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Original Article

In vitro, *ex vivo* and *in vivo* anti-hypertensive activity of *Chrysophyllum cainito* L. extract

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Abstract: *Chrysophyllum cainito* L., a traditional herbal medicine, could have the potential for management of hypertension due to presence of polyphenolic compounds. The extracts and fractions of the pulp of plant were evaluated for *in vitro* (inhibition of angiotensin I converting enzyme/ACE assay), *ex vivo* (isolated aorta relaxation assay) and *in vivo* (salt induced hypertensive rat assay). The alcoholic and aqueous extract (ALE and AQE respectively) of fruit of plant *C. cainito* was having 14.8 and 9.2% yield respectively. The fractionation with ethyl alcohol (EAF) and butanol (BTF) yielded 2.52 & 2.17% respectively from ALE and 0.46 & 0.31% respectively from AQE with respect to fruit pulp dry weight. More phenolic content was found in ALE (3.75±0.15 mg gallic acid equivalent or GAE g^{-1} of dry power of fruit pulp) compared to AQE and maximum in ethyl acetate fraction of ALE (ALE-EAF) (2.32±0.21 mg GAE g^{-1} of dry power of fruit pulp) among all fractions. ACE inhibition activity was found to be maximum in ALE-EAF 62.5±7.34%. While *ex vivo* study using isolated tissue of aorta showed again showed maximum activity (62.82±6.19 and 46.47±8.32% relaxation with 50 µg mL^{-1} and 10 µg mL^{-1} GAE concentration respectively). ALE-EAF reduced the elevated arterial pressure of salt induced hypertensive rat significantly to the level of normotensive animal group. Results of ALE-EAF have shown its potential as a source for novel constituent for the treatment hypertension and should further be studied for isolation of specific constituent for more effectiveness.

Keywords: *ACE inhibition, aorta ring assay, chrysophyllum cainito, extracts, fraction* hypertension, *salt induced hypertensive rat model*

Introduction

In current time, hypertension is a big issue of concern in worldwide population. According to a survey, hypertension is found to affect approximately one third of the Western population which is in turn a risk factor for cardiovascular diseases like stroke, coronary heart disease, peripheral artery disease etc. [1]. Global scenario reveals that only cardiovascular disease accounts one third of deaths of total (17 million deaths a year) and more than half cases among these complications are due to hypertension. According to WHO, approximately 40% of adults aged 25 and above had been diagnosed with hypertension worldwide in 2008. Overall, high-income countries have a lower prevalence of hypertension i.e. 35% (due to good health system) than lower income countries groups at 40%. Other reasons for increasing prevalence of hypertension can also be correlated to exposure to persistent stress, excessive alcohol consumption, use of tobacco unhealthy diet, physical inertness, excess weight and ageing. The projected % of death in 2030 by cardiovascular diseases is going to be 23.7% in comparison to 17.5% in 2008. The annual loss of approximately US$ 250 billion is due to cardiovascular disease including hypertension [1-3]. In such a situation, it becomes important to find a cost effective and therapeutically effective drug for this disease.

Since long time herbs has been the source of prototype for their derivative synthesis [4, 5]. Moreover, modern drug therapies are costly affair, so opting traditional herbal medicine for the management of hypertension and other diseases is wise decision [6-8].

Chrysophyllum cainito L., belonging to family Sapotaceae [9], commonly known as star apple.

It is an ornamental tree and produces large, edible fruits. Star apple is cultivated throughout the Caribbean, Central America, and parts of South America as well as in Southeast Asia, Jamaica [10].

This plant has been reported to have great amount of polyphenolic compounds in various parts of plant e.g. fruit pulp phenolic compounds 387.1±223.2 [149-698] mg/100 g, seed phenolic compounds 73.5±52.0 [25.7-156.4] mg/100 g [10]. This plant has also been reported as a cure to various ailments e.g. antidiabetic [9, 11], antioxidant [12, 13], antifungal [14], anti-inflammatory, antihypersenstivity [15].

Its constituents includes polyphenols [10, 12, 13], like (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, quercetin, quercitrin, isoquercitrin, myricitrin, and gallic acid [15], ferulic, caffeic, sinapic, gallic, ellagic and myricetin [16], volatile constitutents like (*E*)-2-hexenal, 1-hexanol, limonene, linalool, α-copaene and hexadecanoic acid [17]. In addition, potassium (most highly concentration) boron, calcium, iron, manganese was higher than most of the herbs and among 20 amino acids its constitutes aspartic acid, glutamic acid, proline, and lysine to be 37.6, 9.5, 6.1, and 5.4% respectively, of the total amino acids [18]. It is also a source of vitamin E (3050.95-3322.31 μM Trolox (analogue of Vit E)/100 g dry weight) [16].

