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E-mail: cjim_en@cjim.cn

Original Article

Antihypercholesterolemic, Antioxidant and Renal Protective Effects of Mengkudu (*Rubiaceae*) Fruit in Nephropathy-Induced Albino Rats

U.S. Mahadeva Rao[1] and C. Shanmuga Sundaram[2]

ABSTRACT **Objective**: To assess the modulatory impact of alcoholic extract of fruit of Mengkudu (AEFM, *Morinda citrifolia* L., *Rubiaceae*) on renal oxido-lipidemic stress in hypercholesterolemic albino rats. **Methods**: Twenty-four male albino rats were randomly divided into four groups with six rats in each group: group Ⅰ as control, group Ⅱ fed with hypercholesterolemic diet (HCD) for 45 days (4% cholesterol and 1% cholic acid), Group Ⅲ rats fed with HCD for 45 days + AEFM (300 mg/kg body weight/day orally) for last 30 days and group Ⅳ normal rats fed AEFM alone. The blood was collected using ethylenediamine tetraacetic acid (EDTA) as an anticoagulant for various biochemical analysis, and excision of kidney was done for histological analysis. **Results**: The levels of total cholesterol (TC), triacylglycerol (TG), phospholipids (PLs), renal functional parameters and lipid peroxidation products were markedly mitigated in AEFM treated hypercholesterolemic rats (group Ⅲ) compared to group Ⅰ (P<0.01). Activities of both enzymic and non-enzymic free radical scavenging factors were significantly increased in group Ⅲ compared to group Ⅰ (P<0.01). In group Ⅲ the mRNA levels of interstitial endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) genes were obviously up-regulated (P<0.01) and down-regulated in (P<0.05) compared with group Ⅰ. Histomorphological observations also exhibited similar as in group Ⅲ AEFM commendably protects the renal tissues compared with group Ⅰ (P<0.01). **Conclusion**: AEFM can act as nephroprotective agent by attenuating the renal oxidative stress, lipid levels as well as regulating NOS level and by this means protects the kidney in hypercholesterolemic induced nephropathy experimental rats.

KEYWORDS renal markers, lipid profile, antioxidant, nitric oxide synthase, oxidative stress

Hypercholesterolemia and hypertriglyceridemia has been established to be the major risk factors for the progression of various renal diseases.[1] The relationship between hypercholesterolemia and renal damage has been well documented for the past few decades.[2,3] Numerous studies have publicized that the abnormal lipid metabolism (elevated lipid level) acts as an integral factor rather than primary initiator in modulating progressive renal damage.[4,5] It is increasingly recognized that, reactive oxygen species (ROS) and proinflammatory cytokines are involved in the development of renal damage induced by hypercholesterolemia.[6] Free radical species produce tissue damage by initiating lipid, protein and DNA oxidative modifications, owing to elevated oxidative stress which could be involved in hypercholesterolemia-induced renal vasculopathies.[7]

A number of studies have revealed that hypercholesterolemia generally upshots in oxidative stress and since *Mengkudu* or *Noni* fruits saponins, flavonoids, alkaloids, and phytosterol are potent free radical scavengers, they could be reconnoitered as possible supplements for treating hypercholesterolemia-induced oxidative stress in nephrons, hence Mengkudu fruit was picked for the current study. *Morinda citrifolia* L. (*Rubiaceae*), commonly called Mengkudu or Noni or Indian mulberry, is a small evergreen tree or shrub of Polynesian origin.[8] The tree bears a lumpy, green to yellowish-white fruit, normally 5-10 cm in length, with a surface covered in polygonal-shaped sections.[9] Mengkudu fruit has a long history of use as a medicinal plant in parts of Malaysia, Polynesia, and Australia and is considered to be the second most important medicinal

