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Effects of Solvent Extracts of *Peristrophe bicalyculata* Leaves and Stem on Blood Pressure, Kidney and Liver Enzymes of two Kidney-One-Clip Hypertensive Rats

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ABSTRACT

The aqueous, butanolic and methanolic fractions of *Peristrophe bicalyculata* were assessed for their effects on the arterial blood pressure, liver enzymes and some kidney parameters in hypertensive rats. Forty rats were assigned into nine groups of five rats each: control, hypertensive control, reference drug, enalapril (3.8 mg/kg body weight), methanolic, butanolic and aqueous extracts at 100 and 200 mg/kg body weight each. All the rats, except the control group were made hypertensive using the Two Kidney One Clip (2K1C) method. Administration of the extracts started four weeks after induction of hypertension, and lasted for two weeks. Blood pressure was determined before inducing hypertension, and then after the induction on weekly basis. Liver and kidney parameters were determined at the end of the administration. Results revealed significant decrease ($p<0.05$) in blood pressure of the rats that were given the extracts compared to the hypertensive control group. The serum levels of urea and creatinine, as well as alanine aminotranferase (ALT) activity significantly decreased in the rats administered the reference drug and extracts, while the butanolic extract did not have effect on activity of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) compared to the hypertensive control group. However, rats treated with the aqueous extract, especially at a higher dose (200 mg/kg body weight) significantly ($p<0.05$) reduced the blood pressure and all the parameters determined compared to those administered the butanolic and methanolic extracts of the plant. In conclusion, this study has further supported that acclaimed antihypertensive effect and attenuation of liver and kidney damage of *Peristrophe bicalyculata*, with the aqueous extract being the most effective.

Keywords: Antihypertensive, *Peristrophe bicalyculata*, Enalapril, Two-Kidney-One- Clip, Rats

INTRODUCTION

Coronary artery diseases present some of the major health problems across the globe today, with coronary heart disease, stroke and hypertension being the most common. Hypertension is often called a "silent killer" because persons with hypertension are often asymptomatic for years (Aftab, 1995). The renin-Angiotensin Aldosterone System is an important regulator of sodium and water balance, as well as blood pressure homeostasis in man. Angiotensin-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1), which is a part of the system found in the plasma and endothelial cells of animals and humans acts on the decapeptide angiotensin I, to form a highly active vasopressor, angiotensin II. It also degrades the potent vasodilator, bradykinin, to an inactive heptapeptide (Reeves and O'Dell, 1986). Angiotensin-converting–enzyme inhibitors block the activation of the rennin-angiotensin system by preventing the conversion of angiotensin-I to angiotensin-II and could retard the progression of both heart failure and atherosclerosis.

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Most of the antihypertensive drugs so far available do not seem to possess complete curative properties, and research on some indigenous drugs derived from herbs which are used by traditional healers have proved successful in some cases and scientific research on them has led to the discovery of certain potent remedies (de Souza *et al*., 1982) signifying the importance of the study of natural products, many of which are yet to be discovered.

Peristrophe bicalyculata is used by the traditional healers in the treatment of many skin related problems. It is an antidote for snake poison when macerated in an infusion of rice, and as insect repellants. It is also used as horse feed and ploughed into the soil as green manure. The ethanol extract of the plant has been reported to exhibit analgesic, anti-inflammatory and antibacterial properties (Chopra, 1959; Dwivedi, 2002). Although undocumented, the plant is used in South West Nigeria in the treatment of hypertension and other cardiovascular diseases. It was recently discovered to have hypolipidemic effects (Abdulazeez *et al*., 2009), and such effects are known to protect against cardiovascular diseases, including hypertension.

This study aims at determining the efficacy of *Peristrophe bicalyculata* in the management of hypertension and its effects on the liver and kidney of Two-Kidney-One-Clip hypertension in rats.

