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Hypolidemic and Angiotensin-converting Enzyme Inhibitory Effect of *Peristrophe bicalyculata* (Retz.) Nees

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ABSTRACT

The objective of this study was to investigate the effect of *Peristrophe bicalyculata* on lipid levels and activity of angiotensin-converting enzyme in kidneys, lungs and heart of fat-fed obese Wistar rats. The angiotensin-converting enzyme inhibition pattern of *Peristrophe bicalyculata* was also studied. Apparently healthy male Wistar rats were grouped into: control group, fat-fed control, standard control (atorvastatin at 70 mg kg⁻¹) and low dose (100 mg kg⁻¹) and high dose (250 mg kg⁻¹) extract groups, after confirmation of the lethal dose (LD₅₀). All rats, except those in the control group were fat-fed until obesity was confirmed (BMI>0.3) and treatment given for 4 weeks. From our results, serum and liver levels of Total Cholesterol (TC), Triacylglycerol (TG) and Low Density Lipoprotein increased significantly (p<0.05) in obese rats compared to the control rats; while High Density Lipoprotein (HDL) significantly decreased. Administration of atorvastatin and the extract significantly (p<0.05) reduced TC, TAG and LDL levels and increased HDL in serum and liver. The activity of Angiotensin Converting Enzyme (ACE) in kidneys, lungs and heart of obese rats was significantly (p<0.05) higher than that of rats in the control group. The extract reduced ACE activity in the lungs, kidneys and heart by 97, 98 and 96%, respectively, which was 20, 30 and 15 folds that of the standard drug. Studies on the inhibition pattern of the extract showed that *Peristrophe bicalyculata* inhibits ACE by competing with the substrate for the active site, exhibiting a competitive type of inhibition. The K_m of ACE increased from 0.25 to 2.5 mM at 1% concentration and 5 mM at 2% with an unchanged V_{max} and k_i value of 1.1 mg mL⁻¹. In conclusion, this study has confirmed that the aqueous extract of *Peristrophe bicalyculata* may be useful in the reduction of cardiovascular disease risk.

Key words: *Peristrophe bicalyculata*, angiotensin-converting enzyme, obesity, lipoproteins

INTRODUCTION

Changes in life style which accompanies industrialization has a significant impact on the health of people, with obesity becoming one of the greatest health threats of the century. Obesity occurs due to an imbalance between energy intake and expenditure; with genetic, metabolic and behavioural components playing a significant role. It is a major contributor to the increasing incidence of type II diabetes, cardiovascular disease, hepatic and skeletal muscle insulin resistance, as well as some forms of cancer (Ya-ni *et al.*, 2010; Eckel *et al.*, 2006). Consumption of high levels of dietary fat is thought to be a major factor in promoting obesity in humans as well as in animals (Bray and Popkin, 1998). Obesity results in excessive fat accumulation which alters cholesterol, triglyceride and other lipid levels in the plasma and tissues and contributes to elevation of blood

pressure. Reports by Kannel *et al.* (1993) show that 78 and 65% of essential hypertension in men and women, respectively, is due to obesity. The major contributory mechanisms of obesity-related hypertension include hyperinsulinemia, hyperleptinemia, hypercortisolemia, renal dysfunction, altered vascular structure and function, enhanced sympathetic and renin-angiotensin system activity (Tuck *et al.*, 1981; Rocchini *et al.*, 1989).

Obesity is associated with the activation of the renin-angiotensin system (RAS) (Tuck, 1992). Reports have demonstrated a relationship between plasma angiotensin II levels, plasma renin activity (PRA) and activity of Angiotensin-Converting Enzyme (ACE) with BMI in humans (Licata *et al.*, 1994; Cooper *et al.*, 1997). In addition, the renin-angiotensin-aldosterone system is markedly activated in obesity. Components of the renin-angiotensin-aldosterone system are known to be affected by changes in weight. ACE is responsible for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor that plays a vital role in regulation of blood pressure. Furthermore, ACE catalyses the degradation of bradykinin, a vasodilator. Inhibition of RAAS has been an important strategy for the treatment of hypertension (Schmieder *et al.*, 2007) and as a result, ACE inhibitors have found a role as one class of antihypertensive drug. They are not only useful for blood pressure control, but could also provide beneficial action against early risks associated with cardiovascular disorders by preventing the generation of angiotensin II (Linz *et al.*, 1999).

Peristrophe bicalyculata belongs to the family Acanthaceae. It is found in the Sahel part of Mauritania to Niger and the Northern part of Nigeria and distributed throughout tropical Africa and India. It is used in the treatment of different ailments in South-Western Nigeria. It has been reported to have analgesic, anti-inflammatory and antibacterial properties (Rathi *et al.*, 2003). Mansurah (2011) reported the hypolipidemic effect of the plant on poloxamer-407-induced hyperlipidemic rats. The antihypertensive and ACE inhibitory effect of the plant in 2-K-1-clip hypertensive rats have also been demonstrated (Abdulazeez *et al.*, 2010). The result of these findings provokes interest in determining the effect of *Peristrophe bicalyculata* on lipid levels and activity of ACE using fat-fed obese rats as animal models, as well as determining the inhibition pattern of the extract.

