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Original article

Renal protective effect of *Melothria maderaspatana* leaf fraction on uninephrectomized deoxycorticosterone acetate-salt hypertensive rats

Chinnadurai Veeramani[1], Khalid S. Al-Numair[1], Govindasamy Chandramohan[1], Mohammed A. Alsaif[1], Kodukkur Viswanathan Pugalendi[2]

[1]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia.

[2]Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar-608 002. Tamilnadu, India.

Summary. This study was designed to investigate the renal protective effect of ethyl acetate fraction of *Melothria maderaspatana* (EAFM) leaf on uninephrectomized deoxycorticosterone acetate (DOCA)-salt hypertensive rats. A midscapular incision was made on each rat and the left kidney was excised after ligation of the renal artery. The surgical wound was closed using an absorbable suture. After one week recovery period, hypertension was induced by subcutaneous injection of DOCA-salt solution, twice a week, and the rats received a 1% sodium chloride solution as drinking water throughout the experimental period. EAFM or nifedipine was administered orally once a day for 6 weeks. Administration of DOCA-salt significantly increased the mean arterial pressure and heart rate while treatment with EAFM significantly lowered the blood pressure. In DOCA-salt rats, the level of kidney function markers such as urea, uric acid and creatinine were increased significantly and treatment with EAFM brought these parameters to control rats. In addition, the levels of renin, angiotensin II and aldosterone were decreased significantly in hypertensive rats and administration of EAFM brought these parameters to normal levels. The present study indicated that the EAFM provides good blood pressure control and also protect the renal damage by preventing the abnormal activities of plasma renin, angiotensin II and aldosterone as evidenced by decreased plasma levels of urea, uric acid and creatinine.

Keywords: *Melothria maderaspatana*, DOCA–salt, hypertension, renin, angiotensin II

«Effetto protettivo renale dell'estratto di foglie di Melothria maderaspatana su ratti nefrectomizzati ipertesi con sale di desossicorticosterone acetato »

Riassunto. Questo studio è stato progettato per studiare l'effetto protettivo renale dell'estratto fogliare in etile acetato di *Melothria maderaspatana* (EAFM) su ratti nefrectomizzati ipertesi con sale di desossicorticosterone acetato (DOCA). Su ogni ratto è stata fatta una incisione medio-scapolare e dopo legatura dell'arteria renale è stato asportato il rene sinistro. La ferita chirurgica è stata chiusa con una sutura riassorbibile. Dopo il periodo di recupero di una settimana, l'ipertensione è stata indotta mediante iniezione sottocutanea di soluzione di sale di DOCA, 2 volte a settimana, ed i ratti hanno ricevuto una soluzione di cloruro di sodio all'1% come acqua da bere per tutto il periodo sperimentale. Sono stati somministrati per via orale EAFM o nifedipina una volta al giorno per 6 settimane. La somministrazione di sale di DOCA ha aumentato in modo significativo la pressione arteriosa e cardiaca media mentre il trattamento con EAFM ha abbassato significativamente la pressione sanguigna. Nei ratti trattati con sale di DOCA, il livello dei markers della funzione renale come urea, acido urico e creatinina sono aumentati in maniera significativa e il trattamento con EAFM ha portato questi parametri a livello dei ratti di controllo. Inoltre, i livelli di

renina, angiotensina II e di aldosterone sono diminuiti significativamente nei ratti ipertesi e la somministrazione di EAFM ha portato questi parametri a livelli normali. Il presente studio ha mostrato che EAFM fornisce un buon controllo della pressione arteriosa e protegge anche il danno renale, impedendo una anormale attività di renina plasmatica, angiotensina II e aldosterone, come evidenziato dai diminuiti livelli plasmatici di urea, acido urico e creatinina.

