkiabiglobosa fruit husk, heamatological parameters, rats*

INTRODUCTION

Parkiabiglobosa popularly known as African locust bean tree is known as "*Dorawa*" in Hausa, "*Irugba*" in Yoruba, "*Origili"* in Igbo (Ajaiyeoba, 2002). *Parkiabiglobosa* is a large perennial deciduous tree of up to 7-20m in height but can reach 30m of height in exceptional cases with a dense spreading crown (Sabiti and Cobina, 1992). It is a perennial deciduous tree which can reach 30m in exceptional cases. It has dark

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green bipinnate leaves about 30cm long (Orwa, Mutua, Kindt, Jamnadass and Anthony, 2009). The Pinnae are about 17 pairs with 13-60 leaflets which are oblong to linear measuring 0.8-3.0cm x 0.2-0.8cm. Its flowers are red or orange occurring in clustered heads with a diameter of about 7cm hanging on peduncle of 10-35cm in length (Oruwa, *et al*, 2009). The Pods are linear, glabrous brown measuring12-30cm x 15-25cm. It has a very slow growth and begins fruiting in 8 years. At 15-20 years it produces 25-100kg of pods per tree.

The bark, leaves, flowers and pods have innumerable medicinal and food utilizations (Sabiti and Cobbina, 1992). The pods in particular (husk and pulp) are suitable food for humans and are stored in households. The foliage contains saponins, but it is considered palatable to cattle. Flowers are rich in nectar and bee-hieves are often placed on the branches. The locust bean seed are fermented to make a black, strong smelling, tasty food ingredient, high in protein normally use by women in most African communities as cooking ingredient (Sabiti and Cobbina, 1992), referred to as "*Dawa-dawa*" in Hausa land or "*iru*" in Yoruba. It is rich in protein, lipids and vitamin B_2 (Kourouma, Jean, Achille and Clement, 2011). The fat in the bean seed nutritionally contains approximately 60% unsaturated fat. The seeds are used as coffee substitute, they contains up to 29% crude protein and up to 60% saccharine they are rich in vitamin C and high in oil content (Alabi, Akinsulire and Sanyoaolu, 2005). The pulp is eaten raw or made into a refreshing drink and is used as sweetener it can also be pressed into cake for storage. The young seedlings are nutritious and heavily browsed by livestock; other products such as the burnt husk are added to tobacco to increase its pungency (Ajaiyeoba, 2002).

In Gambia the leaves and roots are used in preparing a lotion for sore eyes. A decoction of the bark of *Parkiabiglobosa* is used as bath for fever, as a hot mouthwash to steam and relieve toothache. The pulp bark is used along with lemon for wound and ulcers (Irvine, 1961). *Parkiabiglobosa* has been identified as a source of tannins, saponins, gums, fuel and wood seeds of various species have also been investigated for their protein and amino acid contents (Fatuga, Babatunde and Oyenuga, 1974). It also contains fatty acids with Arachidonic acid being most abundant (Ajaiyeoba, 2002). As it is with most African plants, the tree is use in traditional medical treatment of diseases to relieve diarrhea, the bark is boiled to make tea, for infectious wounds and fever. The bark when macerated has been used in the treatment of hemorrhoids and burns. Its flowers, when gulled and macerated have been used in the treatment of hypertension and in the prevention of leprosy in some communities. Some use the decoction of the seed made by concentrating its extract through boiling to bring about emotional stability. It is also used to treat mouth sores and insect bites (Kourouma, Jean, Achille and Clement, 2011).

MATERIALS AND METHOD

Plant Collection and Identification: The leaf, stem and fruit of *Parkiabiglobosa*

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were collected from Chibok town of Borno State and submitted for identification and authentication by a botanist with the Department of Biological Sciences, University of Maiduguri. A Voucher specimen *P.biglobosa:* 001 was deposited at the department of Veterinary Physiology, Pharmacology and Biochemistry herbarium.

Extract Preparation: The fruit husk was completely dried at room temperature ($37^{O}C$) and then ground into fine powder using pestle and mortar. Two hundred grams (200gm) of the powder was weighed and mixed with one liter of distilled water in a conical flask. The mixture was shaken and heated at $65^{0}C$ for 30 minutes. It was allowed to cool and filtered using Whatman No.1 filter paper. The filtrate was concentrated in a rotary evaporator and stored at $4^{o}C$ until needed.

Experimental Animals: Albino rats of both sexes were used for this study. They were kept in a plastic rat cages and allowed to acclimatize to laboratory environment for a period of two weeks before commencement of experiment. They were fed with growers' mash (Vital feeds Nig. Ltd) and water *at libitum*. The experiments were conducted in accordance with international guiding principles for biochemical research involving animals (C.I.O.M.S. 1985).

