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Renoprotective effect of *Caralluma fimbriata* against high-fat diet-induced oxidative stress in Wistar rats

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ABSTRACT

The current study was designed to evaluate the renoprotective effect of hydro-alcoholic extract of *Caralluma fimbriata* (CFE) against high-fat diet-induced oxidative stress in Wistar rats. Male Wistar rats were randomly divided into five groups: control (C), control treated with CFE (C + CFE), high-fat diet fed (HFD), high-fat diet fed treated with CFE (HFD + CFE), and high-fat diet fed treated with metformin (HFD + metformin). CFE was orally administered (200 mg/kg body weight) to Groups C + CFE and HFD + CFE rats for 90 days. Renal functional markers such as, urea, uric acid, and creatinine levels in plasma were quantified during the experimental period. At the end of the experimental period, activities of transaminases and oxidative stress markers, i.e., reduced glutathione (GSH), lipid peroxidation, protein oxidation, and activities of antioxidant enzymes were assayed in renal tissue. Coadministration of CFE along with HF-diet in Group HFD + CFE prevented the rise in the levels of plasma urea, uric acid, and creatinine, and elevated activities of renal transaminases with decreased protein content of Group HFD ($p < 0.05$). Establishment of oxidative stress in Group HFD, as evident from elevated lipid peroxidation, protein oxidation levels with depleted levels of GSH, and decreased activities of GSH dependent and independent antioxidant enzymes, was prevented in Groups HFD + CFE and HFD + metformin rats. Further, there were no deviations in the studied parameters but there was improved antioxidant status of Group C + CFE from Group C which revealed the nontoxic nature of CFE even under chronic treatment. Thus, CFE treatment effectively alleviated the HF-diet induced renal damage. Hence, this plant could be used as an adjuvant therapy for the prevention and/or management of HF-diet induced renal damage.

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1. Introduction

Obesity is associated with multiple conditions that are known to compromise renal function, including insulin resistance, hyperglycemia, hyperleptinemia, hypertension, dyslipidemia, and metabolic syndrome which can independently have detrimental effects on renal function [1]. Further, obesity-associated insulin resistance is a major risk factor for type 2 diabetes mellitus (DM) and associated long term complications in DM including diabetic nephropathy. Increasing rates of obesity may be due to easily accessible and palatable diets. Unchecked body weight (BW) due to high appetite is also known as a major cause of obesity. Renal abnormalities are evident from increased BW, fat accumulation, and glomerular sclerosis with consumption of high fat (HF) diet [2]. Nutritional or dietary oxidative stress denotes disturbance of the redox state resulting from excess oxidative load or from inadequate nutrient supply favoring pro-oxidant reactions. Postprandial increases of lipid and carbohydrate levels lead to increased oxidative stress and lipid hydroperoxides present in the diet which are absorbed, contributing to the pro-oxidant load [3]. In obesity, oxidized low density lipoprotein levels are increased which have been shown to stimulate monocyte adhesion to the glomerular injury [4]. However, most of these and other conditions resulting in kidney injury are associated with oxidative stress. Hence, oxidative stress is one of the most important factors associated with kidney injury [5]. Clinical trials suggest that there is no effective treatment for obesity/diabetes-induced nephropathy and hence efforts have been focused on traditional herbal medicine to find novel therapeutic agents for the treatment of nephropathy [6]. Recently, herbs and/or natural products have been highlighted as alternatives to the current management of diabetic nephropathy [7].

Caralluma fimbriata (Family: Apocynaceae; CFE), an edible succulent, is distributed throughout the world including India. It is listed in Indian Materia Medica as a vegetable, famine food, appetite suppressant, and thirst quencher. The standardized extract of this plant is marketed under the trade name GensSlim for management of obesity and BW [8]. In addition, it has several phytotherapeutic properties. Our earlier studies showed the beneficial effects of *C. fimbriata* against HF diet-induced insulin resistance in Wistar rats [9].

Metformin, a biguanide drug, is widely used for the treatment of type 2 DM. It is also reported to possess antioxidant properties [10]. Recently, much attention has also been focused on the possible protective role of metformin in diabetic kidney diseases. With this background the present work is planned to investigate the renoprotective effect of *C. fimbriata* in comparison with metformin on the progression of HF-diet induced oxidative stress in Wistar rats.

