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Antihypertensive effect of rhizome part of *Acorus calamus* on renal artery occlusion induced hypertension in rats

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ABSTRACT

Objective: The rhizomes part of *Acorus calamus* (AC) having the calcium inhibitory effect and diuretic activity which may potentiate Na_+ excretion in hypertension induced by occlusion of renal artery. Therefore this study was aimed to investigate the effect of AC on experimentally induced hypertension. **Methods:** Hypertension in rats was induced by clamping the left renal artery for 4h by arterial clamp (2K1C). At the end of experiment animal were anesthetized with ketamine (50 mg/kg). Carotid artery was cannulated which was connected to pressure transducer for estimation of blood pressure. **Results:** Ethyl acetate extract of *Acorus calamus* rhizomes (EAAC) treated rats that underwent hypertension, demonstrated significant ($P < 0.01$) lower systolic blood pressure and diastolic blood pressure when compared with 2K1C rats indicated blood pressure lowering activity. Plasma renin activity was significantly ($P < 0.05$) decreased in EAAC treated rats compared to 2K1C rats. EAAC treated rats that underwent hypertension demonstrated significant ($P < 0.01$) lower mean blood urea nitrogen and creatinine when compared with 2K1C rats. Lipid peroxidation was significantly ($P < 0.001$) decreased, where as nitric oxide level in tissue was significantly elevated in EAAC treated rats. Antioxidant enzymes like glutathione, superoxide dismutase and catalase were significantly ($P < 0.05$, $P < 0.01$, $P < 0.001$) increased in EAAC treated rats when compared to 2K1C rats. **Conclusions:** In conclusions, EAAC treatment attenuated renal artery occlusion induced hypertension via nitric oxide generation and decreases the plasma renin activity.

1. Introduction

Renovascular hypertension is a common secondary hypertension and the most prevalent form of curable hypertension. Although presently used antihypertensive agents have been shown to reduce the incidence of cardiovascular events, but to control elevated blood pressure (BP) continues to be a worldwide public health problem. Hence, newer antihypertensive agents are needed to expand therapeutic options, increase treatment efficacy, decrease side effects, and enhance patient adherence. Renovascular hypertension occurs in humans due to renal artery constriction, usually from atherosclerotic or fibromuscular dysplastic renal disease, since this lowering of renal perfusion pressure causes the kidney to over produce renin, leads to a continual activation of the renin angiotensin-aldosterone system[1,2].

The renin-angiotensin-aldosteron system (RAAS) plays an important role in the control of cardiovascular homeostasis, affecting both blood pressure and fluid volume and is one of the most important ethiological candidates in hypertension. Originally, the RAS was known solely as an endocrine system, in which angiotensinogen of hepatic origin is secreted into the systemic circulation and cleaved by renin and angiotensin-converting enzyme (ACE), to produce the active peptide angiotensin II (AngII). It is now well documented that RAS, including all the components of the enzymatic pathway, may reside within several individual organs or tissues such as kidney, lung, heart and vascular smooth muscle cells, where it is believed to act in a functionally independent paracrine/autocrine fashion [3]. There is a clear link between oxidative stress and the development of angiotensin II - induced hypertension [4,5] but the exact mechanisms are not yet fully understood.

More than a decade ago, it was concluded from indirect evidence that angiotensin II may induce reactive oxygen

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spices (ROS) formation. In addition, there are several interactions between ROS and nitric oxide (NO) leading to a decrease in NO bioavailability [6]. O2− rapidly reacts with NO, yielding ONOO−, which may decompose into nitrate and OH. ONOO− also oxidizes the zinc − thiolate centre of NO synthtase (NOS), resulting in a decrease in NO formation [7]. NO as a biological messenger is known to be involved in diverse physiological and pathophysiological processes in various organ systems. NO is a potential regulator for angiogenesis and many studies have shown that NO is closely involved in the regulation of systemic blood pressure [8].