Acute Toxicity Study: A pilot test was conducted to ascertain the level of toxicity of the plant extract after which the acute toxicity was determined. Twenty five albino rats were used for the acute toxicity studies which were divided into five groups (A to E) of five rats each. Group E served as the control group. The extract was administered orally to the groups A to D using a cannula No. 20 at the dose rate of 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg respectively while group E was administered distilled water. This was done to establish the range of doses of the extract that would produce toxic effect. The rats were observed for 24 hours post treatment for behavioural signs such as excitement, nervousness, dullness, alertness, ataxia and even death. The median lethal dose (LD_{50}) was then calculated using the arithmetic method of Karber (1931) as modified by Aliu and Nwude (1982).

Prolonged Toxicity: Eighty (80) rats were used for the prolonged toxicity study. They were divided into four (4) groups; A-D of twenty (20) rats each. Groups A - C were treated orally once daily with the aqueous extract at the dose rate of 200mg/kg, 400mg/kg and 600mg/kg respectively for a period of 21 days. While group D received distilled water as the control for the same period. The body weight of the rats were measured prior to the administration of the extract and there after weekly. Blood samples were collected from the tail vein of the rats weekly at the region of 0.2cm before the tips for the determination of the following haematological parameters; Red Blood Cell count (RBC), Haemoglobin concentration, (Hb), Pack Cell Volume (PCV), total white blood cell count, as described by Brown (1976) and Coles (1974).

Determination of Red Blood Cell (RBC) count and White Blood Cell (RBC) count: The improved Neubauer method as described by Cole (1986) was used for determination of RBC count. For determination of WBC count, the white blood cell

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pipette was used to draw blood to 0.5 marks. The tips of the pipette was thereafter wiped and used to draw WBC diluting fluid (Turk's Solution) to 11 mark points above the pipette. It was then shaken thoroughly to mix the contents and then allowed to stand for 3 minutes. The counting chamber was charged with the diluting fluid after discarding the first few drops. One minute after charging the chamber, it was then counted with the help of light microscope at ×40 objectives. The cells in the four corner squares were counted and multiplied by 1000 to give the total number of the cells counted in thousand per cubic millimeter ($\times 10^3 mm^3$).

Determination of the Packed Cell Volume (PVC): The packed cell volume was determined using the method of Coles (1986). The blood was collected from the tail vein of rat in to the heparinized capillary tube. The capillary tubes were centrifuged at the rate of 3000 revolutions per minute (rpm) for 5 minutes. The PVC was read and expressed in percentage (%).

Determination of Haemoglobin Concentration (Hb): Colometric method for the determination of haemoglobin concentration was used (Coles, 1986). The reading was compared with the haemoglobin chart and the value of haemoglobin was expressed in grams per 100ml.

Statistical Analysis: All values were expressed as mean ± standard deviation (S.D.), while analysis of variance (ANOVA) was used to analyze the extent of variation among groups and p values d" 0.05 were considered significant (Mead and Curnow, 1983). The computer statistical software Graph Pad InStat® (2000) was used to analyze the data.

RESULTS AND DISCUSSION

Extraction: The extract of *Parkiabiglobosa* fruit husk was dark brown in colour and was soluble in water, the extract yield was 25%w/w.

Acute Toxicity Study: The administration of aqueous extract of *Parkiabiglobosa* fruit husk orally at various doses resulted in mortality of some of the treated albino rats (Table 1). The oral LD_{50} was calculated to be 1120mg/kg b.w. using the arithmetic method of Karber (1931) as modified by Aliu and Nwude (1982).

Effect of Aqueous Extract of Parkiabiglobosa Fruit Husk on Body Weight of Albino Rats: The effect of the extract on the mean body weight of the rats is presented on table 2. There was a dose-dependent significant ($p<0.05$) increase in body weight of the rats treated with the extracts at the doses of 200, 400 and 600mg/kg b.w after 21 days of extract administration when compared to day zero. The control group also shows a significant ($p<0.05$) increase in body weight at day 14 post treatment. After 14 days withdrawal period, there was significant ($p<0.05$) decrease in body weight as compared to day 21. The extract at doses 200, 400 and 600mg/kg produced 45.0%, 27.2% and 29.2% decrease in body weight respectively, while the control group did

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not show any decrease in weight at the 14th day after withdrawal as compared to 21 day post treatment, rather there was an increase in body weight by 1.8%.

