AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

VAASATHI KASHAYAM, A SIDDHA HERBAL FORMULATION ALLEVIATES HYPERTENSION IN DOCA SALT INDUCED HYPERTENSIVE RATS, EVALUATION OF ACUTE, SUB ACUTE TOXICITY, ANTI-OXIDANT AND DIURETIC ACTIVITY

Preyadarsheni K[1]*, Komalavalli T[2], M. Santhanakumar[3]

*[1]PG Research Scholar, [2]Head of the department, Department of PG Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli,

[3]Assistant professor, Department of Pharmacology, Arulmigu Kalaalingam college of Pharmacy, Krishnankoil, India.

Article info

Article History:
Received: 18-05-2025
Accepted: 28-06-2025
Published: 25-07-2025

KEYWORDS:
Preclinical study, *Vaasathi kashayam,* Systemic hypertension, Toxicity studies, Siddha herbal formulation, Antidiuretic, Antioxidant.

ABSTRACT

Vaasathi Kashayam, a Siddha herbal decoction from Agathiyar 2000, is formulated using *Adhathoda vasica* (*Justicia adhatoda* L.) leaves and dry grapes (Vitis vinifera L.). This study evaluates its anti-hypertensive, antioxidant, and diuretic effects. **Methodology**: DOCA-salt-induced hypertensive Wistar albino rats (5 groups, n=6) were treated for 43 days. Blood pressure (BP), serum sodium, and potassium levels were measured. Blood was collected via retro-orbital plexus under ether anesthesia; BP was recorded through cannulated carotid artery using the 2K1C model. Diuretic effect was assessed using 4 groups (n=6) in metabolic cages, measuring total urine volume and Na^+, K^+, Cl^- levels. Cl^- was estimated as NaCl by titration. Antioxidant activity was assessed via DPPH free radical scavenging assay. Acute and subacute toxicity studies followed OECD 423 guidelines. **Results**: Hypertensive rats had SBP/DBP of 173.33±2.33/145.54±1.23mmHg. Low-dose (200mg/kg) *Vaasathi Kashayam* reduced BP to 135.03±2.56/92.63±1.01 mmHg, and high-dose (400 mg/kg) to 106.02±3.46/ 72.05±0.53mmHg, comparable to the standard (hydrochlorothiazide) 103.72±1.25/ 92.63±1.82mmHg. Diuretic activity showed increased urine output (32.53±0.83 and 30.67± 0.06mL) and elevated urinary electrolytes, comparable to Furosemide. Antioxidant activity peaked at 63.15% inhibition (300µg/mL); standard ascorbic acid showed 132.06% inhibition. No significant toxicity or abnormal changes were observed in acute (up to 2000mg /kg) or subacute (28-day) studies. **Conclusion**: *Vaasathi Kashayam* is a safe formulation with significant anti-hypertensive, antioxidant, and diuretic activities, supporting its therapeutic potential in Siddha medicine.

INTRODUCTION

One of the global targets for noncommunicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030 [WHO, 2020]. Hypertension rates are on the rise, partly due to lifestyle changes such as poor diet, sedentary lifestyles, and aging populations. There is a concerning trend of hypertension developing at younger ages, influenced by factors like obesity and stress.

Access this article online	
Quick Response Code	
	https://doi.org/10.47070/ayushdhara.v12i3.2082

Hypertension contributes significantly to healthcare expenditures globally, both directly (treatment costs) and indirectly (loss of productivity, disability). There are disparities in hypertension prevalence and management between countries and within populations, influenced by socioeconomic factors, access to healthcare, and public health infrastructure. Involvement of AYUSH in Management of such serious and most prevailing non-communicable disease is crucial. This trial drug *Vaasathi kashayam* is a Siddha herbal formulation procured from literature Agathiyar 2000 indicated for *Rathatha Pitham* (hypertension). Leaves of *Adhathoda* (*Justicia adhatoda*.L, Acanthaceae) and dry grapes (*Vitis vinefera*.L, Vitaceae) were grinded and made into

decoction. Herbal drugs have long been revered for their potential therapeutic benefits, rooted in centuries of traditional medicine practices across cultures worldwide. In recent years, there has been a resurgence of interest in these natural remedies due to their perceived safety and holistic approach to health. However, the efficacy and safety of herbal drugs must be rigorously evaluated to ensure their reliability and safety in modern medical contexts. Central to the evaluation process are animal studies, which play a crucial role in assessing both the toxicity and efficacy of herbal drugs before human trials commence. This study aims to delve into the intricate balance between toxicity and efficacy in herbal drugs through comprehensive animal experimentation. By meticulously analyzing biochemical changes, physiological responses, and behavioral changes in animal subjects, toxicity and efficacy were elicited aimed at ensuring patient safety and therapeutic efficacy.