1. Faculty of Medicine, Universiti Sultan Zainal Abidin, Kuala Terengganu (20400), Malaysia; 2. Department of Microbiology, Hindustan College of arts and Science, Kelambakkam, Chennai (603103), India
Correspondence to: Dr. U.S. Mahadeva Rao, Tel: 91-01116547654, E-mail: raousm@unisza.edu.my; raousm@gmail.com
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plant in the Hawaiian Islands.[10] The fruits are also eaten as a food, but primarily only in times of famine.[11] However, Lucas interpreted elements of the following ancient Hawaiian chant (recorded in 1861 about the interactions between the Gods Kamapua'a and Pele) as evidence that Noni fruit was once eaten in times of famine.[12] Kamapua'a chanted as follows: "*I have come now from Puna*. *I have seen the women gathering noni*, *scratching noni, pounding noni, marking with noni*."[13,14]

The uniqueness of the present study is to explore the nephroprotective potential of alcoholic extract of Mengkudu fruit (AEFM) by analyzing lipid profile free radical scavenging activity (FRSA) profile (both enzymic and non-enzymic antioxidants), oxidative marker-lipid peroxidation (LPO), renal markers (urea, creatinine and uric acid) over and above histological observations on hypercholesterolemia induced oxido-lipidemic abnormalities in kidney tissues.

METHODS

Collection and Extraction of AEFM

The fruits of Mengkudu were collected in Kuala Terengganu, Malaysia. They were later verified and authenticated at UniSZA Herbarium, Gong Badak Campus, Kuala Terengganu, Malaysia (Voucher No. 00217). The shade-dried and coarsely powdered deseeded fruit material (1 kg) was extracted with 90% ethanol in the cold (48 h). The extract was filtered and distilled on a water bath to get a dark green syrupy mass. It was finally dried in vacuo (52 g). The AEFM was given orally as a suspension based on appropriate concentration in experimental design.

Experimental Animals and Design

Twenty four male albino rats of Wistar strain weighing 180±20 g were used. The animals were housed in large spacious cages. Food and water were given *ad libitum*. Rats were allowed to adapt to their environment condition for at least 10 days before the initiation of experiment. Animal experiments were conducted according to the guidelines of the Institutional Animal Ethics Committee (AUHAEC 78/ FOM/ 2012). Animals were randomly divided into four groups of six rats each.

Group Ⅰ served as control rats fed with normal diet. Group Ⅱ rats fed with hypercholesterolemic diet (HCD) for 45 days (4% cholesterol and 1% cholic acid). Group Ⅲ rats fed with HCD for 45 days[15] in addition also received AEFM (300 mg/kg perday orally) for last 30 days. Group Ⅳ rats fed with normal diet for 45 days in addition also received AEFM (300 mg/kg perday orally) for last 30 days.[16]

On 46th day all the animals were sacrificed by cervical decapitation under sodium pentobarbitone (60 mg/kg) anesthesia. The kidney tissue with renal artery were expurgated immediately, washed with ice-cold saline and then dried with filter paper. The slice of kidney tissue was fixed with 10% HCHO and stained with hematoxylin and eosin stain for histopathological studies. A 10% homogenate of kidney tissue and artery were prepared by using 0.1 mol/L Tris HCl buffer pH 7.4. Blood was collected in tube without anticoagulant for the separation of serum.

Biochemical Analysis

The total lipids (TL) were extracted in chloroform: methanol solvents (2:1 ratio) and purified by Folch's wash procedure[17] and aliquot were used for the estimation of total cholesterol (TC),[18] triacylglycerd (TG)[19] and phospholipids PLs[20] according to the above mentioned method. The antioxidant enzymes viz., super oxide dismutase (SOD),[21] catalase (CAT),[22] glutathione peroxidase (GPx),[23] glutathione reductase (GR),[24] glutathione S-tronsferase (GST),[25] glutathione (GSH),[26] vitamin-C (ascorbic acid)[27] and vitamin-E (tocopherol)[28] were estimated according to the reported methods. The lipid peroxidation product of LPO in kidney tissue was also measured.[29] Renal markers (serum creatinine, urea and uric acid) were determined using commercial kits (Roche Diagnostics Corporation, Basel, Switzerland) according to manufactures instruction. Protein was estimated by the Lowry method.[30]