Acorus calamus L. (AC), family Araceae, have been used in the Indian and Chinese systems of medicine for hundreds of years for its beneficial role in several kinds of diseases especially the central nervous system abnormalities [9,10]. The rhizomes of AC are well reported for anti−oxidative, anti−inflammatory, neuroprotective and calcium inhibitory effects [11-16]. So we hypothesized calcium inhibitory effects and anti−oxidative of AC could be effective in hypertension. However, the antihypertensive effects of this plant have not been reported till date. Addressing this shortfall the present study was aimed to investigate the effect of AC on experimentally induced hypertension in rats.

2. Materials and methods

2.1 Plant materials and preparation of extract

The rhizomes of AC were collected from the local market of Vadodara, Gujarat, India in the month of January 2012, and authenticated by Food and Drug Laboratory, Vadodara, Gujarat, India. The dried rhizomes were coarse powdered and subjected to extraction with ethyl acetate using continuous Soxhlet extractor. The extract was concentrated using rotary evaporator. The dried extract was stored in air tight container in refrigerator below 8oC. Ethyl acetate extract of AC rhizomes (EAAC) was used to antihypertensive investigation.

2.2 Phytochemical evaluation

Phytochemical screening for presence of secondary metabolites in the EAAC was carried out by Dragendroff's test, Mayer's test (for alkaloids), foam test (for saponin) and ferric chloride test (for tannin) [17].

2.3 Animals

The male wistar rats weighing 200 − 250 g were used for the experiment. The rats were kept in polypropylene cages in an air − conditioned room maintained at a comfortable 23± 2 ℃ with a 12 h light−dark cycle. The protocol described herein was approved by the Institutional Animal Ethical Committee (No. 1029/a/07/CPCSEA) and conducted according to the guidelines of CPCSEA.

2.4 Induction of hypertension and blood pressure measurement

Hypertension in rats was induced by previously reported 2K1C method [18]. In brief; rats were anesthetized by ketamine (60 mg/kg) and diazepam (5 mg/kg). An arterial clamp was placed on to the left renal artery of the kidney. The renal artery was occluded for 4 hrs. After 4 hrs, the renal arterial clamp was removed. The right carotid artery cannulated by advancing PE−50 tubing which was connected to pressure transducer on physiograph for measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP).

2.5 Experimental design

The animals were divided in three groups (n = 6). Group 1: Rats underwent all the surgical procedure without renal artery occlusion (Sham operated); Group 2: Rats underwent renal artery occlusion for 4 hrs (2K1C); and Group 3: Rats treated with EAAC (250mg/kg in 1% tween 80) for seven days and on day seven underwent renal artery occlusion for 4 hrs (EAAC).

2.6 Blood collection and tissue preparation

At the end of experiment the blood was collected by retro−orbital puncture and allowed to clot for 10 minutes at room temperature. Clots were centrifuged at 2500 rpm for 10 minutes to separate the serum. Serum creatinine and blood urea nitrogen (BUN) levels were measured by commercial available kits (Transasia Bio−Medical Ltd). The kidney quickly removed, perfuses immediately with ice cold hypertonic saline solution, weighed and homogenized in chilled tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000×g at 00C for 20 min using Remi C−24 high speed cooling centrifuge. The clear supernatant was used for the assay of lipid peroxidation (LPO), catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) [19].

2.7 Measurement of plasma renin activity

PRA was measured with a kit using 125−I Angiotensin I generation (Sorin−Biomedica Diagnostic Division RIA kit). Angiotensin I coated−tube radioimmunoassay (RIA) was performed in two aliquots of the same sample, one incubated at 37 ℃ for generation and one non − incubated; PRA was calculated as ng angiotensin I generated/ml/h.

2.8 Measurement of tissue nitrite level

The level of nitrite level was estimated by previously established method [19]. To 0.5 mL of tissue homogenate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein−free supernatant was used for the estimation of nitrite levels. To 200 μL of the supernatant, 30 μL of 10% NaOH was added, followed by 300 μL of Tris−HCl buffer and mixed well. To this, 530 μL of Griess reagent was added and incubated in the dark for 10−15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

2.9 Statistical analyses

All the values ware expressed as mean± SEM. Statistical significance was tested between more than two groups using one−way ANOVA followed by the Bonferroni multiple comparisons test by using a computer−based fitting program (Prism, Graphpad 5). Differences was considered to be statistically significant when $P < 0.05$.

