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Antihyperlipidemic effect of *Melothria maderaspatana* leaf extracts on DOCA-salt induced hypertensive rats

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ABSTRACT

Objective: To investigate the antihyperlipidemic effect of crude ethanolic extract of *Melothria maderaspatana (M. maderaspatana)* leaf (CEEM) on deoxycorticosterone acetate (DOCA)-salt hypertensive rats. **Methods:** 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. CEEM or nifedipine was administered orally once a day for 6 weeks. **Results:** In DOCA-salt hypertensive rats, the level of plasma and tissues of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) significantly increased and administration of CEEM significantly reduced these parameters towards normality. Further, the levels of low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) significantly increased while high density lipoprotein-cholesterol (HDL-C) decreased in hypertensive rats and administration of CEEM brought these parameters to normality which proved their antihyperlipidemic action. Histopathology of liver, kidney and heart on DOCA-salt induced rats treated with CEEM showed reduced the damages towards normal histology. **Conclusions:** These findings provided evidence that CEEM was found to be protecting the liver, kidney and heart against DOCA-salt administration and the protective effect could attribute to its antihyperlipidemic activities.

1. Introduction

Hypertension, which is characterized by multiple alterations in the structure and function of the cell membrane, is often associated with important metabolic abnormalities including those concerning lipid metabolism. Lipids, as an integral part of the cell membrane, play a decisive role in the modulation of the membrane properties mentioned. Specific lipid-lipid and lipid-protein interactions result in a highly dynamic but precisely controlled architecture of membrane components. Major regulators of membrane architecture are membrane potential, intracellular Ca^{2+} and pH, lipid composition, cell-to-cell contact, and membrane coupling with the cytoskeleton or extracellular matrix. Intermolecular associations in the membrane and at the membrane-cytoskeleton interface are further selectively controlled by specific phosphorylation and dephosphorylation cascades involving both proteins and lipids. This is regulated by the extracellular matrix as well as by the binding of growth factors and hormones to their specific receptors[1,2].

Multiple metabolic abnormalities often accompany essential hypertension. Decreased high-density lipoproteins (HDL) together with increased plasma levels of low-density (LDL) and very low-density lipoproteins (VLDL), as well as

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hypertriglyceridemia, hypercholesterolemia, and insulin resistance, were found in many hypertensive patients[3,4]. There is increasing evidence for a genetic basis for the association of hypertension with insulin resistance and dyslipidemia. In spontaneously hypertensive rats (SHR) and DOCA salt rats, abnormal lipid metabolism were detected[5]. Changes in both circulating and membrane lipids were observed in SHR and DOCA-salt prior to hypertension development[6]. The alterations of membrane cholesterol or phospholipids content, phospholipid distribution, molecular species pattern of particular phospholipid classes, and degree of fatty acid saturation were reported in both human[7,8] and experimental hypertension[9,10]. These changes in membrane lipid composition may modulate membrane function in a long term manner, contributing to abnormal chronic BP regulation.

In our earlier study we have investigated ethanolic extract of *Melothria maderaspatana* (*M. maderaspatana*) and shown to possess antihypertensive activity on DOCA-salt induced hypertensive rats at the optimum dose of 200 mg/kg BW[11]. Previously, there is no reported that the antihyperlipidemic effect of CEEM on DOC-salt induced hypertensive rats. Therefore, in the present study the main aim was to investigate the effects of CEEM on ameliorate the lipid metabolism on DOCA-salt induced hypertensive rats.

2. Materials and methods

2.1. Preparation of leaf extract

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) ℃. The extract was kept in deep freezer until use.

2.2. 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 airconditioned room (25 ± 1) ℃ 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 Animal Ethics Committee, Annamalai University (Registration Number: 66/1999/CPCSEA, Proposal No. 459) and animals were cared in accordance with the Committee for the purpose of control and supervision on experimental animals" (CPCSEA, 2004).

