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ACE Inhibitory Activity And Antihypertensive Effect Of Aspidopterys Cordata in DOCA-Salt-Induced Hypertension In Rats

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ABSTRACT

Hypertension is symptomatically identified with elevated blood pressure and is an instigator of other cardiovascular disorders. Both developing and developed countries are confronting this with various treatment regimens using synthetic drugs and lifestyle modifications. It needs to create plant-based antihypertensive agents to remedy the elevated blood pressure associated with other complications. In the present study, the fractions of Aspidopterys cordatawere screened for antihypertensive activity in vitro (Angiotensin-converting enzyme inhibition assay) and in vivo (DOCA salt-induced hypertension in rat) models. In the results, the methanol fraction of A. cordata exhibited significantly ($p < 0.05$) higher (IC_{50}=32.82µg/ml) ACE inhibitory activity among other fractions when compared to the standard Captopril. Similarly, at the selected doses (200 mg/kg and 400 mg/kg), the methanol fraction significantly decreased (164±2.34&144.2±1.3) the mean systolic blood pressure (MSBP) in a dose-dependent manner, followed by chloroform (173.4±1.14&163.6±2.07) and ethyl acetate (175.8±1.92&164.4±1.94) fractions.

Keywords: Aspidopterys cordata, DOCA salt, hypertension, ACE inhibitory activity, uninephrectomized rats, Mean systolic blood pressure (MSBP)

INTRODUCTION

Hypertension is one of the leading causes of premature death through cardiovascular diseases such as heart failure, atherosclerosis, and dementia in elders (Unger et al., 2020). Hypertension has become a common medical condition worldwide and increases the economic burden (Kearney PM et al., 2007). Around 1.3 billion people of age above 30 years are suffering from elevated blood pressure. Half the patients having hypertension are not clinically diagnosed in the early stages. Unfortunately, only 20 percent of the patients have their high blood pressure under control (Steichen O et al., 2014). Few conditions such as age, diabetes, kidney diseases, and heredity leading to hypertension cannot be modified. But, abnormal lifestyle habits, including consumption of alcohol, an unhealthy diet regime with high salt with trans-fat, and smoking, can be adapted to minimize this risk (Chen B et al., 2011).

Various synthetic medications such as diuretics, ACE inhibitors, Ca^{+2} channel blockers and beta-blockers, etc. but, none of them can permanently reverse the elevated blood pressure (Hariyadi B et al., 2012). Traditionally, few practices and plants such as Rauwolfia, Veratrum are mentioned to treat hypertension. The comparative safety of herbal medicine over synthetic medicine in chronic usage makes people shift towards the traditional treatment for these disorders (Hajjar I et al., 2003; Furtado FF et al., 2017). It is necessary to explore plants for safe and potent antihypertensive agents with improved tolerance in chronic usage. In regards, the current research is aimed to evaluate the antihypertensive property of Aspidopterys cordata, a native plant of India.

MATERIALS AND METHODS

Plant material

Aspidopterys cordata was collected from Kinnerasani Wild Life sanctuary, Bhadradri Kothagudem District Telangana. The plant is confirmed by Botanist Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool; plant specimen has been submitted (RU/BD/VSN-092) for future reference.

Reagents and chemicals

All the chemicals and reagents were procured from Sigma Aldrich (laboratory grade).

Preparation of fractions

A. cordata herb was collected and dried under shade. The dried aerial parts were powdered and extracted with methanol. The dried methanol extract was suspended in water and fractionated with n-hexane, chloroform, ethyl acetate, and methanol independently. The respective fractions were separated, filtered, and dried under a vacuum. The fraction concentrates were kept in a desiccator.

Animals

In this study, male Wistar rats (weighing around180-200gm) were selected and were randomly grouped in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with a standard commercial pelleted rat diet.The experimental procedures and protocols used in this study were reviewed and approved by the Institutional animal ethics committee (IAEC) before experimental studies (1447/PO/Re/S/11/CPCSEA-31/A).

Angiotensin I converting enzyme (ACE) inhibition assay

ACE is a crucial enzyme regulating blood pressure and fluid balance by converting biologically inactive Angiotensin-I into Angiotensin-II, a potent vasoconstrictor. Inhibition of this step can lower blood pressure and be a treatment option for people with hypertension (Chaudhary, S. K et al., 2015).

The assay protocol for this experiment was adopted from the methods described by Jimsheena et al. with slight modifications (Jimsheena, V.K et al., 2011). The test solution (50 µL) from the plant fractions was prepared using phosphate buffer (200 µL, maintaining a pH of 8.3), sodium chloride (0.2 M), and hippuryl-histidyl-leucine(HHL, 6.5mM). This mixturewas incubated at 37°C for 30 min with ACE solutions (100 µL, 0.1 U mL-1), and the progress of the reaction was suspended by the

addition of1 M HCl (50 µL). The hippuric acid generated from the reaction mixture was separated using ethyl acetate with subsequent centrifugation and vacuum evaporation of the solvent. The absorbance was determined for the final product at 228 nm using a spectrophotometer, and the ACE inhibition activity was calculated as

Inhibition % =(A_a-A_b) x100/(A_a-A_c)

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 Ac is the absorbance with HHL without ACE and the sample (control).