MATERIALS AND METHODS

Reagents: HEPES Sodium salt, hippuryl-L-histidyl-L-leucine Solution (HHL), Captopril, ACE from rabbit lungs and citric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

All other chemicals used were of analytical grade.

Collection and identification of plant sample: The leaves and stems of *Peristrophe bicalyculata* used for this research were obtained from Ibadan, Oyo state, Nigeria in the month of February, 2010. The plant was identified and authenticated by the botanist in the herbarium of Ahmadu Bello University, Zaria, Nigeria with a voucher number 2863.

Preparation of plant sample and extraction: The plant was air-dried in the laboratory and made into powder by grinding. One-hundred gram of powdered plant material was added to 500 mL of distilled water and left to stand for 48 h. The extract was sieved using a muslin cloth and then filtered under suction pressure with a Whatman's filter paper. It was then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) and lyophilized (Christ Alpha 1-2 LD, Germany) to obtain the aqueous extract.

Another 500 g of the powdered plant was extracted in n-hexane (defatting) using Soxhlet apparatus, before extracting with methanol (70%). The process was repeated until all soluble

compounds had been extracted as judged by loss of colour of the filtrate. The extract was then suction filtered, concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) and lyophilized. The methanol extract was then dissolved in distilled water (1000 mL) and partitioned in ethyl acetate and n-butanol using separating funnel, to obtain methanol, ethyl acetate, butanol and water fractions which were concentrated and lyophilized.

Determination of ACE inhibitory effect of *P. bicalyculata*: The assay for ACE inhibitory activity was determined using the Cushman and Cheung (1971) with some modifications on the assay conditions. Briefly, the inhibitor solution (extract) was added to 0.1 M potassium phosphate buffer (pH 8.3), which consists of 5 mM hippuryl-histidyl-leucine (HHL), 0.1 M potassium phosphate and 0.3 M NaCl (pH 8.3). Then the enzyme, ACE was added to the mixture and incubated at 37°C for 30 min. The reaction was terminated by adding 0.25 mL of 1 M HCl and then 1.5 mL ethyl acetate was added to extract the hippuric acid formed by the action of ACE. After removal of ethyl acetate by heat evaporation, the residual Hippuric Acid (HA) was dissolved in 1 mL of deionized water and absorbance of the solution taken at 228 nm to determine the hippuric acid concentration. The inhibition will be calculated from the equation:

$$\text{Inhibition (\%)} = \frac{E_c - E_s}{E_c - E_b} \times 100$$

where, E_s is the absorbance of the reaction mixture (absorbance of HA generated in the presence of ACE inhibitor component), E_c is the absorbance of the buffer (the absorbance of HA generated without ACE inhibitors), E_b is the absorbance when the stop solution was added before the reaction occurred (the absorbance of HA generated without ACE, corresponding to HHL autolysis in the course of enzymatic assay), The inhibitory activity is the amount of inhibitor solution needed to inhibit the original ACE activity by 50% (IC_{50}).

Acute toxicity studies: Acute toxicity tests to determine the Lethal Dose (LD_{50}) was carried out as described by Lorke (1983). The aqueous extract *Peristrophe bicalyculata* was found to be non-toxic to the rats even when administered at 5000 mg kg^{-1} .

Experimental design: All experimental protocol were assessed and approved by the Animal Care and Use Committee of Ahmadu Bello University, Zaria. Thirty-five apparently healthy, recently weaned, wistar rats weighing about 100-120 g were purchased from the National Institute of Trypanosomiasis Research (NITR) Kaduna state and housed in well ventilated cages in the animal house of Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria. They were allowed to acclimatize for 2 weeks before commencement of experiment. The rats were divided into 5 groups of 7 rats each:

Group 1: Control group: Given standard diet, without treatment

Group 2: Fat-fed control: Given high fat feed, without treatment

Group 3: Standard control: Given high fat feed, treated with atorvastatin (70 mg kg^{-1})

Group 4: Low dose: Given high fat feed, treated with aqueous extract of *P. bicalyculata* at 100 mg kg^{-1}

Group 5: High dose: Given high fat feed, treated with aqueous extract of *P. bicalyculata* at 250 mg kg^{-1}

The rats were fed for 8 weeks with standard and high fat diet prepared as described by Martins *et al.* (2004). Animals were allowed free access to water and food, freshly provided daily. Body weights of all animals were assessed once weekly throughout the 8 weeks of dietary treatment until obesity was confirmed. Obesity was determined by the Lee index calculated by the cube root of body weight (g) \times 10/naso-anal length (mm), for which a value >0.3 was classified as obese (Campos *et al.*, 2008). The obese rats were then treated orally with the extract and standard drug (atorvastatin) for another 4 weeks.

Sample collection: At the end of the experiment, rats were sacrificed under anaesthesia and blood collected by cardiac puncture. The kidneys, heart and lungs were also collected, cleaned of fatty tissues and blot dried. They were homogenized at 4°C in cold trizma-HCl buffer (pH 7). The homogenate was centrifuged at 5000 g for 15 min at 4°C and the supernatant used for the assay of ACE activity. The blood was allowed to clot and serum separated using pasture pipette into clean and labeled sample bottles for determination of lipid parameters.