Parole chiave: *Melothria maderaspatana,* sale di DOCA, ipertensione, renina, angiotensina II

Introduction

Hypertension affects approximately 25% of the adult population worldwide, and its prevalence is predicted to increase by 60% by 2025 (1). It is the major risk factor for cardiovascular disease and is responsible for the most deaths worldwide. Various genetic and environmental factors are known to be involved in the pathogenesis of primary hypertension, among which excess sodium intake or hypokalemia has long been regarded as the pivotal environmental factor for this disorder (2). Weinberger et al. estimated that approximately 25% of the normotensive population is "saltsensitive", and their arterial pressures are abnormally responsive to excess dietary NaCl, which may be a more accurate predictor of subsequent cardiovascular diseases as well as morbidity than normotension itself (3, 4). Hypertension is a common component of the morbidity associated with renal failure. Salt sensitivity in hypertension is associated with substantial renal, hemodynamic, and metabolic abnormalities that may enhance the risk of cardiovascular and renal morbidity (5). Ethno-medicinal plants have been used traditionally for the treatment of hypertension and its complications. Recent studies have demonstrated that the medicinal plants reduce the activity of blood pressure related renal dysfunction in experimental animals (6, 7).

Melothria maderaspatana (Linn.) Cogn. Syn. *Mukia maderaspatana*, *Cucumis maderaspatana* or *Mukia scabrella* (Family: Cucurbitaceae) is a monoecious plant having scandent or prostrate stems, very hispid, leaves variable in size, densely covered with white hairs. It is widely recommended in the southern part of Sri Lanka for alleviation of various forms of liver disorders (8). *M. maderaspatana* has been reportedly found to exhibit anti-inflammatory (9), antidiabetic (10) and anticancer (11) activities. In our earlier study we investigated the ethanolic extract of *Melothria maderaspatana* and showed it to possess antihypertensive activity in DOCA–salt-induced hypertensive rats (12). In a further study we fractionated the ethanolic extract of *Melothria maderaspatana* through silica gel (100–200 mesh) column using chloroform, ethyl acetate and methanol (solvents with increasing polarity), test the antihypertensive effect on DOCA–salt-induced hypertensive rats and identified compounds from the active fraction by GC–MS analysis. Among the three fractions, EAFM alone significantly lowered the systolic and diastolic blood pressure, with a dose of 60 mg/kg body weight showing maximum activity. By GC–MS analysis, phytochemicals such as coumarin, vallinic acid, p-coumaric acid, gallic acid, caffeic acid, and ferulic acid were identified in EAFM (13). Ferulic acid has been shown to lower arterial blood pressure in spontaneously hypertensive rats (14). Previous studies have no reported that the renal protective effect of EAFM on uninephrectomized DOCA–salt induced hypertensive rats. Hence, the present study, we planned to investigate the renal protective effect of EAFM on uninephrectomized DOCA–salt induced hypertensive rats.

Materials and methods

Animals

Male albino Wistar rats weighing 200–230 g were purchased from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room (25 ± 1°C) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum. Animal handling and experimental procedures were approved by the Institutional Ani-

mal Ethics Committee, Annamalai University (Registration Number: 66/1999/CPCSEA, Proposal No. 459) and animals were cared for in accordance with the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA, 2004).

Chemicals

DOCA–salt was obtained from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from E. Merck or Himedia, Mumbai, India.

Preparation of leaf fractions by column chromatography

Melothria maderaspatana leaf powder was purchased from the local herbal market (Vinayaga herbals), Chidambaram, Cuddalore district, Tamil Nadu, India. Leaves of *M. maderaspatana* were collected from the same local herbal market and the plant was botanically authenticated. A voucher specimen (AU-6054) of the plant has been deposited at the Herbarium of the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu. The leaf powder was sieved and kept in a freezer until use. 100 g of dry fine powder was suspended in 300 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40 ± 5°C. The crude ethanolic extract (CEEM) was further fractionated through silica gel (100–200 mesh) column using chloroform, ethyl acetate and methanol (solvents with increasing polarity) and dried at room temperature. The chloroform (CFM), ethyl acetate (EAFM) and methanolic (MFM) fractions were dissolved in 0.5% dimethyl sulphoxide (DMSO) at different concentrations and used for testing antihypertensive effects in uninephrectomized DOCA–salt hypertensive rats. Of the three fractions, EAFM alone significantly lowered the systolic and diastolic blood pressure (13). The active fraction of EAFM was used in this study.