Reverse Transcriptase-Polymerase Chain Reaction Analysis

The total RNA was isolated from the renal artery using the Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions. The quality and integrity of RNA were confirmed by spectrophotometric analysis (OD260/280). Reverse transcriptase-polymerase chain reaction (RT-PCR) for eNOS and iNOS, mRNA expression was done according to manufacturer's guidelines (Qiagen One Step RT-PCR mix). Briefly, the reaction mixture contained 10 μL of 5x Qiagen one step RT-PCR buffer containing final concentration of 2.5 mmol/L $MgCl_2$, 2 μL of dNTP mix, 5 μL of each sense and antisense primers of eNOS and iNOS,

5 μL of sense and antisense primers of housekeeping β-actin (each of 0.6 μmol/L final concentration), 1.0 μg of template RNA, 2 μL of Qiagen one step RT-PCR enzyme mix and made up to 50 μL with RNase free water. β-actin was used as an internal control.

Primers were designed by primer3 software with the following sequences as: The forward primer of eNOS: 5'-CACACTGCTAGAGGTGCTGGA-3'; The reverse primer: 5'-TGCTGAGCTGACAGAGTAGTA-3'; The forward primer of iNOS: 5'-GCAGGTTGAGG ATTACTTCTTCCA-3'; The reverse primer of iNOS: 5'-GCCCTTTTTTGCTCCATAGGAAA-3'; The forward primer of β-actin: 5'-AGCCATGTA CGTAGCCATCC-3'; The reverse primer of β-actin: 5'-CTCTCAGCTGTGGTGGTGAA-3' (MWG Biotech, Ebersberg, Germany).

Amplification conditions were used in this study consisted of an initial denaturation at 94 ℃ for 5 min followed by denaturation at 94 ℃ for 2 min. Annealing at 58 ℃ for 30 sec and extension at 72 ℃ for 2 min was done for 30 cycles. The cycles were followed incubation at 72 ℃ for 7 min. To compare with the amount of steady state mRNA, 5 μL of each PCR product was resolved onto 2% agarose gel using TBE buffer after electrophoresis, the gel were viewed under UV light and digital images were captured on biorad gel documentation system. The expression of each target gene was standardized with internal control gene expression (β-actin) and represented as a ratio.

Histopathological Studies

A portion of kidney tissue was quickly excised from sacrificed rats and were fixed in 10% HCHO, then dehydrated in the descending grades of isopropanol and xylene. The renal tissue was then embedded in molten paraffin wax and sectioned at 5 μm thickness and was stained with hematoxylin and eosin (H&E). The sections were then viewed under light microscope (Nikon microscope ECLIPSE E400, model 115, Japan) for histopathological changes.

Statistical Analysis

The values were expressed as mean ± standard deviation ($\bar{x} \pm s$) for six animals in each group. Differences between each group were assessed by one way analysis of variance (ANOVA) using SPSS 17 version and least significant difference (LSD) was determined using post hoc test at the level of $P<0.05$.

RESULTS

Effect of AEFM on Serum Lipid Status

The levels of serum lipids are shown in Table 1. Substantial elevation ($P<0.01$) in the levels of serum lipid profile such as TC, TG, and PLs in Group Ⅱ, as compared to Group Ⅰ. Treatment with AEFM in Group Ⅲ considerably decreased ($P<0.01$) the levels of renal TC, TG and PLs when equivalence to Group Ⅱ.

Table 1. Effect of AEFM on Serum Lipid Profile among Groups (mg/dL, $\bar{x} \pm s$, n=6/group)

Group	Cholesterol	Triglycerides	Phospholipids
Ⅰ	95.98 ± 10.09	94.01 ± 8.73	123.19 ± 4.61
Ⅱ	253.50 ± 24.88*	169.16 ± 12.34*	241.02 ± 5.82*
Ⅲ	132.57 ± 82.98△△	129.31 ± 15.22△△	179.88 ± 7.43△△
Ⅳ	111.51 ± 8.13△	100.36 ± 32.51△	140.30 ± 11.11

Notes: *$P<0.01$, compared with Group Ⅰ; △$P<0.05$, △△$P<0.01$, compared with Group Ⅱ

Effect of AEFM on the Renal FRSA

The activities of renal tissue antioxidant enzymes and non enzymic antioxidant were considerably reduced ($P<0.01$) in Group Ⅱ, when matched to Group Ⅰ (Tables 2 and 3). Oral administration with AEFM (Group Ⅲ) brought back the activities of both enzymic and non-enzymic antioxidant to near normal ($P<0.01$).