Determination of ACE Activity: The ACE activity was determined as described by Cushman and Cheung (1971). Organs were homogenised in cold Trizma-HCl buffer (pH = 7.8) (Sigma) and centrifuged at 4°C for 15 min and 5000 xg. The supernatants were used for the assay. Briefly, the sample (0.2 mL) was added to ACE solution (50 μ L) and the reaction started by adding 0.2 mL of 5 mmol L⁻¹ hippuric histidyl leucine. This was incubated at 37°C for 15 min. The reaction was terminated by adding 0.25 mL of 1.0 N hydrochloric acid and then 2.0 mL ethyl acetate to extract the hippuric acid formed by the action of ACE. This was centrifuged at 3600xg for 2 min and 1 mL of upper layer transferred into a microcentrifuge tube and heated by dry bath at 100°C for 15 min to remove ethylacetate by evaporation. The resulting hippuric acid was dissolved in 3.0 mL of distilled water and the absorbance read at 228 nm using a spectrophotometer (Jenway 6100, Dunmow, Essex, U.K.). Activity in tissues was expressed in units which corresponded to 1 μ mol of hippuric acid released by enzymatic hydrolysis of HHL per minute per milligram of tissue.

Determination of the inhibition pattern of *Peristrophe bicalyculata* extract on ACE: To investigate the inhibition pattern of the extract on ACE, known quantities of the extract were added to each reaction mixture containing the substrate (HHL) and ACE. The ACE inhibitory activities were measured with different concentrations of the substrate and the kinetics of ACE in the presence of the extract determined using Lineweaver-Burk plots (Cheng *et al.*, 2009).

Determination of serum and liver lipid profiles: Liver lipids were extracted according to the method of Folch *et al.* (1957). Serum and liver lipid profiles were determined using a reagents kits (Randox Laboratories Ltd., County Antrim, United Kingdom).

Statistical analysis: The results were analyzed for statistical significance by one way ANOVA using the SPSS statistical program and *post hoc* test (LSD) between groups. All data were expressed as Mean \pm SEM. The p-values <0.05 were considered significant.

RESULTS

ACE inhibitory activity of extracts of *Peristrophe bicalyculata*: Results of the ACE inhibitory activity of all the extracts are presented in Table 1. The standard drug, captopril

Table 1: Angiotensin-converting enzyme inhibitory effect of extracts of *P. bicalyculata*

Samples	IC ₅₀ (μg mL ⁻¹)
Captopril	2.31±0.17 ^a
Aqueous extract	11.84±0.88 ^b
Hexane extract	24.38±1.95 ^{c,d}
Methanol extract	34.82±3.58 ^f
Ethyl acetate fraction	34.32±2.06 ^f
Water fraction	15.16±3.82 ^b
Butanol fraction	20.26±1.40 ^d

Values (Mean±SEM) with different superscript letters are significantly at p<0.05 different

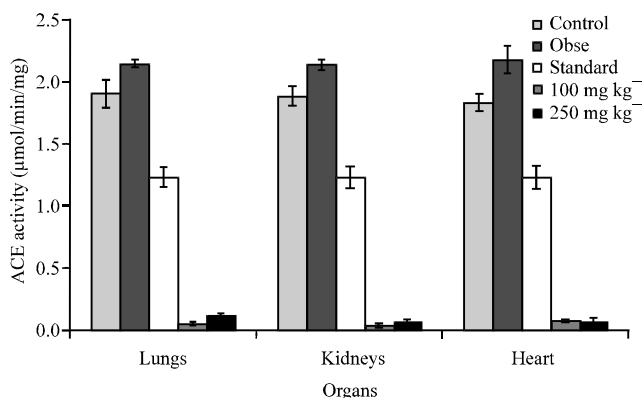


Fig. 1: Effect of aqueous extract of *Peristrophe bicalyculata* on lungs, kidney and heart ACE activity of fat fed obese Wistar rats (n = 35)

inhibited ACE activity at a concentration (2.31±0.17 μg mL⁻¹) significantly (p<0.05) lower than the extracts. The concentration at which the aqueous extract (11.84±0.88 μg mL⁻¹) inhibited the enzyme was not significantly different from that of the water fraction (15.16±3.82 μg mL⁻¹), but it was significantly (p<0.05) lower than the concentration obtained for the butanol fraction (20.26±1.40 μg mL⁻¹) and the hexane extract (24.38±1.95 μg mL⁻¹). However, the ethyl acetate fraction and methanol extract, with IC₅₀ of 34.32±2.06 and 34.82±3.58 μg mL⁻¹, respectively, inhibited the enzyme at the highest concentrations, which were not significantly different from each other.