2.3. Chemicals

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

2.4. 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 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 1 week recovery period the animals were used for further experiments.

2.5. 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.

2.6. Experimental design

The animals were randomly divided into five groups of six animals each. CEEM or nifedipine was suspended in 0.5% dimethyl sulphoxide (DMSO) and administered by intubation (*p.o.*) once in 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 + CEEM (200 mg/kg BW of 0.5% DMSO)

Group 3 DOCA-salt + 1% NaCl

Group 4 DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW of 0.5% DMSO)

Group 5 DOCA-salt + 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 and tissues (liver, kidney and heart) were collected for the measurement of various biochemical parameters.

2.7. Biochemical analysis

Total lipids were extracted from the liver, kidney and heart tissues according to the method of Folch *et al*[12].

Total cholesterol was estimated by the method of Allain *et al*[13]. HDL-C was estimated by the method of Izzo *et al*[14]. Atherogenic index was calculated by the method of Malspina *et al*[15] VLDL-C and LDL-C were calculated by the method of Friedewald *et al*[16]. Triglycerides were estimated by the method of McGowan *et al*[17]. Free fatty acid content was estimated by the method of Falholt *et al*[18]. Phospholipid was estimated by the method of Silversmit and Davis[19].

2.8. Statistical analysis

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

3. Results

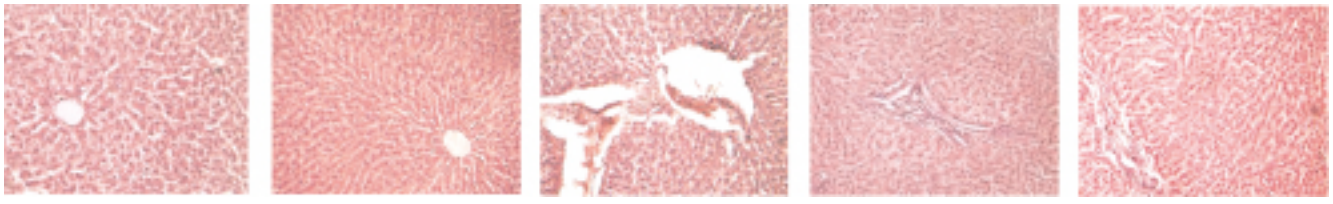
Tables 1, 2, 3 and 4 show the effect of CEEM on TC, TG, FFA and PL in the plasma and tissues of sham-operated and DOCA-salt hypertensive rats. The levels of plasma and tissue TC, TG, FFA and PL increased in DOCA-salt hypertensive rats and administration of CEEM showed a significant reduction in these parameters.

Table 5 shows the effect of CEEM on HDL-C, VLDL-C and LDL-C in the plasma of sham-operated and DOCA-salt hypertensive rats. The levels of LDL-C and VLDL-C significantly increased while HDL-C decreased in hypertensive rats and CEEM administration exhibited significant reduction in LDL-C and VLDL-C and elevation in HDL-C.

Figure 1 shows the histological changes in the liver of sham-operated and DOCA-salt hypertensive rats. DOCA-salt rats showed fatty changes in hepatocytes besides hypertrophy and globular hyalinization, oedema and hydrophic changes. Treatment with CEEM and nifedipine reduced the fatty changes of hepatocytes and hypertrophy.

Figure 2 shows the histological changes in the kidney of sham-operated and DOCA-salt hypertensive rats. DOCA-salt rats showed atrophy of tubular cells with dilated lumen of distal convoluted tubules and congestion of glomerulus. Treatment with CEEM and nifedipine reduced the congestion of glomerulus and focal tubular necrosis.

Figure 3 shows the histological changes in the heart



Sham-operated control rats showing normal hepatocytes and central vein.