The herbs and their extracts, which are polyphenol rich, have been shown to have effect against hypertension and other diseases [7, 19, 20]. In the view of polyphenolic compound to be effective in inhibition of angiotensin I converting enzyme whose activity creates hypertension [7], there is more possibility of the extract of *C. cainito* to be effective against the same condition i.e. hypertension.

In the present research work, the different extracts and fractions of fruit pulp of the plant *C. cainito* was evaluated for anti-hypertensive activity both *in vitro* and *in vivo.* To the best of our knowledge based on vast literature survey, not such work has been performed earlier with the mentioned approach.

Materials and methods

Reagents and chemicals

Rat lung ACE (EC 3.4.15.1) of 2 unit per mg, hippuryl-histidyl-leucine or Bz-Gly-His-Leu (HHL), Folin's reagent were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ethyl acetate, butanol, gallic acid and other chemical used were of analytical grade and used as supplied.

Plant material

Fruits of *Chrysophyllumcainito* were collected from its natural habitat in Hunan province, China, in May, 2013 provided by Wu Shi Pharmacy Ltd. Co., Hubei, China and identified/authenticated by Professor Ding-Xian Han, College of Life Science & Technology, Huazhong University of Science & Technology, China. Fruit pulp was separated from the seeds and cut into small pieces for drying in shades for 120 hours and the dehydrated by lyophilization (freeze drying). Such dried pieces were ground in an electrical grinder and passed through sieve number 5 (4 mm diameter).

Preparation of extract and fractions

The extracts and fractions were prepared by following the methods found in literature with little modifications [21, 22]. The alcoholic extract (ALE) was prepared using coarse, dried powder of fruit of the plant *C. cainito* (250 g) for the hot extraction process (soxhlet) with ethanol (1000 mL) for 20 hours. The aqueous extract (AQE) was prepared from coarse, dried powder of fruit of the plant *C. cainito* (250 g) by the cold maceration process for seven days using 1000 mL mixture of chloroform: water (1:99). After the extraction, marc of both processes were filtered through muslin cloth and concentrated *in vacuo* (rotary evaporator) to approx. 100 mL volume. The concentrated extracts were dried by lyophilization.

The ALE and AQE (20 g each) were suspended in water and were fractionated successively and exhaustively with ethyl acetate and n-butanol using separating funnel. Ethyl acetate, butanol and aqueous fractions from ALE were designated as ALE-EAF, ALE-BTF and ALE-AQF respectively while that of AQE were given the

designation AQE-EAF, AQE-BTF and AQE-AQF, respectively (Figure S1).

Phytochemical screening of ethanolic extracts

The freshly prepared extracts ALE and AQE of *C. cainito* were screened for phytochemical analysis (qualitative) for the presence of class of constituents flavonoids (Lead acetate & Sodium hydroxide Tests), glycosides (Keller Killani test, Borntrager test & Legal test), steroids (Salkowaski reaction, Liberman's reaction & Liberman's Burchard reaction), alkaloids (Mayer's test & Murexide test), tannins (5% $FeCl_3$ & Dilute HNO_3 Tests, carbohydrates (Fehling's test & Benedict's test), proteins amino acids(Millon's test, Xanthoprotein test & Ninhydrin test) using standard procedures available in literature etc. [23, 24].

Determination of total phenol content

The total phenolic content in the extract and fraction was estimated by Folin-Ciocalteu method. 0.5 mL sample was taken after centrifugation at 5000 rpm for 20 minutes. Supernatant was heated at 90°C for 10 minutes. 150 µL of supernatant was mixed with 150 µL distilled water and 1000 µL of complex forming reagent (50:50:1:1 of 2% Na_2CO_3, 2% NaOH, 1.5% $CuSO_4$, 2.5% sodium potassium tartarate) and incubated at 37°C for 10 minutes. Finally 150 µL phenol reagent was added and incubated at 37°C for 30 minutes. Absorbance was taken at 750 nm. Gallic acid was taken as standard (standard, 10-150 μgmL^{-1}). Total phenolic content was estimated as mg Gallic acid equivalents (GAE) g^{-1} of extract [25].