Effect of aqueous extract of *P. bicalyculata* on ACE activity in tissues of fat-fed obese Wistar rats: From the results obtained (Fig. 1), induction of obesity significantly increased (p<0.05) lung ACE activity (2.14±0.03 μmol min⁻¹ mg⁻¹) compared to rats in the control group (1.90±0.11 μmol min⁻¹ mg⁻¹). The extract at 100 and 250 mg kg⁻¹ significantly (p<0.05) reduced lung ACE activity (0.06±0.01 and 0.12±0.05 μmol min⁻¹ mg⁻¹, respectively) compared to obese rats (2.14±0.03 μmol min⁻¹ mg⁻¹) and rats given atorvastatin (1.23±0.08 μmol min⁻¹ mL⁻¹).

Administration of the standard drug, atorvastatin, significantly (p<0.05) reduced kidney ACE activity than that of untreated obese (2.13±0.04 μmol min⁻¹ mg⁻¹) rats. However, kidney ACE levels in rats given the extract at 100 and 250 mg kg⁻¹ (0.04±0.02 and 0.07±0.02 μmol min⁻¹ mg⁻¹, respectively) was significantly lower than rats given the standard drug (1.23±0.09 μmol min⁻¹ mg⁻¹). There was a significant increase (p<0.05) in heart ACE

Table 2: Effect of aqueous extract of *Peristrophe bicalyculata* on serum lipid profile of fat-fed obese Wistar rats

Parameters (mmol L ⁻¹)	Control group	Fat-fed obese rats	Atorvastatin (mg kg ⁻¹)		
			70	100	250
TC	2.99±0.10 ^{ab}	5.25±0.87 ^c	2.83±0.08 ^{ab}	3.12±0.46 ^b	1.74±0.50 ^a
TG	0.72±0.32 ^a	3.89±0.68 ^d	2.06±0.13 ^b	2.77±0.40 ^c	2.37±0.07 ^{bc}
HDL	1.18±0.45 ^d	0.43±0.11 ^a	1.54±0.42 ^d	0.66±0.09 ^{ab}	0.95±0.09 ^{bc}
LDL	2.13±0.78 ^a	6.61±5.57 ^b	2.16±0.05 ^a	3.50±0.87 ^a	2.66±0.97 ^a

Values (Mean±SEM) with different superscript letters along the same row are significantly at p<0.05 different, n = 35

activity of fat-fed obese rats (2.17±0.11 µmol min⁻¹ mg⁻¹) compared to rats in the control group (1.83±0.07 µmol min⁻¹ mg⁻¹) and all other groups. Heart ACE activity was significantly lowered in rats given 100 (0.08±0.03 µmol min⁻¹ mg⁻¹) and 250 mg kg⁻¹ (0.07±0.03 µmol min⁻¹ mg⁻¹) extract compared to obese rats (2.17±0.11 µmol min⁻¹ mL⁻¹) and rats given the standard drug (1.23±0.09 µmol min⁻¹ mL⁻¹).

Effect of aqueous extract of *Peristrophe bicalyculata* on serum lipids of fat-fed obese Wistar rats: Induction of obesity significantly (p>0.05) increased serum levels of Total Cholesterol (TC), Triacylglycerols (TG), Low Density Lipoprotein (LDL) and decreased High Density Lipoprotein (HDL) compared to rats in control group and all other treated rats. The levels of TC, HDL and LDL of rats in the control group was not significantly (p>0.05) different from those treated with atorvastatin. Rats treated with the extract at 250 mg kg⁻¹ (1.74±0.50 mmol L⁻¹) had significantly lower TC level than those given Atorvastatin (2.83±0.08 mmol L⁻¹) and those in the control group (2.99±0.10 mmol L⁻¹). TC level in rats administered with the extract at 100 mg kg⁻¹ (3.12±0.46 mmol L⁻¹) was significantly (p<0.05) higher than those given at 250 mg kg⁻¹, showing a dose-dependent response.

Atorvastatin (2.06±0.13 mmol L⁻¹) reduced TG levels significantly (p<0.05) when compared to fat-fed obese rats (3.89±0.68 mmol L⁻¹), but was significantly (p<0.05) higher than that of control rats (0.72±0.32 mmol L⁻¹). There was no difference in levels of TG in rats administered atorvastatin and those given the extract at 250 mg kg⁻¹ (2.37±0.07 mmol L⁻¹).

Serum LDL levels of obese rats (6.61±5.57 mmol L⁻¹) was significantly (p<0.05) higher than control rats (2.13±0.78 mmol L⁻¹) and all other treated groups. However, the difference was not significant when control rats were compared to rats given atorvastatin (2.16±0.05 mmol L⁻¹) and the extract at 100 (3.50±0.87 mmol L⁻¹) and 250 mg kg⁻¹ (2.66±0.97 mmol L⁻¹).

The extract increased (p<0.05) HDL levels (in a dose-dependent manner) when compared to HDL levels of fat-fed obese rats (0.43±0.11 mmol L⁻¹), but this increase was significantly lower than that of rats given the standard drug (1.54±0.42 mmol L⁻¹) and those in the control group (1.18±0.45 mmol L⁻¹) (Table 2).