Acute toxicity studies

For acute toxicity studies of A. cordata, OECD-425 guidelines were followed. Starting with a dose of 2000 mg/kg, body weight to the first rat, mortality and clinical signs like aggressiveness, restlessness, sedation tremor, ataxia, paralysis, convulsion, prostration, unusual locomotion, etc. were observed for every hour for three hours and, finally periodically until 48 h (short term toxicity). If the animal was survived, then four additional rats were orally administered at dose 2000 mg/kg, sequentially at 48-h intervals. All the experimental animals were maintained under close observation for 14 days (long-term outcomes), and the number of rats that died within the study period was noted (Saleem U et al., 2017). The 50% lethal dose (LD_{50}) value was calculated.

DOCA-salt-induced hypertension and blood pressure measurements

Nephrectomy was performed on rats by making a small incision, and the left kidney was removed,followed by the ligating left renal artery, vein, and ureter. The incision was sutured, and proper post-operative care was followed forseven days. The animals also received 1% sodium chloride solution daily through drinking water and subcutaneous injection of Deoxycorticosterone acetate (DOCA)-salt (20 mg/Kg body weight) twice weekly for five weeks (Bankar, G. R et al., 2011).

Measurement of blood pressure

The tail-cuff method was used for measuring systolic blood pressure weekly to determine the effect of the treatment on the groups (Imenshahidi, M et al., 2013). The animals received prior training, and the blood pressure was measured in triplicates using a pressure transducer(Letica5002 Storage Pressure Meter).After five weeks of treatment, animals were anesthetized with sodium thiopental (45mg/kg body weight, i.p.). The blood pressure was measured from the left common carotid artery after tracheostomy.

Statistical analysis

All values are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) was used for statistical analysis, followed by Tukey-Kramer post-hoc test for multiple comparisons. The differences between the mean were considered significant when the value obtained for "p" was lower than 0.05 ($p < 0.05$). GraphPad Prism TM software, version 6.0 (Graph Pad Software, Inc., San Diego, CA, USA), performed all statistical analysis and plotted the curves.

RESULTS AND DISCUSSIONS

Angiotensin I converting enzyme (ACE) inhibition assay

ACE is one of the prime targets for controlling hypertension, followed by Ca^{+2} channel blockers.The percentage inhibition of the ACE enzyme by the fractions was depicted in the Table1. The results show that the IC_{50} values ranged between 23.94 to 34.22 µg/mL, with A. cordataoffering comparable and higher ACE inhibitory activities. Methanol fractionA. cordataled significantly ($p < 0.05$) higher(IC_{50}=32.82µg/ml) ACE inhibitory activity (Figure 1).

The fractions inhibited the angiotensin-converting enzyme significantly in a dose-dependent manner with IC_{50} values. Methanol fraction exhibited comparatively better inhibition when compared to the standard Captopril. The literature shows that the tannins, flavonoids can interact with the ACE allosterically, and contribute to the antihypertensive activity. In accordance with the previous studies, A. cordata is rich in these polyphenolic compounds.

Acute toxicity studies

From the results, it is evident that the administration of A. cordata was safe up to a dose of 2000 mg/kg. No aforementioned toxic symptoms or mortality were observed at this dose. Hence, the present study selected 1/10th and 1/5th of 2000 mg/kg, i.e., 200 mg/kg and 400 mg/kg as working doses.

DOCA-salt-induced hypertension and blood pressure measurements

DOCA salt treatment significantly increased the mean systolic blood pressure (MSBP) of the experimental animals, and Captopril reverted the blood pressure to normal (Table 2). The effect of the various fractions of A. cordata was studied in different doses (200 mg/kg and 400 mg/kg) for five weeks by measuring the MSBP of the unilaterally nephrectomized rats and compared with the standard (138±1.0) and the negative control (120.8±0.83). At the selected doses (200 mg/kg and 400 mg/kg), the methanol fraction significantly decreased (164±2.34&144.2±1.3) the MSBP in a dose-dependent manner,followed by chloroform (173.4±1.14&163.6±2.07) and ethyl acetate (175.8±1.92&164.4±1.94) fractions (Figure 2).

DOCA salt and 1% NaCl treatment increase the kidney's fluid retention and create significant hypertension in the animals. A. cordata effectively prevented the hypertensive response in rats after administering this aldosterone. The treatment improved osmoregulation and proved to possess antihypertensive properties.

