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Research Article
Moringa, Rosemary and Purslane Leaves Extracts Alleviate Metabolic Syndrome in Rats Induced by High Fat-High Fructose Diet

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Abstract

Background and Objective: Metabolic syndrome is a cluster of metabolic abnormalities characterized by obesity, insulin resistance and dyslipidemia. This study aimed to investigate the impact of moringa, rosemary and purslane leave water extracts on metabolic syndrome in rats. **Materials and Methods:** Phenolic compounds in the plant leaves water extracts were determined by HPLC. Fifty adult male albino rats Sprague-Dawley strain were equally divided into five groups, group (1) Normal rats fed on the balanced diet, group (2) Metabolic syndrome rats fed on High Fat-High Fructose Diet (HF-HFD). The other three groups were fed on HF-HFD and orally administered 200 mg kg^{-1} b.wt. daily of the tested plant's leaves water extracts, respectively, for 12 weeks. Some anthropometric measurements (BMI, Lee index and adiposity index), biochemical parameters such as glucose hemostasis parameters (glucose, Insulin, HOMA-IR and GLP-1), lipids profile (TAGs, TC, LDL-C, HDL-C, free fatty acids, Apo-B and Apo A1), adipokines (leptin and adiponectin), some inflammatory markers (TNF-α and IL-6) and oxidative stress markers (PCC, NO and MDA), some anti-oxidant markers (GSH, CAT and TAOC) as well as, the gene expression level of endothelial nitric oxide synthase were determined. **Results:** The results revealed that feeding rats with HF-HFD for 12 weeks significantly increased anthropometric measurements, some inflammatory markers and oxidative stress markers and worsen glucose hemostasis parameters, lipids profile, adipokines and endothelial function as compared to the normal group. Moreover, co-administration of the tested plant's extracts at the tested dose to HF-HFD fed rats reduced the development of indicators of metabolic syndrome when compared to the metabolic syndrome group. **Conclusion:** The administered plant leaves water extracts at the tested dose could improve the features of metabolic syndrome. Rosemary leaves water extract has more effect in comparison with the other extracts.

Key words: Rosemary, moringa, purslane, HF-HFD, metabolic syndrome

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Metabolic syndrome (MetS) is defined as a condition of visceral obesity and at least two of the following parameters: hyperglycemia, high levels of triacylglycerols (TAGs), low levels of high-density lipoprotein cholesterol (HDL-C) and high blood pressure (BP). Thus, MetS is a complex of metabolic, hormonal and hemodynamic disorders that raises the risk of type 2 diabetes, cardiovascular diseases and fatty liver disease[1].

International Diabetes Federation (IDF) reported that 25-68% of people globally suffer from MetS. Major contributing factors in the development of MetS are abdominal obesity and insulin resistance caused by the trend of consuming fast foods containing high content of saturated fat and carbohydrates[2]. It was shown that animal fats (beef tallow), in addition to, fructose which is widely used as a sweetener in many processed foods and beverages are more efficient for MetS modelling[3]. Such a diet mimics the nutrition of a modern person and is considered the most satisfactory for MetS modelling and the production of the pathogenetic features and the phenomenology of metabolic disorders in MetS, causing oxidative stress and pro-inflammatory responses[4]. The oxidative and inflammatory processes of the metabolic syndrome can be disrupted by phytochemicals found in medicinal plants[5].

Moringa, *Moringa oleifera* Lam. belonging to *Moringaceae* family, is reputed as "Miracle tree" because all the parts fruits, seeds and leaves are nutritionally rich in protein, macro and micronutrients, vitamins, anti-oxidants and show medicinal effects. Moreover, the bioavailability of the nutrients and vitamins from moringa is high especially of vitamin-A precursor and iron. This species has been found appropriate for human as well as animal consumption and its leaves have been proved to demonstrate anti-hyperlipidemic, anti-oxidant, anti-inflammatory and anti-diabetic properties[6].