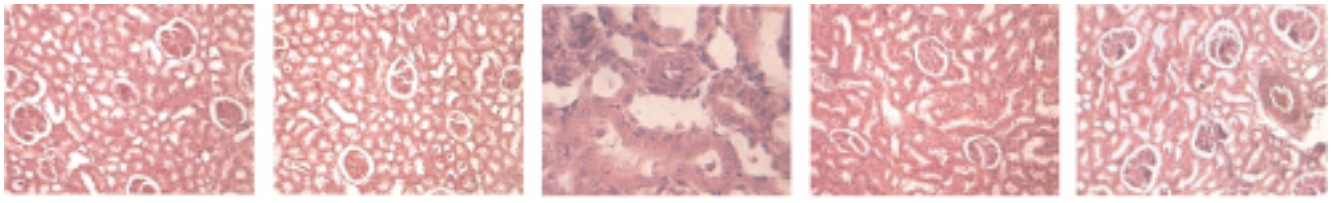
Sham-operated control rats with CEEM showing normal hepatocytes and central vein.

DOCA-salt hypertensive rats showing fatty changes of hepatocytes, hypertrophy, globular hyalinization, oedema and hydrophic changes.

DOCA-salt rats with CEEM showing reduced fatty changes and hypertrophy.

DOCA-salt rats treated with nifedipine showing reduced hydrophic changes and globular hyalinization.

Figure 1. Histopathological changes of liver (H&E, 100×).



Sham-operated control rats showing normal histology of kidney.

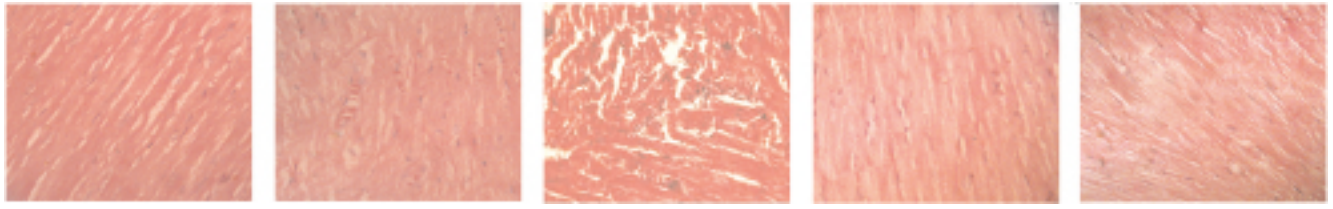
Sham-operated control rats with CEEM showing normal histology of kidney.

DOCA-salt hypertensive rats showing atrophy of tubular cells with dilated lumen of distal convoluted tubules and congestion of glomerulus.

DOCA-salt rats treated with CEEM showing reduced congestion of glomerulus and focal tubular necrosis.

DOCA-salt rats treated with nifedipine showing minimal congestion of glomerulus and focal tubular necrosis.

Figure 2. Histopathological changes of kidney (H&E, 100×).



Sham-operated control rats showing normal histology of cardiac tissue

Sham-operated control rats with CEEM showing normal cardiac myocytes.

DOCA-salt hypertensive rats showing distortion of cardiac myofibril arrangement and thickened arteriole with hypertrophy.

DOCA-salt rats treated with CEEM showing reduced distortion of cardiac myofibril arrangement and hypertrophy.

DOCA-salt rats treated with nifedipine showing minimal distortion of cardiac myofibril arrangement and hypertrophy.

Figure 3. Histopathological changes of heart (H&E, 100×).

of sham-operated and DOCA-salt hypertensive rats. Heart showed distortion of cardiac myofibril arrangement and thickened arterial with hypertrophy in DOCA-salt hypertensive rats. Administration of CEEM and nifedipine reduced distortion of cardiac myofibril arrangement and hypertrophy.

Table 1

Effect of CEEM on total cholesterol, triglycerides, free fatty acids and phospholipids of plasma in sham-operated and uninephrectomized DOCA-salt hypertensive rats.