Effect of Aqueous Extract of Parkiabiglobosa Fruit Husk on Red Blood Cell (RBC) Count, Heamoglobin Concerntration (Hb) and Pack Cell Volume (PCV): The result of the administration of the aqueous extract of *P. biglobosa* fruit husk on red blood cells in rats is presented on table 3. The administration of the extract in all the doses 200, 400 and 600mg/kg in rats did not significantly ($P>0.05$) affect the red blood cells count. The result of the administration of the aqueous extract of *P. biglobosa* fruit husk on heamoglobin concentration of the rats is presented on table 4. Showing that the extract did not significantly ($P>0.05$) affect the heamoglobin count in all the doses of 200, 400 and 600mg/kg throughout the period of the treatment. The result of the administration of the *P. biglobosa* fruit husk on pack cell volume of the rats is presented on table 5 indicating that the extract did not significantly affect the packed cell volume in the doses as stated above.

Effect of Aqueous Extract of Parkiabiglobosa Fruit Husk on White Blood Cell (WBC) Count: The effect of the extract on the mean white blood cells (WBC) count (Table 6) was shown to have a significant ($p<0.05$) increase in the WBC count on day 14 and 21 for all the treated rats at doses 200, 400, 600mg/kg b.w when compared to day zero and control. A significant ($p<0.05$) decrease in the WBC count was recorded following withdrawal. The aqueous extract of *P. biglobosa* fruit husk administered *par os* to albino rats at various doses produced a lethal dose (LD_{50}) value of 1120mg/kg b.w. According to the toxicity range by Clarke E. and Clarke M. (1979) any substance whose oral LD_{50} in rats is between 500-5000mg/kg, is considered to be moderately toxic and could be administered with some degree of safety especially through the oral route where the absorption might not be complete but gradual due to inherent factors limiting absorption in the GIT (Dennis, 1994).

The prolonged administration of the extract resulted in an increase in body weight of the rats at day 21; there was also a decrease in the body weight observed at day 14 withdrawal periods. This implies that the extract has a positive effect on the weight gain of the rats; it could be that the extract is a taste receptor stimulant which stimulates the appetite of the rats thereby increasing feed consumption or the extract could have been responsible for depressing the satiety center leading to continuous consumption of feed, resulting into increase body weight. This result supports the local belief that animals gain weight when fed with the fruit husk of *Parkiabiglobosa.* Hence, it is suggestive that the *P.biglobosa* fruit husk may probably be used as a feed additive with fattening animals, as thus suggested by Okpala (1989). The prolonged administration of the extract did not have a significant ($p>0.05$) effect on the RBC, Hb and PCV, but there was a significant ($p<0.05$) increase in total WBC counts of the treated rats, this may mean a possible stimulation of the immune system. Kashinath (1990) reports that many biological compounds have been known to stimulate the immune functions and Schalm, Jain and Carrol (1975) also report that when there is a persistent antigen load

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in the body, lymphocytes increase. The recent research by Abagale (2011) shows that phytochemical screening that the aqueous extract of *Parkiabiglobosa* fruit husk contains the following phytochemical compounds; phenolics, anthraquinone, glycosides, flavonoids, alkaloids and saponins. Hence the presence of phenolics and saponins might have been responsible for the stimulation of the immune system (Achide, 1987).

Table 1: Oral acute toxicity test of aqueous extract of *Parkiabiglobosa* fruit husk in albino rats

Dose(mg/kg BW)	Dose Difference (DD)	No. of Mortality	Mean Mortality(MM)	(DDxMM)
0	0	0	0	0
200	200	0	0	0
400	200	0	0	0
800	400	2	1	400
1600	800	3	2.5	2000
N = 5				2400

LD_{50} = Highest dosage - That caused death $\frac{\text{Dose difference (DD)X Mean Mortality (MM)}}{\text{Number ofgroups (n)}}$

$LD_{50} = (1600 - \frac{2400}{5})$; $LD_{50} = 1600 - 480$; $LD_{50} = 1120$mg/kg BW

Source: Experimentation, 2015

Table 2: Effect of oral administration of aqueous extract of *Parkiabiglobosa* fruit husk on mean (*) body weight (g) in rats

Dose (mg/kg BW)	Days of Extract Treatment				Days of Extract Withdrawal	
	0	7	14	21	14	
			(Mean±SD)g			
Control	130.1±33.9	145.8±35.8	156.4±34.9	220.0±66.3	224.0±68.2*	(69.2%)
200	120.0±40.1	123.8±40.1	135.3±36.9	235.0±18.9*	162.0±22.6[b]	(48.4%)
400	117.3±36.4	123.4±33.2	130.8±32.1	187.5±59.1*	147.2±16.1[b]	(51.8%)
600	119.9±40.9	127.2±29.1	126.5±25.8	177.9±52.7*	137.4±36.3[b]	(95.8%)