MATERIALS AND METHODS

Collection and identification of plant material

The plant sample was collected from a natural habitat within Ibadan, Oyo State, Nigeria, and identified at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Experimental animals

Forty-five (45) healthy rats of the Wistar strain, weighing between 150 -250 g were obtained from the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Chemicals and reagents

All chemicals and reagents used for the study were of analytical grade.

Preparation of plant extract

The leaves and stem of the plant were air-dried and made into powder by grinding. The methanolic extract of the plant was prepared by defatting 1.845 kg of the powder in n-hexane and macerating in 70% methanol. Each extract was then suction-filtered and the process repeated until all soluble compounds had been extracted, as adjudged by the loss of colour of the filtrate. The total extract was evaporated to dryness *in vacuo* at about 45ºC and further dried to constant weight at the same temperature in a hot air oven. A known amount (132.18 g) of the crude extract was dissolved in sufficient quantity of distilled water and partitioned in n-butanol using separating funnel. A yield of 8.89% of methanolic extract was obtained after extraction, and after partitioning, 18.5% and 4.27% of the aqueous and n-butanol fractions were obtained respectively.

Acute toxicity study

Acute oral toxicity study of the aqueous, methanolic and butanolic extracts of *P. bicalyculata* was carried out as described by Lorke *et al* (1983). Doses of each extract from 10 to 5,000 mg/kg body weight were orally administered after which the animals were observed for forty eight hours for behavioural changes, clinical signs of toxicity and mortality.

Induction of hypertension

Hypertension was induced in forty of the rats using the two kidney one clip (Goldblatt, 1934). The rats were deprived of water and food for twenty-four hours before inducing hypertension, and their blood pressure determined. Briefly, the left flank of each rat was shaved, before anesthesizing with ketamine injection (10 mg/kg body weight, i.m). Incision was made on the left flank and the renal artery of the kidney constricted with a U-shaped

silver Dexon suture material (1.5 metric, 45cm), the incision was sutured and the animals were thereafter returned to their cages.

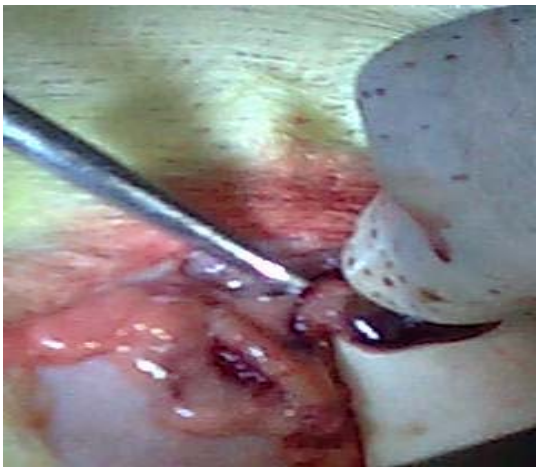
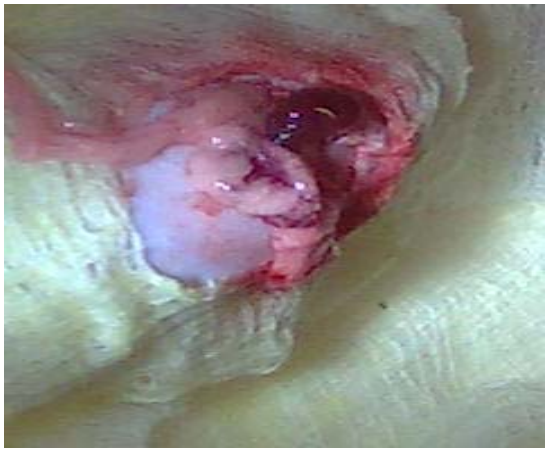


