

AN IN VITRO STUDY OF CINNAMOMUM ZEYLANICUM AS NATURAL INHIBITOR OF ANGIOTENSIN-CONVERTING ENZYME (ACE) ON SHEEP (OVIS ARIES) TISSUESRANJINI H S¹, PADMANABHA UDUPA E G^{1*}, SHOBHA U KAMATH¹, MANJUNATH SETTY², BASAVARAJ HADAPAD³, ASHA KAMATH⁴

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ABSTRACT

Objective: The present study was aimed to find the angiotensin-converting enzyme (ACE) inhibitory activity using the methanolic extract of *Cinnamomum zeylanicum* (as a natural inhibitor) on sheep tissues as the enzyme source.

Methods: Hippuryl-histidyl-leucine (HHL) as a substrate, tissue ACE activity was measured spectrophotometrically at 228 nm. For an incubation period of 30 minutes at 37°C, the linearity of ACE activity of kidney, lung, and testis enzyme was established. A known medicinal plant *C. zeylanicum* was used as natural inhibitor of ACE. In this enzyme assay, inhibitory effect of methanolic extract of *C. zeylanicum* on kidney, lung and testicular ACE was determined. ACE activity was confirmed by captopril, a standard inhibitor of ACE.

Results: In the presence of a methanolic extract of *C. zeylanicum* (10:1), ACE activity was determined and this has inhibited ACE activity very significantly. *C. zeylanicum* leaves extract has reduced sheep kidney, lung, and testis ACE activity by 70.06%, 12.63%, and 20.23%, respectively.

Conclusion: Significant inhibition was observed in the kidney ACE than in lung and testis ACE activity. This can propose that there may be a possible role in controlling blood pressure or reduction in cardiovascular diseases. Some plants with the great medicinal property may be considered as promising sources of natural inhibitors of ACE for medicine and commercial uses. This comprehensive study may show numerous beneficial effects as a potential therapeutic agent for lowering blood pressure.

Keywords: Angiotensin-converting enzyme, Natural angiotensin-converting enzyme inhibitor, Kinetic assay, Hippuryl-histidyl-leucine, *Cinnamomum zeylanicum*, Cardiovascular diseases.

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INTRODUCTION

Angiotensin-converting enzyme (ACE, EC: 3.4.15.1), a ubiquitous endothelial enzyme, interdigitates via physiologically two critical systems, i.e., Renin-angiotensin system (RAS) and bradykinin pathways. The RAS is involved in the regulation of blood pressure, but it is also important at the microvascular level in the regulation of neovascularization. ACE and angiotensin II (a potent vasoconstrictor) [1] are biologically active components [2,3] in RAS. ACE is a bivalent dipeptidyl carboxyl metalloproteinase, endothelial, a luminal surface enzyme that affects multiple systems. Systemic ACE functions in the RAS are controlling fluid balance, blood pressure, and there is also considerable evidence for the potential role of ACE in reproductive functions [4] due to the presence of ACE in testicular Leydig cells. It is also predominantly present in kidney epithelium [5,6]. Some commonly used ACE inhibitors for hypertension, cardiac failure, diabetic nephropathy, acute myocardial infarction [7] are captopril, lisinopril, and enalapril. However, some of them show certain side effects [8]. Excessive use of antihypertensive drugs can cause certain side effects such as cough, hyperkalemia, headache, dizziness, fatigue, nausea, hypotension, and renal impairment [9]. In such cases, maybe, the use of herbal drugs as therapeutic agents may aid to prevent hypertension with enhancement of metabolic health [10]. Large numbers of natural inhibitors such as gooseberry, gokshura, rose petal jam, turmeric, ginger, cinnamon, and cardamom are well known for their hypotensive action [11].

Cinnamon (*Dalchini*) belongs to genus *Cinnamomum*, family Lauraceae, which is largely distributed in India. Its bark/crust and leaves are

widely used as spices in food or to produce essential oils. It attributes to antioxidant, anti-microbial, anti-diarrheal, anti-ulcer, hypolipidemic, and hypoglycemic activities [12]. The chemical constituents containing are essential oils, resinous compounds, cinnamaldehyde, and cinnamate [13]. These contents are responsible for pungent taste and scent. Cinnamon-like antioxidants are added into food to terminate the free radical chain reaction and delay oxidation process [14]. Cinnamon may prevent myocardial infarction-like complications such as oxidative stress-mediated free radicals and high lipid level through antioxidant defense mechanism and its hypolipidemic activity [15]. It is a known medicinal plant used since ancient time to cure different disease conditions in humans as well as in *in vitro* studies as potential ACE inhibitors. This plant has selected for the study because it was used as a source of ACE inhibitors in earlier studies and reported inhibition of ACE [16]. However, detailed studies such as isolation of active components and kinetic studies of ACE inhibition from this medicinal plant are not reported. The interaction between inhibitor and different isoforms of ACE may differ each other and it may be possible to elucidate the same in this study. Hence, this study was initiated to analyze the inhibitory effect of methanolic extract of this medicinal plant on kidney, lung, and testis ACE activity using enzyme kinetics.