Rosemary, *Rosmarinus officinalis* L. belonging to *Labiatae* family, is an evergreen perennial shrub grown native to southern Europe and Asia especially the Mediterranean region[7]. The properties of this plant exist in the richness of active principles that influence almost all organs of the human body. Moreover, it is a rich source of phenolic phytochemicals having significant hypoglycemic, antioxidant, anti-inflammatory, hypolipidemic, anti-atherosclerotic, hypotensive, hepatoprotective and hypocholesterolemic effects[8].

Purslane, *Portulaca oleracea* L. belonging to the *Portulacaceae* family, is an annual herb that is widely spread around the world. Purslane is a nutritious vegetable with high anti-oxidant effects. It is rich in nutrients such as glucose, it is considered an excellent source of α-linolenic acid, essential omega-3 fatty acids, α-tocopherol, ascorbic acid and β-carotene[9]. Purslane leaves include many bioactive compounds like flavonoids and alkaloids which have a variety of pharmacological properties such as anti-oxidant, hypolipidemic, hypoglycemic and anti-inflammatory[10].

The study aimed to investigate the effect of *Moringa oleifera*, *Rosmarinus officinalis* and Portulaca oleracea leaves water extracts on hyperglycemia, hyperlipidemia and insulin resistance in association with some inflammatory markers and oxidative stress in metabolic syndrome induced by high fat-high fructose diet in rats.

MATERIALS AND METHODS

Study site: The study was carried out in an animal house at Biochemistry and Nutrition Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Egypt in November, 2019.

Preparation of water extracts of moringa, rosemary and purslane leaves and determination of phenolic compounds: Leaves of moringa, rosemary and purslane were purchased from the Ministry of Agriculture in Giza, Egypt. Fresh leaves of moringa, rosemary and purslane were collected, dried in the solar furnace at 60°C and grounded by an electric mill to a fine powder then 500 g of powder were soaked in (1.5 L) boiling water. The containers with these contents were undergoing shaking and stirring for 1 h then were left overnight at room temperature (18-22°C). These extracts were filtered through gauze cloth. The filtrates were concentrated at 50°C under reduced pressure using a vacuum pump rotary evaporator to afford a greenish mass of leaves extracts[11-13]. The water extracts of moringa, rosemary and purslane leaves were analyzed for total phenolic compounds in the National Research Centre, Egypt, using HPLC analysis[14].

Diets: The diets used in the present study were, balanced diet prepared as adjusted by Navarrete *et al.*[15] and high fat-high fructose diet containing 21% fat (beef tallow) and 30% fructose prepared according to Lozano *et al.*[16]. Fructose powder was purchased from El-Gomhoreya for Chemicals Company, Cairo, Egypt.

Animal trial: The experimental animals used throughout the present work were 50 adult male albino rats, *Sprague-Dawley* strain weighing 140-160 g, were obtained from El-Salam farm, Giza, Egypt. All rats were randomly housed individually in constantly controlled environments, temperature 25±5°C, humidity 55±10% and 12/12 hrs light/dark cycle were held. All rats were adapted for 7 days, then the animals were divided into 5 groups (10rats/group), the first and second groups served as control groups were fed on a balanced diet and F-HFD, respectively, for 12 weeks and received daily oral doses of distilled water by gastric tube. The other three groups were fed on HF-HFD and orally administered 200 mg kg^{-1} b.wt., daily of plant leaves water extracts by gastric tube for 12 weeks, in the following manner: group (3): Moringa[17], group (4): Rosmary[18] and group (5): Purslane[19].

The blood glucose level was determined the day before scarification after 8 hrs of fasting using (Glucometer 4 Accu-Chek, CA), all rats were weighed and the length was recorded for calculation of BMI and Lee index. The animals were sacrificed under sodium barbiturate anaesthesia and blood samples were collected to separate serum. Visceral adipose tissue and liver were collected using sterile scissors and forceps and washed with 0.9% sterile saline.