Angiotensin I converting enzyme (ace) inhibition assay

ACE inhibition assay was performed on the basis method taken from literature [7, 26] with little modifications. 50 µL solution of extract or fraction was mixed with 200 µL of phosphate buffer (100 mM, pH 8.3) containing 0.2 M NaCl, and 6.5 mMhippuryl-histidyl-leucine (HHL). 100 µL ACE solutions (0.1 U mL^{-1}) was added to start the reaction and mixture was incubated at 37°C for 30 min. 50 µL of 1 M HCl was added to stop the reaction. The Gly-His bond of HHL was then cleaved and the Bz-Gly (hippuric acid) produced by the reaction was extracted with 1.5 mL ethyl acetate. Ethyl acetate supernatant was taken after centrifugation (1200 × g for 15 min), and ethyl acetate was removed by heat evaporation. The extracted product was dissolved in 3 mL volume of distilled water and the absorbance was determined at 228 nm using a spectrophotometer (UNICO UV-2102, Shanghai, China). The inhibition activity was calculated using the following equation

$$Inhibition\,(\%) = \left(\frac{A_a - A_b}{A_a - A_c}\right) \times 100$$

where A_a is the absorbance with ACE and HHL without the sample (positive control, no inhibition and maximum activity); A_b is the absorbance with ACE, HHL and the sample or standard; and A_c is the absorbance with HHL without ACE and the sample (control).

Ex vivo aorta ring assay

The method was followed as per the protocol from literature [27, 28]. The 4-5 mm width rings of isolated thoracic aorta were tied to stainless steel hooks. It is immersed into organ baths containing 10mL Krebs solution at 37°C and oxygenated with O_2:CO_2 at 95:5.2 g weight was given to all tissues for creation of basal tension and changes in basal tension was recorded (Biopac Systems TSD 125c). The contraction of each prepared aorta was maximized by administration of KCl (120 mM) and recorded. The tissues were pre-incubated with extracts or fractions (10 and 50 µg GAE mL^{-1}). Relaxation was expressed as a percentage change from KCl contracted levels i.e. by comparison between maximum vascular contraction before and after addition of samples [28, 29].

In vivo study to evaluate Chrysophyllumcainito extracts/fractions on rats with salt induced hypertension

Male albinos Wistar rats (150-200 g), approximately three months old, were used for *in vivo* experiments in this study. The experimental procedures were in compliance with the Guide for Care and Use of Laboratory Animals (NIH version, revised 1996). All animals were housed (6 per cage) and maintained under standard laboratory conditions (25°C, a normal 12 h:12 h light/dark schedule) with free access to water and food. The animals were given adaptation

Table 1. Treatment-wise allocation of animals

Groups	I	II	III	IV	V	VI	VII
Designation	NT	SIH	S I	S II	T I	T II	T III
Treatment	Water 10 mL kg^{-1} day -1	HS 10 mL kg^{-1} day^{-1}	HS 10 mL kg^{-1} day^{-1} + 10 mg kg^{-1} day^{-1}	HS 10 mL kg^{-1} day^{-1} + 20 mg kg^{-1} day^{-1}	HS 10 mL kg^{-1} day^{-1} + 200 mg kg^{-1} day^{-1}	HS 10 mL kg^{-1} day^{-1} + 500 mg kg^{-1} day^{-1}	HS 10 mL kg^{-1} day^{-1} + 1000 mg kg^{-1} day^{-1}

NT: normotensive group; SIH: salt induced hypertension group; SI and SII: groups for two concentrations of standard (captopril: 10 and 20 mg kg^{-1} day^{-1}); TI, TII and TIII: groups for three concentrations of test drug (ALE-EFA 200 mg kg^{-1} day^{-1}, 500 mg kg^{-1} day^{-1}, 1000 mg kg^{-1} day^{-1}), HS: hypertensive solution (NaCl solution (18% w/v).

Table 2. Preliminary phytochemical screening of *C. cainito*

Test		ALE	AQE
Phytosterols	(a) Salkowaski reaction	+	-
	(b) Liberman'sburchard reaction	+	-
	(c) Liberman's reaction	+	-
Glycoside	(a) Keller killani test	+	+
	(b) Borntrager test	+	+
	(c) Legal test	+	+
Alkaloids	(a) Mayer's test	+	+
	(b) Wagner's Test	+	+
Tannins	(a) 5% $FeCl_3$	+	-
	(b) Dilute HNO_3	+	-
Flavonoids	(a) Lead acetate	+	+
	(b) Sodium hydroxide	+	+
Saponins	Froth Test	+	+
Proteins	(a) Millon's test	-	+
	(b) Xanthoprotein test	-	+
Amino acid	(a) Ninhydrin test	-	+
	(b) Million reagent test	-	+
Carbohydrate	(a) Fehling's test	+	+
	(b) Benedict's test	+	+
Diterpenes	Copper-Acetate Test	-	-
Fats and Fixed Oils	Stain Test	+	-
Resins	Acetone-water Test	-	-

'+' means the class of constituent is present and '-' means class of constituent is absent.

period of at least three weeks to the laboratory environment before commencement of experiments.