METHODS

Hippuryl-histidyl-leucine, Captopril and Bovine serum albumin (BSA) are obtained from sigma chemicals, St. Louis, MO USA. Other chemicals used in this study, are analytical grade (AR) commercial chemicals.

Collection and identification of plant material

Cinnamomum zeylanicum (Bark) was collected from Basaveshwara estate, Shanivarsanthe, Somwarpete Taluk, Kodagu district, Karnataka. This plant was identified with a botanist and submitted the herbarium of *C. zeylanicum* to the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, as well as collected phytochemistry pharmacognosy (PP No: 602A), and the same plant bark was used in the study. Using a blender, dried bark of the plant was ground into a uniform powder and then stored at room temperature.

Estimation of ACE activity

This study was conducted in the Department of Biochemistry, Kasturba Medical College, Manipal, Manipal University, and there was no ethical issue by the Institutional Animal Ethics Committee, KMC, Manipal, Manipal University (IAEC/KMC/39/2014). From local slaughter house, sheep tissues were collected. An amount of 2 g of cleaned tissue was homogenized in 10 ml of 0.1 M phosphate buffer (1:5) and centrifuged at 10 000 g, 4°C for 25 minutes. Supernatant was collected and dialyzed using 0.1 M phosphate buffer [17]. Using BSA as standard, protein content was measured by the method of Lowry *et al.* [18]. Tissue ACE activity was measured by a method modified from Cushman and Cheung using HHL as substrate [17,19]. Tissue ACE activity was also measured similarly in the presence of well-known ACE-inhibitor, i.e., captopril as standard. Hence, the amount of enzyme catalyzing the release of 1 μ mol of hippuric acid per minute at 37°C can be defined as one unit of ACE activity [20,27].

Extraction of powdered plant (Bark) material in methanol

The powder of the plant part is extracted with methanol using a Soxhlet apparatus (60-80°C, 1:50 w/v) until the last extract is colorless. The combined extract is filtered and the filtrate is concentrated and evaporated on a boiling water bath until to afford the 50% extract [21]. Tissue ACE activity in the presence of inhibitor (*C. zeylanicum* [Bark]) was measured from Cushman and Cheung method [17], with 25 μ l of methanolic exact of inhibitor.

RESULTS

The percentage of ACE inhibition was compared in tissues from kidney, lung, and testis in triplicates from natural inhibitor as a methanolic extract of *C. zeylanicum* (10:1) and in captopril as standard drug ACE activity was confirmed with specific ACE inhibitor. In this experimental study, the following graphs, for an incubation period of 30 minutes at 37°C, the linearity of ACE activity of different aliquots from 10 to 50 μ l of kidney, lung, and testis enzyme was established with HHL as substrate.

DISCUSSION

In RAS system, angiotensin II as a potent vasoconstrictor increases angiotensin II (Ang-II) level. One of the causes is an increase in blood pressure leading to hypertension [22,28]. Due to over/long use of synthetic ACE inhibitors, some of the side effects are mentioned. To reduce these side effects, *C. zeylanicum* as natural inhibitor can be used. An acceptable increase in ACE activity was observed in Figs. 1-3 though it is a crude extract where concentration was unknown and also could know the concentration of protein in μ g/ml. *C. zeylanicum* has a potential role in inhibiting the activity of enzymes relevant for hyperglycemia and hypertension [23]. In Tables 1 and 2, data were expressed in median and interquartile range (IQR), and ACE activity in kidney, lung, and testis was 17.77 (17.75, 17.79), 21.69 (21.66, 21.74), and 29.50 (28.90, 29.61) hippuric acid released/g/minute, respectively. ACE activity was also determined in the presence of *C. zeylanicum* in kidney, lung, and testis which was 5.32 (3.55, 5.93), 18.95 (12.66, 20.63), and 23.53 (16.69, 26.1) hippuric acid released/g/minute, respectively, where there is reduction in the ACE activity when compared with the absence of inhibitor. Some studies show that the use of this extract has a significant increase in sperm motility and sperm count [24,30]; however, this study does not have any effect on testicular ACE. ACE activity in the presence of standard drug (50 μ m) captopril was 1.48 (1.23, 1.66), 8.06 (6.71, 9.84), and 14.37 (9.15, 16.08) hippuric acid released/g/minute and there was a significant decrease in the