Literature Review

In a preclinical study by Selvakumar S (2018), the antihypertensive and diuretic effects of *Adhathodai Ilai Chooranam* (ALC) were evaluated in adult male Wistar rats alongside the standard drug captopril. The results indicated that ALC exhibits angiotensin-converting enzyme (ACE) inhibitory properties, alpha-adrenergic receptor antagonism, phosphodiesterase inhibition, and direct stimulation of vascular endothelium to enhance endothelial-derived relaxing factor (EDRF) release, contributing to vasodilation.[26] Pravin Borde (2011) reported that chronic oral administration of myricetin (100 and 300mg/kg for four weeks), a flavonoid isolated from *Vitis vinifera* (Vitaceae), significantly improved blood pressure and oxidative stress markers in DOCA-salt-induced hypertensive rats.[5] Similarly, Giselle Franca da Costa (2020) demonstrated that aqueous extract of grape skin (*Vitis vinifera* L.) effectively prevented the rise in systolic blood pressure in spontaneously hypertensive rats, as measured by the tail-cuff method. Untreated hypertensive rats exhibited blood pressure levels exceeding 200 mmHg, while those treated with grape skin extract showed values below 150mmHg.[6] Siji J. Thandapilly (2012) observed that whole grape powder supplementation led to a notable decrease in blood pressure, enhanced arterial relaxation, improved vascular compliance, and reduced cardiac hypertrophy.[31] In another study, Ifrahim Iqbal Chowdhury evaluated a combined aqueous extract of *Justicia adhatoda* and *Ocimum tenuiflorum*, finding that the blend significantly enhanced antioxidant activity and reduced hyperlipidemia.[15] Among the treatment groups, *Justicia adhatoda (Bashok)* had the most pronounced effect, showing marked improvements in serum lipid profiles, body weight, total protein, LDH levels, and tissue weights. The combination treatment also resulted in the lowest atherogenic index.

MATERIALS AND METHODS

Preparation of Test Drug VK

Equal parts of leaves of Justicia adhathoda and dry fruits of Vitis vinefera (5grams each) were grounded and about 80 ml of water was added to this mixture. This mixture was boiled to 10ml using hot plate and this fresh extract of decoction was used after filtering the residues through filter paper.

Figure 1a Leaves of J.*adhathoda*, 1b Dry fruits of V.vinefera, 1c Preparation of decoction, 1d Using hot plate for boiling followed by filtration using filter paper

Anti-Hypertensive Activity: Deoxycorticosterone acetate salt induced hypertension model

Wistar albino rats having average body weight of 150gm were selected. 2% w/v sodium chloride solution instead of plain water was given until body weight of an average of 200gm was obtained.

Table 1: Grouping for Anti-Hypertensive Activity, CMC-Carboxymethylcellulose, VK- *Vaasathi Kashayam*, DOCA- Deoxycorticosterone Acetate

Methodology

Group	Treatment	Number of Wistar Albino Rats
Group I SHAM/Normotensive	0.25% w/v sodium CMC (10ml/kg, p.o.)	6
Group II Hypertensive	0.25% w/v sodium CMC (10ml/kg, p.o.) + DOCA salt (10mg/kg, s.c., twice in a week)	6
Group III Standard Drug	hydrochlorothiazide (5mg/kg, p.o.) + DOCA salt (10mg/kg, s.c. twice in a week)	6
Group IV Test Group LD	VK (200mg/kg, p.o.) + DOCA salt (10mg/kg, s.c. twice in a week)	6
Group V Test Group HD	VK (400mg/kg, p.o.) + DOCA salt (10mg/kg, s.c. twice in a week)	6

The experimental protocol was carried out over a period of 43 days. Deoxycorticosterone acetate (DOCA) was dissolved in sesame oil and administered twice weekly. For the SHAM control group, only sesame oil was given in the same manner, replacing the test formulation. The following parameters were assessed:

- Serum sodium (Na^+) and potassium (K^+) concentrations
- Blood pressure (BP) using an invasive method on the final day of the study

Estimation of Serum Electrolytes

At the end of the 43-day treatment, blood was drawn from anesthetized rats (ether-induced) via the retro-orbital plexus. Collected samples were centrifuged at 6000 RPM for 15 minutes at 25°C. The resulting supernatant was used to estimate serum Na^+ and K^+ levels using a semi-automated analyzer (RA-50, Bayer Diagnostics) with commercial kits (Auto Span, India). Readings were taken at 500nm and 550nm respectively, and values were expressed in mmol/L.

Blood Pressure Recording: On day 43, rats were anesthetized using intramuscular ketamine (25mg/kg). The left carotid artery was surgically exposed and cannulated with polyethylene tubing filled with 1% heparinized saline under sterile conditions. Blood pressure was recorded in mmHg following the 2K1C model protocol.

Statistical Analysis: Results were expressed as mean ± standard error of mean (SEM). One-way ANOVA followed by Tukey's post hoc test was employed for statistical evaluation. A p-value less than 0.05 was considered statistically significant when comparing the control and hypertensive groups.

Antioxidant Activity – DPPH Assay: To evaluate antioxidant potential, a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed.

Preparation of Stock and Test Solutions: A stock solution was prepared by dissolving 5ml of *Vaasathi Kashayam* (VK) in 95% methanol. Serial dilutions were made to obtain concentrations of 10, 20, 40, 60, 80, 100, 250, and 300 µg/ml. Standard ascorbic acid solutions were also prepared in similar concentrations using methanol as solvent. To each sample, 1 ml of 0.3 mM DPPH in methanol was added to 2.5ml of test solution. The mixtures were incubated at room temperature.

Measurement of Absorbance

After 15 minutes of incubation at 37°C, absorbance was measured at 517nm using a double-beam UV spectrophotometer. The scavenging activity was calculated based on the reduction in absorbance at various concentrations

$$\%\ Scavenging = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Abosrbance of control}} \times 100$$

The 50% inhibitory concentration (IC_{50}) of the test formulation VK for DPPH radical scavenging was determined using linear regression analysis based on the dose-response curve, which plotted percentage inhibition against various concentrations.

Diuretic Activity

Adult Wistar rats of either sex, weighing between 170–200 g, were used for the diuretic evaluation. The animals were kept in standard metal cages with free access to food and water. The diuretic activity was assessed following the procedure outlined by Lipschitz et al. and Kavimani et al. Prior to the experiment, the animals were divided into four groups

(n=6 per group) and subjected to an 18-hour period of fasting and water deprivation.