Plate 1a



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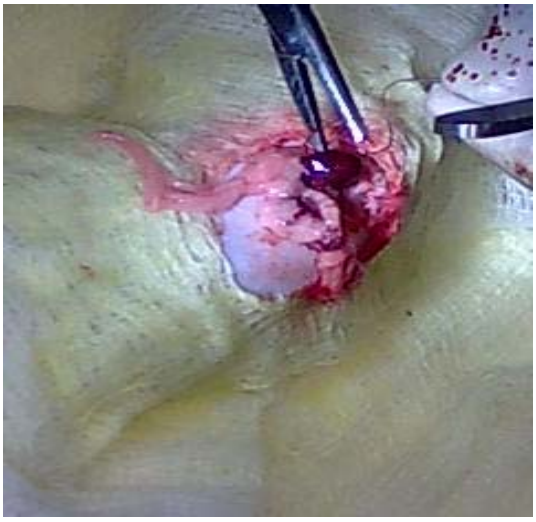

Plate 1b

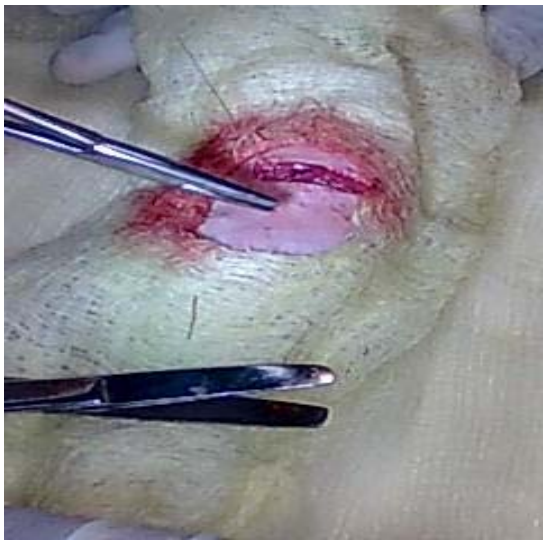

Plate 1d

Plate 1: The different stages in the induction of hypertension; Plate 1a: process after dissection; Plate 1b: the kidney being clipped; Plate 1c: after clipping; Plate 1d: suturing process.

Blood pressure measurement

Blood pressure was taken using a tail-cuff with sphygmomanometer (Ueda Co., Tokyo, Japan).

Animal grouping

A total of forty-five Wistar rats were divided into nine groups, consisting of five rats per group. In the control group (group 1), hypertension was not induced and no further treatment was given throughout the experiment whereas hypertension was induced in other groups as follows: groups 2 (but no treatment given), group 3 (given the reference drug, enalapril (3.8 mg/kg body weight), groups 4 and 5 (given the methanol extract at 100 and 250 mg/kg body weight respectively), groups 6 and 7 (given the butanol extract at 100 and 250 mg/kg body weight respectively) and groups 8 and 9 (given the aqueous extract at 100 and 250 mg/kg body weight respectively).

Five rats were housed per cage, making a total of nine groups and allowed to acclimatize for two weeks before the commencement of experiment. They were all maintained on a commercial preparation of growers mash and water *ad libitum* throughout the experiment. The experiment was carried out following approval from the Animal Care and Use Committee of the University.

Determination of biochemical parameters

Serum alanine aminotrasnferase (ALT), aspartate aminotransferase (AST) were determined as described by Reitman and Frankel (1957) while serum alkaline phosphatase (ALP) activity was determined according to the procedure described by Wright *et al* (1972). Serum urea and creatinine were determined using the Urease-Berthelot and Jaffe-Slote methods respectively.

RESULTS

The result of administration of *P. bicalyculata ad libitum* produced no death in both phases of the experiment; therefore the LD_{50} is above 5,000mg/kg. The effect of the aqueous extract of *P. bicalyculata* in serum urea level as seen in Figure 1.1 was dose dependent, showing a significant ($p<0.05$) decrease on administration of 100 mg/kg of the aqueous extract and a further decrease when 250 mg/kg was administered to the rats compared to hypertensive group and the other extracts. The methanol and butanol extract also significantly ($p<0.05$) reduced serum urea concentration, but was not as effective as the aqueous extract.

