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Effect of *Peristrophe bicalyculata* on Blood Pressure, Kidney and Liver Functions of Two Kidney One Clip (2K1C) Hypertensive Rats

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Abstract: The aqueous, butanolic and methanolic fractions of *Peristrophe bicalyculata* were accessed for their effect on the arterial blood pressure, liver enzymes and some kidney parameters in hypertensive rats. Forty rats were divided into nine groups of five rats each: control, hypertensive control, Standard (enalapril 3.8 mg/kg), methanolic extract (100 and 200 mg/kg), butanolic extract (100 and 200 mg/kg) and aqueous extract (100 and 200 mg/kg). All rats, except control group were made hypertensive using the Goldbatt Two Kidney One Clip (2K1C) method. Administration of extracts began four weeks after induction of hypertension, and lasted for two weeks. Blood pressure was determined before inducing hypertension, and then weekly after the induction. Liver and kidney parameters were determined at the end of experiment. Results showed a significant decrease ($p<0.05$) in blood pressure of the rats given the standard and all extracts compared to the hypertensive control group. The serum levels of urea and creatinine, as well as alanine aminotranferase (ALT) activity significantly decreased in rats given the standard drug, and all extracts, while the butanolic extract did not have effect on activity of aspartate transaminase and alkaline phosphatase compared to the hypertensive control group. However, the blood pressure and other parameters determined were significantly ($p<0.05$) different in rats treated with the aqueous extract, especially at a higher dose (250 mg/kg) compared to those given the butanolic and methanolic extracts of the plant. In conclusion, this work has demonstrated the antihypertensive and hepatoprotective effect of *Peristrophe bicalyculata*, with the aqueous extract being the most effective.

Key words: Antihypertensive, hepatoprotective, *Peristrophe bicalyculata*, two kidney one clip hypertensive rats

INTRODUCTION

Coronary Artery Diseases (CAD) presents 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 (RAAS) 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 renin-angiotensin system by preventing the conversion of angiotensin-I to angiotensin-II and could retard the progression of both heart failure and atherosclerosis.

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 with 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.

The plant *Peristrophe bicalyculata* is used by the traditional healers in the treatment of many skin related problems; it is also used as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellant. The plant 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

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such effects are known to protect against cardiovascular diseases, including hypertension. This study aims at specifically determining the efficacy of *Peristrophe bicalyculata* in the treatment of hypertension and its effects on the liver and kidney, by determining some liver and kidney parameters. The specific objectives of this study include:

- To determine the effect of the aqueous, methanolic and butanolic extracts of *Peristrophe bicalycuta* on blood pressure of 2K1C Hypertensive rats.
- To determine the effect of the extracts on some marker enzymes in the liver such as Alanine transaminase, aspartate transaminase and alkaline phosphatase.
- To determine the effect of the various fractions of the extract on the kidney, by determining creatinine and urea levels in serum.
- To ascertain which of the extracts is most effective in reducing blood pressure.

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 by the botanist at the herbarium in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Preparation of plant extract: The leaves and stem of the plant were air-dried in the laboratory and made into powder by grinding. The methanolic extract of the plant was prepared by defatting 1.845 kg of the powder with n-hexane and macerating in 70% methanol. Each extract was then sunction-filtered and the process repeated until all soluble compounds had been extracted, as judged by 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. 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.

Experimental design: A total of forty-five (45) Wistar rats were divided into nine (9) groups, making five rats (5) per group. In the control group, that is, group 1, hypertension was not induced and no further treatment was given throughout the experiment. Whereas, hypertension was induced in group 2 (but no treatment given), group 3 (given the standard drug, enalapril at 3.8 mg/kg), groups 4 and 5 (given the methanol extract at 100 and 250 mg/kg, respectively), groups 6 and 7 (given the butanol extract at 100 and 250 mg/kg, respectively) and groups 8 and 9 (given the aqueous extract at 100 and 250 mg/kg, respectively). The study was conducted at the Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, between August to December, 2009.

Experimental animals: Apparently healthy rats of the Wistar strain, weighing between 150 to 250 g were obtained from the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The rats were housed five (5) per cage, making a total of nine (9) groups of forty-five (45) rats, allowed to acclimatize for two weeks before the commencement of experiment. They were all maintained on a commercial preparation of growers mash (PLS feeds, Zaria, Kaduna State, Nigeria) and water ad libitum throughout the experiment. All experimental protocol were first of all be assessed and approved by the Animal Care and Use Committee of the University.