2. Methods

2.1. Chemicals

Pyrogallol and 1-chloro-2, 4-dinitrobenzene (CDNB) were obtained from SISCO Research Laboratories (P) Ltd., Mumbai, India. All other chemicals and solvents of analytical grade were procured from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Plant extract

Hydro-alcoholic extract of CFE (brown, dry powder with Batch Number CFE/10011) whole herb was a gift from Green-Chem Herbal Extracts and Formulations, Bangalore, Karnataka, India. The procedure followed by the firm for the preparation of the extract is as follows: the aerial parts of the plant CF were collected, dried in shade, and sized by a crusher to extract material. One hundred grams of the plant material was extracted with 40% aqueous alcohol (40 parts of alcohol and 60 parts of water). Twelve kilograms of the dried herb obtained from 100 kg of the fresh plant gave a final yield of 1 kg of extract having 25% pregnane glycosides. The extract was dissolved in distilled water prior to use.

2.3. Qualitative phytochemical analysis

Phytochemical analysis of CFE was carried out to ascertain the qualitative composition of secondary metabolites such as alkaloids, anthocyanins, anthocyanidins, anthracene glycosides, antraquinones, aucubins, carboxylic acids, catecholic compounds, coumarins, fatty acids, flavonoids, gallic-tannins, phenols, saponins, steroids, and triterpenoids by employing coloration and precipitation test [11].

2.4. Quantitative estimation of total phenols, flavonoids, and saponins

2.4.1. Total phenol content

The total phenolic compound of CFE was assessed using Folin–Ciocalteau phenol reagent method described by Singleton et al [12]. Briefly, 1.0 mL of the extract (1.25 mg) was mixed with 2.5 mL of 10% Folin–Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for 30 minutes. The absorbance was read at 750 nm in a spectrophotometer. The total phenol content was expressed as gallic acid equivalents in milligram per gram of the extract using a standard curve generated with gallic acid (range, 50–500 μg).

2.4.2. Flavonoid estimation

The flavonoid content of CFE was determined using aluminum chloride colorimetric method described by Chang et al [13]. Briefly, 0.5 mL of CFE (2.5 mg) was mixed with 3 mL of 95% methanol, 0.1 mL of 10% (weight/volume) aluminum chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 415 nm against a blank sample. A calibration curve was prepared using quercetin in methanol (range, 10–100 μg). The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract.

2.4.3. Saponins content

The total saponins content of CFE was determined using the method of Hiai et al [14]. A freeze-dried CFE was dissolved in 80% aqueous methanol. To 250 μL of dissolved saponins,

250 μL of vanillin reagent (8% weight/volume in 99.5% ethanol) and 2.5 mL of 72% (volume/volume) sulfuric acid were added slowly by the inner side of the wall of the test tube, mixed, and kept in a water bath at 66°C for 10 minutes and then cooled in ice cold water for 3–4 minutes before the absorbance was measured at 544 nm against a reagent blank. The total saponin content was expressed as diosgenin equivalents in milligram per gram of the extract using a standard curve generated with diosgenin (range, 25–500 μg).

2.5. Animals and HF-diet

Male Wistar rats (170–190 g) used for the present study were procured from Sri Raghavendra Enterprises, Bangalore, India. The animals were acclimatized for 7 days in our animal house (Registered Number 470/01/a/CPCSEA) before dietary manipulation. They were housed two per cage in an air-conditioned room (22 ± 2°C) with 12-hour light/dark cycle and had free access to a standard pellet diet and water. All the procedures were performed in accordance with the Institutional Animal Ethics Committee (Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India). The animals were fed *ad libitum* with a balanced animal pellet diet.

HF diet was procured from the National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. According to Diet Induced Obesity Diets, New Brunswick, NJ, USA, specifications for HF diet (D12492) wherein lard was used as the fat source and the diet contained approximately 60 kcal% of fat, 20 kcal% of protein, and 20 kcal% of carbohydrate. Briefly, 1 kg of the diet composed of casein, 200 g; sucrose, 68.8 g; malto dextrin-10, 125 g; cellulose, 50 g; soybean oil, 25 g; lard, 245 g; L-cystine, 3 g; mineral mix (S10026), 10 g; di-calcium phosphate, 13 g; calcium carbonate, 5.5 g; potassium citrate monohydrate, 16.5 g; vitamin mix (V10001), and 10 g; choline bitartarate, 2 g.

