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ANTIHYPERTENSIVE AND DIURETIC ACTION OF *ADATHODAI ILAI CHOORANAM* - A SIDDHA MONO-HERBAL FORMULATION

S. Selvakumar*[1], S. Visweshwaran[2], S. Sivakumar[3], A. Mariappan[4] and S. Ushakanthan[5]

[1]Siddha Consultant, Sri Amman Siddha Hospital, Perambulur District, Tamil Nadu.
[2]Lecturer, Dept. of Gunapadam, National institute of Siddha Chennai -47.
[3,4]Lecturer, Dept. of Gunapadam, National institute of Siddha, Chennai-47.
[5]Senior Lecturer, Trincomalee Campus, Eastern University, Srilanka.

***Corresponding Author: Dr. S. Selvakumar**
Siddha Consultant, Sri Amman Siddha Hospital, Perambulur District, Tamil Nadu.

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ABSTRACT
Aqueous extract of *Adhathodai Ilai Chooranam* (Adhathoda vasica leaf powder) was evaluated for its antihypertensive activity in renal artery occluded hypertensive rats. Male Wistar rats (200-250 g) randomly divided into six groups (n = 6) were pretreated with aqueous extract of *Adhathodai Ilai Chooranam* for 6 weeks. Hypertension was induced in animals by clamping the renal artery with renal bulldog clamp for 4 h. Ischemia of the kidneys causes elevation of blood pressure by activation of the renin-angiotensin system. Elevated blood pressure of the animals was significantly ($p < 0.05$) decreased by the administration of of *ALC* at dose levels of 250, 500, 1000mg/Kg i.v. Captopril (angiotensin converter enzyme inhibitor ACE-I at the dose of 1mg/kg showed significant ($p < 0.05$) reduction in the elevated blood pressure. Also, *Adathodai Ilai Chooranam* at 500 and 1000 mg/Kg showed significant increase in urine volume as well as sodium and chloride and with mild potassium sparing effect.

KEYWORDS: *Adhathodai Ilai Chooranam,* Adhathoda vasica, Traditional medicine, Herbal medicine.

INTRODUCTION
Hypertension is a Cardiovascular diseases that is globally well known as number one group of 'killer disease and it accounts for 12 million deaths, annually worldwide. Hypertension is one of the leading causes of disability, mortality, and morbidity. It is the most common chronic illness among the world faces.[1,2] Hypertension constitutes a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, myocardium infract, heart failure, renal insufficiency, stroke and dissecting aneurysm of aorta.[3] An elevated arterial pressure though common and asymptomatic, if left untreated, it may emerge out as an important public health issue in developed countries. And can often lead to lethal complication. Presently various drugs and regimes have been introduced which may demonstrate better efficacy but posses side effects. Despite promising role of diuretics to manage fluid overload among chronic kidney disease (CKD) patients, their use is associated with adverse renal outcomes and renal deterioration with diuretic therapy.[4] Recently attention has been focused towards herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases.[5]

MATERIALS AND METHOD

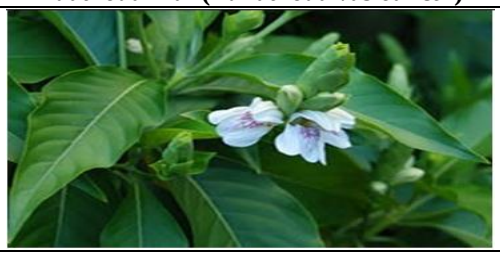
***Adathodai ilai* (*Adhathoda vasica* leaf)**	***AdathodaiIlai Chooranam*(ALC)**
	

Figure 1: Prepared drug Adathodai Ilai Chooranam (ALC).

Collection and preparation of *Adathodai Ilai Chooranam*(ALC)

The single herbal ingredient *Adathodai ilai* (*Adhathoda vasica* leaf) (Family: Acanthacea) was collected from Herbal garden, National Institute of Siddha, Tambaram Sanatorium, Chennai-47 and authenticated by the Botanist of NIS. The Fresh leaves of the Adathodai was collected and it was cleaned well in fresh water. Then middle vein of the leaves were removed, dried in shade. Finally it was finely grounded and sieved using a mesh cloth and the finely powdered drug (*Chooranam*) was stored in an air tight container.