Method of uninephrectomy

Animals were anesthetized by an intraperitoneal injection of ketamine [75 mg/kg body weight (BW)]. A small patch of skin above the left kidney was shaved and cleaned and iodine-based antiseptic was applied. A 1-cm incision was made at the midscapular region. The kidney was freed from the surrounding tissues and gently pulled out. The adrenal gland, which is attached loosely to the anterior pole of the kidney by connective tissue and fat, was gently freed by tearing the attachments, and was put back into the abdominal cavity. The renal artery and ureter were tied by silk thread, severed and then the kidney was removed. The muscle and skin layers were closed separately using a chromic sterile absorbable suture. After a 1-week recovery period the animals were used for further experiments.

Experimental induction of hypertension

Animals were given twice-weekly subcutaneous injections of DOCA (25 mg/kg BW) in dimethyl formamide (vehicle) solution and salt was administered by substitution of 1% NaCl solution for drinking water *ad libitum* throughout the experimental period.

Experimental protocol

The animals were divided into five groups of six animals each. EAFM or nifedipine was suspended in 0.5% DMSO and administered by intubation (p.o.) once a day, between 9 a.m. and 10 a.m., for 6 weeks.

- Group 1 Sham-operated control (0.5% DMSO only).
- Group 2 Sham-operated control + EAFM (60 mg/kg BW of 0.5% DMSO).
- Group 3 DOCA + 1% NaCl.
- Group 4 DOCA + 1% NaCl + EAFM (60 mg/kg BW of 0.5% DMSO).
- Group 5 DOCA + 1% NaCl + nifedipine (20 mg/kg BW of 0.5% DMSO).

After 6 weeks, the animals were anaesthetized, using ketamine (intramuscular injection), and killed between 8 a.m. and 9 a.m. by cervical dislocation. Blood was collected for the measurement of various biochemical parameters.

Blood pressure measurements

Mean arterial pressure and heart rates were determined by the tail-cuff method (IITC, model 31, Woodland Hills, CA, USA). The animals were placed

in a heated chamber at an ambient temperature of 30–34°C for 15 min and 1–9 blood pressure values were recorded from each animal. The lowest three readings were averaged to obtain a mean blood pressure. All recordings and data analyses were done using computerized data acquisition system and software.

Biochemical analysis

Urea, uric acid and creatinine in the plasma were estimated by using the diagnostic kit based on the method of Fawcett (15) and Scott (16), Caraway and Tietz (17) using Jaffe's (18), color reaction respectively. Renin, angiotensin II and aldosterone were measured by a competitive radioimmunoassay by using commercially available kit (19, 20, 21).

Statistical analysis

Statistical evaluation was performed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) using SPSS version 10.0. The significance level was set at $P<0.05$.

