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Research Article

ANGIOTENSIN CONVERTING ENZYME INHIBITION POTENTIAL OF *ANNAPAVALA CHENDHURAM* FOR THE TREATMENT OF HYPERTENSION: AN IN-VITRO ASSAY

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KEYWORDS: *Annapavala chendhuram*, ACE inhibition, hypertension, in-vitro assay, Siddha.

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ABSTRACT

Hypertension is the most noteworthy risk factor for cardiovascular diseases and stroke. Dietary and lifestyle changes play the foremost part to decrease the hazard of hypertension and other related wellbeing complications. Angiotensin Converting Enzyme (ACE) inhibitors play a major role in treating hypertension. *Annapavala chendhuram* is a herbo – mineral Siddha formulation comes under the type of 32 internal medicines of Siddha. Hypolipidemic activity of *Annapavala chendhuram* has been proven by some research studies. Hence, the purpose of the present study was to evaluate the ACE inhibition activity on *Annapavala chendhuram* by using an in-vitro assay. The ACE inhibition assay was evaluated by UV Spectrophotometry technique based on the hydrolysis of histidyl-hippuryl-leucine (HHL) by ACE. About 50µL test sample with varying concentration (100- 500 µg/ml) along with standard captopril (100µg/ml) added with 50µL of ACE and some process had continued. The present study indicates that the test drug *Annapavala chendhuram* was effective in inhibiting the enzyme ACE dose-dependently. Maximum percentage inhibition of about 53.24±8.403% was observed at 500µg/ml when compared to that of the Captopril, a standard ACE enzyme inhibitor agent with the maximum inhibition 86.98 ± 6.375 at the concentration of 100µg/ml. It was concluded that the test drug *Annapavala chendhuram* possess significant anti-hypertensive property in protein denaturation assay. So, further in-vitro evaluation of ACE inhibitory activity on Siddha herbal preparations and clinical trials will be the need of the hour.

INTRODUCTION

One of the largest single risk factors of deaths worldwide is hypertension. Cardiovascular disease and stroke are the world's biggest killers, attributing for a combined 15.2 million deaths in 2016. Globally these diseases have remained the leading causes of death within the last 15 years. Hypertension is that the most noteworthy risk factor for cardiovascular diseases and stroke.[1] Dietary and lifestyle changes play the foremost part to decrease the hazard of hypertension and other related wellbeing complications. However, therapeutic treatment may prove essential for patients whose lifestyle changes prove ineffective and inadequate.[2] Hypertension treatments have widely evolved from low sodium dietary regimens to an unlimited arsenal of contemporary pharmaceuticals. There are many choices for the treatment of hypertension. Some treatments include diuretics, β-blockers, calcium channel blockers and hypertension receptor blockers, the most common of which are angiotensin-converting enzyme inhibitors.[3]

The Angiotensin-Converting Enzyme (ACE), the component of the renin-angiotensin-aldosterone system (RAAS) plays a major role in regulating the blood pressure. Regulation of blood

pressure level by RAAS is via renin, angiotensin and aldosterone release.[4] Renin is released from juxtaglomerular apparatus of the kidney which splits angiotensinogen into angiotensin I. ACE is important for converting angiotensin I to angiotensin II, a known potent vasoconstrictor. Angiotensin II stimulates both the synthesis and release of aldosterone from the adrenal cortex leading torise in blood pressure level via sodium retention.[5] Thus, the concept of inhibiting ACE became a preferred and effective therapeutic approach in treating hypertension and other cardiovascular diseases. There are a plenty of synthetic ACE inhibitors available in the contemporary pharmaceuticals market like captopril and enalapril, have been used extensively as antihypertensive drugs. However, synthetic ACE inhibitors cause various adverse effects like cough, skin problems, nausea, Hyperkalemia, fatigue, dizziness, hypotension, renal impairment etc. so cheaper, safer alternatives are desirable.[6]

Siddha system is that the ancient system of medicine mainly practiced within the southern part of India. It is one among the earliest traditional medicine systems within the world which treats not only the body but also the mind and also the soul.[7] Some of the drugs in traditional medicine were used for a long time ago to decrease the blood pressure level. But the scientific data on anti-hypertensive therapeutics is limited. *Annapavala chendhuram* is a Siddha herbo–mineral formulation comes under the type of 32 internal medicines of Siddha. Hypo-lipidemic activity of *Annapavala chendhuram* has proven by some research. Hence, the aim of the present study was to explore the potential of ACE inhibition activity on *Annapavala chendhuram* by using an in-vitro assay.