Normotensive rats were randomly divided into seven groups of six animals each. One group, neutral control, received tap water using a gastric pipe and served as normotensive (NT) group. Second group was assigned for salt-induced hypertension (SIH) group which is generated by administration of 10 mL 18% NaCl kg^{-1} rat body weight day^{-1}. Gavaging was carried out daily for 30 days with in specified time (10.00 to 11.00 AM). Two positive control groups received 18% NaCl solution and either captopril 10 mg kg^{-1} day^{-1} (SI) or 20 mg kg^{-1} day^{-1} (SII) by gavage. Three groups received 18% NaCl solution and the selected extract or fraction plant at 200, 500 and 1000 mg kg^{-1} day^{-1} by gavage (**Table 1**). Systolic and diastolic arterial pressure (SAP and DAP) were measured by tail cuff method (Kent Scientific, Torrington, CT) for evaluation along with body weight and water consumption [3, 30, 31].

Statistical analysis

The results in this study were expressed as mean ± SD except that of *in vivo* which were expressed as mean ± SEM and were analyzed using analysis of variance (ANOVA) followed by Tucky's test. A significant difference was established with respect to group in comparison, when the $P<0.05$.

Results

Preparation of extracts and fractions and phytochemical screening

In this experimental study, qualitative phytochemical analysis of extracts of *C. cainito* showed the presence of phytosterol, glycosides, tannins, alkaloids, flavonoids, saponins, fats and carbohydrates in alcoholic extract (ALE) while aqueous extract (AQE) showed the absentia of phytosterols, tanins & fats and extra presence of proteins and amino acids in comparison to ALE (**Table 2**).

Three fractions of the each extract were prepared according to the affinity of the constituent towards partitioned phases of organic solvents (ethyl acetate, butanol) represented as AEF and BTF respectively and constituent left in aqueous phase was abbreviated as AQF. The yield of extract with respect to dried powder and yields of fraction with respect to extract and dried powder were calculated for each

Table 3. % yield of extract and fractions of fruit pulp o*f C. cainito*

	W_1	E	Y_1	W_2	W_3	F	Y_2	Y_3
	G	G	% (w/w)	G	G	g	% (w/w)	% (w/w)
ALE	250	37	14.8					
ALE-EAF	250			337.83	20	8.52	42.6	2.52
ALE-BTF	250			337.83	20	7.34	36.7	2.17
ALE-AQF	250			337.83	20	3.91	19.55	1.15
AQE	250	23	9.2					
AQE-EAF	250			543.47	20	2.54	12.7	0.46
AQE-BTF	250			543.47	20	1.73	8.65	0.31
AQE-AQF	250			543.47	20	14.8	74	2.72

E: Amount of extract obtained; F: Amount of fractions obtained; GAE: Total phenol content in form of gallic acid equivalents; W_1: Initial amount coarse powder taken; W_2: Amount of coarse powder required to obtain 20 g extract; W_3: Extract amount taken for fractions preparation; Y_1: Yield of extracts (E) wrt 250 g coarse powder (W_1); Y_2: Yield of fractions (F) wrt 20 g extract (W_2); Y_3: Yield of fractions (F) wrt amount of coarse powder required to obtain 20 g extract (W_3).

Table 4. Total phenolic content of extracts and their fractions

Extracts/Fractions	Phenolic content (GAE mg g-1)
ALE	40.7±4.2
ALE-EAF	62.5±7.34
ALE-BTF	21.4±3.54
ALE-AQF	15.5±5.3
AQE	27.3±6.3
AQE-EAF	49.1±3.54
AQE-BTF	51.53±4.2
AQE-AQF	17.2±1.4

The value is average of three samples with standard deviation.

extracts and their respective fractions have been given in **Table 3**.