Results and discussion

Hypertension has become a significant problem in many developing countries and is primarily responsible for stroke, heart disease, heart failure, kidney disease and blindness (22). Current studies has been shown that the mechanisms by which DOCA plus salt administration increases blood pressure may result from an alteration in renin–angiotensin and nitric oxide levels, increased oxidative stress and damage to kidneys (23). Table 1 shows the effect of EAFM at different concentrations (30, 60 and 120 mg/kg BW) on mean arterial pressure and heart rate in sham-operated and DOCA–salt hypertensive rats. Consistence with previous reports (24), we also observed that the mean arterial pressure and heart rate significantly increased in DOCA–salt hypertensive rats. Oral administration of EAFM to hypertensive rats significantly lowered the mean arterial pressure and heart rate. Since EAFM at a dose of 60 mg/kg BW gave the maximum improvement on blood pressure, it was fixed as the optimum dose for further work. The lower dose of EAFM (30 mg/kg BW) was not effective, because its concentration might not have been enough to counteract the blood pressure in DOCA–salt induced rats. The higher concentration of EAFM (120 mg/kg BW) might have resulted in the production of by-products that interfere with the antihypertensive activity, and consequently decrease its effect. Hence, 60 mg/kg BW EAFM is optimum for antihypertensive activity. The extract of EAFM has been found to contain phytochemicals such as coumarin, vallinic acid, p-coumaric acid, gallic acid, caffeic acid and ferulic acid (13). Several epidemiological studies have shown a significant inverse association between dietary phenolic compounds and long-term mortality from coronary heart disease (25, 26). *In-vivo* biological actions of ferulic acid, one of the phenolic compounds found in EAFM, may support the observed antihypertensive effect of EAFM (14). Ferulic acid has been shown to lower

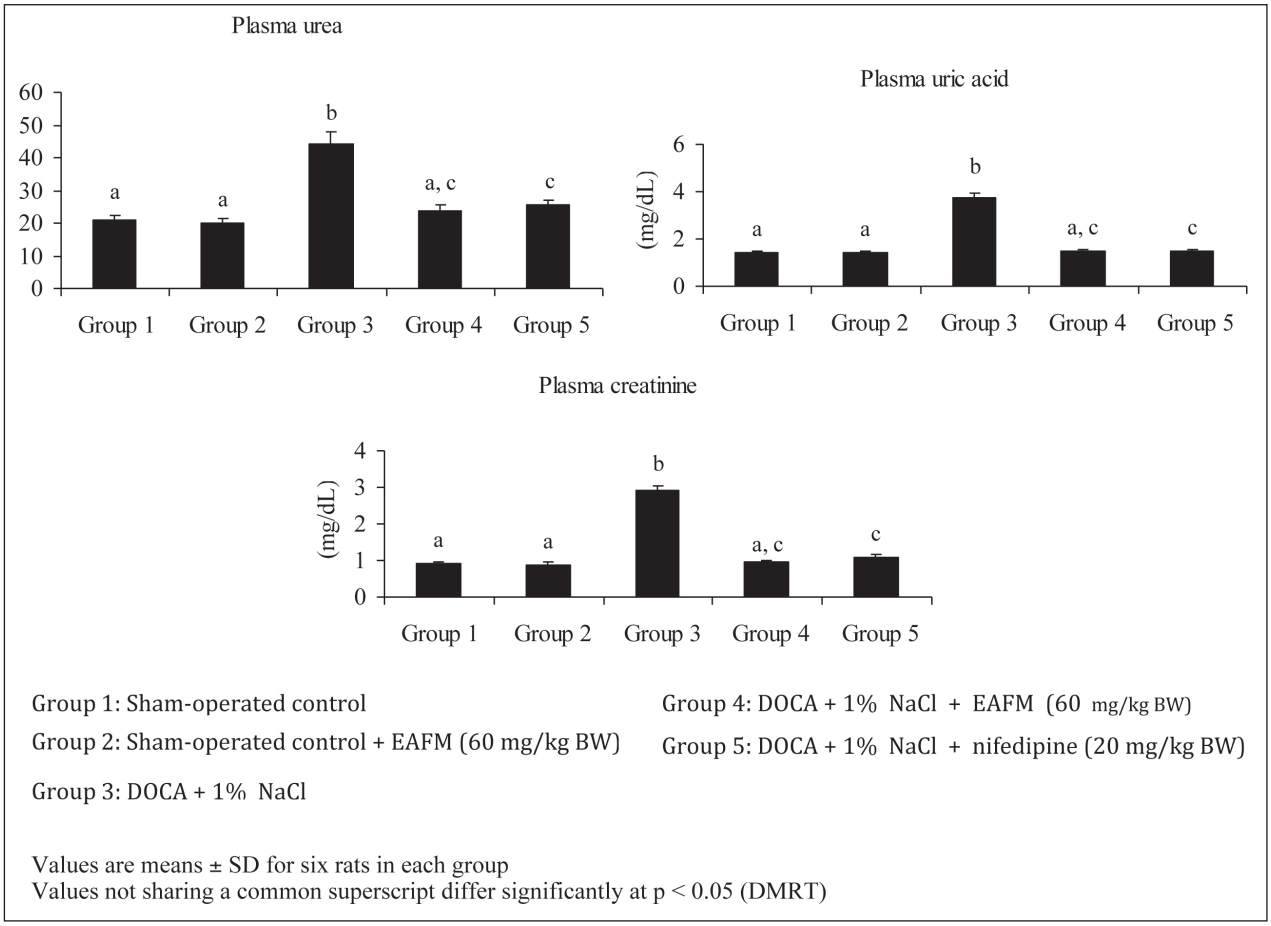
Table 1. Effect of EAFM on the mean arterial pressure and heart rate in the sham-operated and ninephrectomized DOCA-salt hypertensive rats.