Effect of aqueous extract of *Peristrophe bicalyculata* on liver lipids of fat-fed obese wistar rats. Induction of obesity in rats increased TC, TG and LDL, while HDL level decreased significantly (p<0.05) as shown on Table 3. TC levels decreased significantly (p<0.05) in obese rats administered with atorvastatin (7.80±0.07 mmol L⁻¹) and the extract at both doses and the difference was not significant, although significantly lower than rats in the control group (1.36±0.17 mmol L⁻¹). The TG and LDL level of rats treated with Atorvastatin and the extract also decreased significantly when compared to fat-fed obese rats, but the values were significantly (p<0.05) higher than rats in the control group.

Table 3: Effect of aqueous extract of *Peristrophe bicalyculata* on liver lipid profile of fat-fed obese Wistar rats

Parameters (mmol L ⁻¹)	Control group	Fat-fed obese rats	Atorvastatin (70 mg kg ⁻¹)	<i>P. bicalyculata</i>	
				100 mg kg ⁻¹	250 mg kg ⁻¹
TC	1.36±0.17 ^a	16.94±1.03 ^c	7.80±0.07 ^b	9.34±0.62 ^b	8.29±0.70 ^b
TG	0.68±0.28 ^a	10.23±0.76 ^c	4.92±0.22 ^{bc}	5.88±0.15 ^b	5.20±0.45 ^b
HDL	0.70±0.07 ^b	0.26±0.05 ^a	0.61±0.05 ^b	0.54±0.04 ^b	0.74±0.10 ^b
LDL	1.09±0.27 ^a	17.63±0.41 ^d	9.44±0.15 ^b	11.72±0.39 ^c	9.91±0.75 ^b

Values (Mean±SEM) with different superscript letters along the same row are significantly at p<0.05 different, n = 35

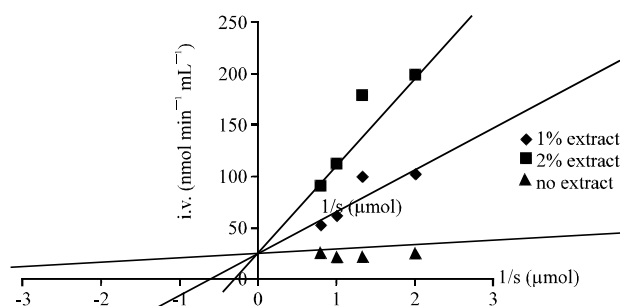


Fig. 2: Lineweaver–Burk plots for the inhibition of angiotensin-converting enzyme by aqueous extract of *Peristrophe bicalyculata*

Liver lipid levels of HDL of rats in the control group (0.70 ± 0.07 mmol L⁻¹), those treated with atorvastatin (0.61 ± 0.05 mmol L⁻¹) and the extract at both doses (0.54 ± 0.04 and 0.74 ± 0.10 mmol L⁻¹ for 100 and 250 mg kg⁻¹, respectively) was not significantly different from one each other, but significantly ($p < 0.05$) lower than that of fat-fed obese rats.

Determination of ACE inhibition pattern: The inhibition kinetics of the aqueous extract of *Peristrophe bicalyculata* was analyzed by Lineweaver-Burk plots as shown as Fig. 2. Our result shows that the inhibitory pattern of *Peristrophe bicalyculata* is competitive with respect to HHL. The extract inhibits ACE activity by competing with the substrate for the active site. Also *Peristrophe bicalyculata* exhibited a dose-dependent inhibitory effect on ACE, as it increased the K_m of ACE from 0.25 to 2.5 mM for 1% extract and 5 mM for 2% extract while the V_{max} remained unchanged (0.063 nmole min⁻¹ mL⁻¹). The K_i was found to be 1.1 mg mL⁻¹.

DISCUSSION

Effect of the aqueous extract of *P. bicalyculata* on tissue ACE activity of obese rats:

Angiotensin-converting enzyme, a decapeptidyl peptidase, is widely distributed not only in the cardiovascular system, but also in various non-cardiovascular tissues, such as the vascular endothelial cells of the lung, distal tubular epithelial cells and human alveolar macrophage (Sharifi *et al.*, 2003). The results of the present study shows elevated levels of ACE in kidneys, lung and heart of fat-fed obese rats. This agrees with reports by Sharifi *et al.* (2003) that ACE activity increases in all diseases that involve proliferation of endothelial cells, including diabetes, obesity and hypertension. Also, Abdulazeez *et al.* (2010) demonstrated that serum and tissue ACE activity were significantly elevated in two-kidney-one-clip hypertensive rats. According to Barton *et al.* (2000) obesity increases the risk of cardiovascular diseases, including hypertension, by altering the

endothelin and Renin Angiotensin Aldosterone System (RAAS), while Chan *et al.* (2005), reported that an increase in ACE activity in the obese condition is due to the activation of sympathetic nervous system by adipose tissue derived hormones which result in production of renin that convert Angiotensinogen to Angiotensin I, which is then converted to angiotensin II by ACE. Thus, from our results it is evident that increased ACE activity in obese rats may be due, in part, to alteration in RAAS.