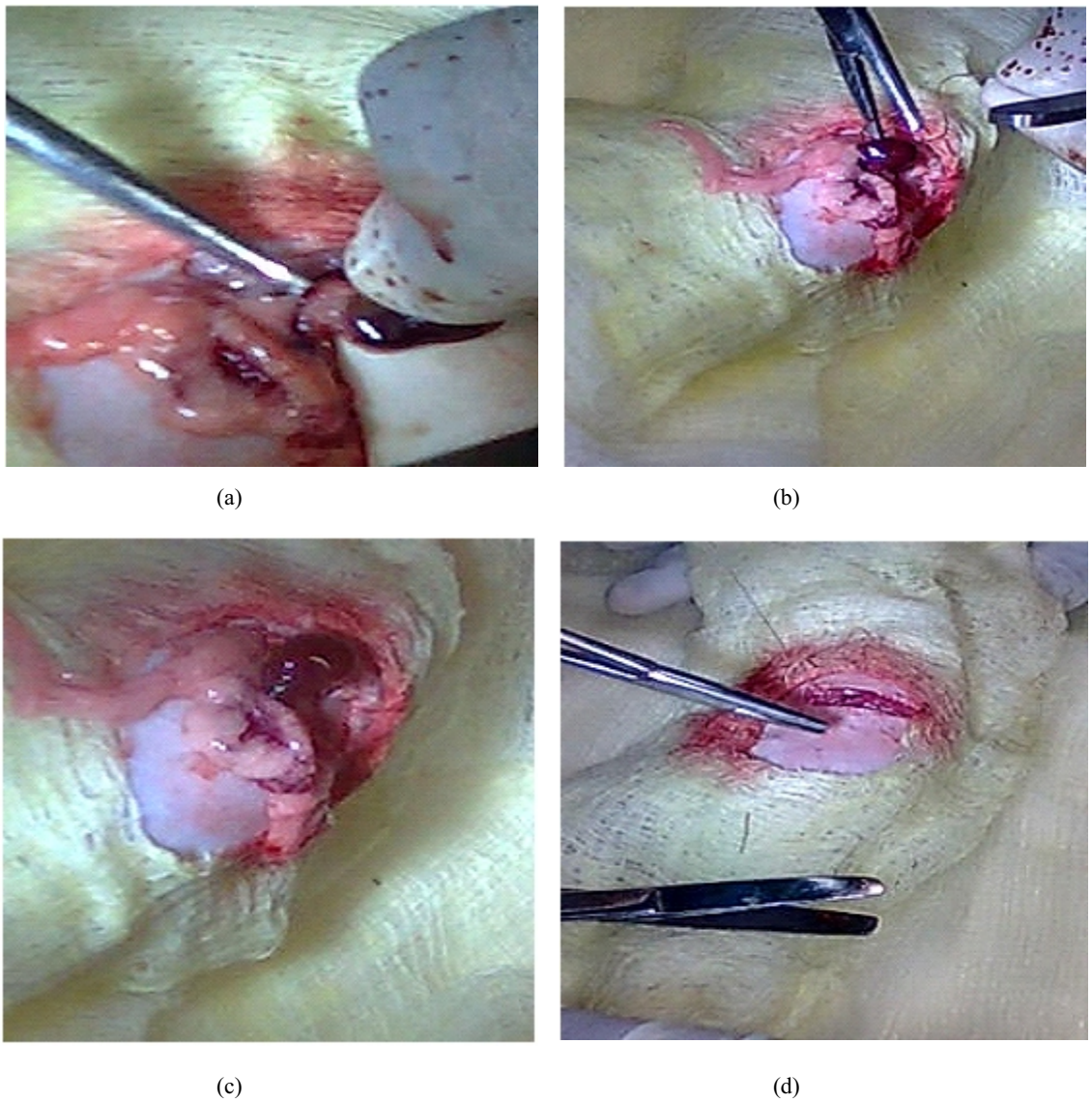
Acute toxicity studies: Acute oral toxicity study of the aqueous, methanol and butanolic extracts of *Peristrophe bicalyculata* was carried out in Wistar rats weighing 150 to 250 g as described by Lorke *et al.* (1983). Doses of each extract from 10 to 5,000 mg/kg were orally administered and animals observed for up to forty eight hours for behavioural changes, toxicity and mortality.