MATERIALS AND METHODS

Ingredients of Annapavala Chendhuram [8]

S. No	Tamil Name	Botanical/Chemical name	Quantity
1.	*Annabedhi*	Ferrous sulphate	227 gm
2.	*Kodipavalam*	*Corallium rubrum*	170 gm
3.	*Elumichai*	*Citrus limon*	Required amount

Procurement of the raw drug

The raw drug *Annabedhi* and *Kodipavalam* were procured from the authenticated raw drug shop at Chennai. Lemon was procured from the vegetable market, Tambaram, Chennai.

Authentication of raw drugs

Department of Geology, University of Madras, Chennai, authenticate the *Kodipavalam* and *Annabedhi* samples by analyzing the microscopical characters and literature sources.

The purification process of raw drugs

- ***Annabedhi*:[9]**

  Annabedhi was soaked in Lemon juice for 3 days and made it dry under the dark in an earthen vessel.

- ***Kodipavalam*:[9]**

  Kodipavalam was soaked in the lemon juice for 3 days in an earthen vessel. It was kept under the sunlight for 3 days. Then 4th day *Kodipavalam* was washed with the water and wiped with a cloth.

Purification of raw drugs

Fig 1: Before purification – Annabedhi

Fig 2: After purification - Annabedhi

Fig 3: Before purification – *Kodipavalam*

Fig 4: After purification - *Kodipavalam*

Preparation method of *Annapavala chendhuram* [8]

The trial drug *Annapavala chendhuram* was prepared in the Gunapadam Laboratory, National Institute of Siddha, Chennai. The purified *Kodipavalam* and *Annabedhi* were made into a fine powder separately and both powders were placed in *Kalvam* (mortar). It was ground well with lemon juice for six hours. Then it was made into *Villai* (pellets) and dried in sunlight. The pellets were put into an earthen vessel which was closed with another vessel and 7 clay cloth made to the margin of earthen vessels and the set up was dried in sunlight for one day. After that, it was put in a deep pit and *Pudam* process (incineration process) was done with 300 cow dung cakes. Once it cools the *Pudam* was opened and the pellets were taken out. The pellets were collected after cooling, weighed, finely powdered and stored in a clean, dry, airtight glass container.

Dose: 120 – 260 mg.

Adjuvant: Honey, Ghee, butter or *Impurallegiyam*.

Indication: *Kasam, Swasakasam, Shayam, Rakthaushnam, Pithaushnam, Mega ushnam* and *Aththijuram*. This *Chenduram* may be given for all diseases with suitable *Anubanam* as an alternative medicine.

Preparation method of *Annapavala chenduram*

Fig 5: Fine powder of *Annabedhi*

Fig 6: Fine powder of *Kodipavalam*

Fig 7: Grinding of *Annabedhi*

Fig 8: *Villai* in clay sealed earthen vessel Kodipavalam with lemon juice

Fig 9: Incineration with 300 cow dung cakes

Fig 10: Final grinding of APC

Angiotensin Converting Enzyme (ACE) inhibition assay [10]

ACE inhibition assay of the sample *Annapavala chendhuram* was performed based on the well-established protocol using UV- Spectrophotometry technique based on the hydrolysis of histidyl-hippuryl-leucine (HHL) by ACE. About 50µL test sample with varying concentration (100- 500 µg/ml) along with standard captopril (100 µg/ml) added with 50µL of ACE followed by incubation under 37°C for a brief time. Next phase of the reaction was initiated by addition of 150µL of the substrate (8.3 mM of HHL) with incubation at 37°C for 10 min time. Followed by this about 50µL of 1 M HCl was added to the reaction mixture to halt the reaction process. Hippuric acid thus formed will be eliminated by extraction with 1.5 mL ethyl acetate to the study mixture. Excess ethyl acetate was removed by heat evaporation. The final extracted product was dissolved in 3mL volume of DD water and the absorbance was determined at 228 nm using a spectrophotometer. The percentage inhibition activity was calculated using the following equation. The experiment was performed in triplicate.

$$\% \text{ Inhibition} = \left(\frac{\Delta \text{ control absorption} - \Delta \text{ sample absorption}}{\Delta \text{ control absorption}}\right) \times 100$$

Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test.