Table 2: Grouping for diuretic activity

Groups	Treatment	Adult Wistar Rats
Group I Control	Normal saline 10 mg/kg, i.p	6
Group II Standard	Furosemide 10 mg/kg, i.p	6
Group III LD	VK 200 mg/kg, p.o.	6
Group IVHD	VK 400 mg/kg, p.o.	6

Immediately following administration, the animals were placed in metabolic cages (two per cage) specifically designed to separate urine from feces. They were maintained at a controlled room temperature of 25 ± 0.5°C. Throughout the observation period, the rats were denied access to both food and water. After 5 hours, total urine output was collected and analyzed. The concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) in the urine were measured using flame photometry. Chloride concentration, expressed as NaCl, was estimated by titration with 2.096 g/L silver nitrate solution, using a drop of 5% potassium chromate as an indicator.

Acute Oral Toxicity – OECD 423 Oral Class Method: As per OECD guideline 423, acute oral toxicity was assessed using the class method. Wistar rats were divided into four groups, each consisting of three animals. On the first day, *Vaasathi Kashayam* (VK) was administered orally at escalating doses of 5mg/kg, 50mg/kg, 300mg/kg, and 2000mg/kg body weight. The animals were monitored weekly for a period of 14 days. Observations included physical and behavioral responses, activity in home cages, reactions when handled, and mortality, if any.

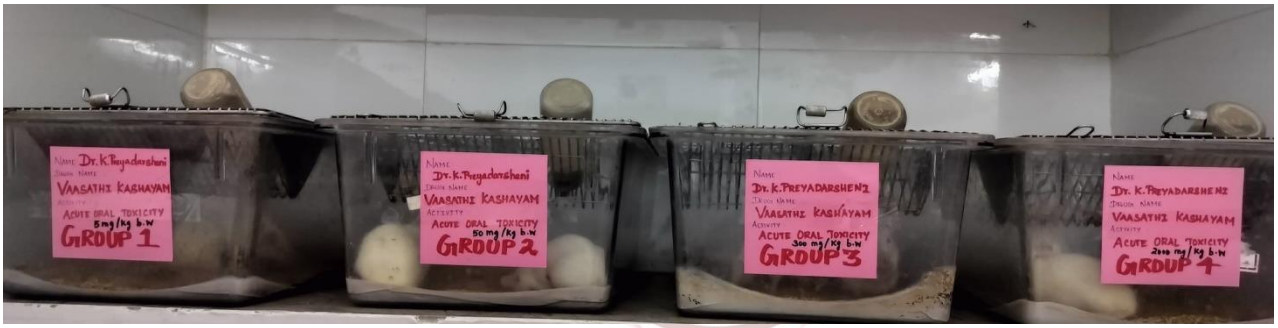


Figure 2: Grouped Wistar rats for acute oral toxicity

Subacute Oral Toxicity Study

The subacute toxicity evaluation was conducted over a 28-day period. A total of 30 animals were randomly divided into five groups (n = 6 per group): Control, Vehicle control (administered with palm jaggery), and three test groups receiving *Vaasathi Kashayam* (VK) at low (LD), medium (MD), and high (HD) doses. The animals were monitored daily, and the following parameters were recorded throughout the study and assessed at the end:

- Body weight changes
- Gross organ appearance and weight
- Food and water consumption
- Hematological and biochemical parameters
- Serum electrolyte levels

Histopathological Evaluation – Subacute Toxicity

Tissue Collection and Fixation: At the conclusion of the treatment period, heart tissues were excised from all experimental groups. Immediately after removal, the tissues were immersed in 10% buffered formalin to preserve cellular architecture by stabilizing proteins and preventing autolysis.

Tissue Processing: Post-fixation, tissues were sequentially dehydrated using graded alcohol concentrations to eliminate moisture. The specimens were then treated with xylene to clear the tissue, facilitating paraffin wax infiltration. Once cleared, the hearts were embedded in paraffin blocks to provide structural integrity for microtome sectioning.

Sectioning and Staining: Thin sections approximately 5 µm in thickness were obtained from the paraffin blocks using a rotary microtome. These sections were mounted on glass slides and subjected to hematoxylin and eosin (H&E) staining. Hematoxylin stained cell nuclei and other acidic structures blue to purple, while eosin imparted a pink hue to cytoplasmic and other basic components.

Microscopic Analysis: The stained sections were observed under a light microscope for detailed histological examination. Cellular morphology and tissue integrity were assessed to identify any

pathological alterations resulting from the administration of the test formulation.

RESULTS

Anti-Hypertensive Activity: Hypertensive groups showed 173.33±2.33 SBP and 145.54±1.23 DBP. Rats with LD (Low dose) of *Vaasathi kashayam* elicited 135.03±2.56 SBP and 92.63±1.01 DBP while HD (High dose) showed *106.02±3.46 SBP and 72.05±0.53 DBP* which is in par with standard drug group (hydrochlorothiazide) *103.72±1.25 SBP and 92.63±1.82 DBP*. SHAM group showed 113.35±2.62 SBP and 82.46±3.42 DBP.

Table 3: Results of anti-hypertensive activity serum sodium, potassium levels expressed in mmol/L and SBP-DBP (mmHg) at the end of 43rd day

Group	Serum Level		Blood Pressure in mm/Hg	
	Sodium	**Potassium**	**Systolic**	**Diastolic**
Normal	146.65 ±3.69	5.65±0.23	113.35±2.62	82.46±3.42
Hypertension	**115.81±4.21**	**5.26±0.32**	**173.33±2.33**	**145.54±1.23**
Hydrochlorothiazide	159.42±8.42	4.45±0.23	103.72±1.25	92.63±1.82
Low dose	**164.62±2.87**	**4.92±0.46**	**135.03±2.56**	**92.63±1.01**
High dose	**123.11±0.32**	**5.16±0.01**	**106.02±3.46**	**72.05±0.53**

Anti-Oxidant Activity: Highest DPPH scavenging activity of 63.15% at concentration 300 and the lowest percentage of inhibition 27.13% at concentration 20 were noted while ascorbic acid (standard) showed highest percentage of inhibition 132.06% at 300 and the lowest percentage of inhibition 87.09% at concentration 20.

