Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2015; 3(5B):1936-1940 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Angiotensin Converting Enzyme (ACE): Inhibition of Sheep Kidney and Lung ACE In vitro by *Rauwolfia serpentina* and *Allium sativum*

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Abstract: Regulation of blood pressure is one of the important functions of Angiotensin I converting enzyme (ACE, EC. 3.4.15.1). ACE is widely distributed and appears to have several important physiological functions including its major role in blood pressure regulation. In the treatment of congestive heart failure, coronary artery disease, diabetic nephropathy etc.ACE inhibitors are used. The current study was aimed to find the effect of aqueous extracts of *Rauwolfia serpentina* leaves and *Allium sativum* cloves on sheep kidney and lung ACE. The method is Hippuryl-Histidyl-Leucine (HHL) as substrate, sheep kidney and lung ACE activity was measured and the hippuric acid released was measured spectrophotometrically at 228 nm. Aqueous extracts of *Rauwolfia serpentina* leaves and *Allium sativum* cloves were used in the enzyme assay to determine their effect on kidney and lung ACE. The results of linearity of ACE activity in kidney and lung was established with HHL as substrate for the incubation period of 30 min at 37°C. ACE activity was confirmed with specific ACE inhibitors like Captopril, Lisinopril, and Enalapril.25 µl of *R. serpentina* leaf extract reduced ACE activity by 68% and 57% in kidney and lung respectively. 25 µl of *A. sativum* cloves extract reduced ACE activity by 50% and 60% in kidney and lung respectively. In conclusion, use of medicinal plants is gaining considerable significance as a drug to treat hypertension. The significant inhibition of kidney and lung ACE activity by these two plant products suggests their possible role in controlling blood pressure as a mode of treatment for cardiovascular diseases, when used as supplement medicine.
Keywords: Angiotensin Converting Enzyme (ACE), Blood pressure, ACE Inhibitors, *Rauwolfia serpentina*, *Allium sativum.*

INTRODUCTION

Angiotensin Converting Enzyme (EC-3.4.15.1) is a zinc metalopeptidase that hydrolyses carboxyl terminal dipeptide of many oligopeptide substrates, inclusive of angiotensin I (Ang I) and bradykinin [1]. This enzyme is most commonly related with the control of blood pressure [2, 3]. The octapeptide Angiotensin II is one of the most potent vasoconstrictors [4]. ACE is widely distributed in various body tissues, in which predominantly present in kidney epithelium [5, 6]. In Renin – Angiotensin – Aldosterone system (RAS), ACE and angiotensin II are biologically active components of RAS. These components play a key role in maintenance of blood pressure [7, 8]. ACE inhibitors competitively inhibit the angiotensin converting enzyme which is used to treat hypertension, cardiac failure, diabetic nephropathy, acute myocardial infarction [9].

Hypertension is a word used to denote chronic disease i.e., high blood pressure due to the exertion of force of blood against the walls of arteries but sustained hypertension over time is a major risk factor for cerebro vascular, coronary artery and renal diseases. It is considered to be one of the major morbidity and mortality factors due to its association with different organs and its severe consequences including myocardial infarctions, strokes and heart failure [10]. Excessive use of antihypertensive drugs can cause certain side effects like cough, hyperkalemia, headache, dizziness, fatigue, nausea, hypotension and renal impairment was been reported [11]. In such cases, use of herbal drugs as therapeutic agents is helped to prevent hypertension and there will be enhancement of metabolic health [12].

Widely used ACE inhibitors include Captopril, Lisinopril and Enalapril, however show certain side effects as mentioned above. A large number of natural inhibitors are well known for their hypotensive action. Some of these include Gooseberry, Gokshura, Rose petal jam, Turmeric, Ginger, Cinnamon, and Cardamom etc [13]. *Allium sativum* commanly known as Garlic, is a member of the Alliaceae family, may be one of the known medicinal plant, used since ancient time to cure

different disease conditions in humans. Garlic is a principal medicinal, which is used to lower blood pressure and cholesterol, fight infections, and prevent cancer [14]. *Rauwolfia serpentina* (Sarpagandha), is a member of the Apocynaceae family, an important medicinal plant in the pharmaceutical world due to the presence of its immense therapeutic properties. It is generally used in medicine and used mainly for the treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia, and epilepsy[15].This study was initiated to analyze inhibitory effect of these aqueous extract of medicinal plants on kidney and lung ACE activity using enzyme kinetics.

MATERIALS AND METHODS

Sheep kidney tissue, Sheep lung tissue, *Rauwolfia serpentine* leaves, *Allium sativum* cloves, Hippuryl- L- Histidyl - L- Leucine (HHL, Sigma) Captopril (Sigma), Lisinopril (Sigma) Enalapril (E. Merck). Other reagents used were analytical grade commercial chemicals.

Collection of sheep tissues (kidney and lung) were obtained from local slaughter house. Only parts of the tissue free from any disease process were used in the study. Tissue extract was prepared by weighing 2g of cleaned tissue and homogenized using 0.1M phosphate buffer after which it was cold centrifuged at 10,000g to collect the supernatant. This was then dialyzed using cellulose membrane against same buffer. Protein content in the extract was measured by the method of Lowry *et al*, [20] using Bovine Serum Albumin (BSA) as standard.