Induction of hypertension: Hypertension was induced in forty of the rats using the two kidney one clip Goldblatt method of inducing hypertension. The rats were deprived of water and food about 24 h before induction of hypertension, and their blood pressure taken. 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 m, 45 cm), the incision was sutured and the animals returned to their cages (Fig. 1).

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

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

Determination of activity of Liver enzymes: Serum alanine aminotrasnferase (ALT) or serum glutamate pyruvate transaminase (SGPT), Aspartate transamninase (AST) or serum glutamic a-oxoglutrate transaminase (SGOT) and serum alkaline phasphatase (ALP) were determined as described by Reitman and Frankel (1957).



(a) (b)

(c) (d)

Fig. 1: The different stages in the surgery process; (a) shows the process after dissection; (b) shows the kidney being clipped; (c), after clipping and (d) demonstrates the suturing process

Determination of kidney parameters: Serum urea was determined using the Urease-Berthelot method and serum Creatinine by Jaffe-Slote Method.

RESULTS

Results of LD_{50} determination of *Peristrophe bicalyculata* administered *ad libitum* recorded no death in both phases of the experiment, therefore the LD_{50} was considered to be above 5,000 mg/kg.

The effect of the aqueous extract of *Peristrophe bicalyculata* on serum urea level as seen in Fig. 2 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 (Fig. 3) decreased significantly ($p<0.05$) in rats given the aqueous extract than the hypertensive control group, it was not dose dependent, also there was no significant ($p<0.05$) decrease in serum creatinine level of rats given butanol extract and those in the hypertensive group.

This study also revealed a significant decrease ($p<0.05$) in serum level of alanine aminotranferase (ALT) in rats given the aqueous, methanol and butanol extracts

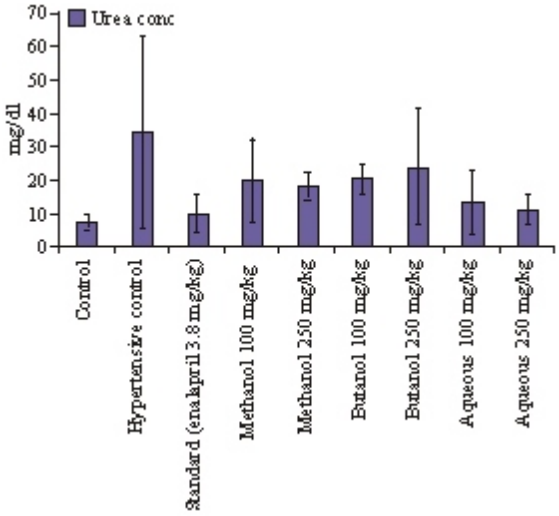


Fig. 2: Effect of the plant extracts on serum urea concentration of two kidney one clip hypertensive rats

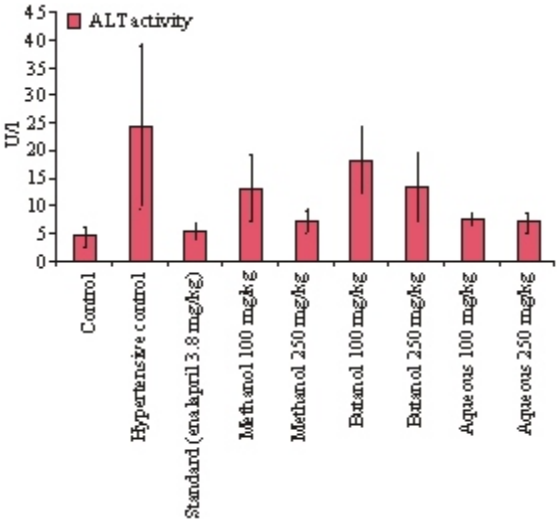


Fig. 4: Effect of the plant extracts on serum ALT activity of two kidney one clip hypertensive rats

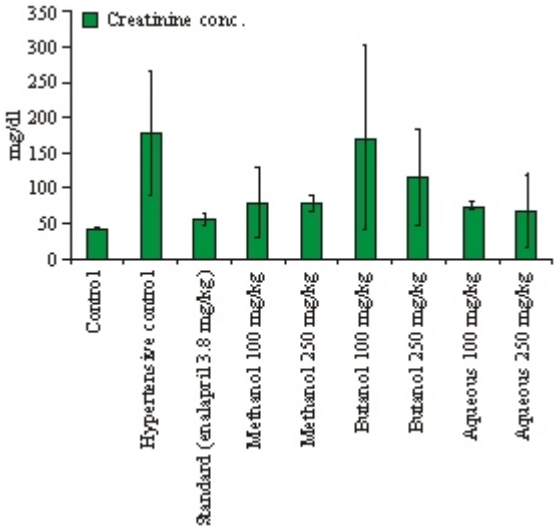


Fig. 3: Effect of the plant extracts on serum creatinine concentration of two kidney one clip hypertensive rats