Table 4: Results of Anti-oxidant activity – Absorbance under 517nm expressed in Mean ± Std Err Mean (SEM), percentage of inhibition DPPH radical scavenging property expressed in %

Concentration	Ascorbic acid (Standard) drug		Absorbance	% inhibition
	Absorbance	% inhibition		
20	1.02 ± 0.0010	87.09 %	1.201 ± 0.005	27.13%
40	0.976 ± 0.0020	92.01%	1.102 ± 0.010	30.01%
60	0.842 ± 0.0131	98.10%	0.925 ± 0.022	42.50%
80	0.722 ± 0.0042	113.41%	0.802 ± 0.110	52.01%
100	0.662 ± 0.0120	111.50%	0.781 ± 0.014	59.25%
250	0.541 ± 0.0010	122.10 %	0.652 ± 0.102	61.14%
300	0.480 ± 0.010	132.06%	0.422 ± 0.010	63.15%
Ic 50 values		Ic_{50}= 6.3µg/ml		Ic_{50} = 24.03µg/ml

Diuretic Activity: Single dose administration of *Vaasathi kashayam* as 200mg/Kg LD and 400mg/Kg HD and standard Furosemide (10mg/kg) have increased the urinary output which were 32.53±0.83, 30.67±0.06 and 42.40±0.06 along with an increase in concentration of sodium, potassium and chloride ions in urine.

Table 5: Results of Diuretic activity - Total urine volume 24h expressed in Mean ± Std Err Mean (SEM), Total Sodium, Potassium and chloride levels in mmol/L

Group	Urine Volume Mean ± Std.Err.Mean	Total Na+ (m moles/L)	Total K Potassium+ (m moles/L)	Total Cl- (M moles/L)
Normal	28.71±0.05	104.59±4.99	124.97±4.07	98.10±0.87
Standard (furosemide)	42.40±0.06**	164.70±5.08**	148.07±6.95**	149.83±0.91**
Test group VK LD	32.53±0.83**	144.60±5.64**	145.17±6.05**	117.86±0.09**
Test group VK HD	30.67±0.06**	119.48±3.07**	139.20±3.38**	101.04±0.10**

Acute Oral Toxicity

Table 6: Results of acute oral toxicity

Acute Oral Toxicity 14 Days		Group I	Group II	Group III	Group IV
	Dose(mg/kg)	**5mg/kg**	**50mg/kg**	**300mg/kg**	**2000mg/kg**
Physical and Behavioural Examinations	Observation sign	Normal	Normal	Normal	Normal
	No. of animals affected	0	0	0	0
Home Cage Activity (Functional and Behavioural Observation)	Body position	3	3	3	3
	Respiration	3	3	3	3
	Clonic involuntary Movement	3	3	3	3
	Tonic involuntary Movement	3	3	3	3
	Palpebral closure	3	3	3	3
	Approach response	3	3	3	3
	Touch response	3	3	3	3
	Pinna reflex	3	3	3	3
	Tail pinch response	3	3	3	3
	Observation	Normal	Normal	Normal	Normal
Hand Held Observation (Functional and Behavioral Observation)	Reactivity	3	3	3	3
	Handling	3	3	3	3
	Palpebral closure	3	3	3	3
	Lacrimation	3	3	3	3
	Salivation	3	3	3	3
	Piloerection	3	3	3	3
	Pupillary reflex	3	3	3	3
	Abdominal tone	3	3	3	3
	Limb tone	3	3	3	3
	Observation	Normal	Normal	Normal	Normal
Mortality		0	0	0	0

Oral class method showing no significant changes in physical & behavioral examinations, home cage activity, hand held observation and zero mortality (0/3 even in high dose group IV 2000mg/kg b.wt.) Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

From acute toxicity study, it was observed $^{ns}p>0.05$ that the administration of *Vaasathi kashayam* at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So, No-Observed-Adverse-Effect-Level (NOAEL) at *Vaasathi kashayam* is 2000 mg/kg