The significant reduction in tissue ACE activity observed when rats were given aqueous extracts of *Peristrophe bicalyculata* agrees with Abdulazeez *et al.* (2010), who reported that administration of the aqueous extract of *Peristrophe bicalyculata*, significantly reduced serum and tissue ACE activity in hypertensive rats and suggested that the extract may contain ACE inhibitor(s), which inhibit the synthesis of Angiotensin II from Angiotensin I or degrade bradykinin. Our present result further proves the presence of an inhibitor, which is active in the aqueous extract of *Peristrophe bicalyculata*.

The significant reduction ($p < 0.05$) in ACE activity in rats given the aqueous extract when compared with the group given 70 mg kg^{-1} atorvastatin indicate that the extract contains potent ACE inhibitors. However, atorvastatin, known to inhibit HMG-CoA reductase, prevent the production of cholesterol and retards or even reverse progression of coronary artery disease, might have reduced ACE activity based on the fact that it causes weight loss. This is because, it has been demonstrated that ACE activity reduces with weight loss (Engeli *et al.*, 2005). This is however, inconclusive since the effectiveness of atorvastatin is dose-related.

Effect of the aqueous extract of *P. bicalyculata* on lipoprotein levels in liver and serum of obese Wistar rats:

The increase in TC, TG and LDL cholesterol levels in serum of fat-fed obese rats agrees with studies by Ono *et al.* (2006) and Xia *et al.* (2010) who reported that high fat diet increases lipoprotein levels. Mosa-Al-Reza *et al.* (2012) reported elevated TC, TG and LDL with a decreased HDL level in fat-fed rats. It is also well established that one of the main risk factors of atherosclerosis is hyperlipidemia (Genest Jr. *et al.*, 1992), as an atherogenic profile includes elevated serum TC, LDL and TC levels and reduced concentrations of HDL (Nishina *et al.*, 1993). Thus, the result of the present study is in accordance with various reports that obesity increases the risk of cardiovascular diseases (Haslam, 2007). Also, the increase in hepatic TC and TG in the fat-fed rats observed in the present study correlates with studies by Shefer *et al.* (1992), who reported a 2-fold increase in hepatic cholesterol in rats relative to control when both were fed a high-fat atherogenic diet containing cholic acid. Also, diets supplemented with cholesterol and cholic acid has been shown to markedly increase liver weights (two-fold), hepatic triglycerides (3.7 fold) and cholesterol (12 fold) concentrations in geese (Eder, 1999). Inclusion of saturated fatty acids in the diet has also been shown to produce hypercholesterolemic effect in rats (Zulet *et al.*, 1999).

Increased LDL cholesterol with decreased HDL cholesterol usually increases the serum total cholesterol. This is because the plasma clearance of cholesterol is often impaired when HDL levels are low. Triacylglycerols levels have also been found to increase with increase in plasma cholesterol. Atherogenicity therefore develops when LDL cholesterol, triacylglycerols and total cholesterol are elevated relative to plasma HDL. Elevated HDL-cholesterol improves the transportation of cholesterol from the plasma to the liver for biotransformation and excretion, thereby preventing atheroma formation and blood vessel occlusion (Ojiakor and Nwanjo, 2005).

The inhibition of HMG-CoA reductase by the standard drug, atorvastatin, causes a decrease in TC, TG and LDL, while HDL is increased (Chan *et al.*, 2002; Mansurah, 2011) as seen in our

current study. In the current study, the elevated serum and liver TC, TG and LDL levels in obese rats were markedly decreased when rats were given aqueous extract of *Peristrophe bicalyculata*, showing its lipid-lowering effect. *Peristrophe bicalyculata* has been reported to lower TC, TG and LDL cholesterol in P-407-induced hyperlipidemic rats (Mansurah, 2011). Although, the mechanism of the plant is unknown, it is possible it may have inhibited lipoprotein lipase (Wassan *et al.*, 2003; Megalli *et al.*, 2005) or increase fecal excretion of bile acids and cholesterol (Moriceau *et al.*, 2000; Soppimath *et al.*, 2001).

Inhibitory pattern of *Peristrophe bicalyculata*: Our result shows that the inhibitory pattern of *Peristrophe bicalyculata* is competitive with respect to the substrate, HHL. This shows that the extract inhibits ACE activity by competing to the substrate for the active site. Research from various studies on the ACE inhibition types of different peptides or compounds has shown that they may exhibit non-competitive (Nakagomi *et al.*, 2000; Saiga *et al.*, 2006) or mixed non-competitive inhibition (Hou *et al.*, 2003). However, our result agrees with reports of Wu and Ding (2001) and Gouda *et al.* (2006) that most ACE inhibitory peptides and compounds are competitive inhibitors.

In conclusion, this study has demonstrated that the aqueous extract of *Peristrophe bicalyculata* is hypolipidemic. It also inhibits ACE activity in obese condition, thus, it may be useful in the management of hypertension.