Preparation of drug solution

Adathodai ilai chooranam and Aquazide tablet were powdered, triturated and suspended in 0.5% CMC in distilled water. Urea was powdered, triturated and dissolved in distilled water. All solutions were prepared freshly and stored in glass bottles**.** The chemicals and reagents such as Captopril (Tridoss Laboratories Pvt. Ltd., India), Urethane (Himedia Laboratories Pvt. Ltd., India), Heparin (Merlin Pharma Pvt. Ltd., India), Hydrochlorothiazide (Ajanta Pharmaceuticals Pvt. Ltd., India.), Urea (Research Lab, India), Electrolyte estimation kit (Coral Clinical Systems, India) and all the chemicals of analytical grade were purchased from local vendors of Chennai, India.

Experimental animals and grouping

Thirty six adult male Wistar rats (200-250 g) were randomly divided into six groups (n = 6). Group I served as control and were administered distilled water (10 ml/Kg, p.o.). In Group II rats the renal artery was occluded for 4 hour by using renal bulldog clamp and they served as occluded control and were given distilled water (10 ml/Kg/p.o.). Group III, Group IV and Group V were administered with ALC 250, 500, and 1000 mg/Kg/day, p.o. respectively and Group VI rats were treated with Standard drug Captopril (1 mg/Kg) for a period of 6 weeks.

All the animals were procured from Animal house of Vels college of Pharmacy, Chennai, India and were housed in groups of 5-6 in standard polypropylene cages with wire mesh top at standard environmental condition at temperature of 25 ± 2ºC and relative humidity of 45-55% under 12 hr: 12 hr light dark cycle in the institutional animal house. Animals had free access to standard pellet rodent diet and water was provided ad libitum. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (Reg.No XIII/IAEC/CPCSEA/VEL CP/22/08.08.2013).

Anti-hypertensive activity

Invasive measurement of hemodynamic changes

At the end of the treatment animals were anaesthetized by urethane (1.25 g/Kg, IP). A small incision was given on the left side of the peritoneal cavity of animal to expose the left kidney. The renal artery was occluded for 4 hour by using renal bulldog clamp. The jugular vein was cannulated for the administration of drugs. The carotid artery was cannulated and connected to the physiograph through the pressure transducer to measure the blood pressure using eight channel recorder power lab. After the stabilization of BP, the renal bulldog clamp was removed, BP was stabilized for 10 min, and 1/10th of the administered dose of the ALC extract, i.e. 25, 50, and 100 mg/kg was given respectively through jugular vein. The parameters like BP, systolic BP (SBP), diastolic BP (DBP) and mean arterial BP (MABP) were recorded for each animal after and before the removal of renal bulldog clamp, and at 15, 30, 45, and 60 min after the treatments. All the parameters for normal control group were recorded without clamping the renal artery. Changes in blood pressure of treated groups were compared with the control groups.

Diuretic activity of *Adathodai Ilai chooranam*

Male Wistar rats (200-250 g) were randomly divided into six groups (n = 6) and were withdrawn food and water fifteen hours prior to the experiment. Group I animals served as normal control and were given distilled water (10 mL/Kg, p.o.). Group II rats were administered Urea (1 g/Kg, p.o.) and Group III, Group IV and Group V were treated with *Adathodai ilai chooranam* (ALC) 250, 500, and 1000mg/kg.p.o. respectively. Group VI was treated with standard drug hydrochlorothiazide (1 mg/Kg, p.o.) Additionally, normal saline (5 mL/100 g, p.o.) was given by gavage to the animals from all treatment groups. Three animals per group were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine for 5hr. The sodium, potassium, and chloride content of urine were analyzed at 5 hr and 24 hr after the treatment by using respective standard electrolyte estimation kit procedures in auto-analyzer.

RESULTS

Table 1: Effect of *Adathoda Ilai Chooranam* on Mean Arterial Blood Pressure in renal artery-occluded hypertensive rats.