2.6. Experimental design

The experimental rats were divided into five groups, each comprising eight rats: control rats (C); control rats treated with CFE (200 mg/kg BW/d) in distilled water orally by gastric intubation for 90 days (C + CFE); HF-diet fed rats (HFD); HF diet fed rats treated with CFE (200 mg/kg BW/d) in distilled water orally by gastric intubation for 90 days (HFD + CFE); and HF diet fed rats treated with metformin (20 mg/kg BW/d) in distilled water orally for 90 days (HFD + Met). Rats from Groups HFD, HFD + CFE, and HFD + Met were fed with HF diet for 90 days and rats from Group C and C + CFE were fed a normal pellet diet. Rats from Groups C and HFD received distilled water as a vehicle control.

2.7. Sample collection

During the experimental period at 30-day intervals, blood was collected from 12-hour fasted rats with a capillary tube from the retino-orbital plexus in fresh vials containing EDTA (10 mg/mL) and centrifuged at 8000*g* for 5 minutes. Plasma was separated and used for the estimation of creatinine, urea, and uric acid. At the end of the experimental period, i.e., 90 days, the animals were fasted overnight and sacrificed by cervical dislocation. The kidneys were excised into ice cold saline, thoroughly rinsed, and the weight was recorded.

2.8. Renal functional markers

Renal functional markers in plasma, i.e., urea, uric acid, and creatinine were quantified using commercial kits (Excel Diagnostic kits, Hyderabad, India). Ten percent renal homogenate prepared in 0.1M Tris-HCl buffer (pH 7.4) was centrifuged at 18,514*g* for 45 minutes in the Laboratory centrifuge 5804 R model (Eppendorf India Ltd), rotor number 14030. The clear supernatant obtained was used for the assay of the renal transaminases, i.e., glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) following the method of Reitman and Frankel [15].

2.9. Oxidative stress markers

Ten-percent tissue homogenate prepared in 0.15M KCl at 4°C was used for the estimation of lipid peroxidation (LPO), protein oxidation (PO), and reduced glutathione (GSH). LPO in the kidney was estimated colorimetrically by measuring the thiobarbituric acid reactive substances using the method of Utley et al [16]. The extent of LPO was expressed as nmoles of malondialdehyde formed/g tissue, using a molar extinction coefficient of malondialdehyde as 1.56 × 105M/cm. GSH was determined according to the method of Ellman [17] using 5,5-dithiobis (2-nitrobenzoic acid), which readily gets reduced by sulfhydryl compounds forming a highly colored anion having a maximum absorbance at 412 nm. Protein carbonyl content was measured by forming hydrazone derivatives using 2, 4-dinitrophenylhydrazine which were quantified spectrophotometrically at 370 nm according to the method of Levine et al [18]. The protein concentration in the tissue homogenate and supernatant were measured by the method of Lowry et al [19].

2.10. Antioxidant enzyme assays

Ten-percent tissue homogenate in 0.15M KCl was prepared using a Potter–Elvehjem (Sigma-Aldrich, India) homogenizer at 4°C and centrifuged in the cold (4°C) at 18,514*g* for 45 minutes. The clear supernatant was used for assay of antioxidant enzymes. Glutathione peroxidase (GPx) was assayed using the method of Rotruck et al [20]. A known amount of the enzyme was allowed to react with H_2O_2 in the presence of GSH for a specified time period and the remaining GSH was measured using the method of Ellman [17]. The activity was expressed as μg of GSH consumed/min/mg protein. Glutathione-s-transferase (GST) activity was assayed by monitoring the increase in the absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate according to the method of Habig et al [21]. The activity was expressed as μmoles of CDNB-GSH conjugate formed/min/mg protein using millimolar extinction coefficient of CDNB-GSH conjugate as 9.6. Glutathione reductase (GR) activity was assayed by following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) spectrophotometrically at 340 nm according to the method of Pinto and Bartley [22]. The activity was expressed as μmoles of NADPH oxidized/min/mg protein by using millimolar extinction coefficient of NADPH as 6.22.