Anthropometric measurements: BMI and Lee index was calculated using equations as described by Novelli *et al.*[20] and adiposity index was calculated using the equation as described by Fernandez *et al.*[21]:

$$\text{BMI}\left(\text{g cm}^{-2}\right) = \frac{\text{Body weight (g)}}{\text{Length 2 (cm}^2\text{) from nose to anus}}$$

$$\text{Lee index} = \frac{\sqrt[3]{\text{Body}}\ \text{weight (g)}}{\text{Length (cm) from nose to anus}}$$

$$\text{Adiposity index} = \frac{\text{Weight of visceral fat (g)}}{\text{Final body weight (g)}} \times 100$$

Biochemical analysis: Serum insulin and glucagon-like peptide-1 (GLP-1) levels were determined by the ELISA kits (BioSource, Europe S.A., Nivelles and Belgium) Cat. No.: MBS724709 and (RayBiotech, Inc., Norcross, GA, United States) CAT.: # EIA-GLP1, EIAM-GLP1, EIAR-GLP1, respectively. Serum Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated through the following Eq.[22]:

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting blood glucose level}\left(\text{mg dL}^{-1}\right) \times \text{Fasting insulin level}\ \left(\text{ng mL}^{-1}\right)}{405}$$

Serum-free fatty acids (FFAs), apolipoprotein A1 (Apo A1) and apolipoprotein B (Apo B) levels were determined by ELISA kits: (Bioassay Technology Laboratory, Shanghai. China) Cat. No.: E0738Ra, (Cusabio kit, USA) CAT. No.: CSB-E08105r and Life span Biosciences, Inc. (Seattle, WA, USA) CAT. No.: LS-F4843, respectively. Serum leptin, adiponectin, tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and protein carbonyl content levels (PCC) were determined by ELISA kit (Cusabio kit, USA) CAT. No.: CSB-E07433r, (Ray Biotech, Inc., Norcross, GA, United States) CAT.: ELR-Adiponectin, (Cusabio kit, USA) CAT. No.: CSB-E11987r, (Quantikine Elisa USA and Canada, R and D Systems, Inc.) CAT. No.: R6000B and (Bio Source, Europe S.A., Nivelles and Beligium) CAT. No.: MBS726854, respectively. Serum triacylglycerols (TAGs), Total Cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) levels were measured using the enzymatic colorimetric assay kits according to manufacturer technique.

Liver Nitric Oxide (NO) level, malondialdehyde (MDA) level, reduced glutathione (GSH), catalase (CAT) EC 1.11.1.6 activity and total antioxidant capacity (TAOC) level were measured using the enzymatic colorimetric assay kits according to manufacturer technique.

Gene expression measurement of eNOS in liver tissue by RT-qPCR: Gene expression measurement of mRNA levels of Endothelial Nitric Oxide Synthase (eNOS) and the housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in liver tissue was done using the real-time quantitative polymerase chain reaction (RT-qPCR) CAT. No. # K0251. Total RNA was extracted from 100 mg of each liver tissue sample using TRIzol total RNA extraction reagent according to the methodology of TRIzol kit CAT. No.: 15596026. Reverse transcription of the extracted mRNA was done by using Revert Aid First Strand cDNA Synthesis Kit CAT. No.: #K1621. Relative quantification of expressed genes calculated according to Derveaux *et al.*[23] as follows:

$$R = 2^{-\Delta\Delta Ct}$$

The primer sequences (forward and reverse) used for eNOS and GAPDH were designed as described by Zhao *et al.*[24]

Table 1: Forward and reverse sequences of the primers used for eNOS and GAPDH genes

Gene	Primers	
eNOS	Forward Sequence	5-TATTTGATGCTCGGGACTGC-3
	Reverse sequence	5-AAGATTGCCTCGGTTTGTTG-3
GAPDH	Forward Sequence	5-TGGAGTCTACTGGCGTCTT-3
	Reverse sequence	5-TGTCATATTTCTCGTGGTTCA-3

eNOS: Endothelial nitric oxide synthase, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

and Mohammadi *et al.*[25], respectively. The sequences of the designed primers were presented in Table 1.