Although serum creatinine levels (Figure 1.2) significantly ($p < 0.05$) decreased on administration of the aqueous extract when compared with the hypertensive group, it was not dose dependent, as the different on administering the low dose (100 mg/kg) and high (250 mg/kg) dose was not significant. However, there was no significant ($p<0.05$) decrease in serum creatinine level when butanol extract was administered to the animals compared with the hypertensive group.

This study also revealed a change in serum level of alanine aminotranferase (ALT) as shown below (figure 1.3). The effect of the butanol and methanol extracts on ALT was dose-dependent and significantly ($p<0.05$) decreased at 200 mg/kg body weight when compared with the hypertensive group, however, both doses of the aqueous extract were significantly ($p<0.05$) lower when the aqueous extract was administered.

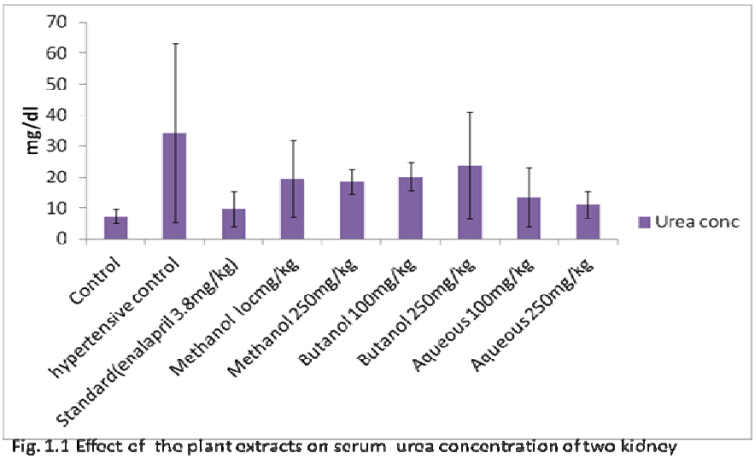

Fig. 1.1 Effect of the plant extracts on serum urea concentration of two kidney one clip hypertensive rats.

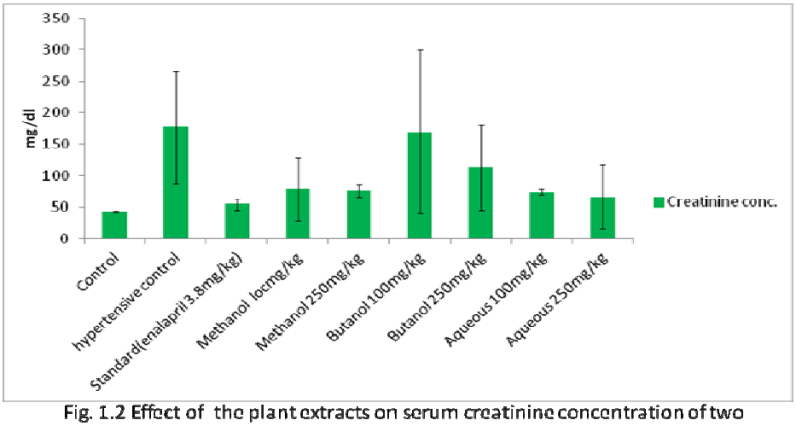

Fig. 1.2 Effect of the plant extracts on serum creatinine concentration of two kidney one clip hypertensive rats.

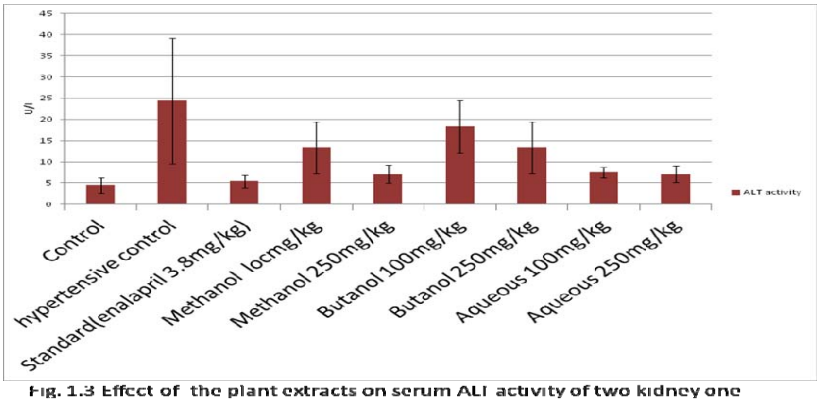

Fig. 1.3 Effect of the plant extracts on serum ALT activity of two kidney one clip hypertensive rats.

