



Effect of methanolic leaf extract of *Parkia biglobosa* on some biochemical indices and hemodynamic parameters in rats

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ABSTRACT

To provide justification for the use of plant in ethnomedicine, the leaf extract of *P. biglobosa* was investigated for its effects on some biochemical indices, antioxidant status and hemodynamic parameters in rats. 80% methanolic extract of *P. biglobosa* leaf (PBE, 75mg/kg body weight) was administered once daily to experimental animals over a 30-day period. The effect on biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), albumin, total-, direct bilirubin, urea, creatinine and lipid profile was assessed. Hepatic activity of glutathione peroxidase (GPx) and levels of lipid peroxidation (LPO) and reduced glutathione (GSH) were quantified to gain insight into the antioxidant status. Effect on hemodynamic parameters was investigated in other groups of rats pretreated with PBE (50- or 75 mg/kg) or ramipril (10 mg/kg) for fifteen consecutive days. PBE reduced serum ALT and AST levels after 10 days of treatment but with no change in hepatic activities of enzymes. Significant reduction ($P < 0.01$) in serum levels of direct- and total bilirubin, cholesterol, triglyceride, LDL-cholesterol was also observed after 15 days of treatment in rats while other indices were not significantly affected. Improved antioxidant status was shown by the significantly reduced LPO and increased GPx activity. Furthermore, PBE demonstrated hypotensive effect by reducing the mean arterial blood pressure and heart rate in rats. The results of this study strongly indicate the hypotensive effect and the safety of PBE to vital organs at the dosages evaluated and there by scientifically support its traditional use.

Keywords: *Parkia biglobosa*, extract, biochemical indices, hemodynamic

INTRODUCTION

Parkia biglobosa (Jacq.) Benth., commonly called the African locust bean is a widespread savannah tree that grows predominantly in the tropical regions of Africa. The tree is known as *Igi iru* or *Irugba* among the Yoruba people of South-western Nigeria where the seeds are fermented to make a strong smelling and tasty soup condiment rich in protein popularly called *Iru*. *Parkia biglobosa* tree was largely prescribed in traditional medicine for its multiple medicinal virtues and the leaves, bark and seeds were prescribed for the treatment of arterial hypertension, piles, amoebiasis, bronchitis, cough, burn, zoster, and abscess [1]. There is increasing importance of natural products in the areas of drug discovery, mostly as sources of new lead compounds of novel chemical structure and as the active ingredients of useful treatments derived from traditional systems of medicine. Phytochemical screening has revealed the presence of saponins, tannins, phenolics and cardiac glycosides in *Parkia biglobosa* [2] and the in vitro antioxidant property of the crude ethanolic extract of the leaf and stem bark was reported [1]. However, the effects of *P. biglobosa* on hemodynamic parameters *in vivo* and on extracardiac tissues still remain largely unknown. This study was therefore designed to investigate the effects of *P. biglobosa* leaf extract on blood pressure of normotensive rats and on antioxidant parameters and biochemical markers of tissue toxicity with a view to providing pharmacological basis for the traditional use of plant in ethnomedicine.

EXPERIMENTAL SECTION

Chemicals

Glutathione, Thiobarbituric acid, 5', 5'-Dithiobis-(2-nitrobenzoate) DTNB, and Hydrogen peroxide were purchased from Sigma Chem., Co. (London, UK). All other chemicals were of analytical grade and were obtained from British Drug Houses, (Poole, UK). The water used was glass distilled.

Plant material

Fresh leaves of *Parkia biglobosa* were collected in Isua-Akoko, Ondo State, Nigeria. Botanical identification and authentication was carried out by Dr. Ugboogu A.O and Mr Shasanya O.S at the herbarium of the Forestry Research Institute (FRIN) Ibadan, Oyo state, Nigeria where a voucher specimen (no 109603) was deposited.

Preparation of methanolic extract of *Parkia biglobosa* leaf

The leaves were air-dried at room temperature and ground to fine powder using a blender. A 500 g sample of the powdered material was macerated in 1200 ml of a mixture of methanol and water (4:1) for 48 hours. The filtrate was concentrated using rotary evaporator and then subjected to freeze-drying. The residue was kept at -20°C for future use.