Statistical analysis: Data were statistically analyzed by Statistical Package for Social Science (SPSS) version 16.0. Values were presented as Mean±Standard Error (SE). Statistical differences between groups were performed using one way ANOVA, the mean difference was significant at the level ($p<0.05$) according to Levesque[26].

RESULTS

HPLC analysis of bioactive components in moringa, rosemary and purslane leaves water extracts: The phenolic compounds identified in moringa leaves water extract, were 30% rutin, 21% apigenin-7-glucoside, 18% caffeic acid, 11% p-hydroxybenzoic acid, 5% catechin, 5% p-coumaric acid, 4% gallic acid, 3% protocatechuic acid, 2% chlorogenic acid and 1% syringic acid as shown in Fig. 1. In the case of rosemary leaves water extract, the phenolic compounds identified were 58% rosmarinic acid, 21% apigenin-7-glucoside, 6% protocatechuic acid, 5% rutin, 4% p-coumaric acid, 3% chlorogenic acid, 2% cinnamic acid and 1% quercetin as shown in Fig. 2. The phenolic compounds identified in purslane leaves water extract were 28% gentisic acid, 19% rutin, 19% p-hydroxybenzoic acid, 18% apigenin-7-glucoside, 6% chrysin, 4% chlorogenic acid, 3% protocatechuic acid, 1% gallic acid, 1% caffeic acid and 1% ferulic acid as shown in Fig. 3.

Effects of moringa, rosemary and purslane leave water extracts' oral doses on anthropometric measurements and glucose hemostasis parameters: The obtained results in Table 2 showed that the BMI, Lee index and the adiposity index were significantly ($p<0.05$) increased in the metabolic syndrome group. BMI value, which is considered as an indicator for obesity, was elevated from 0.581±0.018 g cm^{-2} in the normal group to 0.756±0.016 g cm^{-2} in the metabolic syndrome group.

Long term consumption of HF-HF diet gradually deteriorates insulin activity leading to insulin resistance which results in higher values in blood glucose 120.48±1.09 mg dL^{-1} and insulin 6.61±0.036 ng mL^{-1} concentrations as well as in HOMA IR index 1.97±0.007 have been observed in the metabolic syndrome group when compared to the normal group values (79.45±0.569 mg dL^{-1}, 2.15±0.029 ng mL^{-1} and 0.424±0.006, respectively). While, the GLP-1 level was significantly decreased in the metabolic syndrome group as compared to the normal group.

On the other hand, the groups orally treated with moringa, rosemary and purslane leave water extracts showed a significant decrease in obesity indicators BMI, lee index and adiposity index when compared to the metabolic syndrome group. The BMI value was declined to 0.636±0.015, 0.636±0.016 and 0.661±0.006 g cm^{-2}, respectively. Lee index and adiposity index were also reduced in the three orally treated groups when compared to the metabolic syndrome group. The results demonstrated a non-significant difference between the tested plant's water extracts in these parameters.

The parameters of glucose homeostasis were significantly improved in all the groups orally treated with moringa, rosemary and purslane leave water extracts. However, the best results were obtained by rosemary leaves water extract, where the glucose, insulin and HOMOA IR index values were decreased (88.52±0.885 mg dL^{-1}, 2.26±0.047 ng mL^{-1} and 0.494±0.010, respectively) while the GLP-1 level was increased.

Effects of moringa, rosemary and purslane leave water extracts' oral doses on lipids profile: The statistical analysis of data in Table 3, clarified that high fat-high fructose diet caused an alteration in lipid profile resulting in dyslipidemia which is manifested by a significant ($p<0.05$) increase in TAGs, TC, LDL-C, FFAs and apolipoprotein B levels as compared to the normal group and a significant ($p<0.05$) decrease in high-density lipoprotein-cholesterol and apolipoprotein A1 levels when compared to the normal group. Values of TAGs and LDL-C were increased from 106.35±1.24 and 80.65±2.04 mg dL^{-1}, respectively in the normal group to 137.40±0.924 and 121.64±1.62 mg dL^{-1}, respectively in the metabolic syndrome group. However, the HDL-C value was decreased in the metabolic syndrome group (37.84±0.436 mg dL^{-1}) as compared to the normal group (62.76±0.703 mg DL^{-1}).