S.no.	Groups	Treatment	Mean Arterial Blood Pressure (MABP) in mmHg at different time interval				
			MABP after removing clip	5 min	15 min	30 min	60 min
1	Group I	Distilled water	--------	82.46±4.28	83.12±4.26	82.53±5.32	83.80±5.10
2	Group II	Distilled water	126.32±6.35	115.67±4.92[a]	117.31±3.06[a]	104.15±4.82[a]	102.22±5.12[a]
3	Group III	ALC250mg/ kg.	121.15±4.62	74.51±5.02*	76.34±4.00*	72.20±4.80*	68.10±4.45*
4	Group IV	ALC 500mg/kg.	116.11±5.31	70.36±4.31*	68.00±4.11*	66.82±4.98*	72.32±5.42*
5	Group V	ALC 1000mg/kg	115.83±2.68	61.39±5.22*	63.81±5.92*	60.30±5.42*	60.21±5.73*
6	Group VI	Captopril 1mg/ kg.	120.44±4.00	47.45±3.00*	38.77±4.78*	35.52±3.81*	28.34±3.45*

Values in the results are expressed as mean± SEM, (n=6), [a]$p<0.05$ significantly different in comparison with Normal control, *$p<0.05$ significantly different in comparison with Negative control.

Table 2: Effect of *Adathoda ilai chooranam* on pulse pressure, systolic BP, diastolic BP, and heart rate in Normal rats.

Treatment	Pulse Pressure (mm Hg)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Heart Rate (beats/ min)
Normotensive rats	32.11±0.44	116.8±0.61	94.2±0.43	435.6±1.00
Normotensive+ AIC 250mg/kg	31.80±0.32[a]	98.7±0.34*	87.0±0.41*	434.4±0.91[b]
Normotensive+ AIC 500mg/kg	18.21±0.50*	77.2±0.81*	65.4±1.8*	426.1±0.40*
Normotensive+ AIC 1000mg/kg	16.5±0.52*	65.2±1.42*	58.5±0.51*	424.8±0.55*

Values in the results are expressed as mean± SEM, (n=6), **p<0.001* versus control; [a]*p<0.01* versus control; [b]*p<0.05* versus control.

Table 3: Effect of *Adathoda Ilai Chooranam* on pulse pressure, systolic BP, diastolic BP, and heart rate in Ischemia and reperfusion induced rats.

Treatment	Pulse Pressure (mm Hg)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Heart Rate (beats/ min)
Normotensive rats	31.5±0.22	117.54±1.02	98.1±0.34	432.8±0.62
Renal IR	44.2±0.42[a]	142.7±0.32[a]	111.8±0.25[a]	445.3±0.65[a]
Renal IR + AIC 250mg/kg	42.7±0.05[b]	134.8±0.48*	105.7±0.82*	441.2±0.72[b]
Renal IR + AIC 500mg/kg	38.4±0.32*	129.1±0.42*	97.2±0.74*	444.3±0.48*
Renal IR + AIC1000mg/kg	37.0±0.34*	123.2±0.52*	98.5±0.42*	440.1±0.41*
Renal IR + Captopril1mg/kg	32.4±0.28*	118.15±1.04*	97.2±0.34*	430.8±0.64*

Values in the results are expressed as mean± SEM, (n=6), [a]*p<0.001* versus control; **p<0.001* versus IR; [b]*p<0.01* versus IR group. (Ischemia and reperfusion-IR).

Table 4: Effect of *Adathoda Ilai Chooranam* on Urine Volume, Sodium, Chloride and Potassium.

Group	Treatment	Urine volume	Sodium	Chloride	Potassium
Group I	Distilled water (10 mL/Kg, p.o.)	1.52±0.25	55.04±0.08	10.90±1.10	92.10±1.22
Group II	Urea (1 g/Kg, p.o.)	3.15±0.56**	101.01±0.92**	11.00±1.24	97.00±0.90**
Group III	AIC-250mg/kg.p.o.	3.12±0.67**	89.10±3.04**	12.41±0.72	94.22±3.34
Group IV	AIC-500mg/kg.p.o.	3.55±0.62**	100.22±0.70**	13.37±0.90*	121.40=7.23***
GroupV	AIC-1000mg/kg.p.o.	4.42±0.55***	98.20±0.68**	14.28±1.46*	92.05±0.14
Group VI	Hydrochlorothiazide(1mg/Kg,p.o.)	4.81±0.20*	63.12±0.07*	16.13±0.80	91.42±1.23

Values are expressed as mean ± SEM (n = 6). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with normal control (one-way ANOVA followed by Dunnett's test.