Sub-Acute Toxicity

Sub-Acute Oral Toxicity Study	Normal	Vehicle	Low Dose	Mid Dose	High Dose

		Control	Control	VK	VK	VK
Weight in Grams (Mean± SD)	1st day	184.07±0.17	184.02±0.05	209.06±0.03	184.02±0.02	194.06±0.04
	7th day	195.08±0.04	193.07±0.04	213.08±0.02	185.09±0.09	198.02±0.02
	14th day	201.05±0.22	203.08±0.09	216.02±0.04	187.03±0.02	199.94±0.07
	21st day	209.01±0.07	212.04±0.07	218.05±0.03	187.07±0.04	204.06±0.02
	28th day	217.10±0.08	213.06±0.09	220.06±0.04	190.02±0.02	206.76±0.04
Organ Weight (Physical Parameter) In Gram (Mean± SD)	Heart	2.91±0.08	2.01±0.03	2.07±0.20	2.12±0.08	2.19±0.03
	Liver	4.32±0.27	4.06±0.34	4.34±0.32	3.79±0.39	4.28±0.46
	Lungs	1.9±0.28	1.49±0.32	1.44±0.35	1.39±0.27	1.33±0.58
	Kidney L	1.04±0.06	0.97±0.24	0.96±0.13	1.04±0.24	1.62±0.18
	Kidney R	1.08±0.03	0.94±0.18	0.92±0.25	1.06±0.12	1.12±0.27
Haematological Parameters (Mean± SD)	RBC Million/mm^3	4.7±0.02	4.9±0.02	5.2±0.02	5.1±0.02	4.6±0.04
	WBC Thousand/mm^3	7.9±0.02	8.7±0.01	7.3±0.03	6.5±0.02	6.4±0.02
	Haemoglobin gm/dl	13.67±0.07	13.07±0.08	12.90±0.02	12.48±0.09	11.87±0.12
	Differential count %					
	Neutrophils	49.07±0.50	51.05±0.01	50.21±0.06	53.45±0.21	53.37±0.12
	Eosinophils	3.06±0.09	3.16±0.20	3.19±0.16	3.07±0.16	3.21±0.17
	Monocyte	9.17±0.10	7.39±0.31	8.26±0.12	8.47±0.12	9.16±0.12
	Lymphocyte	38.04±0.03	38.64±0.07	37.09±0.07	38.07±0.04	36.08±0.10
Biochemical Parameter (Mean± SD)	SGPT (U/L)	07.67±0.09	25.68±0.03	34.88±0.26	37.38±0.13	42.47±0.08
	SGOT(U/L)	17.68±0.09	23.66±0.35	19.48±0.15	25.26±0.28	29.73±0.14
	Urea (mg/dl)	13.32±0.08	14.71±0.03	19.71±0.12	26.69±0.10	40.46±0.08
	Creatinine (mg/dl)	0.69±0.30	0.72±0.32	0.79±0.20	0.82±0.12	0.92±0.14
	Total Bilirubin (mg/dl)	0.95±0.23	0.76±0.24	0.76±0.23	0.75±0.24	0.77±0.42
Food Intake In Gram (Mean± SD)	1st Day	21.02±0.05	22.04±0.07	19.66±0.05	22.91±0.07	21.72±0.06
	7th Day	21.67±0.04	22.07±0.05	19.93±0.04	21.8±0.06	22.99±0.04
	14th Day	21.95±0.03	21.38±0.03	20.02±0.03	21.59±0.05	22.87±0.07
	21st Day	19.5±0.07	21.33±0.04	20.08±0.07	20.73±0.03	21.55±0.03
	28th Day	23.05±0.04	21.93±0.07	21.3±0.05	20.94±0.04	22.81±0.05
Water Intake in ml	1st Day	12.46±0.06	12.55±0.06	13.28 ±0.01	12.47±0.06	11.34±0.16
	7th Day	11.58±0.80	11.62±0.14	12.21 ±0.07	11.79±0.08	12.03±0.11

(Mean± SD)	14th Day	13.68±0.15	13.27±0.04	13.26 ±0.61	12.74±0.09	11.94±0.31
	21st Day	11.48±0.16	12.86±0.02	12.36 ±0.01	11.41±0.04	12.02±0.23
	28th Day	12.03±0.06	12.85±0.05	13.39±0.04	13.05±0.09	12.73±0.14
Electrolytes (mmol/L) (Mean± Sd)	Sodium (mmol/L)	135.85±0.13	137.30±0.13	137.65±0.05	138.37±0.09	138.17±0.12
	Chloride (mmol/L)	101.04±0.08	99.24±0.18	101.67±0.09	98.12±0.08	102.04±0.05
	Potassium (mmol/L)	3.95±0.10	4.07±0.13	4.04±0.03	4.02±0.01	3.98±0.02

Table 6 Results of Subacute toxicity after 28 days of drug administration showing no significant abnormal changes. Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dennett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