Animals

Albino rats (wistar strain) of either sex weighing between 180-220g, purchased from the Central Animal house of University of Ibadan, Ibadan, Nigeria were used for the study. They were housed in the primate colony of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The animals were kept in wire mesh cages under controlled light cycle (12h light/12h dark), fed with commercial rat chow (Vital Feeds Nigeria Limited) ad libitum and liberally supplied with water. All animal experiments and protocol conform to the guidelines of National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use.

Experimental design

Toxicological study

Age-matched albino rats weighing 200±20 gram were divided into eight (8) groups of six (6) animals each. Groups were designated *Day 0-7* based on the number of days treated with extract.

Day 0: Animals in this group received distilled water only and served as the control.

Day 1: Animals received 75 mg/kg of PBE by gavage once for a single day.

Day 5: Animals received 75 mg/kg of PBE by gavage once daily for five (5) consecutive days.

Day 10: Animals received 75 mg/kg of PBE by gavage once daily for ten (10) consecutive days.

Day 15: Animals received 75 mg/kg of PBE by gavage once daily for fifteen (15) consecutive days.

Day 20: Animals received 75 mg/kg of PBE by gavage once daily for twenty (20) consecutive days.

Day 25: Animals received 75 mg/kg of PBE by gavage once daily for twenty-five (25) consecutive days.

Day 30: Animals received 75 mg/kg of PBE by gavage once daily for thirty (30) consecutive days.

Animals were euthanized by decapitation to harvest blood and the liver tissue which were used for various biochemical analyses.

Hypotensive effect of extract

Age-matched normotensive albino rats weighing 200±20 gram were divided into four (4) groups with each group comprising five (5) animals.

Group 1: Animals received distilled water only throughout and served as the control.

Group 2: Animals received 50 mg/kg of PBE by gavage once daily for fifteen (15) consecutive days.

Group 3: Animals received 75 mg/kg of PBE by gavage once daily for fifteen (15) consecutive days.

Group 4: Animals received 10 mg/kg ramipril by gavage once daily for fifteen (15) consecutive days. The choice of the dosages was based on a previous study on PBE which showed the effectiveness of the extract at the 50-75 mg/kg body weight range (data not shown).

Blood pressure determination

To determine the hypotensive effect of PBE and the standard ramipril in rats, animals were anaesthetized with a mixture of urethane (25%) and alpha-chloralose (1%). At state of anaesthesia, the animal were laid supine and femoral artery exposed and cannulated with a small polyethylene catheter connected to a pressure transducer (Becton Dickinson, Sandy, UT, USA) which in turn was connected to a polygraph 7D model of Grass Polygraph (Grass Instrument Company, Quincy, Massachusetts, USA). The speed of the equipment was 10mm/sec. The blood

pressure was then calculated as recorded in form of tracing by the polygraph after a 20-min stabilization period. The rats' body temperature was maintained at 37°C with a heating pad throughout the experiment. On the tracing, the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to the upper border represent the systolic pressure. Each centimeter (cm) change on the tracing paper corresponds to 20mmHg pressure change in the grass polygraph. The mean arterial blood pressure (MAPB) was calculated as shown: $MAPB = DP + 1/3 (SP - DP)$

Where; DP= Diastolic pressure and SP=Systolic pressure. Heart rate (beats/min) corresponds to the number of strokes within a distance of 600mm (60cm) on the polygraph recordings.

Biochemical Parameters

The activities/levels of ALT, AST, ALP and serum lipid profile were estimated using assay kits from Randox Laboratories Ltd., UK according to the instructions of the manufacturer.

Antioxidant Status

Estimation of Reduced Glutathione (GSH) Level

Post mitochondrial fraction (PMF) reduced glutathione (GSH) level was assayed by measuring the rate of formation of chromophoric product in a reaction between 5,5-dinitrobenzyl-2-nitrobenzoic acid and free sulphhydryl groups (such as GSH) at 412 nm as described by Beutler *et al.* [3].

Assessment of Lipid Peroxidation

Extent of lipid peroxidation in the PMF was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale [4].

Glutathione Peroxidase Activity (GPx)

PMF reduced glutathione (GSH) level was assayed based on the rate of H₂O₂ consumption as described by Rotruck *et al.* [5].

Protein Estimation

Protein concentration was measured by the method described by Lowry *et al.* [6] using bovine serum albumin (BSA) as standard.