3. Results

3.1 Phytochemical evaluations

Percentage yield of EAAC rhizomes were 5.70 % w/w. Preliminary phytochemical screening of EAAC revealed the presence of secondary metabolite like tannins, alkaloids, and saponin.

3.2 Effect of EAAC on blood pressure

2K1C group of rats demonstrated significant ($P < 0.001$) increased in SBP (Figure 1A) and DBP (Figure 1B) when compared with sham operated animals, which was significantly ($P < 0.01$) decreased in EAAC group of rats when compared with 2K1C group.

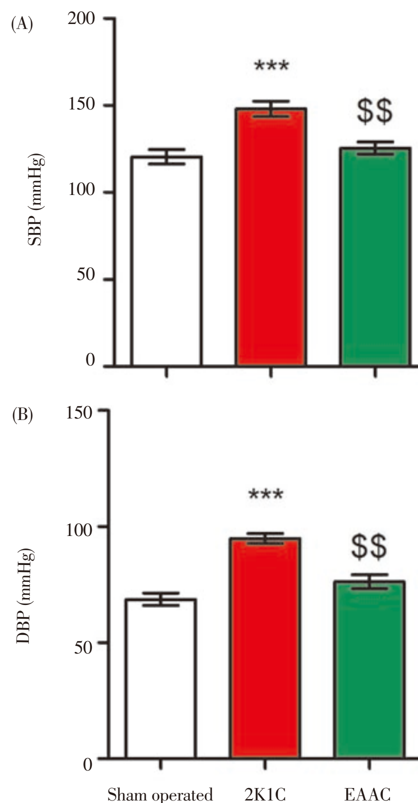

Figure 1 Effect of EAAC on SBP (A) and DBP (B).
Values are mean± SEM (n = 6), analyzed by one-way ANOVA followed by Bonferoni's multiple comparison tests. ***denote P < 0.001 for chance differences vs sham operated and $$ denote P < 0.01 for chance difference vs 2K1C.

3.3 Effect of EAAC on plasma renin activity

We observed significant ($P < 0.001$) elevation of PRA in 2K1C group when compared with sham operated group. EAAC treated rats demonstrated significant ($P < 0.05$) reduction in PRA when compared with 2K1C group (Figure 2).

3.4 Effect of EAAC on kidney function

In 2K1C group of rats serum creatinine and BUN were significant ($P < 0.001$) higher when compared with sham operated group, which was significantly ($P < 0.01$) lower in EAAC group when compared with 2K1C group (Figure 3A, 3B).

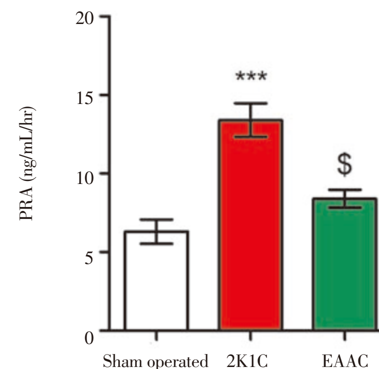

Figure 2 Effect of EAAC on plasma renin activity.
Values are mean± SEM (n = 6), analyzed by one-way ANOVA followed by Bonferoni's multiple comparison tests. ***denote P < 0.001 for chance differences vs sham operated and $ denote P < 0.05 for chance difference vs 2K1C.