Name of the groups	TC (mg/dL)	TG (mg/dL)	FFA (mg/dL)	PL (mg/dL)
Sham-operated control	82.16 ± 5.74[a]	57.52 ± 3.06[a]	55.84 ± 4.06[a]	80.53 ± 6.43[a]
Sham-operated control + CEEM (200 mg/kg BW)	79.35 ± 3.36[a]	55.27 ± 2.38[a]	53.46 ± 3.81[a]	78.08 ± 5.68[a]
DOCA-salt + 1% NaCl	166.73 ± 8.82[b]	160.39 ± 8.58[b]	123.58 ± 8.64[b]	148.91 ± 10.06[b]
DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW)	84.47 ± 5.24[a]	61.29 ± 5.57[a]	59.57 ± 4.25[a]	84.27 ± 6.73[a]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	132.64 ± 6.18[c]	118.36 ± 6.75[c]	88.45 ± 5.67[c]	106.68 ± 7.49[c]

Values are means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2

Effect of CEEM on total cholesterol, triglycerides, free fatty acids and phospholipids in the liver of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

Name of the groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Sham-operated control	4.37 ± 0.36[a]	3.63 ± 0.24[a]	8.48 ± 0.54[a]	24.57 ± 1.64[a]
Sham-operated control + CEEM (200 mg/kg BW)	3.97 ± 0.28[a]	3.61 ± 0.31[a]	7.89 ± 0.42[a]	23.77 ± 1.85[a]
DOCA-salt + 1% NaCl	6.46 ± 0.46[b]	7.37 ± 0.54[b]	15.39 ± 0.97[b]	45.85 ± 3.03[b]
DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW)	4.59 ± 0.23[a]	3.87 ± 0.37[a]	8.74 ± 0.67[a]	25.38 ± 1.84[a]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	5.86 ± 0.35[c]	5.47 ± 0.30[c]	10.48 ± 0.84[c]	36.26 ± 2.07[c]

Values are means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 3

Effect of CEEM on total cholesterol, triglycerides, free fatty acids and phospholipids in the kidney of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

Name of the groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Sham-perated control	4.80 ± 0.32[a,c]	3.52 ± 0.30[a]	4.63 ± 0.34[a]	16.57 ± 1.02[a]
Sham-operated control + CEEM (200 mg/kg BW)	4.45 ± 0.36[a]	3.43 ± 0.29[a]	4.18 ± 0.39[a]	15.82 ± 1.08[a]
DOCA-salt + 1% NaCl	7.83 ± 0.57[b]	6.87 ± 0.54[b]	9.86 ± 0.56[b]	30.46 ± 2.45[b]
DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW)	4.97 ± 0.38[a]	3.87 ± 0.33[a]	4.86 ± 0.36[a]	17.57 ± 1.54[a]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	6.19 ± 0.52[c]	5.13 ± 0.29[c]	7.43 ± 0.52[c]	23.58 ± 1.64[c]

Values are means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 4

Effect of CEEM on total cholesterol, triglycerides, free fatty acids and phospholipids in the heart of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

Name of the groups	(mg/g wet tissue)			
	TC	TG	FFA	PL
Sham-operated control	2.57 ± 0.20[a]	4.64 ± 0.35[a]	5.47 ± 0.38[a]	12.07 ± 1.10[a]
Sham-operated control + CEEM (200 mg/kg BW)	2.44 ± 0.18[a]	4.04 ± 0.37[a]	5.03 ± 0.41[a]	11.58 ± 0.96[a]
DOCA-salt + 1% NaCl	4.73 ± 0.32[b]	6.15 ± 0.59[b]	10.42 ± 1.07[b]	22.17 ± 2.12[b]
DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW)	2.74 ± 0.21[a]	4.82 ± 0.28[a]	5.83 ± 0.54[a]	13.03 ± 1.17[a]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	3.75 ± 0.27[c]	5.37 ± 0.39[c]	8.54 ± 0.68[c]	18.66 ± 1.51[c]

Values are means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 5

Effect of CEEM on HDL-C, VLDL-C and LDL-C in the plasma of sham-operated and uninephrectomized DOCA-salt hypertensive rats.