Statistical Analysis

All values are expressed as mean ± SEM of six or five animals. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Toxicological investigation of drugs and herbal products is important owing to the selective toxicity of some drugs to specific tissues. Consequently, we investigated the effect of PBE on some biochemical markers of tissue toxicity even though its cardioprotective effect was earlier established in our laboratory (unpublished data). **Table 1** depicts the effect of PBE on serum biochemical indices of rats treated with the extract over a 30-day period. Serum albumin was not significantly affected by PBE until the 25th day of treatment when a significant decrease ($P < 0.05$) was observed. Serum levels of total- and direct bilirubin were significantly decreased ($P < 0.001$) after 10 days of PBE consumption. Serum bilirubin provides useful information on how well the liver is functioning [7]. As a product of heme metabolism, it functions in-vivo as a powerful anti-oxidant, anti mutagen, anti-complement and an endogenous tissue protector [8]. Decreased extracellular bilirubin levels have been correlated with contemporary increase in plasma antioxidant capacity and decrease in oxidative stress conditions. Extract-dependent decrease in total bilirubin levels may therefore suggest antioxidative and hepatoprotective potency [8].

In the present study, the rate of creatinine and urea clearance was not affected by treatment with extract for up to 10 days or more. Serum creatinine and urea levels are sensitive and reliable biochemical indices for evaluation of renal function in animal models [9]. PBE at the study dose had no deleterious effect on renal function as it did not impair renal clearance of serum markers up to 30 consecutive days of treatment. Abnormally increased serum levels are suggestive of compromised renal clearance and impairment to the kidney function such as in acute glomerulonephritis, nephrosclerosis and even tubular necrosis [10].

Table 1: Levels of some serum indices in rats treated with PBE over a period of 30 days.

Treatment Duration Days	Albumin (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
0	40.30±4.6	1.41±0.16	1.94±0.21	18.08±2.1	0.78±0.12
1	38.42±3.1	1.34±0.13	1.57±0.16***	16.00±2.0	0.767±0.10
5	38.90±4.4	0.71±0.09***	0.63±0.07***	13.46±3.1*	0.72±0.05
10	38.52±3.5	0.67±0.05***	0.61±0.05***	14.90±1.0	0.72±0.06
15	39.73±1.9	0.64±0.08***	0.73±0.09***	15.51±2.5	0.78±0.05
20	36.25±3.7	0.98±0.10***	1.26±0.04***	18.89±1.8	0.97±0.15
25	33.09±5.9*	1.09±0.13***	1.41±0.23***	17.80±3.0	0.97±0.03
30	29.31±2.8***	1.20±0.09**	1.32±0.13***	17.50±1.7	0.85±0.1

Values are expressed as mean ± SD (n = 6). PBE, *P. biglobosa* leaf extract at 75 mg/kg

*Significantly different from day 0 ($P < 0.05$), **significantly different from day 0 ($P < 0.01$), ***significantly different from day 0 ($P < 0.001$).