*= Significant ($p<0.05$) as compare to day zero, [b] = Significant ($p<0.05$) as compare to day 21
(*) Mean ± S.D based on 5 observations

Source: Experimentation, 2015

Table 3: Effect of oral administration of aqueous extract of *Parkiabiglobosa* fruit husk on mean (*) red blood cells count ($x10^6/mm^3$) in rats

Extract Dose (mg/kg BW)	Days of Extract Treatment			Days of Extract	Withdrawal
	0	7	14	21	14
			(RBC ± SD) $X10^6/mm^3$		
Control	5.60±0.41	5.70±0.48	5.74±0.48	5.70±0.48	5.75±0.48
200	5.98±0.81	5.39±0.68	5.33±0.62	5.20±0.42	5.85±0.49
400	6.43±0.86	6.66±0.94	6.34±0.79	6.64±0.94	6.48±0.89
600	6.59±0.89	6.30±0.59	6.40±0.59	6.33±0.59	6.34±0.59

$p>0.05$ not significant; (*) Mean ± SD; n=5

Source: Experimentation, 2015

Table 4: Effect of oral administration of aqueous extract of *Parkiabiglobosa* fruit husk on mean (*) hemoglobin concentrations (g/dl) in rats

Extract Dose (mg/kg BW)	Days of Extract Treatment			Days of Extract	Withdrawal
	0	7	14	21	14
			(Hb± SD) g/dl		
Control	10.83±1.14	10.88±1.15	10.89±1.16	10.93±1.17	10.84±1.14
200	11.87±1.41	11.48±1.89	11.20±1.40	11.34±1.63	11.98±1.59
400	11.48±1.30	11.79±1.90	11.30±1.24	11.54±1.35	11.10±1.41
600	11.23±1.10	11.18±1.58	11.36±1.28	11.34±1.25	11.98±1.68

$p>0.05$ not significant when compared to day zero
(*) Mean ± SD based on 5 observations

Source: Experimentation, 2015

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Table 5: Effect of oral administration of aqueous extract of *Parkiabiglobosa* fruit husk on mean (*) packed cell volume (PCV) in rats

Extract Dose (mg/kg BW)	Days of Extract Treatment				Days of Extract Withdrawal
	0	7	14	21	14
			(PCV±SD)%		
Control	43.1±2.13	43.4±2.67	43.0±2.10	43.6±2.98	43.4±2.50
200	43.7±2.25	43.0±2.13	43.4±2.50	43.8±2.68	43.5±2.50
400	43.1±2.13	43.4±2.50	43.8±2.68	43.0±2.13	43.0±2.13
600	43.0±2.13	43.2±2.13	43.5±2.50	43.4±2.50	43.3±2.50

p>0.05 is not significant when compared to day zero; (*) Mean + based on 5 observations

Source: Experimentation, 2015

Table 6: Effect of oral administration of aqueous extract of *Parkiabiglobosa* fruit husk on mean (*) white blood cells count (WBC) in rats

Extract Dose (mg/kg BW)	Days of Extract Treatment				Days of Extract Withdrawal
	0	7	14	21	14
			(WBC±SD)X10^3/mm^3		
Control	8.40±1.4	8.41±1.4	8.52±1.4	8.60±1.4	8.40±1.4
200	7.41±0.8	7.60±0.8	14.20±2.1*	16.0±2.4*	9.40±1.5[b]
400	13.38±1.8	13.53±1.8	17.40±2.8*	19.20±3.1*	9.61±1.5[b]
600	12.51±1.1	12.80±1.1	16.30±2.5*	18.41±2.9*	9.60±1.4[b]

*= Significant ($p<0.05$) increase compare to day zero.

[b]=Significant ($p<0.05$) decrease compare to day 21

(*) Mean ± based on 5 observations

Source: Experimentation, 2015

CONCLUSION

This study was conducted to evaluate the toxic effect of the aqueous extract of *Parkiabiglobosa* fruit husk on haematological parameters in albino rats. The oral acute toxicity was examined using the standard method of Karber as modified by Aliu and Nwude (1982). The prolonged toxicity was evaluated by estimating the haematological parameters such as Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin concentration (Hb), White Blood Cells (WBC) count and the body weight. The findings show that the oral LD_{50} of the extract was 1120mg/kg b.w. indicating that the extract is moderately toxic. The extract appears to have fattening property since it has a positive response on weight gain. Hence, the aqueous extract of Parkiabiglobosa fruit husk can be said to be safe but also with a significant ($p<0.05$) increase in total serum WBC count which may mean a possible stimulation of the immune system.

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