There was no significant ($p>0.05$) decrease in the level of AST when methanolic extract was administered at the low dose (100 mg/kg) but decrease was significant ($p>0.05$) at high dose (250mg/kg), showing a dose-effect relationship. There was however no significant difference between methanol extract administered at 250 mg/kg and aqueous extract administered at the same dose (Figure 1.4).

The standard drug significantly reduced serum urea and creatinine levels, as well as ALT, ASP and ALP activity when compared to the hypertensive control group. However, its effects on serum creatinine, ALT and AST levels were not significantly different from the control group.

The serum ALP activity was significantly reduced ($p<0.05$) by the standard drug, methanol (at 250 mg/kg) and both doses of the aqueous extract compared to the hypertensive control group. However, administration of the butanol extract did not reduce serum ALP activity when compared to hypertensive control group. A dose-effect relationship was observed when methanol extract was administered, as the high dose significantly ($p<0.05$) decreased ALP compared to the hypertensive group (Fig 1.5).

The induction of hypertension significantly increased the blood pressure in all operated rats from the first to the fourth week, when no treatment was given to them. Administration of the standard drug (enalapril) for two weeks significantly decreased the blood pressure compared to the hypertensive control group. The effect of the methanol extract was dose-dependent, as the low dose did not significantly decrease blood pressure, when compared to the hypertensive rats, while administration at a high dose significantly decreased blood pressure (Figure 1.6). Compared to rats in the hypertensive group and those given the butanol (Figure 1.7) and methanol extracts, administration of the aqueous extract (Figure 1.8) at both low and high doses significantly decrease blood pressure.

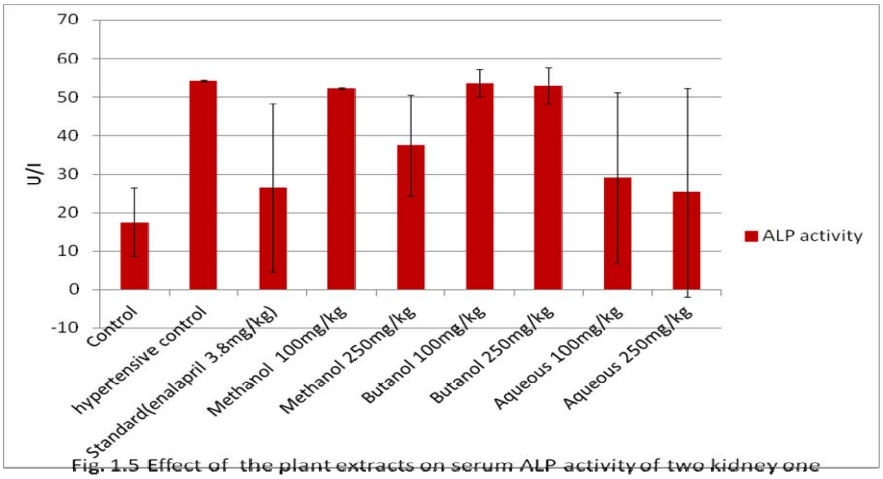

Fig. 1.5 Effect of the plant extracts on serum ALP activity of two kidney one clip hypertensive rats.