Superoxide dismutase activity was assayed according to a modified procedure adopted by Soon and Tan [23], based on its ability to inhibit the oxidation of pyrogallol. Change in absorbance was monitored at 420 nm for 5 minutes and the activity was expressed as the amount of enzyme required to inhibit the oxidation of pyrogallol by 50% which was equal to one unit. The activity was expressed as units/min/mg of protein. Catalase (CAT) activity was assayed by following the decomposition of H_2O_2 spectrophotometrically at 240 nm according to the method of Beers and Sizer [24]. The activity was expressed as mmoles of H_2O_2 decomposed/min/mg protein.

2.11. Statistical analysis

The results were expressed as mean ± standard error. Data were analyzed for significant difference using Duncan's Multiple Range test ($p < 0.05$) using SPSS/PC statistical program (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA) [25].

3. Results

3.1. Phytochemical profile of CFE

The phytochemical screening of hydro-alcoholic extract of CF whole plant revealed the presence of alkaloids, anthocyanins, carboxylic acids, coumarins, flavonoids, gallic-tannins, phenols, saponins, and steroids indicating the presence of pharmacologically important phytochemicals (Table 1) whereas quantitative phytochemical analysis of CFE (Table 2) showed an appreciable amount of polyphenolic compounds (60 mg gallic acid equivalents/g extract) followed by saponins (6.2 mg diosgenin equivalents/g extract) and flavonoids (2.2 mg quercetin equivalents/g extract).

3.2. Absolute and relative kidney weight

As shown in Table 3, Group HFD showed significantly higher absolute and relative weight of kidney (11.3% and 18.9%, respectively) compared with Group C whereas Groups HFD + CFE and HFD + Met showed ameliorating effects of respective treatments against HF diet-induced gain in absolute and relative kidney weight.

3.3. Renal functional markers and transaminases

Plasma concentrations of urea, uric acid, and creatinine at 0 days, 30 days, 60 days, and 90 days of the experimental period in five groups are presented in Table 4. In comparison with Group C, Group HFD showed increased levels of urea (34.6%), uric acid (152.5%), and creatinine (50.0%) at 90 days of the experimental period. The Groups HFD + CFE and HFD + Met showed a significant reduction in urea (31.9%/33.1%), uric acid (46.9%/48.4%), and creatinine (40.7%/41.7%) compared with Group HFD by the end of the experimental period respectively. CFE/Met coadministration along with HF diet in Groups HFD + CFE and HFD + Met ameliorated the HF diet induced hike in the renal GOT (29.6%) and GPT (28.4%) compared with Group C. No significant variation was observed in the activities of renal transaminases between Groups C and C + CFE (Table 5).

Table 2 – Quantitative phytochemicals profile of *Caralluma fimbriata*.

Total polyphenolic compounds (mg gallic acid equivalents/g extract)	Flavonoids (mg quercetin equivalents/g extract)	Saponins (mg diosgenin equivalents/g extract)
60.0 ± 0.28	2.2 ± 0.10	6.2 ± 0.20

Data are presented as expressed as mean ± standard error of the mean ($n = 3$).

Table 3 – Absolute and relative kidney weight in five experimental groups.

Groups	Kidney tissue weight	
	Absolute (g)	Relative (g/100 g body weight)
Control	2.04 ± 0.04[a]	0.53 ± 0.02[a]
C + CFE	2.00 ± 0.03[a]	0.58 ± 0.01[b]
HFD	2.27 ± 0.07[b]	0.63 ± 0.02[c]
HFD + CFE	1.96 ± 0.02[a]	0.54 ± 0.01[a]
HFD + Met	1.96 ± 0.02[a]	0.57 ± 0.01[b]

Data are presented as mean ± standard error of the mean ($n = 8$ animals). Values with different superscripts within the column are significantly different at $p < 0.05$ (Duncan's multiple range test).

3.4. Renal oxidative stress markers and antioxidants

Figure 1 shows the renal oxidative stress markers. Group HFD showed significantly higher levels of LPO (149.7%) and PO (16.3%) and low levels of renal GSH (38.1%) as compared with

Table 1 – Qualitative phytochemical profile of *Caralluma fimbriata*.

Phytochemicals	Presence (+)/absence (–)	Phytochemicals	Presence (+)/absence (–)
Alkaloids	+	Coumarins	+
Anthocyanins	+	Fatty acids	–
Anthocyanidins	–	Flavonoids	+
Anthracene glycosides	–	Gallic-tannins	+
Antraquinones	–	Phenols	+
Aucubins	–	Saponins	+
Carboxylic acids	+	Steroids	+
Catecholic compounds	–	Triterpinoids	–

Table 4 – Effect of *Caralluma fimbriata* (CFE)/metformin (Met) administration on plasma urea, uric acid, and creatinine in high-fat (HF) diet fed Wistar rats.