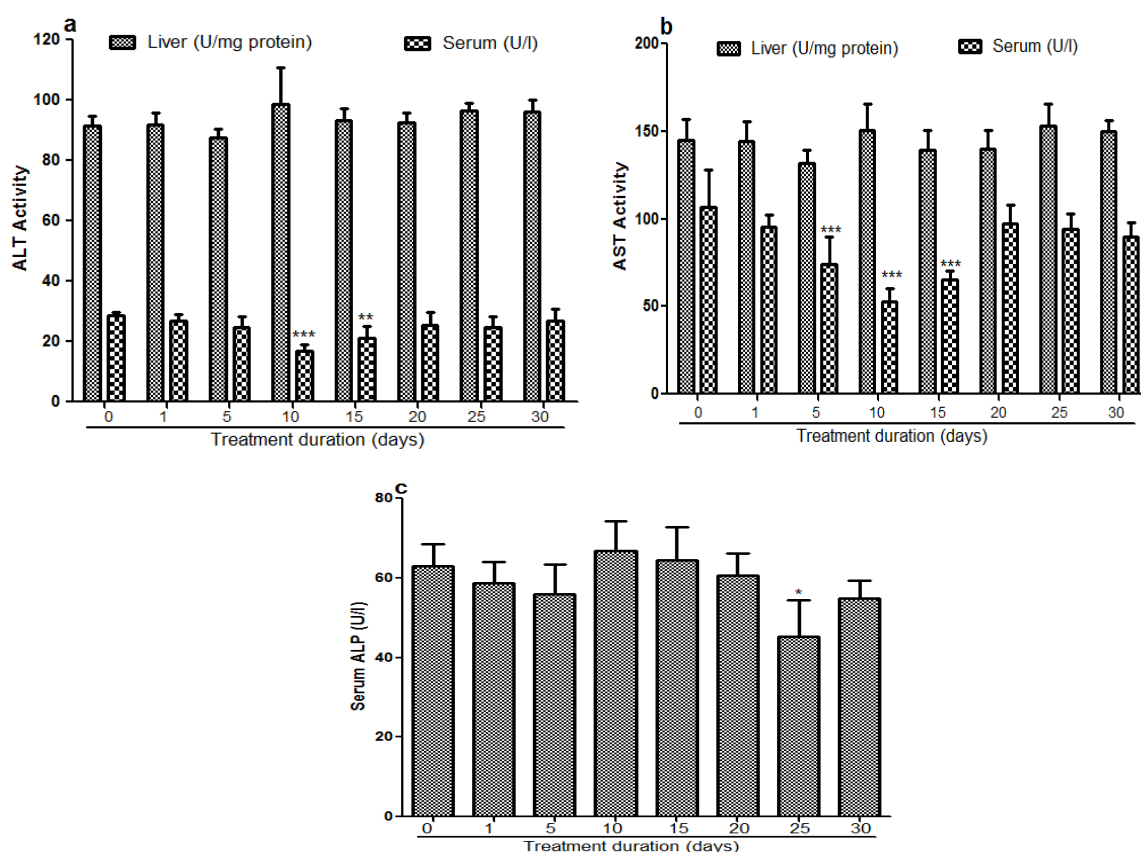


Figure 1: Activities of marker enzymes in rats treated with PBE over a period of 30 days. (a) serum and hepatic ALT, (b) serum and hepatic AST (c) serum ALP activity.

Values are expressed as mean ± SD (n = 6). PBE; *P. biglobosa* leaf extract at 75 mg/kg, ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs day 0.

Fig 1 shows the levels of ALT, AST and ALP in serum and hepatic tissue of rats. These are marker of hepatic tissue toxicity often released into blood stream upon membrane damage [11]. Hepatic levels of enzymes were unchanged throughout the duration of treatment with PBE but statistically significant decrease in serum levels of enzymes was observed between 10 and 15 days of treatment with PBE. The decreased levels of serum enzymes observed after consumption of *P. biglobosa* extract might suggest that the release of these enzymes is inhibited. Probably, one or more constituents of plant are involved in stabilizing membrane integrity of tissues keeping the membrane intact.