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REFERENCES

- Abdulazeez, A.M., C.A. Awasum, Y.S. Dogo and P.N. Abiayi, 2010. Effect of *Peristrophe bicalyculata* on blood pressure, kidney and liver functions of two kidney one clip (2K1C) hypertensive rats. *Br. J. Pharmacol. Toxicol.*, 1: 101-107.
- Barton, M., R. Carmona, H. Morawietz, L.V. Duscio and W. Goettsch *et al.*, 2000. Obesity is associated with tissue-specific activation of renal angiotensin-converting enzyme *in vivo*: Evidence for a regulatory role of endothelin. *Hypertension*, 35: 329-336.
- Bray, G.A. and B.M. Popkin, 1998. Dietary fat intake does affect obesity. *Am. J. Clin. Nutr.*, 68: 1157-1173.
- Campos, K.E., G.T. Volpato, I.M.P. Calderon, M.V.C. Rudge and D.C. Damasceno, 2008. Effect of obesity on rat reproduction and on the development of their adult offspring. *Braz. J. Med. Biol. Res.*, 41: 122-125.
- Chan, D.C., G.F. Watts, P.H. Barrett, T.A. Mori, L.J. Beilin and T.G. Redgrave, 2002. Mechanism of action of a 3-Hydroxy-3-methylglutaryl coenzyme a reductase inhibitor on apolipoprotein B-100 kinetics in visceral obesity. *J. Clin. Endocrinol. Metab.*, 87: 2283-2289.
- Chan, N.N., P.C. Tong, A.P. Kong and J.C. Chan, 2005. Obesity hypertension: The rationale for renin-angiotensin system blockade. *Hong Kong Med. J.*, 11: 217-220.

- Cheng, F.Y., T.C. Wan, Y.T. Liu, C.M. Chen, L.C. Lin and R. Sakata, 2009. Determination of angiotensin-I converting enzyme inhibitory peptides in chicken leg bone protein hydrolysate with alcalase. *J. Anim. Sci.*, 80: 91-97.
- Cooper, R., C. Rotimi, S. Ataman, D. McGee and B. Osotimehin *et al.*, 1997. The prevalence of hypertension in seven populations of west African origin. *Am. J. Public Health*, 87: 160-168.
- Cushman, D.W. and H.S. Cheung, 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem. Pharmacol.*, 20: 1637-1648.
- Eckel, R.H., R. Kahn, R.M. Robertson and R.A. Rizza, 2006. Preventing cardiovascular disease and diabetes: A call to action from the American diabetes association and American heart association. *Diabetes Care*, 29: 1697-1699.
- Eder, K., 1999. Fatty acid composition of tissue lipids of growing geese fed cholesterol and cholic acid. *J. Anim. Physiol. Anim. Nutr.*, 81: 141-149.
- Engeli, S., J. Bohnke, K. Gorzelniak, J. Janke and P. Schling *et al.*, 2005. Weight loss and the renin-angiotensin-aldosterone system. *Hypertension*, 45: 356-406.
- Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Genest Jr., J., J.R. McNamara, J.M. Ordovas, J.L. Jenner and S.R. Silberman *et al.*, 1992. Lipoprotein cholesterol, apolipoprotein A-I and B and lipoprotein (a) abnormalities in men with premature coronary artery disease. *J. Am. Coll. Cardiol.*, 19: 792-802.
- Gouda, K.G.M., L.R. Gowda, A.G.A. Rao and V. Prakash, 2006. Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11s globulin of soybean (*Glycine max*). *J. Agric. Food Chem.*, 54: 4568-4573.
- Haslam, D., 2007. Obesity: A medical history. *Obes. Rev.*, 8: 31-36.
- Hou, W.C., M.H. Lee, F.L. Hsu and Y.H. Lin, 2003. Inhibitory activities of semicarbazide-sensitive amine oxidase and angiotensin converting enzyme of pectonhydroxamic acid. *J. Agric. Food Chem.*, 51: 6362-6366.
- Kannel, W.B., R.J. Garrison and A.L. Dannenberg, 1993. Secular blood pressure trends in normotensive persons. *Am. Heart J.*, 125: 1154-1158.
- Licata, G., R. Scaglione, A. Ganguzza, S. Corrao and M. Donatelli *et al.*, 1994. Relationship between fasting serum insulin, plasma renin activity and diastolic blood pressure in young obese subjects. *Am. J. Hypertens.*, 7: 314-320.
- Linz, W., P. Wohlfart, A.S. Bernward, T. Malinski and G. Wiemer, 1999. Interactions among ACE, kinins and NO. *Cardiovasc Res.*, 43: 549-561.
- Lorke, D., 1983. A new approach to practical acute toxicity testing. *Arch. Toxicol.*, 54: 275-287.
- Mansurah, A., 2011. Effect of *Peristrophe bicalyculata* on lipid profile of P-407-induced hyperlipidemic Wistar rats. *J. Med. Plants Res.*, 5: 490-494.
- Martins, A.C.P., K.L.A. Souza, M.T. Shio, P.C.F. Mathias, P.I. Lelkes and R.M.G. Garcia, 2004. Adrenal medullary function and expression of catecholamine-synthesizing enzymes in mice with hypothalamic obesity. *Life Sci.*, 74: 3211-3222.
- Megalli, S., F. Aktan, N.M. Davies and B.D. Roufogalis, 2005. Phytopreventive anti-hyperlipidemic effects of *Gynostemma pentaphyllum* in rats. *J. Pharm. Pharmaceut Sci.*, 8: 507-515.