Effect of AEFM on the Levels of Renal LPO

The levels of renal LPO of HCD induced control and experimental rats were illustrated in Table 4. A significant raise ($P<0.01$) in LPO levels of renal tissues were observed in HCD fed groups as compared to the control group. Treatment with AEFM (Group Ⅲ) caused significant drop ($P<0.05$) in the levels of LPO products, when compared to untreated hypercholesterolemic rats (Group Ⅱ).

Effect of AEFM on the Serum Levels of Renal Markers

Table 5 represents the serum levels of kidney markers of HCD induced control and experimental rats. A concomitant elevation ($P<0.01$) in the levels of renal marker such as serum urea, creatinine and uric acid were observed in Group Ⅱ on comparison with Groups Ⅰ. Group Ⅲ significantly ($P<0.01$) restored the activity of kidney markers to near normal levels compared to Group Ⅰ. Group Ⅳ didn't show any significant change in renal lipid profile, antioxidant status and renal functional parameters as compared

Table 2. Effect of AEFM on the Activities of Renal Enzymic Antioxidant among Groups ($\bar{x} \pm s$, n=6/group)

Group	SOD	CAT	GPx	GR	GST
Ⅰ	4.89 ± 0.11	73.89 ± 5.02	24.94 ± 2.30	1.86 ± 0.67	0.66 ± 0.14
Ⅱ	2.99 ± 0.66*	53.91 ± 6.22*	21.21 ± 1.10*	1.56 ± 0.36*	0.51 ± 0.09*
Ⅲ	3.55 ± 0.85△	65.04 ± 4.06△	22.84 ± 1.16△	1.72 ± 0.33△	0.59 ± 0.08△
Ⅳ	3.77 ± 0.27	69.82 ± 5.88	24.02 ± 1.34	1.83 ± 0.22	0.65 ± 0.05

Notes: SOD: Units/mg protein, one unit is equal to amount of enzyme that inhibits the auto- oxidation reaction by 50%; CAT: μ moles of H_2O_2 consumed/min/mg protein; GPx: μ g of GSH oxidized/min/mg protein; GR: μ mol NADPH oxidized/min/mg protein; GST: nmol cDNB-GSH conjugate/min/mg protein. *P<0.01, compared with Group Ⅰ; △P<0.01, compared with Group Ⅱ

Table 3. Effect of AEFM on the Activities of Renal Non-Enzymic Antioxidant among Groups ($\bar{x} \pm s$, n=6/group)

Group	GSH	Vit-C	Vit-E
Ⅰ	9.44 ± 1.54	2.02 ± 0.59	1.92 ± 0.24
Ⅱ	7.11 ± 0.59*	1.44 ± 0.35*	1.28 ± 0.30*
Ⅲ	8.09 ± 0.64△	1.81 ± 0.27△	1.67 ± 0.26△
Ⅳ	9.01 ± 1.96	2.10 ± 0.41	1.89 ± 0.29

Notes: GSH, Vit C, Vit: μ g/mg protein. *P<0.01, compared with Group Ⅰ; △P<0.01, compared with Group Ⅱ

with Group Ⅰ.

Effect of AEFM on the mRNA Expression of NOS in Renal Artery

Figures 1 and 2 showed the mRNA expression of eNOS and iNOS among groups. The mRNA expression levels of eNOS and iNOS gene was significantly decreased (P<0.05) and increased (P<0.01) respectively in the renal artery of Group Ⅱ (lane 3) as compared to Group Ⅰ (lane 2). Oral treatment with Group Ⅲ substantially up-regulated and down-regulated (P<0.01) the mRNA expression levels of eNOS and iNOS respectively in renal artery (lane 4) when compared to Group Ⅱ (lane 3). Group Ⅳ doesn't showed any changes in mRNA expression levels of eNOS and iNOS.