Tissue (kidney and lung) ACE activity was measured with Hippuryl-Histidyl-Leucine (HHL) as substrate by a method modified from Cushman and Cheung [16]. The reaction mixture (0.175 ml) contained 0.1 ml of 5mM HHL in 0.2M phosphate buffer of pH 8.3 containing 600mM sodium chloride and tissue extract (10-50 µl). After 30 minutes of incubation at 37ºC, the reaction was arrested by adding 0.175ml of 1M HCl. Hippuric acid (HA) released was extracted with 2ml ethyl acetate and resuspended in ethanol and measured spectrophotometrically at 228nm.Also, substrate saturation was performed in the presence of 20µl of kidney extract and 40µl of lung extract with substrate concentration varying from 1mM - 8 mM, K_m and V_{max} values for ACE were measured. Tissue ACE activity was also measured similarly, in the presence of well known ACE inhibitors Captopril, Enalapril and Lisinopril [17].One unit of ACE activity is defined as the amount of enzyme catalyzing the release of 1nanomole of Hippuric acid per minute at 37ºC [18]**.**

Inhibitor extract was prepared, 4g of serpentine leaves and 4 g of garlic cloves were homogenized (separately) in 10ml of 0.1 M phosphate buffer (1:2.5 aqueous extract) and cold centrifuged at 10,000g for 20 minutes. The supernatant from each were taken and used for ACE inhibition assays.Tissue (kidney and lung) ACE activity in presence of inhibitor was measured from Cushman and Cheung method with 25µl each of Sarpagandha and Garlic extracts. Also, Substrate saturation in presence of inhibitor was measured as mentioned with 20µl and 40µl of kidney and lung tissue extract respectively and with 25µl of each inhibitor makes the total reaction volume to 0.175ml using 0.2M phosphate buffer. One Inhibitory potency is equivalent to the decrease in one unit of ACE activity [18].

RESULTS

The linearity of kidney and lung ACE activity was established with HHL as substrate for an incubation period of 30min at 37°C was 26.0±1.18 and 24.4 ±0.96 nmoles of hippuric acid released/ml/min respectively (Figure 1; Table 1). K_m for the kidney ACE activity was measured as 5.0mM, V_{max} was 24nm of HA /min. K_m for the lung ACE activity was measured as 4.8mM, V_{max} was 24.8nM of HA/min (Figure 2).ACE activity was determined in presence of inhibitors, 7.45±0.97, 6.75 ±1.47 on kidney and lung in case of *R.serpentina and* 12.75±0.85, 11.90 ±1.38 on kidney and lung in case of *A.sativum* (Table 2) and their was a significant decrease in (1:2.5 aqueous extract) ACE activity (Figure 3 & 4). In kidney inhibitory potency of *Allium sativum* was found to be 15 inhibitory units with 50% inhibition and *Rauwolfia serpentina* was 14 inhibitory units with 68% inhibition. In lung, inhibitory potency of *Allium sativum* was found to be 13 inhibitory units with 60% inhibition and *Rauwolfia serpentina* was 17 inhibitory units with 57% inhibition (Table 2). Hence these two plant products inhibited ACE activity very significantly.

Table-1: ACE activity* in Sheep kidney and lung tissues

Tissue	ACE Activity*
Kidney	26.0±1.18
Lung	24.4 ±0.96

*ACE activity is expressed as nm of Hippuric acid released/ml/min.
Data are expressed as Mean±SD; n=6

Table-2: ACE activity* in Sheep kidney and lung tissues in presence of inhibitors

Inhibitors	ACE Activity in kidney	ACE Activity in lung	% Inhibition kidney	% Inhibition lung
Rauwolfia serpentine	7.45±0.97	6.75 ±1.47	68	57
Allium sativum	12.75±0.85	11.90 ±1.38	50	60

*ACE activity is expressed as nm of Hippuric acid released/ml/min.
Data are expressed as Mean±SD; n=6