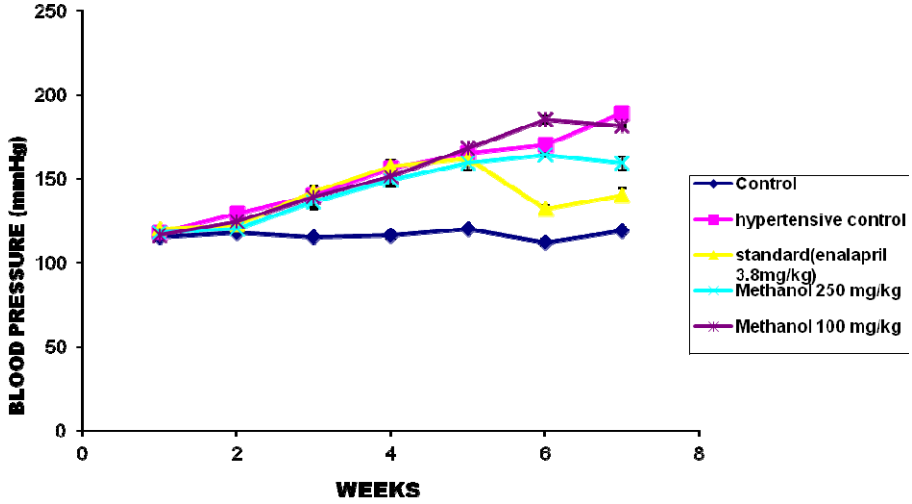

Figure 1.6: Effect of the methanolic extract of *Peristrophe bicalyculata* on blood pressure of two kidney one clip hypertensive rats.

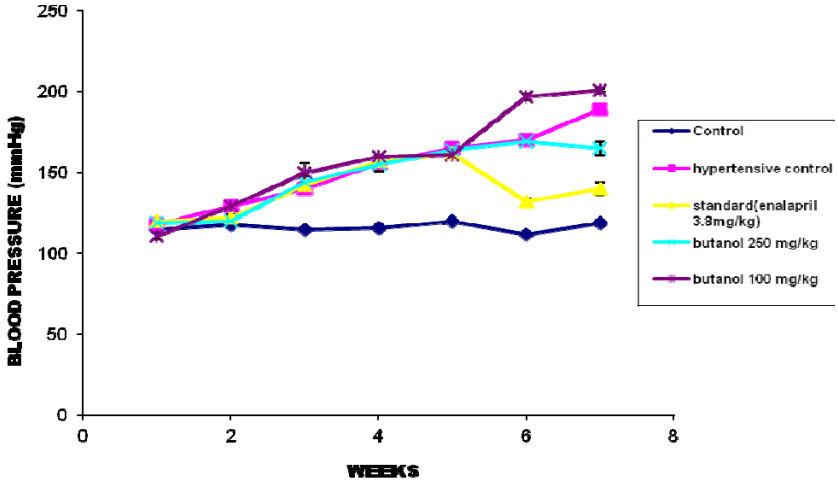

Figure 1.7: Effect of the butanolic extract of *Peristrophe bicalyculata* on blood pressure of two kidney one clip hypertensive rats.

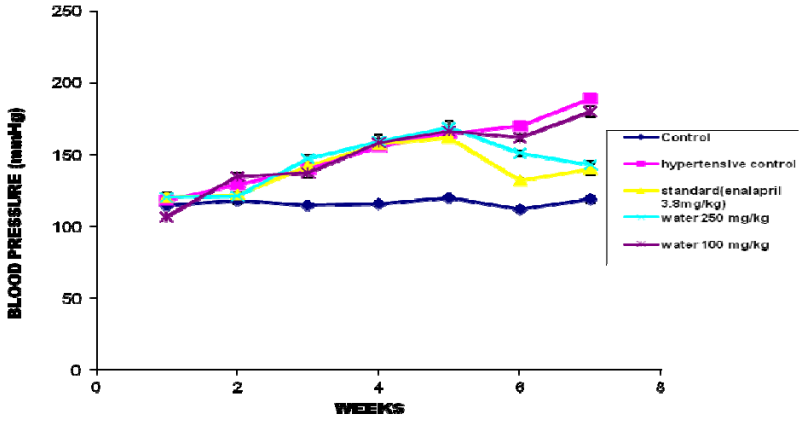

Figure 1.8: Effect of the aqueous extract of Peristrophe bicalyculata on blood pressure of two kidney one clip hypertensive rats.