Name of the groups	Mean arterial pressure (mm Hg)		Heart rate (bpm)	
	0 week	6th week	0 week	6th week
Sham-operated control	96.57 ± 4.60	100.67 ± 6.00 [a]	345.54 ± 6.89	358.82 ± 6.66 [a]
DOCA-salt + 1% NaCl	95.02 ± 5.45	186.91 ± 9.66 [b]	350.83 ± 7.25	405.68 ± 10.05 [b]
DOCA-salt + 1% NaCl + EAFM (30 mg/kg BW)	97.46 ± 6.08	157.27 ± 8.73 [c]	355.54 ± 6.17	386.58 ± 8.73 [c]
DOCA-salt + 1% NaCl + EAFM (60 mg/kg BW)	93.55 ± 5.45	105.50 ± 6.48 [a]	355.64 ± 7.20	362.34 ± 6.94 [a]
DOCA-salt + 1% NaCl + EAFM (120 mg/kg BW)	94.99 ± 5.77	153.72 ± 8.48 [c]	353.67 ± 6.35	380.75 ± 8.88 [c]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	95.54 ± 5.78	107.17 ± 6.80 [a]	345.28 ± 7.10	363.87 ± 8.15 [a,d]

Values are means ± SD for six rats in each group
Values not sharing a common superscript differ significantly at p < 0.05 (DMRT)
bpm–beats per minute

arterial blood pressure in spontaneously hypertensive rats (14).

Kidneys play a central role in regulation of body salt and water. Disordered regulation of transport in the kidneys is responsible for the altered balance of salt and water in pathophysiological states including some experimental models of hypertension (27). Hypertension is a risk factor for the faster progression of renal damage and reduction of BP is an efficient way of preventing or slowing the progression of this damage. International guidelines recommend lowering BP to 140/90 mmHg or less in patients with uncomplicated hypertension, and to 130/80 mmHg or less for patients with diabetic or chronic renal disease. The process of renal target organ damage is hypertensive nephrosclerosis, a pathophysiologic process marked by progressive reduction in kidney function with very little associated clinical signs and symptoms until moderate to severe renal damage is present. The pathogenesis of hypertensive renal damage involves mediators from various extracellular systems, including the RAS (28). The hypertensive rats induce elevation of the plasma levels of urea, uric acid and creatinine which are considered as significant markers of renal function (29). Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine bases, adenine and guanine; creatinine is endogenously produced and released into body fluids and its clearance is measured as an indicator of glomerular fitration rate (30, 31). Figure 1 shows the levels of kidney function markers namely urea, uric acid and creatinine in the plasma of sham-operated and DOCA-salt hypertensive rats. The levels of kidney function markers such as urea, uric acid and creatinine were increased significantly in the plasma of DOCA-salt hypertensive rats and may be due to kidney dam-