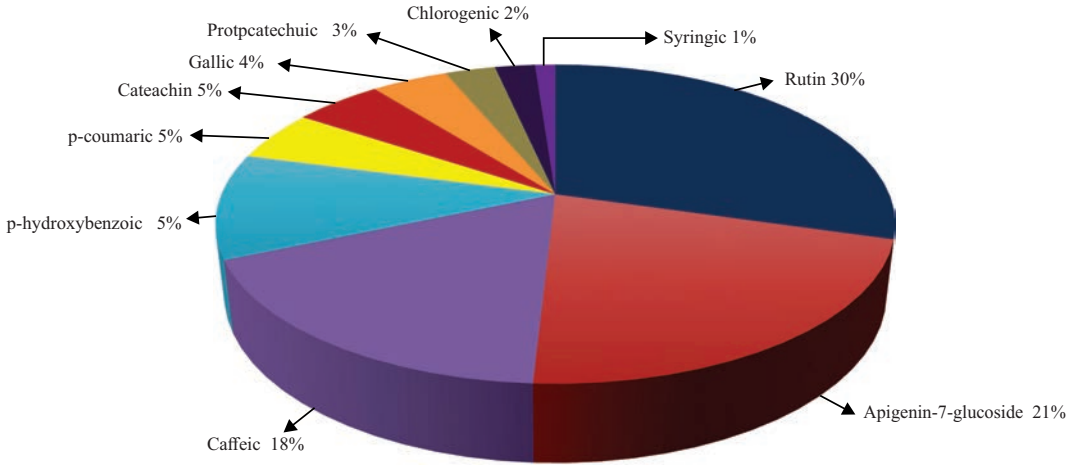

Fig. 1: Phenolic compounds in moringa leaves water extract (µg mL^{-1})

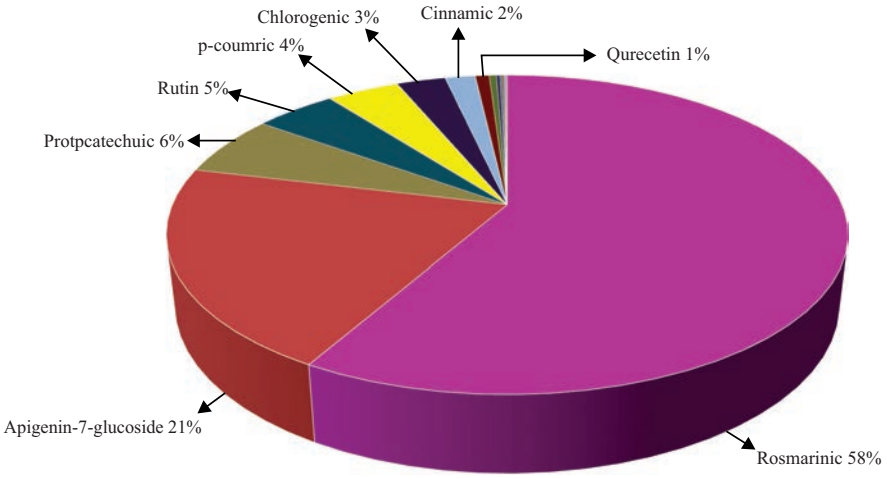

Fig. 2: Phenolic compounds in rosemary leaves water extract (µg mL^{-1})