Parameter	Urea (mg/dL)				Uric acid (mg/dL)				Creatinine (mg/dL)			
Experimental period (d)	0	30	60	90	0	30	60	90	0	30	60	90
Control	43.12 ± 0.30^{a}	43.41 ± 0.33^{a}	43.92 ± 0.28^{a}	44.23 ± 0.28^{a}	2.05 ± 0.007^{a}	2.12 ± 0.006^{a}	2.20 ± 0.005^{a}	2.34 ± 0.006^{b}	0.69 ± 0.003^{a}	0.70 ± 0.002^{a}	0.71 ± 0.004^{a}	0.72 ± 0.006^{a}
C + CFE	42.51 ± 0.27^{a}	42.43 ± 0.26^{b}	42.21 ± 0.22^{b}	42.05 ± 0.24^{b}	2.12 ± 0.004^{b}	2.16 ± 0.004^{a}	2.31 ± 0.008^{b}	2.22 ± 0.005^{b}	0.68 ± 0.002^{a}	0.67 ± 0.003^{b}	0.65 ± 0.002^{b}	0.63 ± 0.006^{b}
HFD	42.23 ± 0.23^{a}	45.84 ± 0.30^{c}	51.23 ± 0.34^{c}	59.52 ± 0.24^{c}	2.14 ± 0.005^{b}	3.05 ± 0.006^{b}	4.55 ± 0.007^{c}	5.91 ± 0.009^{e}	0.69 ± 0.002^{a}	0.78 ± 0.002^{c}	0.92 ± 0.001^{c}	1.08 ± 0.005^{c}
HFD + CFE	42.95 ± 0.23^{a}	42.22 ± 0.27^{b}	41.54 ± 0.25^{d}	40.52 ± 0.18^{d}	2.10 ± 0.004^{b}	2.52 ± 0.007^{c}	2.93 ± 0.005^{d}	3.14 ± 0.005^{d}	0.69 ± 0.003^{a}	0.68 ± 0.004^{b}	0.66 ± 0.003^{b}	0.64 ± 0.006^{b}
HFD + Met	43.04 ± 0.28^{a}	42.35 ± 0.24^{b}	41.45 ± 0.31^{d}	39.83 ± 0.36^{e}	2.13 ± 0.005^{b}	2.44 ± 0.008^{d}	2.82 ± 0.004^{e}	3.05 ± 0.010^{c}	0.69 ± 0.002^{a}	0.68 ± 0.003^{b}	0.65 ± 0.004^{b}	0.63 ± 0.005^{b}

C = control rats; C + CFE, control rats treated with *Caralluma fimbriata*; HFD = high-fat diet fed rats; HFD + CFE = high-fat diet fed rats treated with CFE; HFD + Met = high-fat diet fed rats treated with metformin.
Data are presented as mean ± standard error of the mean (n = 8 animals). Values with different superscripts within the column are significantly different at $p < 0.05$ (Duncan's multiple range test).
C = control rats; C + CFE, control rats treated with *Caralluma fimbriata*; HFD = high-fat diet fed rats; HFD + CFE = high-fat diet fed rats treated with CFE; HFD + Met = high-fat diet fed rats treated with metformin.

Table 5 – Effect of *Caralluma fimbriata* (CFE)/metformin (Met) on the activities of renal transaminases and protein content in High-fat (HF) diet fed Wistar rats.

Parameters	GOT (μg of pyruvate formed/min/mg protein)	GPT (μg of pyruvate formed/min/mg protein)	Protein (mg/g tissue)
Control	1.62 ± 0.15^{a}	0.81 ± 0.15^{a}	4.46 ± 0.025^{a}
C + CFE	1.58 ± 0.18^{a}	0.83 ± 0.12^{a}	4.31 ± 0.023^{b}
HFD	2.10 ± 0.28^{b}	1.04 ± 0.23^{c}	4.83 ± 0.043^{c}
HFD + CFE	1.77 ± 0.15^{c}	0.94 ± 0.12^{b}	4.44 ± 0.030^{a}
HFD + Met	1.71 ± 0.18^{d}	0.91 ± 0.13^{b}	4.43 ± 0.032^{a}

Data are presented as mean ± standard error of the mean values of eight rats per group. Means in the same column not sharing a common superscript are significantly different ($p < 0.05$).
C = control rats; C + CFE, control rats treated with *Caralluma fimbriata*; HFD = high-fat diet fed rats; HFD + CFE = high-fat diet fed rats treated with CFE; HFD + Met = high-fat diet fed rats treated with metformin.