Phenolic contents of extract and fractions

Every extract and fractions thereof were characterized for the phenolic content by the method mentioned iDetermination of total phenol contentn "Determination of Total Phenol Content" and were expressed in terms of standard gallic acid equivalents (GAE). GAE was found to 3.75±0.15, 2.32±0.21, 0.31±0.02, 1.59±0.08, 2.42±0.34, 0.88±0.14, 0.61±0.05, 1.39±0.13 in ALE, ALE-EAF, ALE-BTF, ALE-AQF, AQE, AQE-EAF, AQE-BTF and AQE-AQF respectively (**Table 4**). It was noteworthy that phenolic content in ALE was higher than AQE, moreover, ethyl acetate fraction (EAF) of ALE takes maximum of the phenolic content from both ALE and AQE which suggested the affinity of the polyphenolic compounds for the organic phases of for extraction or fractionation.

Kubola and coworkers carried out the phytochemical analysis of some of wild fruits of Thai including *C. cainito*, found the phenolic content of green fruit and ripened fruit of *C. cainito* reported to be 88±3.70 and 28.54±0.73 mg GAE g^{-1} [32]. The study of antioxidant activity methanolic and ethylacetate extracts of fresh fruits of *C. cainito* were characterized for different polyphenolic compounds out of which gallocatechin was highest in concentration and had highest antioxidant activity [12].

Einbond and coworkers suggested that the presence of sugars and ascorbic acid aqueous fractions can mask the antioxidant activity of polyphenols that may be present in the fraction [13].

ACE-inhibition activity of extracts and fractions of C. cainito

In antihypertensive studies, ACE inhibition assay is an important characteristic to be determined as ACE is involved in two hypertension cascades i.e. conversion of angiotensin I to angiotensin II and bradykinin degradation [33]. In ACE inhibition assay all extracts and fractions were evaluated taking constant GAE (50 μg mL^{-1}) for each. Constant GAE was prepared by taking the amount of extract or fraction in which 50 μg GAE was present (13.33, 21.55, 161.29, 31.44, 20.63, 56.49, 81.79, 35.93 mg of ALE, ALE-EAF, ALE-BTF, ALE-AQF, AQE, AQE-EAF, AQE-BTF and AQE-AQF, respectively) and mixing in 1 mL of buffer. In many reports, polyphenols are suggested to be the main class of constituent to inhibit the ACE [7, 34, 35] by sequestration of the enzyme cofactor (Zn^{2+} ion) as a mechanism of action [34, 36]. So it became logical to compare/evaluate the extracts and fractions on the same level of GAE.

The result obtained by ACE inhibition assays with different extracts and fractions were given in **Figure 1**. Results showed that ACE inhibition

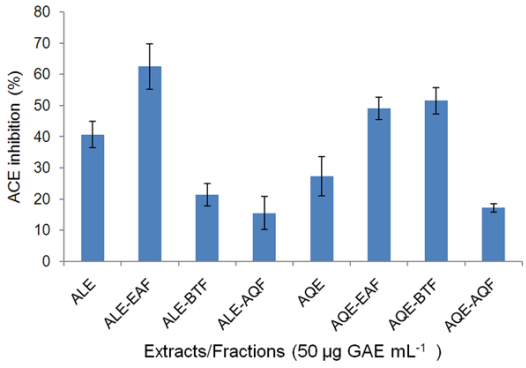

Figure 1. ACE inhibition (%) efficacy of extracts and their fractions with equal phenolic content (50 µg GAE mL^{-1}). The value is average of three samples with respective standard deviation.

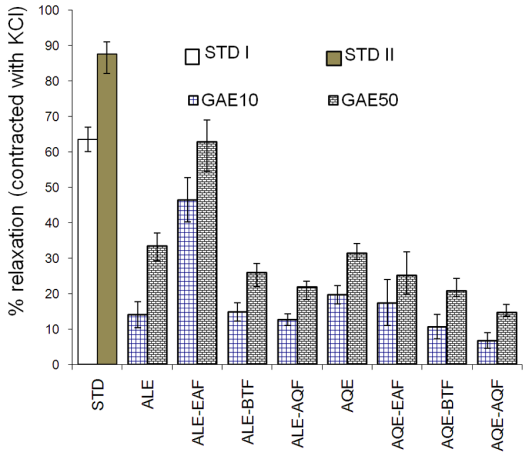

Figure 2. Effect of extracts and their fractions with two concentration of phenolic content (10 and 50 µg GAE mL^{-1}) on % relaxation (from KCl contraction) on aorta smooth muscle tissue. STD I and II are the captopril concentrations 0.25 and 0.5 µg mL^{-1} and GAE 10 and 50 represents the phenolic amount (10 and 50 µg GAE mL^{-1}) of various extracts. The values are average of three samples with respective standard deviation as error bars.

activity was maximum in ALE-EAF and comparative in AQE-EAF and AQE-BTF. Here one point is to note that the inhibition was better in EAF fraction than their corresponding extracts suggesting the affinity of the ACE inhibitors for the organic phase EAF during fractionation process. The Similar affection was observed in a study and showed good ACE inhibition with the ethyl acetate fraction (IC_{50} 7.5 mg mL^{-1}) and aqueous fraction (IC_{50} 2.5 mg mL^{-1}) of aqueous extract of plant *Rosa rugosa* flowers [37].