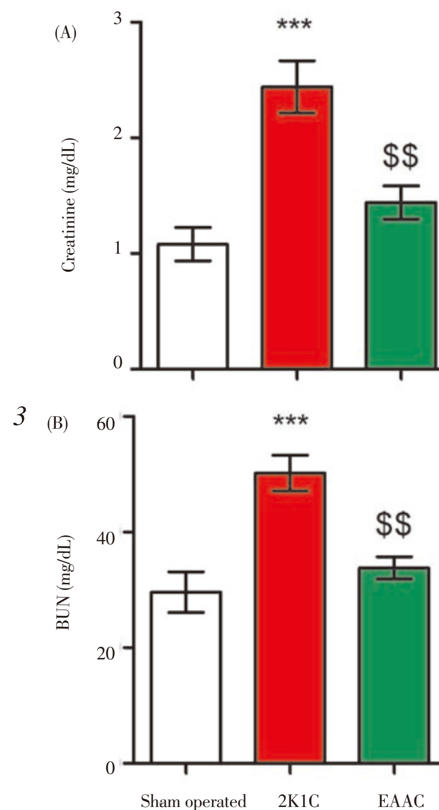

Figure 3 Effect of EAAC on serum creatinine (A) and BUN (B).
Values are mean± SEM (n = 6), analyzed by one-way ANOVA followed by Bonferoni's multiple comparison tests. ***denote P < 0.001 for chance differences vs sham operated and $$ denote P < 0.01 for chance difference vs 2K1C.

3.5 Effect of EAAC on antioxidant enzyme

2K1C group of rats established significant ($P < 0.001$) increase in MDA level when compared with sham operated group. We observed significant decrease in activities of GSH, catalase and

SOD in 2K1C group of rats when compared with sham operated group (P < 0.01, P < 0.001, P < 0.001 respectively). Treatment with EAAC found to significant (P < 0.001) decrease MDA level and significant increase in activities of GSH (P < 0.05), catalase (P < 0.001) and SOD (P < 0.01) when compared with 2K1C group (Figure 4A–D).

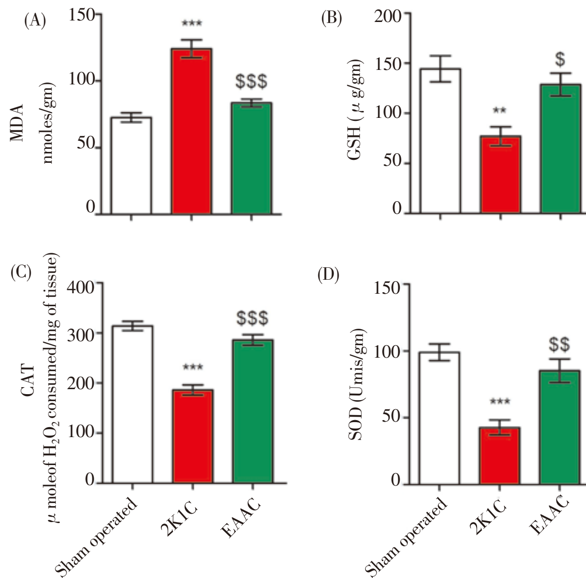

Figure 4 Effect of EAAC on antioxidant enzyme like MDA level (A), GSH (B), catalase (C) and SOD (D).
Values are mean± SEM (n = 6), analyzed by one–way ANOVA followed by Bonfferoni's multiple comparison tests. ***denote P < 0.001 for chance differences vs NC, ** denote P < 0.01 for chance differences vs sham operated and \$\$\$ denote P < 0.001 or \$\$ denote P < 0.01 or \$ denote P < 0.05 for chance difference vs 2K1C.

3.6 Effect of EAAC on tissue nitrate level

We observed significant (P < 0.001) lower NO level in 2K1C group when compare with sham operated group which was significantly (P < 0.01) higher in EAAC group when compare with 2K1C group (Figure 5).

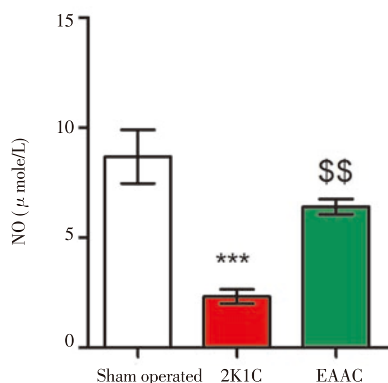

Figure 5 Effect of EAAC on nitric oxide level.
Values are mean± SEM (n = 6), analyzed by one–way ANOVA followed by Bonfferoni's multiple comparison tests. ***denote P < 0.001 for chance differences vs sham operated and \$\$ denote P < 0.01 for chance difference vs 2K1C.