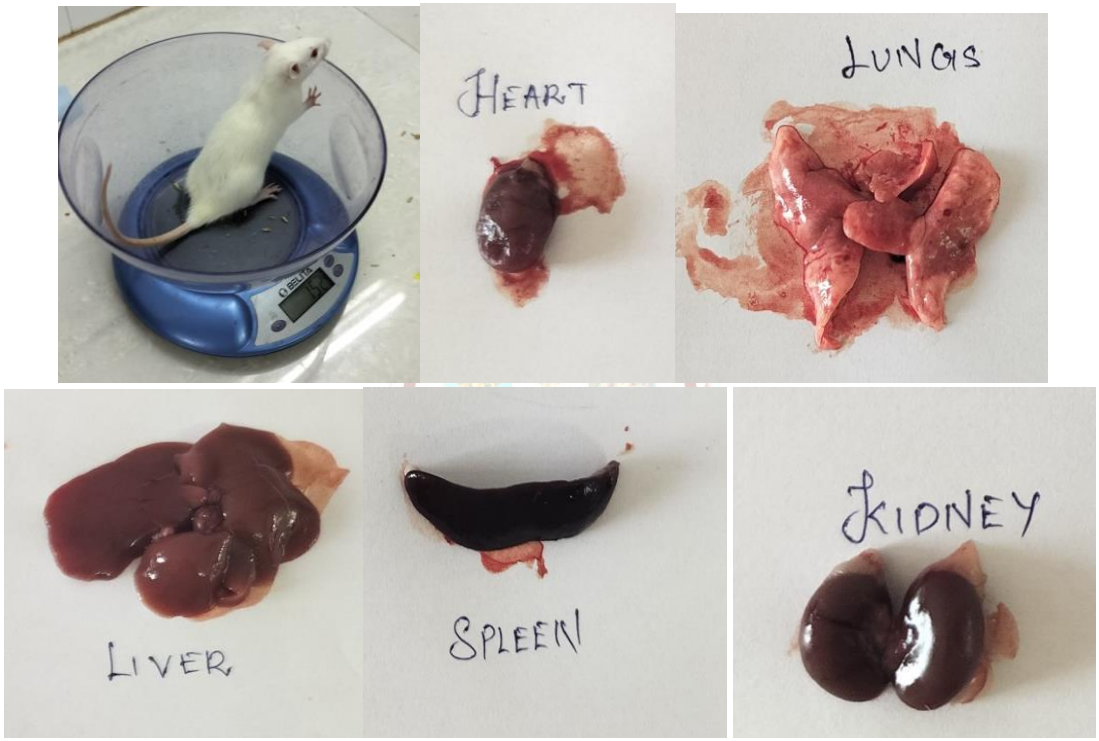


Figure 3a: Weighing of wistar rats after sub-acute toxicity, 3b-f: Gross appearance of organs after sub-acute toxicity revealing no abnormal inflammatory changes.

Vaasathi Kashayam administration resulted in a statistically significant (p<0.05) increase in body weight across all treated groups. The impact on vital organs such as the kidneys, heart, liver, and lungs were assessed. There were no significant differences (p>0.05) in organ weights, even at higher doses, and macroscopic examination revealed no notable changes in organ coloration. Hematological analysis indicated no significant alterations (p>0.05), although hemoglobin and RBC levels showed an increase in the treated groups. Biochemical parameters- including SGPT, SGOT, ALP, urea, and creatinine- were compared between the control group (Group I) and treatment groups (II, III, and IV). The observed changes were statistically significant (p<0.05), as presented in Table 7 and Figures 3b–f.

Histopathological Findings – Sub Acute Toxicity

Group	Histopathology	Heart Muscle, Myocardium

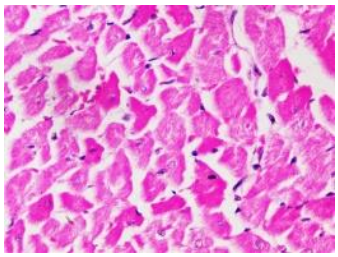
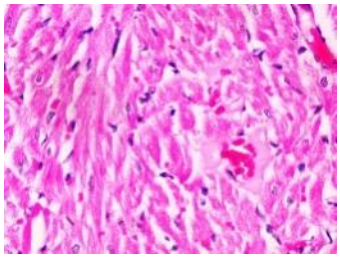
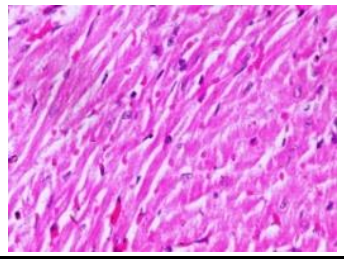
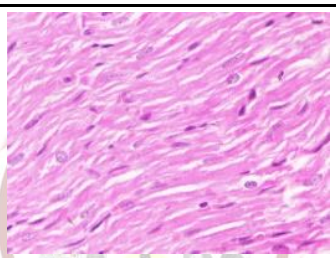
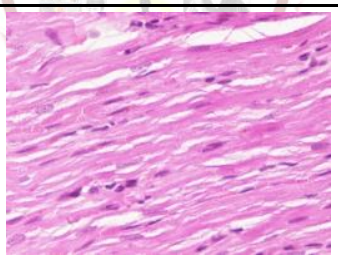
Group I SHAM/Normotensive		Not damaged Showed normal cells.
Group II Hypertensive		Damaged Myocardium with focal haemorrhage, compared to control group.
Group III Standard Drug		Not damaged Muscle fiber was still good Showed normal cells.
Group IV Test Group LD		Not damaged compared to toxic group Muscle fiber was still good
Group V Test Group HD		Not damaged Muscle fiber was still good. Showed normal cells

Figure 4 showing histopathology results of all 5 groups.

DISCUSSION

Mineralocorticoids like DOCA salt cause retention of sodium and water in the body. This retention continues until a point where the kidneys are unable to handle the increased pressure, leading to escape diuresis (increased urine output despite high sodium levels). Previous studies have shown that the administration of mineral corticoid together with salt results in sodium retention, potassium depletion, hypertension, extensive tissue damage and even death, whereas activating natriuretic systems and suppressing sodium- and water-retaining systems to increase sodium excretion. *Vaasathi kashayam* results in a significant reduction in systolic and diastolic blood pressure. After 43 days of DOCA salt treatment twice a week followed by *Vaasathi kashayam* administration on daily basis, there was a significant decrease in serum sodium levels and a simultaneous increase in serum potassium levels. This indicates that *Vaasathi kashayam* may indeed be promoting the excretion of excess sodium and the retention of potassium, which helps in reducing hypertension and preventing tissue damage. *Vaasathi kashayam* appears to counteract the hypertensive effects of mineralocorticoids by promoting sodium excretion and potassium retention. This mechanism aligns with the observed changes in serum electrolyte levels and the significant reduction in blood pressure noted in the study. Based on the results of the DPPH radical scavenging assay, *Vaasathi kashayam* (VK) demonstrated concentration-