Effect of AEFM on Histopathological Changes in Renal Tissue

Figure 3 depicts the histopathological changes in renal tissue of HCD induced control and experimental rats. Transection of renal tissue (Group Ⅰ) rendered normal architecture of glomeruli and tubules of nephron (Figure 3A). Transection of Group Ⅱ depict swelling of renal tubules and tubular epithelial denudation with casts in the widened lumens (Figure 3B). Transection of Group Ⅲ exhibited mild tubular epithelial impairment when compared to Group Ⅱ (Figure 3C). Group Ⅳ (Figure 3D) exhibited no changes in the morphology/ structure of renal tissue.

Table 4. Effect of AEFM on the Levels of LPO among Groups ($\bar{x} \pm s$, n=6/group)

Group	TBARS	Hydroperoxide
Ⅰ	2.44 ± 0.74	78.42 ± 3.71
Ⅱ	3.86 ± 0.92*	129.08 ± 6.76*
Ⅲ	2.55 ± 0.02△	100.37 ± 3.78△
Ⅳ	2.32 ± 0.44	81.93 ± 2.08

Notes: Units: nmoles of MDA formed/min/mg protein. *P<0.01, compared with Group Ⅰ; △P<0.01, compared with Group Ⅱ

Table 5. Effect of AEFM on the Levels of Serum Renal Markers among Groups (mg/dL, $\bar{x} \pm s$, n=6/group)

Group	Urea	Creatinine	Uric acid
Ⅰ	25.04 ± 1.89	0.98 ± 0.87	1.95 ± 0.26
Ⅱ	39.54 ± 3.84*	1.42 ± 0.67*	3.05 ± 0.47*
Ⅲ	31.88 ± 2.98△	1.25 ± 0.35△	2.25 ± 0.58△
Ⅳ	27.66 ± 1.11	1.08 ± 0.26	2.04 ± 0.69

Notes: Units: nmoles of MDA formed/min/mg protein. *P<0.01, compared with Group Ⅰ; △P<0.01, compared with Group Ⅱ

Group Ⅳ presented normal renal architecture which is similar to that of Group Ⅰ.

DISCUSSION

The objective of the present study to assess the modulatory impact of AEFM on serum lipid levels, renal FRSA status, serum levels of renal functional markers, mRNA expression of eNOS and iNOS levels and renal histopathology in high cholesterol induced rats. It has been persuasively proven that rats with chronic hyperlipidemia and hypercholesterolemia develop glomerulosclerosis without immune complex disease.[31–33] In this contemporaneous study it has been found that there was a noteworthy elevation in the levels of serum lipid profile such as TC, TG and PLs in HCD fed rats, as compared to normal rats owed to the augmented absorption of lipids (serum) which reflect in elevated renal lipid levels. These results are well supported by several erstwhile studies.[15,34] Oral

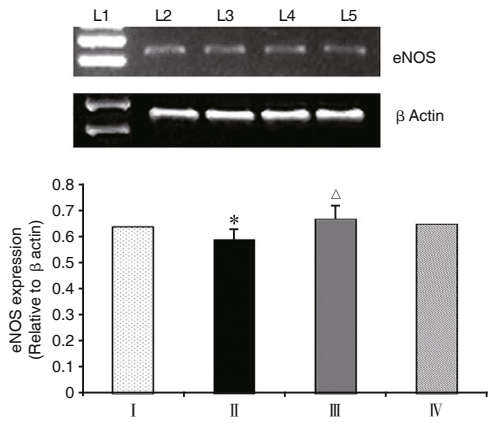

Figure 1. Effect of AEFM on mRNA Expression of eNOS among Groups ($\bar{x} \pm s$, n=6/group)

Notes: Lane 1 represents DNA marker; Lane 2 represents experimental animal Group Ⅰ; Lane 3 represents experimental animal Group Ⅱ; Lane 4 represents experimental animal Group Ⅲ; Lane 5 represents experimental animal Group Ⅳ; Densitometric analysis of the bands is expressed as net intensity ratio corrected for the corresponding β-actin contents. $^{*}P$<0.05, compared with group Ⅰ; $^{\triangle}P$<0.01, compared with group Ⅱ