CONCLUSION

In the present study, the fractions of A. cordata exhibited antihypertensive activity screened through In vitro and In vivo models.ACE is one of the powerful enzymes involved in the pathophysiology of hypertension, and inhibition of this enzyme is also an effective strategy to control high blood pressure. The In vivo results found that the chronic oral administration of various fractions of A. cordata succeeded in normalizing the MSBP in unilaterally nephrectomized rats after five weeks of treatment. It concludes the antihypertensive property of A. cordata.

Conflict of Interest: The authors declare no conflict of interest.

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Table 1. Percentage inhibition of the ACE enzyme by A. cordata

Conc. µg/ml	n-hexane fraction	Ethyl acetate fraction	Chloroform fraction	Methanol fraction	Captopril
100	19.2±1.3	37.2±1.3	45.6±1.14	73.8±1.3	87.4±1.5
50	15.4±1.34	28.6±1.9	36.6±2.4	64.4±1.94	73.92±0.83
25	14.4±1.14	16.6±1.14	22.6±1.14	48.2±3.5	57.4±0.83
12.5	10.8±1.3	12.4±1.14	18.2±1.3	34.8±5.2	40.8±1.94

6.5	8.4±1.34	8.2±1.3	12.4±1.3	22.2±1.64	28.8±1.48
IC_{50} µg/ml	**52.79**	**42.91**	**41.3**	**32.82**	**27.12**

Table 2. Effect of A. cordataon MSBP of the unilaterally nephrectomized rats

Treatment	MSBP
Negative control	120.8±0.83
Disease control	212±1.58
Positive control	138±1.0 ****
n-Hexane fraction 200µg/ml	205.6±2.7 ns
n-Hexane fraction 400µg/ml	204.8±1.64 ns
Ethyl acetate fraction200µg/ml	175.8±1.92 **
Ethyl acetate fraction 400µg/ml	164.4±1.94 ****
Chloroform fraction 200µg/ml	173.4±1.14 **
Chloroform fraction 400µg/ml	163.6±2.07 ****
Methanolfraction 200µg/ml	164±2.34 ***
Methanol fraction 400µg/ml	144.2±1.3 ****

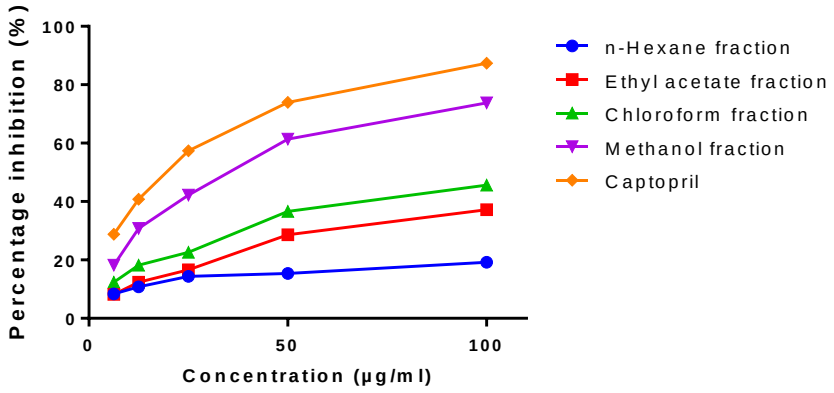

Figure 1. Percentage inhibition of the ACE enzyme by A. cordata

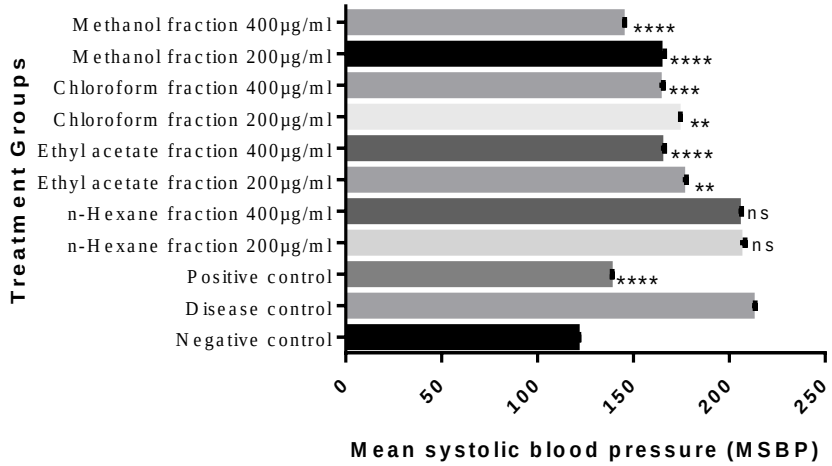

Figure 2. Effect of A. cordataon MSBP of the unilaterally nephrectomized rats