Group 1: Sham-operated control

Group 2: Sham-operated control + EAFM (60 mg/kg BW)

Group 3: DOCA + 1% NaCl

Group 4: DOCA + 1% NaCl + EAFM (60 mg/kg BW)

Group 5: DOCA + 1% NaCl + nifedipine (20 mg/kg BW)

Values are means ± SD for six rats in each group

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Figure 1. Effect of EAFM on urea, uric acid and creatinine in the plasma of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

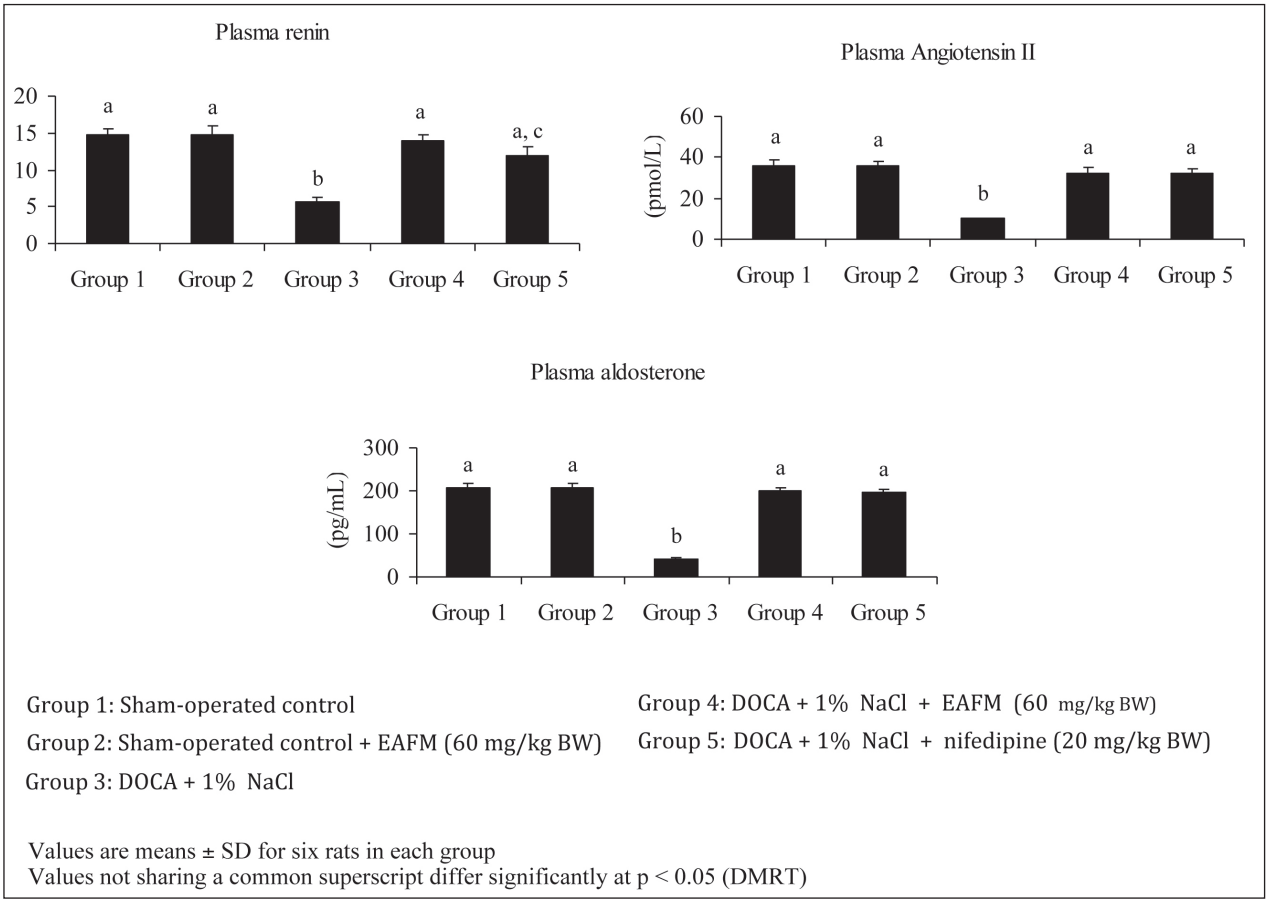


Figure 2. Effect of EAFM on renin, angiotensin II and aldosterone levels by radioimmunoassay in the plasma of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