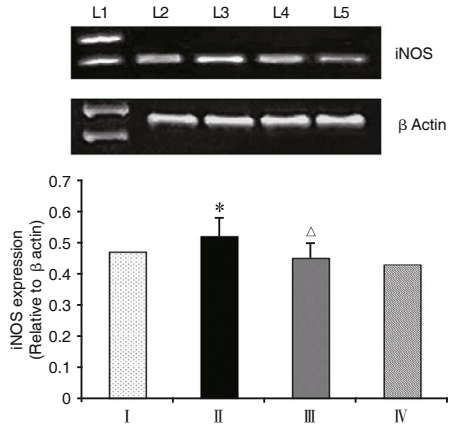

Figure 2. Effect of AEFM on mRNA Expression of iNOS among Groups ($\bar{x} \pm s$, n=6/group)

Notes: Lane 1 represents DNA marker; Lane 2 represents experimental animal Group Ⅰ; Lane 3 represents experimental animal Group Ⅱ; Lane 4 represents experimental animal Group Ⅲ; Lane 5 represents experimental animal Group Ⅳ; Densitometric analysis of the bands is expressed as net intensity ratio corrected for the corresponding β-actin contents. $^{*}P$<0.01, compared with Group Ⅰ; $^{\triangle}P$<0.01, compared with Group Ⅱ

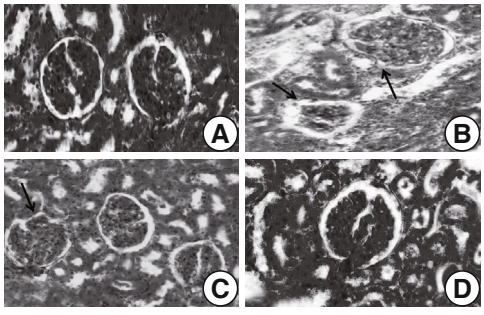

Figure 3. Effect of AEFM on Histopathological Modifications in Renal Tissue among Groups

Notes: A: Group Ⅰ showed normal architecture of glomeruli and tubules of nephron (H&E, 100×); B: Group Ⅱ portrayed swelling of renal tubules and tubular epithelial denudation with casts in the widened lumens (glomerular hypertrophy) of renal tissue; C: Group Ⅲ exhibited mild tubular epithelial damage when compared to (Group Ⅱ); D: Group Ⅳ displayed normal renal architecture similar to that of control rats (H&E, 100×) (Scale bar-50 μm).

administration of AEFM significantly decreases the levels of TC, TG, PLs when compared to HCD induced rats which may be due to antihypercholesterolemic effect.[35]

It was postulated that cholesterol-induced renal damage might occur due to increased oxidative stress (imbalance between oxidant and FRSA) in renal tissue.[36] In animal model feeding of high cholesterol diet produces severe hypercholesterolemia and glomerular lesion by increased oxidative stress in kidney.[37,38] Quite a lot of reports have revealed that hypercholesterolemia worsen the antioxidant defense systems of various organs principally renal tissue.[39,40] During HCD condition the activities of renal antioxidant enzymes and non enzymic antioxidant were markedly bottled-up. These results were parallel to the previous report that during HCD condition there is a concomitant increase in the levels of free radicals (ROS) which leads to oxidative stress and finally ends up in diminished activities of these antioxidant enzymes.[15,36]

Treatment with AEFM, the activities of these antioxidant enzymes in renal tissue were reverted back to near normal. Earlier it has been reported that AEFM has an FRSA owing to the presence of its saponins, flavonoids and phytosterol[41] and hence antioxidants alleviate the hypercholesterolemic induced oxidative stress in animal models, mainly due to their free radical scavenging capabilities. Martinet and his coworkers[42] have demonstrated that HCD induces ROS overproduction which could in turn initiate LPO. During HCD condition there will be prominent upsurge in ROS generation this in turn convert the normal LDL to oxidized LDL (oxLDL) which is an imperative contributory factor for glomerulosclerosis.[43] In this present study it has been found that a concomitant elevation in LPO levels were observed in HCD fed groups matched to the normal group, because of increased ROS generation. These results are compatible with several previous studies.[39,44] AEFM treatment restored the level of LPO and equivalence to

normal rats due to its antioxidant capacity.