Name of the groups	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
Sham-operated control	46.88 ± 3.06[a]	11.50 ± 0.67[a]	23.78 ± 2.28[a]
Sham-operated control + CEEM (200 mg/kg BW)	47.64 ± 3.40[a]	11.05 ± 0.52[a]	20.66 ± 0.57[a]
DOCA-salt + 1% NaCl	27.57 ± 2.03[b]	32.08 ± 1.89[b]	107.08 ± 5.61[b]
DOCA-salt + 1% NaCl + CEEM (200 mg/kg BW)	43.64 ± 3.16[a]	12.26 ± 1.23[a]	29.37 ± 1.86[a,c]
DOCA-salt + 1% NaCl + nifedipine (20 mg/kg BW)	36.07 ± 3.03[c]	23.67 ± 1.49c	73.70 ± 3.64[d]

Values are means ± SD for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

4. Discussion

Abnormality in lipids and lipoprotein metabolism play a central role in the pathogenesis of hypertension. The presence of high BP and hyperlipedimia is so common in patients with hypertension and animal models of hypertension that many have argued that the high BP itself may play a role in altering lipid metabolism, resulting in abnormalities. Our result showed a decrease in the levels of TC, TG, LDL-C, VLDL-C, PL and increase in the level of HDL-C in hypertensive rats after treatment with CEEM. High levels of total cholesterol and LDL cholesterol are major risk factors for cardiovascular diseases, whereas increased HDL-cholesterol is associated with a decrease in cardiovascular disease risk. It is well known that HDL-cholesterol plays an important role in the transport of cholesterol from the periphery to the liver by the "reverse cholesterol transport" pathway. Several studies show that an increase in HDL cholesterol is associated with a decrease in coronary risk[20,21] and most of the drugs that decrease total cholesterol also decrease HDL cholesterol[22]. But in the present study the extract decreased the total cholesterol and LDL cholesterol and enhanced the HDL cholesterol signi cantly.

This is an important advantage in treatment of hypercholesterolemia especially among Indians where low HDL cholesterol is the prevalent lipoprotein abnormality[23]. High levels of TC and more importantly, LDL cholesterol are major coronary risk factors[24,25]. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries, a rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence is bad for health and a direct risk factor for coronary heart disease[26,27]. Administration of CEEM lowered both total and LDL cholesterol in experimental rats, which could be reducing the incidence of coronary events[28]. The decrease of serum TG level is an important finding of this experiment. Recent studies also show that triglycerides are independently related to coronary heart disease[29] and most of the antihypercholesterolemic drugs do not decrease triglycerides levels, but CEEM lowered it significantly and this effect might be related to increase the endothelium bound lipoprotein lipase which hydrolyses the triglycerides into fatty acids.

Phospholipids are vital components of biomembrane and play an important role in the transport of triacylglycerol. FFA and TC also promote the synthesis of phospholipids. These phospholipids and FFA are important for the maintenance of cellular integrity, microviscosity and survival[30]. Increased levels of plasma phospholipids and FFA were observed in hypertensive rats, which might be due to membrane damage caused by increased lipid peroxidation. Increased lipid peroxidation thought to be consequence of oxidative stress that occurs when the dynamic balance between pro-oxidant and antioxidant mechanism is impaired[31]. *M. maderaspata* is also being recognised as a potent antioxidant. Increased antioxidant levels may decrease lipid peroxidation, thereby preventing membrane damage that leads to decreased plasma and tissue phospholipids and FFA levels.

In conclusion, our findings the treatment with CEEM has played a major role in lipid metabolism that may be due to its antilipidperoxidive effect and the present study strongly supports the efficacy of CEEM in controlling lipid parameters in serum and liver, kidney and heart of DOCA-salt hypertensive rats. The biochemical findings were supported by histopathological study.

Conflict of interest statement

There are no conflicts of interest.

Acknowledgements

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