Group C. Coadministration of CFE/Met along with HF diet in Groups HFD + CFE and HFD + Met for 90 days prevented the increased LPO and PO and decreased GSH levels in kidney.

Table 6 shows the activities of antioxidant enzymes in kidney of the five experimental groups. The activities of GPx, GR, GST, superoxide dismutase, and CAT were significantly lowered (23.7%, 15.2%, 40.7%, 24.3%, and 28.3%, respectively) in Group HFD rats compared with Group C. Coadministration of CFE/Met along with HF diet in Groups HFD + CFE and HFD + Met for 90 days prevented HF diet-induced decline in the activities of enzymes in kidney. However, CFE administration to control rats (Group C + CFE) caused a slight increase in the activities of these antioxidant enzymes.

4. Discussion

The present study revealed that CFE contains pharmacologically important phytochemicals. Numerous epidemiological studies suggest that herbs/diets rich in phytochemicals and antioxidants execute a protective role in health and disease. Quantitative phytochemical analysis of CFE (Table 1) showed an appreciable amount of polyphenolic compounds followed by saponins and flavonoids. Pregnane glycosides, flavanone glycosides, megastigmane glycosides, bitter principles, saponins, and various other flavonoids are reported as the key phytochemical ingredients in *Caralluma* species [26]. Polyphenols are the major plant compounds with antioxidant activity [27]. Saponins have been reported to possess a wide range of biological activities due to its antioxidant activity. Flavonoids are reported to be beneficial against obesity and diabetes [28]. The presence of these phytochemicals in CFE and synergism among these compounds may be the most likely option for the protective effect of CFE against HF diet-induced metabolic alterations in the present study.

Lifestyle factors and diet play a key role in the development of kidney disease in several stages including progress of obesity and metabolic syndrome and occurrence of obesity-related glomerulopathy. These are associated with increased