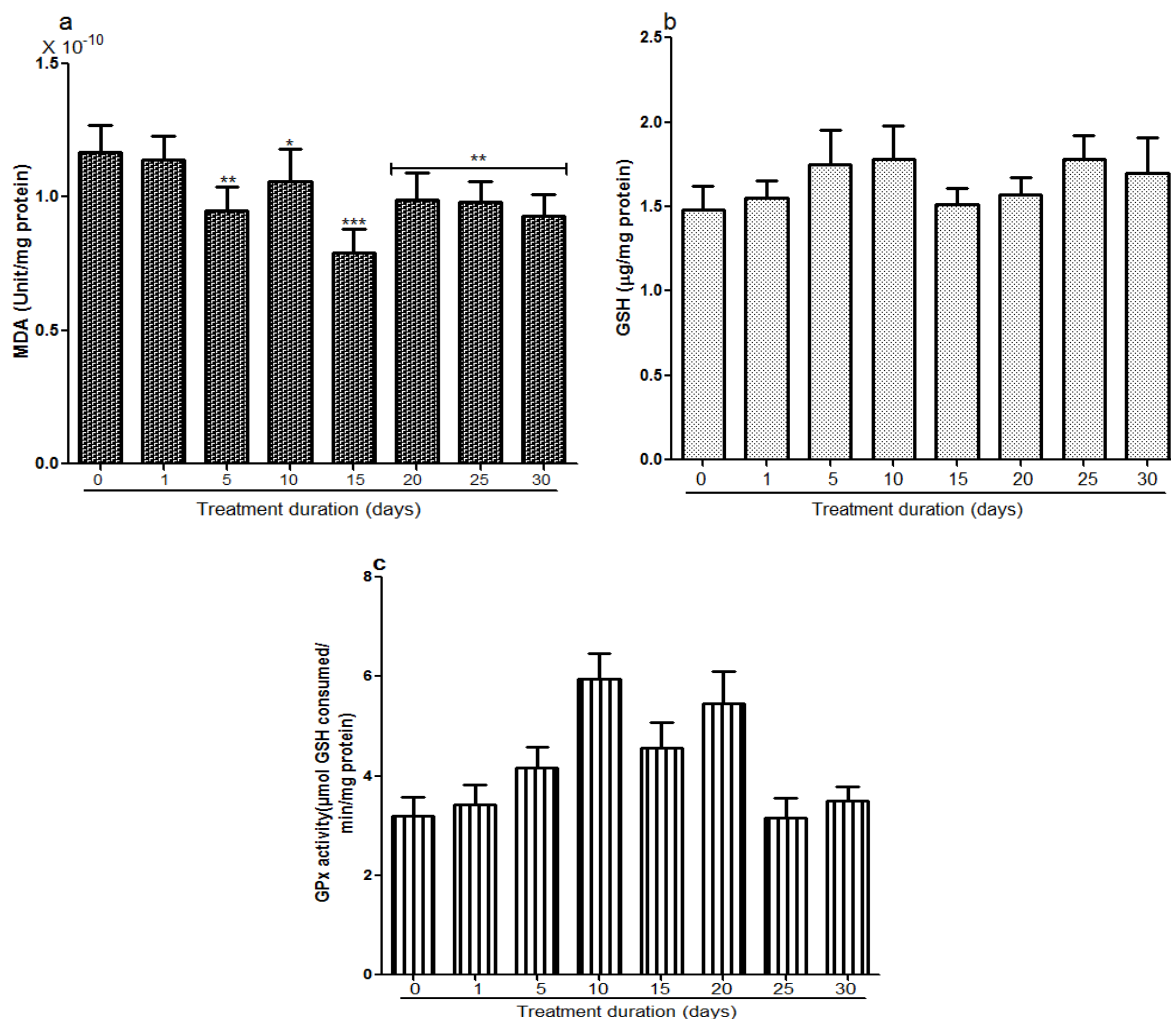


Figure 2: Hepatic antioxidant status of rats treated with PBE over a period of 30 days. (a) malondialdehyde level (b) reduced glutathione level (c) glutathione peroxidase activity.

Values are expressed as mean \pm SD ($n = 6$). PBE; *P. biglobosa* leaf extract at 75 mg/kg. ** $P < 0.01$, *** $P < 0.001$ vs day 0.

Table 2: Serum lipid profile of rats treated with PBE over a period of 30 days

Treatment Duration Days	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
0	80.04 \pm 6.3	138.55 \pm 12.5	44.15 \pm 1.40	5.29 \pm 0.63
1	76.84 \pm 7.4	140.58 \pm 11.4	41.68 \pm 1.95	5.00 \pm 0.54
5	67.45 \pm 5.8**	127.82 \pm 9.6	38.03 \pm 2.7**	4.99 \pm 0.8
10	56.35 \pm 5.9***	128.26 \pm 8.8	24.01 \pm 2.1***	5.25 \pm 0.7
15	39.46 \pm 4.3***	103.30 \pm 8.2***	14.32 \pm 2.1***	5.33 \pm 0.4
20	60.23 \pm 4.7***	73.60 \pm 4.3***	34.58 \pm 2.6***	5.46 \pm 0.6
25	48.68 \pm 6.2**	42.14 \pm 5.2***	36.50 \pm 3.9***	5.33 \pm 0.8
30	49.57 \pm 5.8***	37.08 \pm 3.5***	38.90 \pm 3.41***	5.25 \pm 0.9

Values are expressed as mean \pm SD ($n = 6$). PBE; *P. biglobosa* leaf extract at 75 mg/kg, *significantly different from day 0 ($P < 0.05$), **significantly different from day 0 ($P < 0.01$), ***significantly different from day 0 ($P < 0.001$).