Copious investigations have suggested the important role of abnormal lipid metabolism (high cholesterol level) as an integral factor in progressive renal damage.[6,45] Scheuer and his colleague[46] reported that oxidative stress is a pathogenetic factor in lipid-induced nephropathy. Nephrons are the basic unit of renal tissue and used for filtering the ultra-filtrate especially for excretion of renal markers.[47] During HCD condition, more amount of ROS are generated, which in turn damage nephron (depletion in nephron count) that leads to decreased filtration rate and thereby increasing the levels of renal markers functional parameters such as urea, uric acid and creatinine in serum and hence the level of marker enzymes were notably elevated in HCD fed rats. Similar observations were reported earlier in hypercholesterolemic rats.[2,48] Oral supplementation with AEFM significantly reduced the levels of renal markers because of its FRSA thus protect nephron from ROS induced damage.

Hypercholesterolemia has been found to decrease nitric oxide (NO) availability in the renal circulation and renal artery.[49] NO is reported to play an important role in the body and involve in the control of renal function.[50] Especially in the health status, the eNOS are identified as a protective NO-generating enzyme and contribute to the maintenance of the normal renal structure.[51] The NO derived from eNOS usually plays a protective effect, however, the one comes from iNOS might be associated in the renal injury.[52] Over-expression of iNOS and excessive NO are proposed as one of the causes of vascular dysfunction in kidney by activating inflammatory cytokines.[53] Renal NO level is significantly decreased and peroxynitrite level is significantly enhanced in cholesterol-fed animal model.[54,55] In agreement with above statement it has been found out markedly down-regulation and up-regulation of mRNA expression in the levels of both eNOS and iNOS gene respectively in renal artery of hypercholesterolemic induced rats. It might be because of enhanced production of superoxide (O_{2^-}), which then reacts with NO to form peroxynitrite ($ONOO^-$) and thereby decreasing NO level as well as increased oxLDL. OxLDL is suggested to be a potential regulator of NO generation. It has the capacity of regulating eNOS and iNOS activity via inhibiting Akt-mediated eNOS serine 1177 phosphorylation, preventing eNOS-Hsp90 interaction, up regulating caveolin (Cav-1) and activating endothelial arginase Ⅱ (Arg Ⅱ).[56] The overexpression of iNOS may result in a reduced expression of eNOS.[57] Accordingly, the iNOS up regulation most likely contributes to eNOS reduction in the present model as well (both are inversely proportional to each other).

However on treatment with AEFM substantially up regulation and down-regulation in the mRNA expression level of eNOS (maintain normal NO level) and iNOS (decrease inflammatory cytokines level) were noted in renal artery when compared to HCD induced rats, owing to its antioxidant property (SOD) and thereby suppression the production of O_{2^-} radical as well as attenuating the level of vascular endothelial growth factor (VEGF) in the renal tissue of AEFM treated hypercholesterolemia rats. Thus the renal protective effect of AEFM was associated with the activity of regulating the NO signaling pathway due to its antioxidant capacity in hypercholesterolemic rats.

Hypercholesterolemia and the resulting athero sclerosis have been implicated in the pathophysiology of glomerulosclerosis.[58,59] Histological examination of the control renal tissue portrayed normal glomeruli and tubules of nephron. In the case of HCD induced rats the mild swelling of renal tubules and tubular epithelial denudation with casts in the widened lumens (glomerular hypertrophy). In the previous section it has also been reported that increase in renal markers was due to increased nephron damage by free radical produced during HCD condition which might be the reason for this significant morphological alteration in renal tissue. The present results are well correlated with previous studies.[6,15,59] After treatment with AEFM renal tissue showed mild tubular epithelial damage when compared to HCD rats due to its FRSA. AEFM alone treated rats exhibited normal renal architecture similar to that of control rats.

From the above results it could be hypothesized that consumption of AEFM significantly regulates serum lipid levels, renal oxidative stress, renal markers, mRNA expression of NOS and morphology of renal tissue mainly due to its antihypercholesterolemic and antioxidant properties and it has been conjectured that AEFM can effectively relieve the renal oxido-

lipidemic injury associated with hypercholesterolemia.

Conflict of Interests

The authors declare that they have no conflict of interests.

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