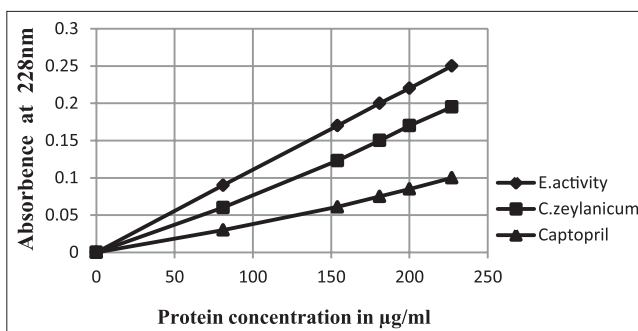


Fig. 1: Kidney

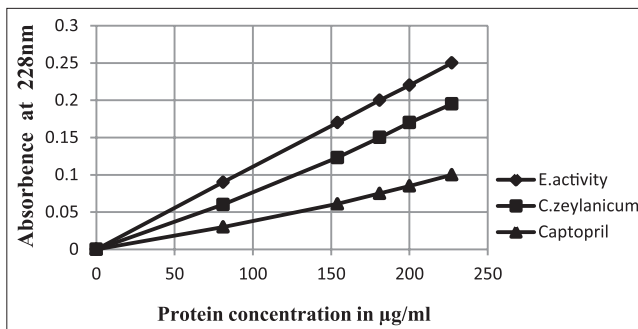


Fig. 2: Lung

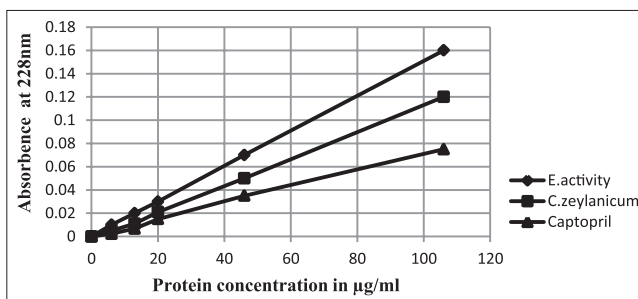


Fig. 3: Testis

ACE activity in 1% of inhibitor extract. Hence, ACE activity is more in kidney than in lung and testis and also as ACE activity decreases, percentage of inhibition increases. Probably, then, *C. zeylanicum* as inhibitor might be more effective toward kidney ACE isoform in which can help to correlate the different functions and actions of Ang-II with different isoforms of ACE [29]. Hence, the percentage of ACE inhibition in the presence of *C. zeylanicum* was 70.06%, 12.63%, and 20.23% in kidney, lung, and testis, respectively. Percentage (%) of ACE inhibition in the presence of captopril was 91.67%, 62.84%, 51.28% in kidney, lung, and testis, respectively. Hence, this plant has inhibited ACE activity very significantly. The median value of ACE activity in all tissues in the presence of inhibitor was found to be significant when compared to control ($p < 0.01$) in Table 1. The median value of ACE activity in all tissues was statistically significantly different from other two groups ($p < 0.01$) in Tables 1 and 2. Hence, ACE has been inhibited significantly by 70% which is almost near to standard drug (captopril), i.e., 91.7% [27] which is a crude extract. Hence, we can expect much higher inhibition if we could purify the active component from different solvents. Therefore, this plant will be a good antihypertensive agent by inhibiting ACE activity in different tissues.

C. zeylanicum is one of the most well-known natural antioxidants and has been shown to possess various biological properties such as antibiotic, antioxidative, anti-diabetic, anti-influenza, and apoptosis-inducing properties [25]. Use of antioxidant containing compounds can

Table 1: ACE activity in tissues and percentage (%) of ACE inhibition in presence of *Cinnamomum zeylanicum*

Category	ACE activity in absence of inhibitor	ACE activity in presence of inhibitor	% of inhibition in presence of inhibitor
Kidney	17.77* (17.75,17.79)	5.32* (3.55,5.93)	70.06
Lung	21.69* (21.66,21.74)	18.95* (12.66,20.63)	12.63
Testis	29.50* (28.90,29.61)	23.53* (16.69,26.1)	20.23

*ACE activity is expressed as μm of hippuric acid released/g/minute, *data is expressed in median (IQR), *number of trials carried is in triplicates (n=3). *p<0.05, ACE: Angiotensin converting enzyme, IQR: Interquartile range, *C. zeylanicum*: *Cinnamomum zeylanicum*

Table 2: ACE activity and percentage (%) of ACE inhibition in presence of captopril

Category	ACE activity in presence of captopril	% of inhibition in presence of captopril
Kidney	1.48* (1.23,1.66)	91.67
Lung	8.06* (6.71,9.84)	62.84
Testis	14.37* (9.15,16.08)	51.28

*ACE activity is expressed as μm of hippuric acid released/g/minute, *data were expressed in median (IQR), *number of trials carried is in triplicates (n=3),

*p<0.05, ACE: Angiotensin converting enzyme, IQR: Interquartile range

be beneficial toward some studies not only to maintain antioxidants levels in the body but also to treat the long-term complications that can arise [13,26]. Therefore, the search for more effective and safer hypotensive compounds from medicinal plants has continued to be an important area of active research.

CONCLUSION

Using medicinal plants, the study was undertaken in a systematized method in which they may have the property of reducing blood pressure. Due to medicinal properties of *C. zeylanicum*, there was significant inhibition in the kidney than in lung and testis. Inhibition of ACE activity by this plant suggests that there may be a possible role in controlling blood pressure or reduction in cardiovascular diseases. However, this medicinal plant can be considered as one of the promising sources of a natural inhibitor of ACE for medicine and commercial uses. This comprehensive study may show numerous beneficial effects as a potential therapeutic agent for lowering blood pressure.

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