Level of malondialdehyde in the hepatic tissue was quantified to assess the extent of membrane lipid peroxidation. PBE decreased lipid peroxidation reactions in the hepatic membrane after only five days of treatment and non significant increase in GSH level was also observed during this period. Significant surge in glutathione peroxidase activity was caused by PBE between 5 and 20 days of treatment (fig 2). The depletion of GSH seems to be a prime factor that permits lipid peroxidation [12] and this is common during oxidative stress conditions. In this study, the non depletion but rather increase (albeit non significant) in hepatic GSH level of PBE treated-rats suggests its non toxicity at the study dose and may explain the observed significant reduction in hepatic lipid peroxidation and consequent boosting of the activity of the selenium-containing antioxidant glutathione peroxidase enzyme in rats. From the present findings, it can be inferred that PBE enhances the antioxidant status in treated rats and reduces free

radical production by augmenting GPx level and inhibiting membrane lipid peroxidation possibly through one or more of its constituent principles.

As shown in **Table 2**, statistically significant decrease ($P < 0.01$) in the serum levels of both total cholesterol and its LDL fraction was observed as early as 5 days of treatment with PBE. Serum triglyceride level was lowered significantly after 15 days of treatment with extract while serum HDL level was not affected. Serum lipid lowering activity of drugs and natural product is desirable as toxic side effects are associated with the use of synthetic hypolipidemic drugs [13]. The reduction of total cholesterol and the associated decrease of its LDL fraction (Table 2) in PBE-treated rats is the target of several hypolipidemic drugs owing to the association of the latter with arteriosclerosis, heart-attack and peripheral vascular disease [14]. The observed hypolipidemic effect of PBE might result from constituent phytochemicals notably the saponins [15,16] and phenolics [17]. The mechanisms responsible for the observed hypolipidemic effect of PBE could be explored further in future studies.