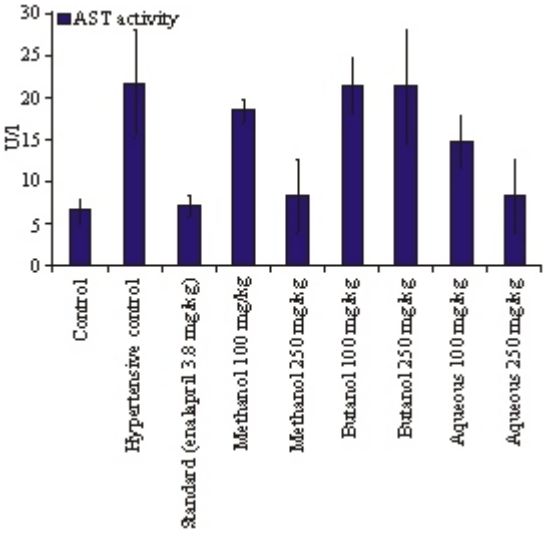


Fig. 5: Effect of the plant extracts on serum AST activity of two kidney one clip hypertensive rats

(Fig. 4). 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.

The effect of the aqueous and methanol extract on serum AST level were significantly lower ($p>0.05$) when compared to that of rats in the hypertensive control group, and was dose-dependent There was however no significant difference between methanol extract administered at 250 mg/kg and aqueous extract administered at the same dose (Fig. 5).

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. 6).

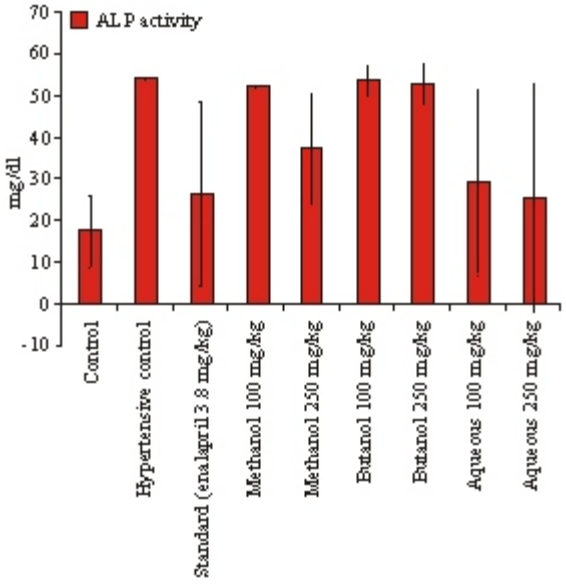


Fig. 6: Effect of the plant extracts on serum ALP activity of two kidney one clip hypertensive rats

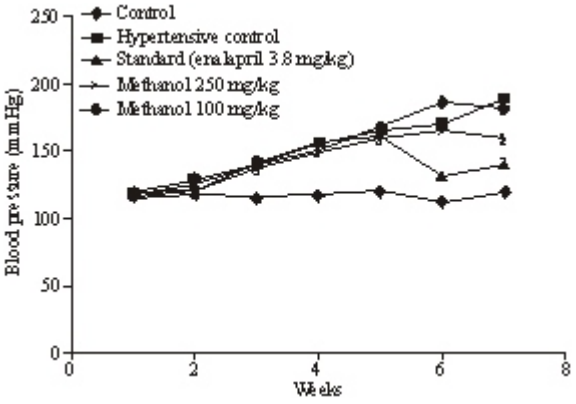


Fig. 7: Effect of the methanolic extract of *Peristrophe bicalyculata* on blood pressure of two kidney one clip hypertensive rats

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 it (Fig. 7). On the other hand, administration of the aqueous extract (Fig. 9) at both low and high doses significantly decreased blood pressure than in rats within the hypertensive control group and those given the butanol (Fig. 8) and methanol extracts.