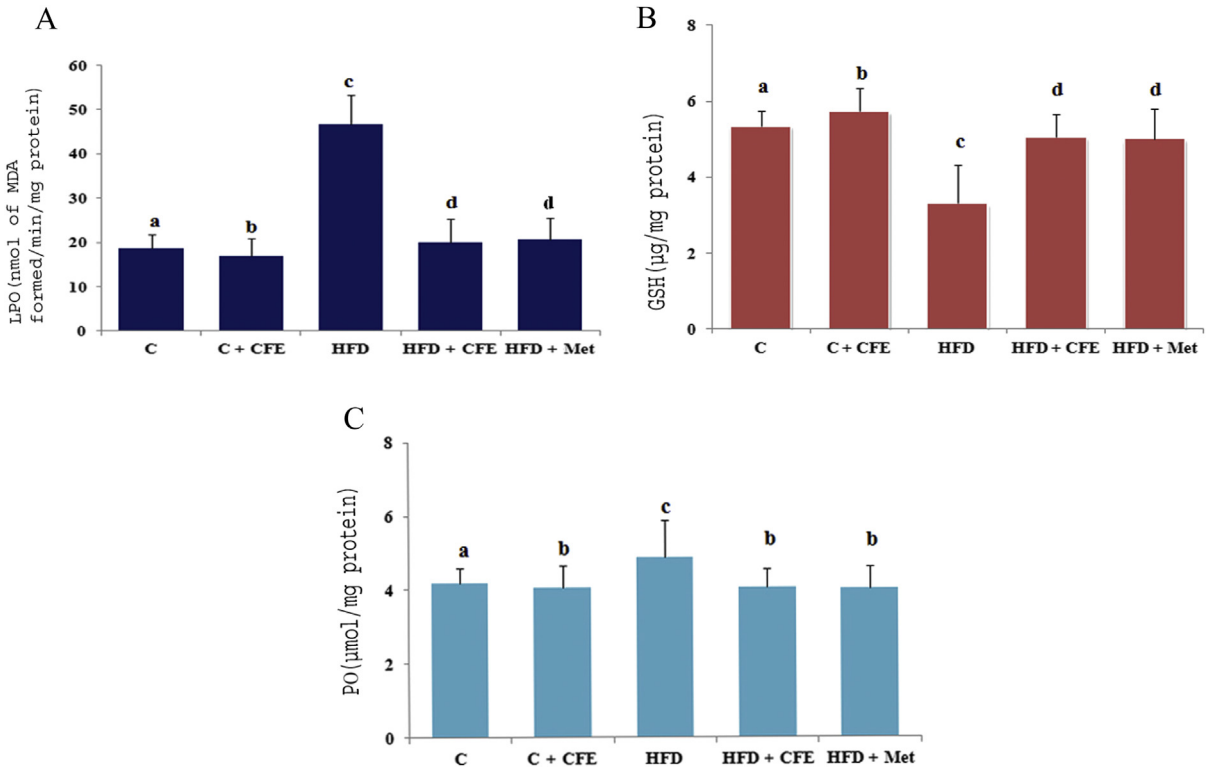

Figure 1 – (A) Lipid peroxidation (LPO); (B) reduced glutathione (GSH); and (C) protein oxidation (PO) in renal tissue of all experimental groups of rats. Values are mean ± standard error of the mean ($n = 8$ animals in each group). Means in the same column not sharing a common superscript are significantly different ($p < 0.05$). C = control rats; C + CFE, control rats treated with *Caralluma fimbriata*; HFD = high-fat diet fed rats; HFD + CFE = high-fat diet fed rats treated with CFE; HFD + Met = high-fat diet fed rats treated with metformin; MDA = methylenedioxyamphetamine.

Table 6 – Effect of *Caralluma fimbriata* (CFE)/metformin (Met) on the activities of antioxidant enzymes in high-fat (HF) diet fed Wistar rats.

Parameters	Control	C + CFE	HFD	HFD + CFE	HFD + Met
GPx (µg of GSH consumed/min/mg protein)	5.27 ± 0.09^{a}	5.83 ± 0.06^{b}	4.02 ± 0.08^{c}	5.53 ± 0.03^{d}	5.01 ± 0.06^{e}
GR (µmol NADPH oxidized/min/mg protein)	32.16 ± 0.37^{a}	32.73 ± 0.32^{a}	27.28 ± 0.36^{b}	32.79 ± 0.29^{a}	32.78 ± 0.22^{a}
GST (µmol CDNB-GSH conjugate formed/min/mg protein)	71.92 ± 0.11^{a}	71.13 ± 0.31^{a}	42.66 ± 0.31^{b}	71.19 ± 0.20^{a}	71.72 ± 0.32^{a}
SOD (U/mg protein)	21.42 ± 0.26^{a}	24.53 ± 0.20^{b}	16.22 ± 0.29^{c}	21.26 ± 0.22^{a}	22.06 ± 0.18^{d}
CAT (mmol H_2O_2 consumed/min/mg protein)	34.54 ± 0.37^{a}	35.76 ± 0.13^{b}	24.75 ± 0.24^{c}	31.99 ± 0.42^{d}	32.85 ± 0.21^{d}

Data are presented as mean ± standard deviation values of eight rats per group. Means in the same row not sharing a common superscript are significantly different ($p < 0.05$).
C = control rats; CAT = catalase; C + CFE, control rats treated with *Caralluma fimbriata*; GPx = glutathione peroxidase; GR = glutathione reductase; GST = glutathione-s-transferase; HFD = high-fat diet fed rats; HFD + CFE = high-fat diet fed rats treated with CFE; HFD + Met = high-fat diet fed rats treated with metformin; SOD = superoxide dismutase.