age caused by the oxidative stress by increasing the formation of superoxide. Administration of EAFM to hypertensive rats these renal function markers significantly reverted to near sham-operated control levels and thus reduction may be due to have antioxidant activity (32) and BP lowering effect of this extract.

The renin angiotensin aldosterone system (RAAS) is crucially involved in maintaining hypertension and a raised sympathetic activity has an important influence on initiating the activation of RAAS (33). Thus, during malignant hypertension as a consequence of kidney injury renin is released that leads to the formation of angiotensin II and aldosterone. Ang II also regulates sodium transport by epithelial cells in intestine and kidney (34). There has also been a growing appreciation of the organ-specific roles exerted by Ang II acting as a paracrine factor (35). In addition to its physiological roles, locally produced Ang II induces inflammation, cell growth, mitogenesis, apoptosis, migration, and differentiation, regulates the gene expression of bioactive substances, and activates multiple intracellular signaling pathways, all of which might contribute to tissue injury. Recent attention has been focused on findings that local Ang II levels are differentially regulated in the kidney. Because there often is not clear evidence for markedly elevated circulating renin or Ang II concentrations, identification of local RAS activity is essential for understanding the mechanisms mediating pathophysiological functions. In particular, the Ang II contents in renal tissues are much higher than can be explained on the basis of equilibration with the circulating concentrations (36, 37). Furthermore, the demonstration of much higher concentrations of Ang II in specific regions and compartments within the kidney indicates selective local regulation of in-

trarenal Ang II (37, 38). Thus, it is now apparent that intrarenal Ang II levels are regulated in a manner distinct from circulating Ang II concentrations. It has also been revealed that Ang II produced locally in the kidney exerts an important regulatory influence on renal hemodynamics and functions as a paracrine factor (38, 39). Further studies demonstrate that reduced renal function and its structural changes are associated with inappropriate activation of the intrarenal Ang II, leading to the development of hypertension and renal injury (37). The locally activated renin-angiotensin system plays an important role in the process of renal damage. Although high salt intake and DOCA-salt rats suppresses the plasma renin-angiotensin-aldosterone system, the locally activated system plays an important role in the process of renal damage in DOCA-salt hypertensive rats. Kim et al. (40) reported that administration of TCV-116, a specific angiotensin II antagonist, protected against renal functional and morphological deterioration without changing the blood pressure, suggesting an important role of angiotensin II for renal impairment in this rat model. Figure 2 shows the effect of EAFM on renin, angiotensin II and aldosterone levels in the plasma of sham-operated and DOCA-salt hypertensive rats. The plasma levels of renin, angiotensin II and aldosterone significantly decreased in DOCA-salt rats, which might be due to locally activated RAAS causing hypertension. Administration of EAFM brought these parameters to normality, as the extract protect the renal damage in DOCA-salt hypertensive rats as evidenced by decreased levels of urea, uric acid and creatinine.

In conclusion, EAFM provides good blood pressure control and also protect the renal damage by preventing the abnormal activities of plasma renin, angiotensin II and aldosterone as evidenced by decreased plasma levels of urea, uric acid and creatinine.

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Correspondence:
Dr. K.V. Pugalendi, M.Sc., M.Phil., Ph.D.
Professor and Head
Department of Biochemistry & Biotechnology,
Annamalai University, Annamalainagar – 608 002,
Tamilnadu, India.
Tel: +91-4144-238343.
Fax : +91-4144-239141.
E-mail: chinnaveeramani@gmail.com