A commercial antihypertensive drug, captopril, was taken as standard and its effect on enzyme kinetics was observed (Figure S2). IC_{50} of captopril was determined and found to be 11.68±2.59 ng mL^{-1}. The value is near to the value found in literature i.e. 0.05 µg mL^{-1} [34], 15.16 ng mL^{-1} [37]. As the ACE inhibition was better in ALE-EAF, AQE-EAF and AQE-BTF so IC_{50} of these fractions were determined with six different gallic acid equivalent concentrations (GAE). IC_{50} was found to be best in ALE-EAF (34.26±4.64 µg GAE mL^{-1}) while AQE-EAF and AQE-BTF were having lesser capability to inhibit ACE (IC_{50} 67.76±5.1, 144.4±3.74 µg GAE mL^{-1} respectively).

Ex vivo aortic ring assay

Along with ACE inhibition changes in vascular tone on isolated tissue (*Ex vivo*) of aortic wall usually give more closer idea of antihypertensive capability of the compounds so aortic ring assay was also included in this study. Again the concentration of the extracts and fractions were taken according to the gallic acid equivalents (10 and 50 µg mL^{-1}) assuming the constituent to be phenolic in nature as found in literature.

Stimulation of aortic rings with 120 mM KCl resulted in a sustained contraction equivalent to 5363±541 mg was taken as maximum. The additions of the extracts or fractions of *C. cainito* caused the relaxant response at various levels. Results of % of relaxation of aortic endothelium (**Figure 2**) showed that the maximum activity was in ALE-EAF 62.82±6.19 and 46.47±8.32 % with higher (50 µg mL^{-1}) and lower (10 µg mL^{-1}) GAE concentration respectively which was in comparison with standard captopril 0.5 and 0.25 µg mL^{-1} (87.64±3.4 and 63.54±5.54% respectively.) ALE-EAF was selected for further *in vivo* studies as it was showing its effectiveness at each level i.e. phenolic content, *in vitro* and *ex vivo*.

In vivoevaluation of selected fraction from plant C. cainito using salt induced hypertensive rat model

The salt induced hypertensive (SIH) rat model was used to study the antihypertensive activity of the ethyl acetate fraction of alcoholic extract (ALE-EAF). High salt diet induces a cytochrome P450 isoform which has been estimated by metabolic and immunological techniques. Otherwise these are either absent or present with negligible concentration in normal group of animals. Increased salt intake also increase

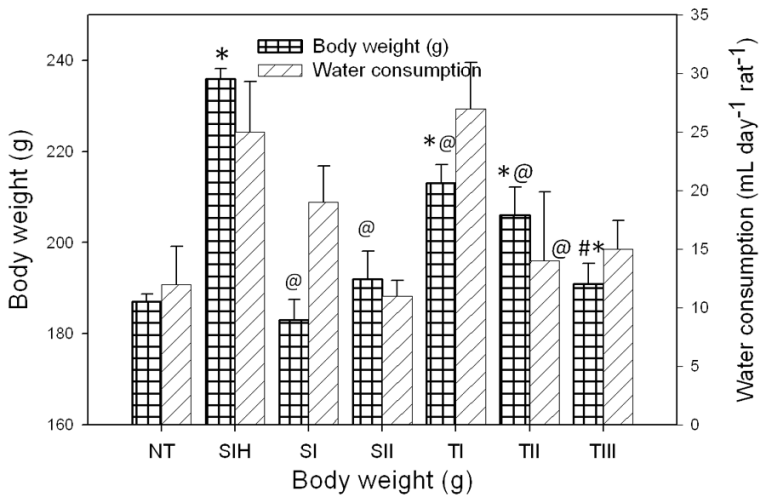

Figure 3. Effect of fraction of plant *C. cainito* (ALE-EAF) with three concentrations (200, 500 and 1000 mg kg^{-1} equivalent to 0.464, 1.16 and 2.32 mg GAE kg^{-1} respectively) on Body weight and Water consumption of SIH rats. The value is average of six animals with respective standard error of mean (SEM) as error bars. *, @ and # represents the significant difference ($p<0.05$) in comparison to sham group (NT), negative control (SIH group) and lowest dose of fraction (TI) for body weight. There was significant difference in water consumption in among any comparison.