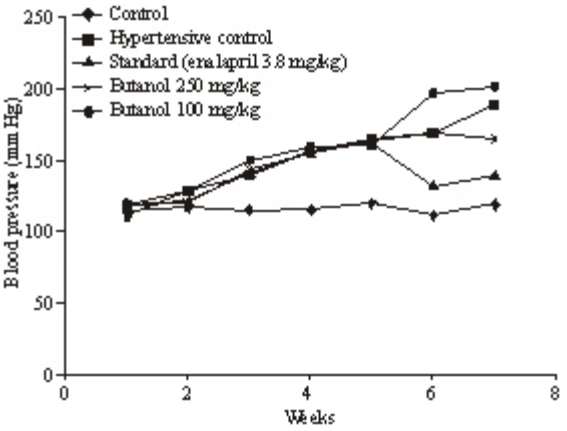


Fig. 8: Effect of the butanolic extract of *Peristrophe bicalyculata* on blood pressure of two kidney one clip hypertensive rats

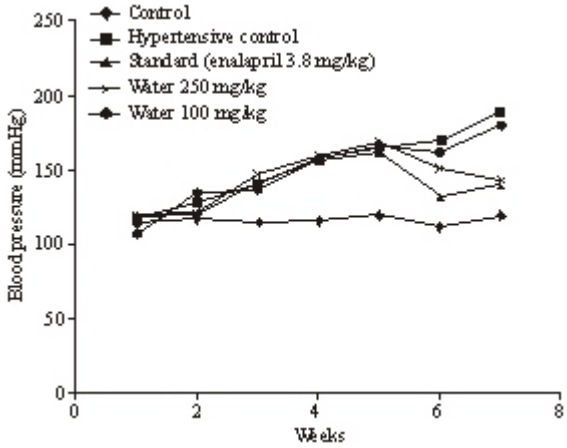


Fig. 9: Effect of the aqueous extract of *Peristrophe bicalyculata* on blood pressure of two kidney one clip hypertensive rats

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 was 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 Liver Function Tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. These enzymes leak into the blood when damage is done to the liver, and their levels increase 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 oxabacctain 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 shows 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 level in blood may reflect a balance/imbalance 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), and this may be responsible for 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, this study has demonstrated the blood pressure lowering effect of *Peristrophe bicalyculata*, and thus its efficacy in the treatment of hypertension. It has also shown the hepatoprotective effects of the plant, with the aqueous extract, especially at a higher dose of 250 mg/kg body weight being most effective. From the study, we may suggest that the mechanism of action of the plant may be through the RAAS, since the hypertension induced was renin-angiotensin system dependent.

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REFERENCES

Abdulazeez, A.M., A.J. Nok and H. Gwani, 2009. Effect of *Peristrophe bicalyculata* on lipid profile of P-407-induced hyperlipidemic wistar rats. An unpublished thesis submitted to the Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Aftab, K., 1995. Pharmacological screening of natural products for their anti-hypertensive action. Thesis submitted to the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan.

Badyal, D.K., H. Lata and A.P. Dadhich, 2003. Animal models of hypertension and effect of drugs. Indian J. Pharmacol., 35: 349-362.

Chopra, H., 1959. Medicinal Herbs of Chhattisgarh, India Having Less Known Traditional Uses. Retrieved from: http://www.botanical.com/ (Accessed: July 7, 2008).

Dwivedi, S., 2002. Ethnomedicinal Uses of Some Plant Species by Ethnic and Rural Peoples of Indore District of Madhya Pradesh, India. Retrieved from: http://www.pharmainfo.net/ (Accessed date: September 2, 2008).

De Souza, N.J., B.N. Ganguli and J. Reden, 1982. Strategies in the discoveries of drugs from natural sources. Ann. Rep. Med. Chem., 17: 301-310.

Goldblatt, H., J. Lynch, R.F. Hanzel and W.W. Summerville, 1934. Studies on experimental hypertension-1: The production of persistent elevation of systolic blood pressure by means of renal ischaemia. J. Exp. Med., 59: 347-379.

Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54(4): 275-287.

Matti, M., E. Tiula, T. Alikoski and V. Manninen, 1995. Effects of hypertension and dyslipidemia on the decline in renal function. Hypertension, 26: 670-675.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Reeves, P.G. and B.L. O'Dell, 1986. Effects of dietary zinc deprivation on the activity of angiotensin-converting enzyme in serum of rats and guinea pigs. J. Nutr., 116: 128-134.

Zimmerman, H.J., 1978. Hepatoxicity: The Adverse Effect of Drugs and Chemicals on the Liver. Apple tonCentury Crofts, New York, pp: 173-175, 353.