dependent scavenging activity against DPPH radicals, with the highest inhibition observed at 63.15%. This suggests that VK contains compounds capable of neutralizing free radicals, which are known to cause oxidative damage to biomolecules such as nucleic acids, proteins, and lipids. The significant scavenging activity of VK against DPPH radicals indicates its potential as an antioxidant. The findings suggest that by reducing oxidative damage, VK could potentially slow down disease progression in conditions where oxidative stress plays a significant role. Diuretics are valuable not only in treating conditions like hypertension but also in managing fluid overload in congestive heart failure (CCF) and acute left ventricular failure. By reducing plasma volume and venous return to the heart, diuretics help alleviate symptoms such as pulmonary congestion, peripheral edema, orthopnea, and paroxysmal nocturnal dyspnea. Extract of VK administered has been shown to increase urinary output in a dose-dependent manner. This means that higher doses of VK led to greater volumes of urine being excreted. The increase in urinary output induced by VK was accompanied by increased excretion of electrolytes such as sodium (Na+), potassium (K+), and chloride (Cl-). This pattern of electrolyte excretion is similar to that seen with standard diuretics like furosemide. VK at doses of 200mg/kg and 400mg/kg showed diuretic activity comparable to furosemide, a well-known loop diuretic used in clinical practice. Furosemide is effective in promoting the excretion of sodium and water from the body, and VK appears to exhibit similar saluretic effects. Based on the acute and subacute toxicity studies conducted according to OECD guidelines on Wistar albino rats, *Vaasathi kashayam* (VK) demonstrated favorable safety profiles. The acute toxicity study did not show any mortality in the tested animals. This indicates that VK has a high acute safety margin, meaning it is unlikely to cause severe toxicity even at relatively high doses in a single administration. In the subacute toxicity study, VK was administered repeatedly over a period of 28 days. During this period, there were no observed deaths among the rats. Additionally, comprehensive assessments including functional observations, hematological (blood-related) parameters, and biochemical investigations did not reveal significant changes that would indicate toxicity. The absence of significant changes in biochemical parameters (such as liver enzymes, kidney function markers, etc.) and hematological parameters (such as red blood cells, white blood cells, etc.) further supports the safety of VK during prolonged administration. Based on these findings, it can be concluded that *Vaasathi kashayam* is well-tolerated in rats when administered over an extended period. This establishes a foundation for considering VK as safe for long-term use in potential therapeutic applications, aligning with its traditional use and supporting further clinical investigations.

CONCLUSION

In conclusion, the toxicological evaluation of *Vaasathi Kashayam* (VK) confirms its favorable safety profile, indicating that it can be administered over prolonged periods without causing significant adverse effects on major organ systems or physiological functions in animal models. The findings of this study offer supportive data on the formulation's purity, quality standards, and its potential as an antihypertensive and antioxidant agent. These results strengthen the basis for considering VK as a viable therapeutic option or supplement for managing hypertension and conditions linked to oxidative stress. Moreover, the study highlights VK's promising diuretic activity, demonstrated by enhanced urinary output and increased electrolyte excretion, with no notable signs of acute or subacute toxicity. Future investigations are recommended to isolate and identify the active constituents responsible for these pharmacological effects, explore their mechanisms in various disease models, and validate efficacy and safety through clinical trials in human subjects.

Acknowledgement

I would like to give my remarkable gratefulness to my guide and head of the department Prof. Dr. T. Komalavalli MD(S) Ph.D., and the head of my institution Dr. B. Malarvizhi MD(S)., for permitting me to perform this animal study. I express profound gratitude to animal handling advisor Mr. Santhanakumar M. Pharm, and Dr. A.Manoharan MD(S) Ph.D., for their advice and support throughout the study. I'm grateful for the insightful comments offered by Dr. G.Subash chandran MD (S), Ph.D. Finally, I would like to express my heartfelt gratitude towards my parents, friends and juniors for their love and guidance in this endeavor.

REFERENCES

1. Alugoju Phaniendra. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 2015; 30: 11–26.
2. Ancy P, Padmaja V, Radha K, Jose J, Hisham A. Diuretic activity of the roots of Flacourtia indica. Int J Pharm Pharm Sci. 2013; 5(1): 79-83.
3. Babu CS, Kalaivani P, Ranju V, Sathiya S, Anbarasi C, Mahadevan MV, Vijayakumar H, Sunil AG, Thanikachalam S. Venthamarai chooranam, a polyherbal Siddha medicine, alleviates hypertension via AT_1R and eNOS signaling pathway in 2K1C hypertensive rats. Exp Biol Med (Maywood). 2014; 239(6): 758-769.