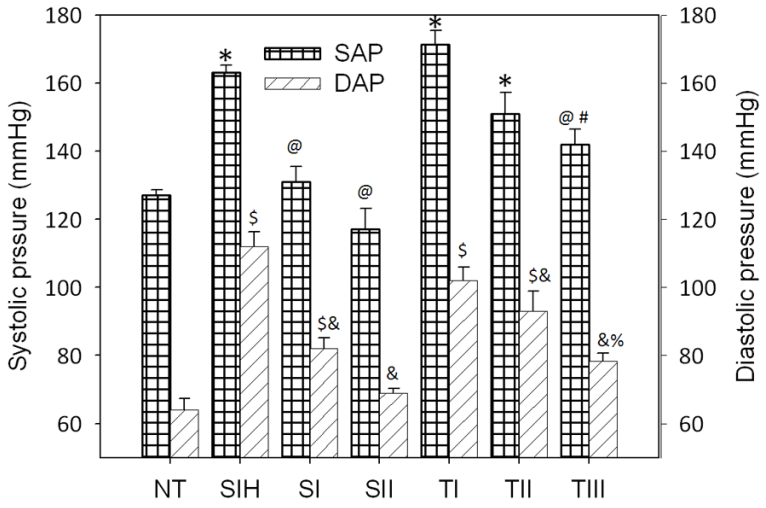

Figure 4. Effect of fraction of plant *C. cainito* (ALE-EAF) with three concentrations (200, 500 and 1000 mg kg^{-1} equivalent to 0.464, 1.16 and 2.32 mg GAE kg^{-1} respectively) on atrial blood pressure (systolic and diastolic) of SIH rats. The value is average of six animals with respective SEM. *, @ and # represents the significant difference ($p<0.05$) in comparison to sham group (NT), negative control (SIH group) and lowest dose of fraction (TI) for systolic arterial pressure (SAP). $, & and % represents the significant difference ($p<0.05$) in comparison to sham group (NT), negative control (SIH group) and lowest dose of fraction (TI) for diastolic arterial pressure (DAP).

rate of renal salt excretion and prevents retention of salts resulted in volume expansion and hypertension as a consequence. High salt intake and the conspicuous high renal epoxygenase activity and urinary excretion of 5,6-epoxyeicosatrienoic acids (EET) and dihydroxyeicosatrienoic acids, together with inhibition of sodium salt absorption (at proximal and distal nephron) of EET suggested that cytochrome P450 (salt-inducible) arachidonic acid epoxygenase may be one of the functionally significant components of the kidney's adaptive response to an increased salt intake [38]. It can be hypothesized that alcoholic fraction (ALE-EAF) may inhibit one or more cause of the hypertension biochemical pathway.

First of all body weight and water consumption of the normotensive (NT), salt induced hypertensive (SIH), captopril (S) and ALE-EAF (T) were checked. It was found that body weight and water consumption both are significantly higher than the NT group. While S and T group showed reverse of the SIH effect to extent up to NT (**Figure 3**).

While **Figure 4** showed similar kind of effect with systolic and diastolic arterial pressure (SAP, DAP respectively) i.e. both SAP and DAP in SIH are significantly higher than the NT group. While S and T group showed reverse of the SIH effect to extent up to NT.

Conclusion

Hypertension, a causative factor for cardiovascular diseases, needs a handy cure as a cost effective herb. *Chrysophyllumcainito* L., the star apple is an ornamental tree and involved as cure to various ailments reported mainly due to polyphenolic compounds present in various parts of this plant.