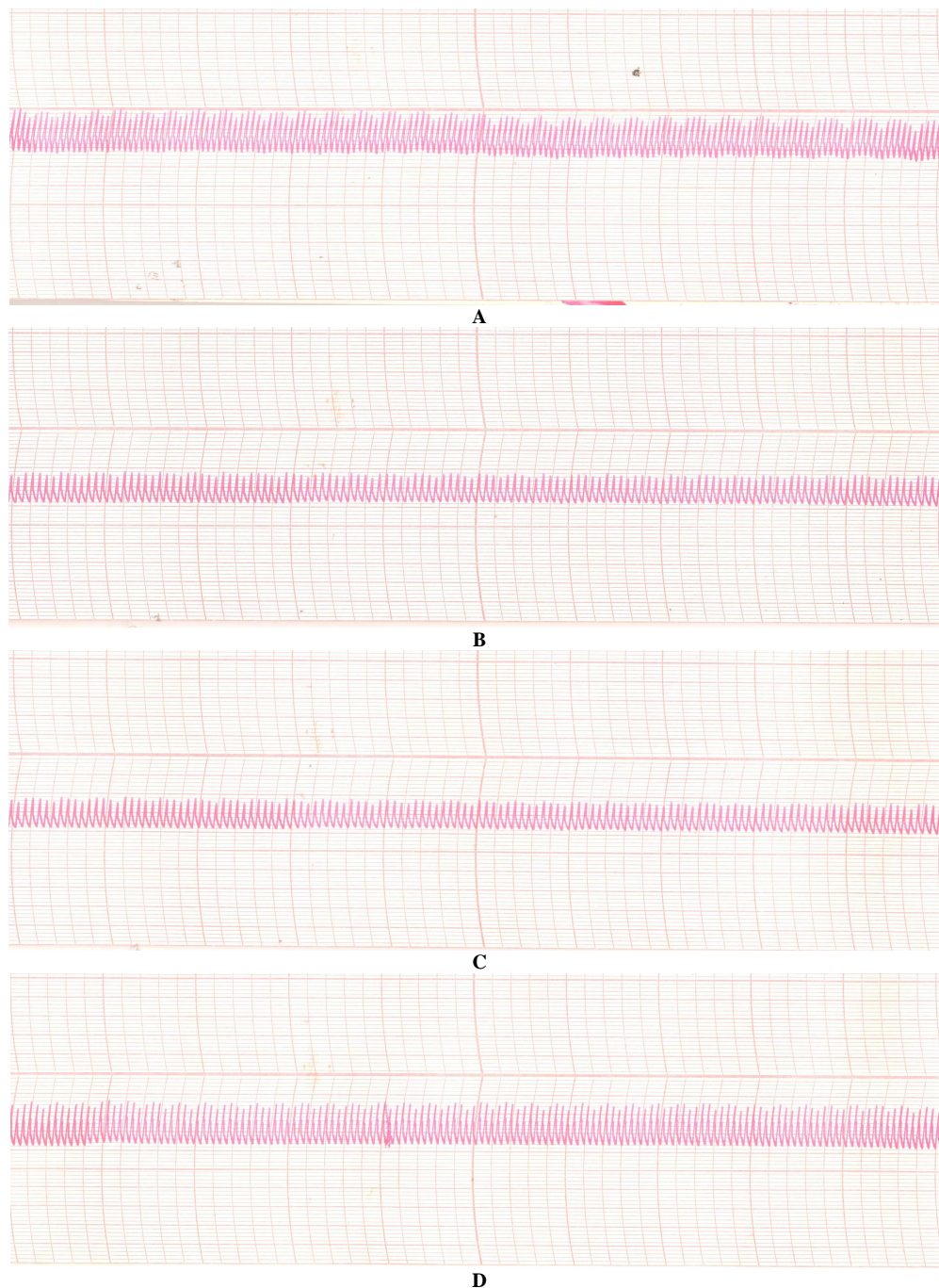


Figure 3: Representative cardiac polygraphs of control (A), PBE 50 mg/kg (B), PBE 75 mg/kg (C) and ramipril 10 mg/kg (D) treated rats.

Table 3: Effect of *P. biglobosa* leaf extract (PBE) on hemodynamic parameters in rats

Treatment Groups	Systolic pressure (mm Hg)	Diastolic Pressure (mm Hg)	MAPB (mm Hg)	Heart Rate (Beats/min)
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
Control	125.0 \pm 8.6	73.3 \pm 3.5	90.6 \pm 5.2	404.0 \pm 8.0
PBE 50mg/kg	55.0 \pm 2.9***	38.0 \pm 1.2***	41.5 \pm 2.0***	320.3 \pm 16.0**
PBE 75 mg/kg	58.7 \pm 4.8***	33.3 \pm 3.5***	37.4 \pm 3.3***	328.2 \pm 8.1**
Ram 10 mg/kg	74.0 \pm 3.8***	31.3 \pm 3.7***	45.8 \pm 3.5***	368.0 \pm 8.0*

Values are expressed as mean \pm SD (n = 5), *significantly different from control (P < 0.01), **significantly different from day 0 (P < 0.01), ***significantly different from control (P < 0.001).

The polygraph tracings and the blood pressure values of rats pretreated with the reference drug, ramipril and aqueous-methanolic extract of *P. biglobosa* leaf are presented in **fig 3** and **table 3** respectively. Treatment with 50- and 75 mg/kg of extract induced hypotensive effect in normotensive rats as observed by the statistically significant decrease (P<0.001) in both systolic and diastolic blood pressure as well as the mean arterial blood pressure. Comparable effect was observed for the angiotensin-converting enzyme (ACE) inhibitor standard drug, ramipril. The heart rate of rats was also significantly decreased by PBE.

The blood pressure is determined by the rate of blood flow produced by the heart (cardiac output) and the resistance of arterioles to blood flow. The observed hypotensive effect of ramipril is consistent with earlier reports on ACE inhibitors [18,19]. PBE had similar effect to ramipril action on hemodynamic parameters but it could not be ascertained from the present study whether similar mechanisms are involved. It is not unlikely that the hemodynamic effect of PBE is related to a direct action of the extract on the heart rate since the latter was significantly affected by PBE treatment in rats. The peptidyl dipeptide hydrolase, ACE is known to contain a zinc ion in its active sites which coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subjected to a nucleophilic attack [20]. Free hydroxyl groups of phenolic compounds have been suggested to be important structural moieties to chelate the zinc ions, thus inactivating the ACE activity [21]. The aromatic hydroxyl groups of flavonoids may demonstrate an ACE inhibitory activity due to the generation of chelate complexes with zinc ions within the active center of ACE and several flavonoids have been proven to demonstrate competitive inhibition towards ACE [22,23]. We speculate that the hypotensive effect of PBE could be due to the interaction of one or more of its constituent phenolics with ACE.

In conclusion, the present study demonstrates, for the first time, the hypotensive effect *P. biglobosa* leaf. It also reveals the safety of *P. biglobosa* leaf extract to some vital organs at the dosages evaluated. Further investigation is however required in the case of this study to ascertain the active principle of PBE responsible for the hypotensive effect as well as the underlying mechanism(s).

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