DISCUSSION

The two kidney one clip model of inducing hypertension is renovascular. This is a very commonly used model of hypertension, where renin-Angiotensin Aldosterone System (RAAS) plays an important role. Experimentally, renal hypertension is produced by renal artery constriction, which activates peripheral RAAS and sympathetic nervous System, resulting in decreased blood volume which leads to sympathetic stimulation, and thus renin secretion by the kidneys. Renin converts angiotensinogen to angiotensin-I. Angiotensin-I is converted to angiotensin-II by angiotensin converting enzyme (ACE). Angiotensin-II is a potent vasoconstrictor which increases blood pressure and causes the release of aldosterone leading to salt and water retention resulting in increased blood volume and hypertension. In the Two kidney one clip (2K1C) hypertension, as described by Goldblatt (1934), the renal artery is constricted on only one side with the other artery (or kidney) left untouched. This results in a sustained increase in blood pressure due to increased plasma renin activity (PRA). This explains the increase in blood pressure four weeks after the surgery, and the hypertension induced was renin-angiotensin dependent, because salt and water retention did not occur since the other kidney is intact (Badyal et.al., 2003). The decrease in blood pressure observed as the extract was administered is evident of the efficacy of the use of *Peristrophe bicalyculata* as an antihypertensive drug. Our studies also demonstrated that the antihypertensive effect if the plant may be due to its effect on RAAS, since the model used was RAAS-dependent.

It is a known fact that the liver contains various enzymes which are essential in determining the effectiveness of the liver. These enzymes leak into the blood when damage is done to the liver, and can be measured in the serum or plasma. The significant increase in the level of liver enzymes after induction of hypertension is evident that hypertension may have caused acute liver damage, thus allowing these enzymes leak into the blood. Alanine aminotransferase (ALT) also called serum glutamic pyruvate transamiase (SGPT), Aspartate Aminotransferase (ASAT) also called serum glutamic oxaloacetate transaminase (SGOT) and alkaline phosphatise are present in hepatocytes (liver cells) and any damaged to the liver affect their concentration in blood; hence they are regarded as marker enzymes (Zimmerman, 1978). ALT is associated with not only with the liver it is also present in red cells, cardiac and skeletal muscles and is so not specific to the liver (Zimmerman, 1978).

The decrease in the liver enzymes observed after treating the hypertensive rats, is evident of the hepatoprotective effect of *Peristrophe bicalyculata*. The significant ($p<0.05$) decrease in these enzymes on administering the aqueous extract compared to the methanol and butanol extract, shows that the type solvent used may have effect on the possible antihypertensive component of the plant. And at this point, we may attribute this to the polarity, as the aqueous extract is most polar, followed by the methanol and then butanol extract with the least polarity.

Urea is the main excretory product of protein metabolism, and its in blood represents mainly a balance between urea formation from protein catabolism and urea excretion by the kidney, thus the significant ($p<0.05$) increase of serum urea in hypertensive rats shows hypertension created an imbalance between its formation and excretion.

Studies of altered creatine and creatinine metabolism are generally performed when renal damage is suspected. In renal failure, creatinine is retained with other non-protein nitrogen constituents of the blood, though creatinine is less regularly affected (Matti *et. al.*, 1995), thus the significantly high level of creatinine in hypertensive rats.
These increases in urea and creatinine can be attributed to the hypertension, which is known to accelerate the decline in renal function even in people without renal disease (Matti *et. al*., 1995).

In conclusion, the present study has demonstrated the efficacy of *Peristrophe bicalyculata* in the treatment of hypertension. It has also shown that the

aqueous extract at a higher dose of 250 mg/kg body weight is most effective than other fractions and that the mechanism of action of the plant may be through the RAAS.

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