The aqueous and alcoholic extract of fruit of plant *C. cainito*were found to contain some main class of constituents i.e. alkaloids, glycosides, tannins, phytosterols, flavanoids with produced with good yield i.e. 14.8% with alcoholic extract (ALE) and 9.2% with aqueous extract (AQE). The extracts were fractionated with organic solvents i.e. ethyl alcohol (EAF) and butanol (BTF) with yield 2.52, 2.17% respectively from ALE and 0.46, 0.31% respectively from AQE with respect to fruit pulp dry weight. Total phenolic content was maximum in ALE compared to AQE and its ethyl acetate fraction of ALE (ALE-EAF) among all fractions i.e. 3.75±0.15 and 2.32±0.21 mg gallic acid equivalent (GAE) g^{-1} respectively. In *vitro* study i.e. ACE inhibition activity was also maximum in ALE-EAF and comparative in AQE-EAF and AQE-BTF taking equal phenol content (50 μg mL^{-1}) in each extract. While *ex vivo* study using isolated tissue of aorta showed again showed maximum activity (62.82±6.19 and 46.47±8.32% relaxation with 50 μg mL^{-1} and 10 μg mL^{-1} GAE concentration respectively) which was in equivalent with standard captopril 0.5 and 0.25 μg mL^{-1} (87.64±3.4 and 63.54±5.54% respectively). *In vivo* study using salt induced hypertensive rat model also showed significant effectiveness for the fraction (ALE-EAF) and the standard to bring the pressure same as that of normotensive animal group.

The effectiveness of the fraction ALE-EAF at each level of evaluation i.e. *in vitro* to *in vivo* via *ex vivo* was comparable to existing standard drug. Here we can conclude that this plant as such, its extract and more precisely the ethyl acetate fraction of alcoholic extract could be good source for the effective and novel constituent to treat this concerned and threatening cause of cardiovascular diseases.

Disclosure of conflict of interest

None.

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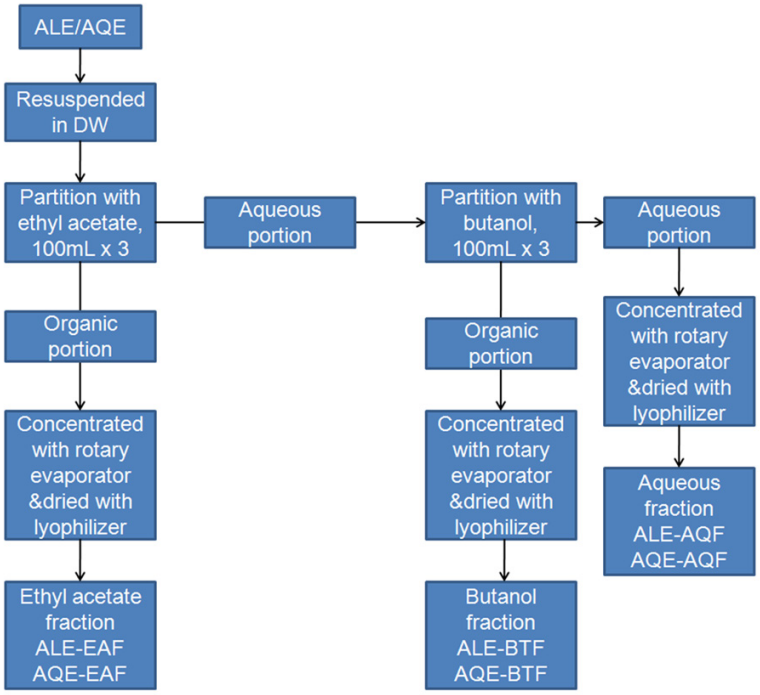

Figure S1. General scheme of fractionation procedures from both extracts (ALE and AQE) employed for *C. Cainito.*

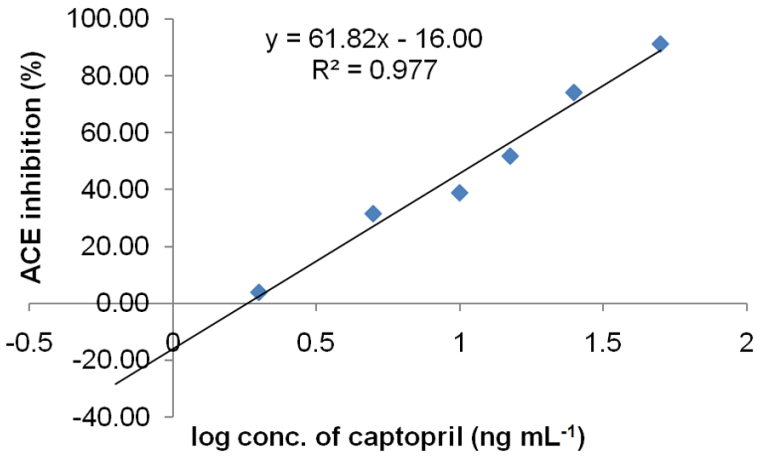

Figure S2. ACE inhibition of captopril.