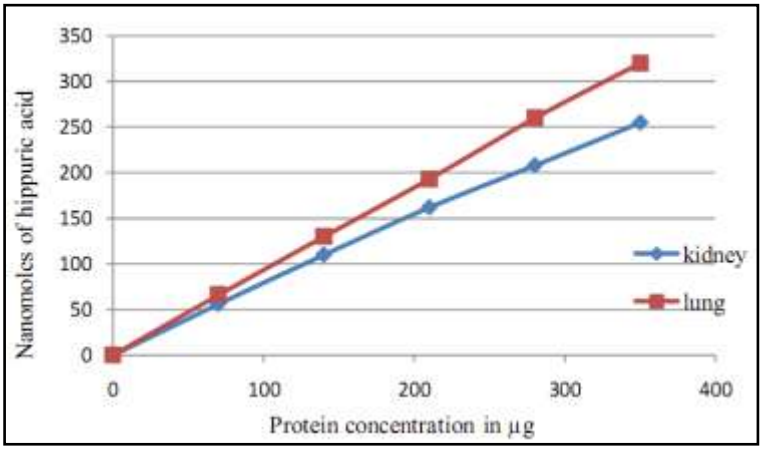

Fig-1: Effect of enzyme concentration

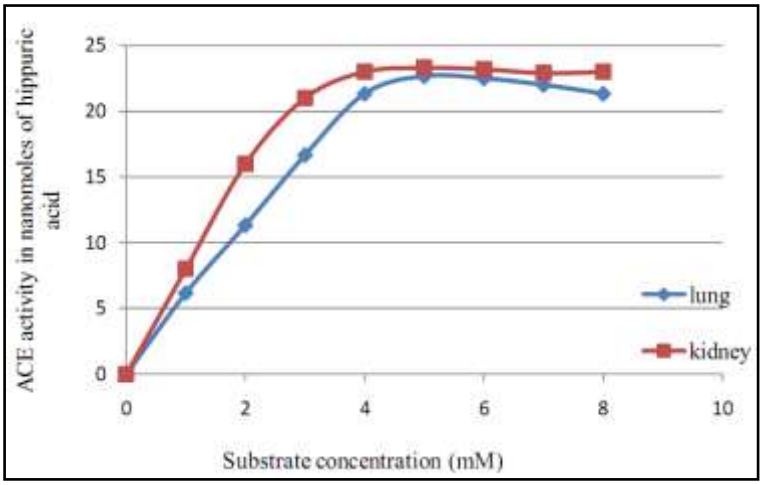

Fig-2: Substrate Saturation Curve

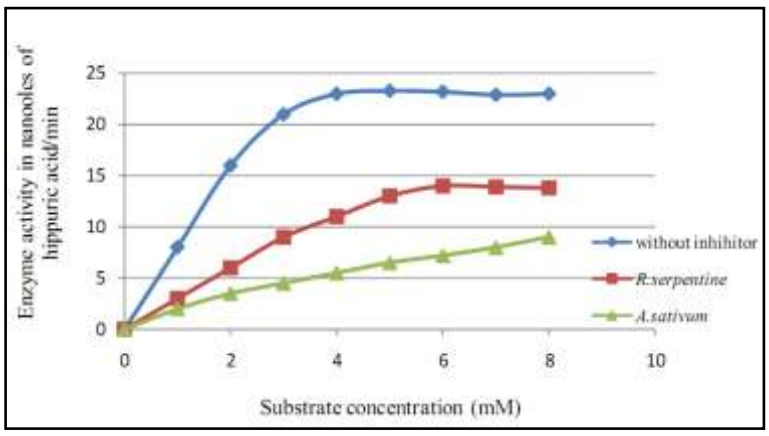

Fig- 3: Substrate saturation in presence of *R.serpentine*

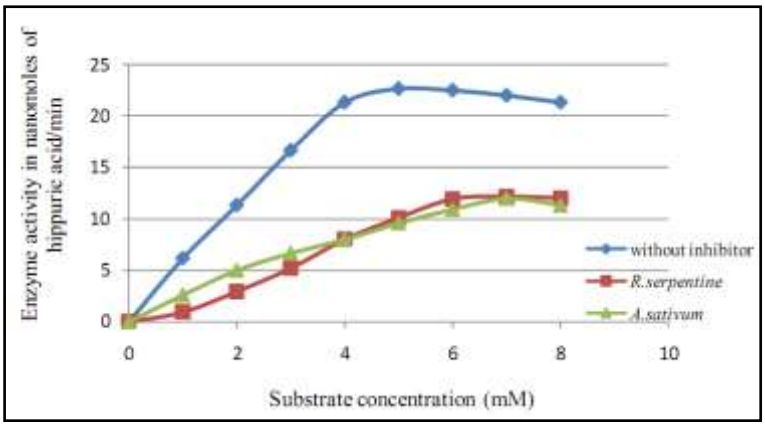

Fig-4: Substrate saturation in presence of *A.sativum*

DISCUSSION

Linearity in the figure 1 has indicated a significant ACE activity in the lung and kidney, which is found to be high in kidney [Table1].The Substrate concentration found to be 5mM when velocity of the reaction reached half of the Vmax, which is also reported in earlier studies [19]. Hence, the experiment has further carried out by taking substrate concentration constant at 5mM.Substrate saturation curve in presence of inhibitors has showed high Km value with maximum velocity being unchanged, which may be refers to the inhibitory components present in the crude extract probably show competitive type of inhibition. Therefore, these plants will be good antihypertensive agents by inhibiting ACE activity.

CONCLUSION:

This study was conducted in a systematic manner using medicinal plants in which, they may reduce blood pressure. Due to its medicinal properties, the two plants likely have some possible mechanism by which *Allium sativum* exert renoprotective properties could be through inhibition of ACE activity and *Rauwolfia serpentina* is effective in treating liver disease, cancer and mental illness also. Our study may supports that, the aqueous extract of two plants can be used for treating hypertension as their extracts are good inhibitors of ACE by enzyme kinetics. Thus, these plants can be an alternative treatment for hypertension.

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