DISCUSSION

In this study, treatment with *Adathodai Ilai Chooranam* (250, 500, and 1000 mg/Kg/day, p.o.) for six weeks showed significant ($p < 0.05$) and dose dependent decrease in BP, Systolic BP, Diastolic BP, and Mean Arterial Blood Pulse at different time intervals when compared with occluded control group. Captopril tablet (1 mg/Kg) showed significant ($p < 0.05$) reduction in BP, Systolic BP, Diastolic BP, and Mean Arterial Blood Pulse as compared with occluded control. Reduction in Mean Arterial Blood Pressure (MABP), pulse and blood pressure in normotensive rats is a direct indication of hypotensive effect. MAP, pulse and blood pressure and heart rate raised significantly in renal hypertensive rats ($p<0.001$ versus control), whereas MAP, blood pressure, pulse pressure and heart rate decreased dose-dependently in renal hypertensive rats after intravenous administration of 250, 500 and 1000 mg/kg of ALC,

suggesting that ALC has antihypertensive and negative chronotropic effects (p<0.001 versus renal IR; p<0.01 versus renal IR group) (Ischemia and reperfusion-IR) as shown in Table-1, Table-2 and Table-3.[6]

The administration of *Adathodai Ilai Chooranam* (500 and 1000 mg/Kg) showed significant ($p < 0.05$ and $p < 0.01$ respectively) increase in urine volume, sodium, chloride and potassium content as compared with normal control group at 5h. The administration of urea (1 g/Kg, p.o.) and hydrochlorothiazide (10 mg/Kg, p.o.) showed significant ($p < 0.001$) increase in sodium content, chloride and potassium content of urine as compared with the normal control group at 5 hr. Further the administration of Urea (1 g/Kg, p.o.) and hydrochlorothiazide (10 mg/Kg, p.o.) showed a significant ($p < 0.01$ and $p < 0.001$ respectively) increase in Urine volume as compared with normal control group at 5h. In rats, renal hypertension is induced by clamping the left renal artery. After reopening of the vessel, accumulated renin is released into circulation. The protease renin catalyzes the first and rate-limiting step in the formation of angiotensin II leading to acute hypertension. Blockade of renin angiotensin system is one of the important mechanisms for antihypertensive effect in this regards.[7,8] One of the primary functions of kidneys is to regulate Na^+ and water excretions, and consequently, they play a dominant role in the long-term control of BP.[9] Diuretics are frequently prescribed to control blood pressure and for symptomatic relief of fluid overload.[10,11] To be clinically effective, however, such compounds must induce the loss of sodium. This helps to reduce the volume of blood circulating through the cardiovascular system. Potassium sparing diuretics may also be able to increase the effectiveness of more proximally acting diuretics. Potassium sparing diuretics reduce the incidence of serious ventricular arrhythmias in patients with heart failure (particularly those who are on digoxin) and hypertension (particularly in patients with left ventricular hypertrophy).[12,14]

In the present study, *Adathodai Ilai Chooranam* at a dose of 1000 mg/Kg showed weak diuretic activity with increase in Urine volume, Sodium and chloride. However there was no significant difference in excretion of potassium at 250, 500mg/Kg of ALC. This may indicate the potassium sparing action of ALC in low and mid doses. From the above discussion it may be concluded that the observed antihypertensive effect was possibly due to the inhibition of ACE, functioning as an antagonist at vascular α-receptors, inhibition of phosphodiesterase or the direct action on vascular endothelium to increase the release of EDRF, and thereby, producing the vasodilation. *Adathodai ilai chooranam* may aid in inhibiting ACE and produce diuretic effect which can be further contributed to its antihypertensive effect.

CONCLUSION

Adathodai ilai chooranam has been traditionally used for the treatment of various ailments in siddha system of medicine. However, the pharmacological or clinical studies for antihypertensive and diuretic property have not yet been reported. Hence, the present research was undertaken to evaluate the antihypertensive and diuretic activity of *Adathodai ilai chooranam* a Siddha preparation. In conclusion this study provides further experimental evidence that justifies the Siddha medicinal claims towards the use of this plant in the treatment of hypertension.

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