4. Discussion

High blood pressure is a risk factor for stroke, coronary heart disease and renal vascular disease. The control of hypertension through diet has been the focal point of public health and mass media attention. The usual method for controlling hypertension is the use of long–term drug therapy. Drugs have many side effects that can complicate the clinical problem. That is why medical professionals and even most of patients prefer herbal medicine and preventive strategies.

2K1C model was used extensively for a better understanding of the relationship among the renin angiotensin system (RAS), hypertension and cardiovascular disorder. Irrespective of species, the 2K1C model is characterized by an increase in blood pressure immediately after clipping, which parallels the release of active renin concentration. The contribution of the RAS to the development and maintenance of 2K1C hypertension has been well established. The plasma and renal renin levels were found to increase in both the early and chronic phase of 2K1C hypertensive rats. Earlier studies indicated that in early phase of 2K1C hypertensive animals, increased activity of PRA and renin–angiotensin–aldosterone is responsible for increasing blood pressure [20]. We observed the significant elevation of PRA level in 2K1C group consistence with increased in SBP (Figure 1A) and DBP (Figure 1B) that is long–established previous report [20]. EAAC decreased SBP and DBP in hypertensive animal might be because of decreased plasma renin activity that what we observed in our study. Depending on the severity of ischemia after renal artery occlusion can lead to injury and dysfunction of the kidney. In present study increased serum creatinine and blood urea nitrogen because of ischemic renal injury [19], and reversal with EAAC might be as a result of protection against ischemic renal injury

It is well known that ROS contribute to the pathogenesis of numerous cardiovascular diseases including hypertension and NAD(P)H oxidase being the predominant source of ROS. Activation of this enzyme leads to a variety of intracellular signalling events that ultimately promote endothelium dysfunction, vascular smooth muscle cell proliferation, pro–inflammatory genes expression and reconstruction of the extracellular matrix. Angiotensin II via activation of the AT1 receptor, stimulates NAD(P)H oxidases activity in vascular smooth muscle cells increasing superoxide anion formation and nitric oxide inactivation a causative factor of hypertension. Furthermore, many Ang II effects seem to be mediated by enhanced ROS production. In humans, hypertension is also considered a state of oxidative stress that can contribute to the development of atherosclerosis and other hypertension–induced organ damage. SOD, CAT and GSH are the three primary antioxidant enzymes among the endogenous systems for removal of reactive oxygen species. The three enzymes are also the first set of defences for the body against oxidant–induced cytotoxic challenges. Assessment of antioxidant activities and lipid peroxidation by products in hypertensive subjects indicate an excessive amount of ROS and a reduction of antioxidant mechanism activity in both blood as well as in several other cellular systems [21]. Present study reveals that there is a significant decrease in antioxidant enzyme like of SOD, CAT, GSH and increase in the level of MDA in kidneys of 2K1C group. This is an indication of oxidative stress generation. Pre–treatment of EAAC decreased the oxidative stress which might be a major mechanism to restore the endothelial dysfunctions.

Abnormality in endothelium function which is characterized by decreased in NO concentration, is an important risk factor in hypertension. Endothelial dysfunction in releasing

endothelium-derived relaxation factors such as NO and impaired endothelium-dependent relaxation has been demonstrated in several animal models of hypertension and clinical studies [22 - 24]. It suggested that abnormality of NO pathway in hypertension may be due to lower NO production and/or higher NO degradation. Moreover, an increase in reactive oxygen species generation and lower level of antioxidants were detected in hypertensive subjects [20]. In addition, lower NO production may be due to reduced L-argenine or endothelium nitric oxide synthase expression in hypertensive rats [25]. It is also important that antioxidant properties of AC might suppress the trapping of NO by ROS. AC is also reported to block calcium channel caused vasodilation resulted in increases of shear stress, which may stimulates NO synthase expression and release NO from endothelium that what we observed elevated NO by EAAC treatment in our study [26 - 28].

5. Conclusion

EAAC treatment attenuated experimentally induced hypertension via nitric oxide generation and decrease in plasma renin activity.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgments

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