- Moriceau, S., C. Besson, M.A. Levrat, C. Moundras, C. Remesy, C. Morand and C. Demigne, 2000. Cholesterol-lowering effects of guar gum: Changes in bile acid pools and intestinal reabsorption. *Lipids*, 35: 437-444.
- Mosa-Al-Reza, H., S. Saeed, D.A. Sadat and A. Marziyeh, 2012. Comparison of the beneficial effects of guar gum on lipid profile in hyperlipidemic and normal rats. *J. Med. Plants Res.*, 6: 1567-1575.
- Nakagomi, K., R. Yamada, H. Ebisu, Y. Sadakane, T. Akizawa and T. Tanimura, 2000. Isolation of acein-2, a novel angiotensin-I converting enzyme inhibitory peptide derived from a tryptichydrolysate of human plasma. *FEBS Lett.*, 467: 235-238.
- Nishina, P.M., S. Lowe, J. Verstuyft, J.K. Naggert, F.A. Kuypers and B. Paigen, 1993. Effects of dietary fats from animal and plant sources on diet-induced fatty streak lesions in C57BL/6J mice. *J. Lipid Res.*, 34: 1413-1422.
- Ojiakor, A. and H. Nwanjo, 2005. Effect of vitamin E and C on exercise-induced oxidative stress. *Global J. Pure Appl. Sci.*, 12: 199-202.
- Ono, Y., E. Hattori, Y. Fukaya, S. Imai and Y. Ohizumi, 2006. Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats. *J. Ethnopharmacol.*, 106: 238-244.
- Rathi, A., C.V. Rao, S. Khatoon and S. Mehrotra, 2003. Ethnopharmacological evaluation of *Peristrophe bicalyculata* Nees for anti-inflammatory and analgesic activity. *Nat. Prod. Sci.*, 9: 195-199.
- Rocchini, A.P., V. Katch, D. Kveselis, C. Moorehead, M. Martin, R. Lampman and M. Gregory, 1989. Insulin and renal retention in obese adolescents. *Hypertension*, 14: 367-374.
- Saiga, A., T. Okumura, T. Makihara, S. Katsuda, F. Morimatsu and T. Nishimura, 2006. Action mechanism of an angiotensin I-converting enzyme inhibitory peptide derived from chicken breast muscle. *J. Agric. Food Chem.*, 54: 942-945.
- Schmieder, R.E., K.F. Hilgers, M.P. Schlaich and B.M. Schmidt, 2007. Renin-angiotensin system and cardiovascular risk. *Lancet*, 369: 1208-1219.
- Sharifi, A.M., N. Akbarloo, B. Heshmatian and A. Ziai, 2003. Alteration of local ACE activity and vascular responsiveness during development of 2K1C renovascular hypertension. *Pharmacol. Res.*, 47: 201-209.
- Shefer, S., L.B. Nguyen, G. Salen, G.C. Ness and I.R. Chowdhary *et al.*, 1992. Differing effects of cholesterol and taurocholate on steady state HMG-CoA reductase and cholesterol 7 α -hydroxylase activities and mRNA levels in the rat. *J. Lipid Res.*, 33: 1193-1200.
- Soppimath, K.S., A.R. Kulkarni and T.M. Aminabhavi, 2001. Chemically modified polyacrylamide-g-guar gum-based crosslinked anionic microgels as pH-sensitive drug delivery systems: Preparation and characterization. *J. Control Release*, 75: 331-345.
- Tuck, M.L., J. Sowers, L. Dornfeld, G. Kledzik and M. Maxwell, 1981. The effect of weight reduction on blood pressure, plasma renin activity and aldosterone levels in obese patients. *N. Eng. J. Med.*, 304: 930-933.
- Tuck, M.L., 1992. Obesity, the sympathetic nervous system and essential hypertension. *Hypertension*, 19: 167-177.
- Wassan, K.M., S. Ramaswamy, M. Kwong, I.J. Goldberg, T. Wright and T.P. Johnston, 2003. Poloxamer 407-mediated alterations in the activities of enzymes regulating lipid metabolism in rats. *J. Pharm. Pharmaceut. Sci.*, 6: 189-197.

- Wu, J. and X. Ding, 2001. Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptides derived from soy protein on spontaneously hypertensive rats. *J. Agric. Food Chem.*, 49: 501-506.
- Xia, D., X. Wu, Q. Yang, J. Gong and Y. Zhang, 2010. Anti-obesity and hypolipidemic effects of a functional formula containing *Prunus mume* in mice fed high-fat diet. *Afr. J. Biotechnol.*, 9: 2463-2467.
- Ya-ni, Y., Y. Qiong-Fen, N. Fu, L. Xiao-Wei and L. Fang-Gen, 2010. Effect of four Bifidobacteria on obesity in high fat diet induced rats. *World J. Gastroenterol.*, 16: 3394-3401.
- Zulet, M.A., A. Barber, H. Garcin, P. Higuieret and J.A. Martinez, 1999. Alterations in carbohydrate and lipid metabolism induced by a diet rich in coconut oil and cholesterol in a rat model. *J. Am. Coll. Nutr.*, 18: 36-42.