INTRODUCTION
Angiotensin Converting Enzyme (EC-3.4.15.1) is a zinc metallopeptidase 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 cerebrovascular, 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 commonly 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 37°C, the reaction was arrested by adding 0.175ml of 1M HCl. Hippuric acid (HA) released was extracted by a method modified from Cushman and Cheung method with 25µl 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). 

\[ K_m = \text{24nm of HA/min} \]

\[ V_{max} = \text{24nm of HA/min} \]

In kidney inhibitory potency of \( R.serpentina \) was 24.8±0.96 on kidney and lung inhibitory potency of \( R.serpentina \) was 14 inhibitory units with 57% inhibition (Table 2) and their was a significant decrease in (1:2.5 aqueous extract) ACE activity (Figure 3 & 4). In kidney inhibitory potency of \( Allium sativum \) and \( R.serpentina \) 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**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ACE Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>26.0±1.18</td>
</tr>
<tr>
<td>Lung</td>
<td>24.4 ±0.96</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>ACE Activity in kidney</th>
<th>ACE Activity in lung</th>
<th>% Inhibition kidney</th>
<th>% Inhibition lung</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rauwolfia serpentine</em></td>
<td>7.45±0.97</td>
<td>6.75 ±1.47</td>
<td>68</td>
<td>57</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>12.75±0.85</td>
<td>11.90 ±1.38</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

*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.serentina
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.

REFERENCES