oxidative stress and apoptosis in the kidney, leading to inflammation and deterioration of renal function [29]. In the present study we found that HFD caused significant elevation of plasma urea, creatinine, and uric acid by chronic feeding of a HF diet is an indication of defective kidney function in Group HFD rats which are in agreement with earlier reports [30]. Whereas studies on hypercalorie/cafeteria diet fed obese rats showed no change in serum urea with a significant rise in creatinine and uric acid levels [31]. Decreased renal protein content with enhanced activities of tissue GOT and GPT observed in Group HFD rats indicates enhanced catabolism of proteins ultimately resulting in increased production of urea in these animals. In addition to this, decreased renal clearance due to impaired renal function under HF diet fed conditions might have aggravated the situation. Coadministration of CFE/Met along with HF diet has given protection from HF diet-induced kidney dysfunction as it normalized the increased levels of plasma urea, creatinine, and renal transaminases in Groups HFD + CFE and HFD + Met. These protective properties of the extract may be due to its ameliorative activity on metabolic alterations observed under HF diet fed conditions and may also be because the plant is very rich in phyto-chemical ingredients with antioxidant properties [32]. A similar observation of the renoprotective role of CFE was

reported against increased levels of serum creatinine and uric acid in hypercalorie/cafeteria diet fed rats [33].

HF diet-induced oxidative stress as evidenced by elevated renal LPO, PO, with a decreased GSH level in Group HFD rats is in agreement with an earlier report [34]. Kume et al [35] reported that renal injury observed under HF diet fed conditions might be due to HF diet-induced alteration of renal lipid metabolism by an imbalance between lipogenesis and lipolysis along with systemic metabolic abnormalities and subsequent renal lipid accumulation and LPO. Damaged renal tissue acts as sources of reactive oxygen species (ROS) and activates the process of LPO. Previous studies strongly suggest that oxidative stress induced by HF diet enhances lipid peroxidation and diminishes the activities of antioxidant enzymes [36]. GSH represents the first line of defense against free radicals and is also responsible for the maintenance of protein thiols and acts as a substrate for GPx and GST. GPx activity is considered to symbolize the initial protective response required for adjusting the H_2O_2 concentration under physiological condition as well as after oxidative insult. Furthermore, it has been postulated that high intake of dietary fat enhances ROS overproduction. ROS themselves can reduce the activity of antioxidant enzymes such as CAT and GPx [37]. A significant decrease in the level of GSH in Group HFD rats could be the result of decreased synthesis or increased degradation/utilization of GSH by increased oxidative stress and decreased regeneration as evident from the lower activity of GR.

The antioxidant potential of CFE against HF diet-induced oxidative stress is evident from lower levels of PO, LPO, higher GSH levels, and increased activities of antioxidant enzymes seen in Group HFD + CFE when compared with Group HFD. A significant increase in the GSH content in kidneys of CFE-treated HF diet-fed rats indicates that CFE treatment activated the compensatory mechanism against the oxidative stress, which might have either increased the biosynthesis of GSH or lowered the utilization of GSH due to decreased oxidative stress or both. Explanations of possible mechanisms underlying the antioxidant potential of a drug include the prevention of GSH depletion and destruction of free radicals due to its rich antioxidant phytochemical profile. The antioxidant potential of this plant against oxidative stress in the liver has been well documented [9]. Hence, extracts from *Caralluma* species are the object of increasing interest for nutraceutical companies [38]. The significant attenuation of oxidative stress in Group HFD + Met indicates a protective role of metformin against HF diet-induced oxidative stress in kidney. These findings are in agreement with earlier reports in diabetic rats [39]. Various investigations show that Met decreases intracellular ROS and protects tubular injury by restoring the biochemical alterations and regulation of oxidative stress on renal tubules. Therefore Met may exert some of its beneficial effects by improvement of renal oxidative stress under HF diet fed conditions. Thus, we hypothesize that the possible explanation for reducing renal injury following the consumption of CFE can be a useful add-on therapy to curtail oxidative stress in HF diet-induced Wistar rats. Similar to CFE treatment, Met treated HF diet fed rats also showed protection from HF diet induced oxidative stress.

5. Conclusion

In view of these findings, it is possible to conclude that HF diet feeding results in pronounced oxidative stress leading to kidney malfunctioning which may lead to renal damage. CFE/Met treatment significantly ameliorated the renal dysfunction and oxidative stress observed under HF diet-fed conditions. Based on the results of the present and our earlier study [9], it is suggested that the renoprotective effect of CFE may be attributed to its antiobesity properties like controlling BW and obesity-associated metabolic disorders, i.e., hyperglycemia, insulin resistance, hypertriglyceridemia, and hyperleptinemia along with its antioxidant potential. Thus, hydro-alcoholic extract of CF would seem useful as an adjuvant for the prevention and/or management of oxidative stress in kidney.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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