4. Badyal DK, Lata H, Dadhich K. Animal models of hypertension and effect of drugs. Indian J Pharmacol. 2003; 35: 349–362.
5. Borde P, Mohan M, Kasture S. Effect of myricetin on deoxycorticosterone acetate (DOCA)-salt-hypertensive rats. Nat Prod Res. 2011; 25(16): 1549-1559.
6. da Costa GF, Ognibene DT, da Costa CA, Teixeira MT, Cordeiro VDSC, de Bem GF, Moura AS, Resende AC, de Moura RS. Vitis vinifera L. grape skin extract prevents development of hypertension and altered lipid profile in spontaneously hypertensive rats: role of oxidative stress. Prev Nutr Food Sci. 2020; 25(1): 25-31.
7. da Costa KCM, Oliveira LdS, Silva JC, Santana TS, de Freitas RA, Bressan AFM, Gómez-Alonso S, Pérez-Navarro J, Pertuzatti PB, Giachini FR. Enhancing vascular health and lowering blood pressure in spontaneously hypertensive rats through Syrah grape (Vitis vinifera) pomace: the role of phenolic compounds. Nutrients. 2024; 16(14): 2312.
8. Dr. S. Venkatrajan. Agathiyar 2000. 1st ed. Chennai; Siddha Publication; 2014. p. 218.
9. Dr. Shanmugavelu. Noi Naadal Noi Mudhal Naadal Thirattu-Part 2. Chennai; Indian Medicine Department; 1967. p. 102-103.
10. Bomfim GHS, Musial DC, Miranda-Ferreira R, Nascimento SR, Jurkiewicz A, Jurkiewicz NH, Moura RS. Antihypertensive effects of the Vitis vinifera grape skin (ACH09) extract consumption elicited by functional improvement of P1 (A1) and P2 (P2X1) purinergic receptors in diabetic and hypertensive rats. Pharma Nutrition. 2019; 8: 100146.
11. Gulcin I. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012; 86: 345-391.
12. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007; 35: 1147-1150.
13. Huang HY, Huang JJ, Tso TK, Tsai YC, Chang CK. Antioxidant and angiotension-converting enzyme inhibition capacities of various parts of Benincasa hispida (wax gourd). Nahrung. 2004; 48: 230-233.
14. Hussain F, Islam A, Bulbul L, Moghal MR, Hossain MS. In vitro thrombolytic potential of root extracts of four medicinal plants available in Bangladesh. Anc Sci Life. 2014; 33(3): 162-164.
15. Iqbal Chowdhury I, Rahman MA, Hashem MA, Bhuiyan MMH, Hajjar D, Alelwani W, Makki AA, Haque MA, Tangpong J, Bakhtiar MTB. Supplements of an aqueous combination of Justicia adhatoda and Ocimum tenuiflorum boost antioxidative effects and impede hyperlipidemia. Anim Model Exp Med. 2020; 3(2): 140-151.
16. K Bharathi, S Semalatha, Tamilselvan R. Anti-hypertensive activity of Siddha herbo-mineral formulation Eladhi Chooranam in spontaneously hypertensive rats. Eur J Pharm Med Res. 2022; 9: 218-222.
17. Kandasamypillai. History of Siddha Medicine. Vol. 1. Chennai; Thamarai Noolagam; 1998. p. 917.
18. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 17th ed. Pune; Nirali Prakashan; 2007.
19. Kasote DM, Hegde MV, Katyare SS. Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s), and implications of antioxidant therapy. Biofactors. 2013; 39: 392-406.
20. Kolatkar SB, Kulkarni SD, Joglekar GV. Quantitative evaluation of blood pressure responses in dogs to various vasoactive agents under the influence of commonly used anaesthetics. Indian J Pharmacol. [Details incomplete].
21. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89: 217–233.
22. Kumar EA, Kumar DA, Venkatesh P, Ramu VA, Prabakaran L. Effect of diuretic activity of Baliospermum montanum (Wild) Muell in male albino rats. Int J Pharm Bio Sci. 2012; 2(8): 49-54.
23. Lohar DR. Protocol for Testing: Ayurvedic, Siddha and Unani Medicines. Ghaziabad; Pharmacopoeial Laboratory for Indian Medicine; [No date].
24. Nassiri-Asl M, Hosseinzadeh H. Review of the pharmacological effects of Vitis vinifera (grape) and its bioactive compounds. Phytother Res. 2009; 23(9): 1197-1204.
25. Patel JM, Patel NM, Patel AA, Patel J, Patel S. Comparative diuretic activity of root and aerial part methanolic extracts of Echinops echinatus Roxb. Int J Pharm Bio Sci. 2011; 3(5): 168-172.
26. Selvakumar S, Visweshwaran S, Sivakumar S, Mariappan A, Ushakanthan S. Antihypertensive and diuretic action of Adathodai Ilai Chooranam—a Siddha mono-herbal formulation. Int J Pharm Sci Rev Res. 2018; 50(2): 28-32.
27. Shankar M, Gowrishankar NL. Preliminary phytochemical and diuretic potential of methanolic extract of Azima tetracantha Lam. leaf. Int J Res Ayurveda Pharm. 2011; 1(4): 275-278.

28. Sies H. Oxidative stress: oxidants, antioxidants. Exp Physiol. 1997; 82: 291-295.
29. Szent-Giörgyi A. Lost in the twentieth century. Annu Rev Biochem. 1963; 36: 1-15.
30. Tehreem S, Rahman S, Bhatti MS, Uddin R, Khan MN, Tauseef S, El-Seedi HR, Bin Muhsinah A, Uddin J, Musharraf SG. A UPLC-DAD-based bio-screening assay for the evaluation of the angiotensin converting enzyme inhibitory potential of plant extracts and compounds: pyrroquinazoline alkaloids from Adhatoda vasica as a case study. Molecules. 2021; 26(22): 6971.
31. Thandapilly SJ, LeMaistre JL, Louis XL, Anderson CM, Netticadan T, Anderson HD. Vascular and cardiac effects of grape powder in the spontaneously hypertensive rat. Am J Hypertens. 2012; 25(10): 1070-1076.
32. Thiyagarajan. Sarakku Suthi Muraigal. 1st ed. Chennai; Siddha Maruthuva Nool Veliyitu Pirivu, Indian Medicine and Homoeopathy Dept.; 2008. p. 6-13.
33. World Health Organization. Hypertension [Internet]. Geneva: WHO; [cited 2025 Jul 3]. Available from: https://www.who.int/health-topics/hypertension

Cite this article as:
Preyadarsheni K, Komalavalli T, M.Santhanakumar. Vaasathi Kashayam. A Siddha Herbal Formulation Alleviates Hypertension in DOCA Salt Induced Hypertensive Rats, Evaluation of Acute, Sub Acute Toxicity, Anti-Oxidant and Diuretic Activity. AYUSHDHARA, 2025;12(3):91-102.
https://doi.org/10.47070/ayushdhara.v12i3.2082
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Preyadarsheni K
PG Research Scholar,
Department of PG Pothu Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli,
Email:
preyadarshenikrishnan@gmail.com