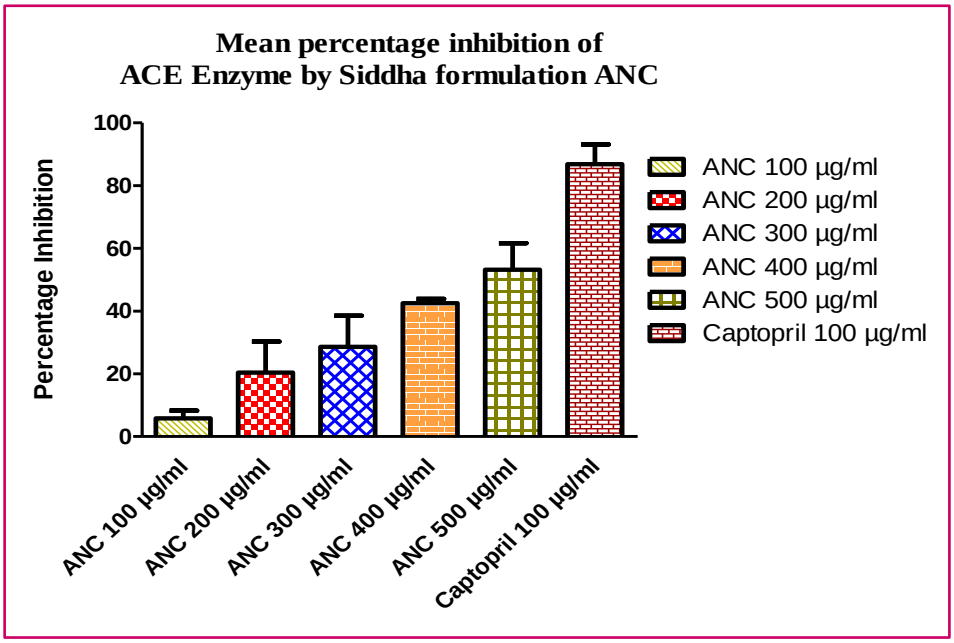
RESULTS

The result obtained from the present study indicates that the test drug, *Annapavala chendhuram* was effective in inhibiting the enzyme ACE dose-dependently. Maximum percentage inhibition of about 53.24±8.403% was observed at 500µg/ml when compared to that of the Captopril, a standard ACE enzyme inhibitor agent with the maximum inhibition 86.98 ± 6.375 at the concentration of 100µg/ml.

Table 1: Percentage Inhibition of ACE at various concentration

Concentration in µg/ml	Percentage Inhibition of ACE
ANC 100	5. 80 ± 2.444
ANC 200	20. 4 ± 9.953
ANC 300	28. 65 ± 8.809
ANC 400	42. 51 ± 1.435
ANC 500	53. 24 ± 8.403
Captopril (100 µg)	86.98 ± 6.375

Graph 1: Percentage Inhibition of ACE at various concentration



ANC – *Annapavala chendhuram*

DISCUSSION

Hypertension, a worldwide illness, is a major factor in cardiovascular diseases that affects a large population of adults. Risk factors of hypertension are high salt and spicy food intake, alcohol consumption and tobacco use, low Calcium and Potassium intake, psychological stress, heredity, intake of fatty food which causes obesity and hyperlipidemia leading to atherosclerosis in blood vessels and physical inactivity. As per Siddha, *Pithadosha* is predominant in elevating the hypertension. In contemporary medicine, one of the most effective medications for the treatment of hypertension is Angiotensin-Converting Enzyme (ACE) inhibitors. Meanwhile, traditional medicines have been used for treating illnesses. Therefore, they can be important resources to develop new drug candidates.[11] Some studies revealed that the test drug *Annapavala chendhuram* possess hypolipidemic activity.[12] Hence, ACE inhibition evaluation was done on *Annapavala chendhuram*. This study indicated 53.24% at 500µg/ml. It may suggest for the prehypertensive stage. The standard drug captopril was used as a positive control. The first oral ACE inhibitor, captopril was considered a breakthrough in managing blood pressure and was also an early example of structure-based drug design.[13] Other synthetic ACE inhibitors which are widely used for clinical use in the treatment of hypertension include enalapril, alacepril, and lisinopril.[14] Due to the side effects of synthetic ACE inhibitors, traditional Siddha drug *Annapavala chendhuram* is the safe and affordable for management of hypertension.

CONCLUSION

Natural products of animals and plants origin have been used by humans since ancient times either in the pure forms or crude extracts to treat many diseases. Today mankind is looking towards the traditional medicines due to low side effects. This study concluded that the test drug *Annapavala chenduram* possess significant anti-hypertensive property in protein denaturation assay. Many studies revealed that herbals possess the activity of ACE inhibition. So, further in-vitro evaluation of ACE inhibitor activity on Siddha herbal preparations and clinical trials will be the need of the hour.

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