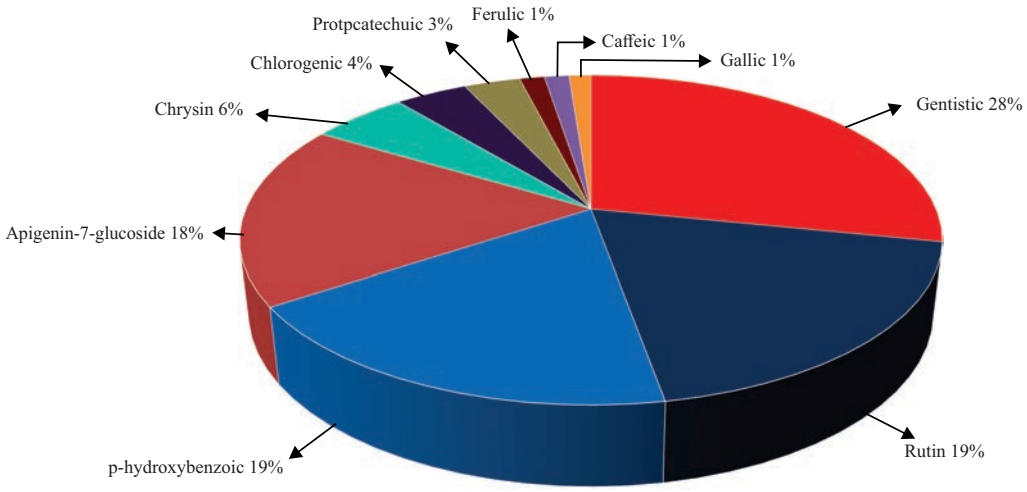

Fig. 3: Phenolic compounds in purslane leaves water extract (µg mL^{-1})

Table 2: Effects of moringa, rosemary and purslane leaves water extracts' oral doses on anthropometric parameters and glucose hemostasis in metabolic syndrome rats

Groups parameters	Normal	Metabolic syndrome	HF-HFD+moringa	HF-HFD+rosemary	HF-HFD+purslane
BMI (g cm^{-2})	0.581±0.018^{c}	0.756±0.016^{a}	0.636±0.015^{b}	0.636±0.016^{b}	0.661±0.006^{b}
Lee index ($\sqrt[3]{g}$/cm)	0.309±0.003^{c}	0.343±0.003^{a}	0.322±0.002^{b}	0.320±0.002^{b}	0.323±0.002^{b}
Adiposity index (g %)	2.39±0.121^{b}	3.39±0.173^{a}	2.32±0.239^{b}	2.51±0.233^{b}	2.16±0.139^{b}
Glucose (mg dL^{-1})	79.45±0.569^{c}	120.48±1.09^{a}	88.50±1.24^{b}	88.52±0.885^{b}	90.99±1.03^{b}
Insulin (ng mL^{-1})	2.15±0.029^{e}	6.61±0.036^{a}	3.81±0.019^{c}	2.26±0.047^{d}	3.97±0.027^{b}
HOMA-IR index	0.424±0.006^{e}	1.97±0.007^{a}	0.832±0.011^{c}	0.494±0.010^{d}	0.891±0.013^{b}
GLP-1 (pg mL^{-1})	233.52±2.09^{a}	108.39±1.40^{e}	200.43±2.39^{c}	216.78±0.539^{b}	194.18±0.606^{d}

Values expressed as Mean±SE. There was no significant difference between means have the same superscript in the same row at p<0.05

Table 3: Effects of moringa, rosemary and purslane leaves water extracts oral doses on lipids profile in metabolic syndrome rats

Groups parameters	Normal	Metabolic syndrome	HF-HFD+moringa	HF-HFD+rosemary	HF-HFD+purslane
TAGs (mg dL^{-1})	106.35±1.24^{c}	137.40±0.924^{a}	119.31±0.912^{b}	118.66±0.547^{b}	119.78±0.877^{b}
TC (mg dL^{-1})	134.60±1.05^{d}	212.56±1.89^{a}	161.31±1.64^{b}	159.89±1.77^{b}	154.18±1.85^{c}
LDL-C (mg dL^{-1})	80.65±2.04^{c}	121.64±1.62^{a}	107.77±2.03^{b}	104.60±0.868^{b}	106.33±0.831^{b}
HDL-C (mg dL^{-1})	62.76±0.703^{a}	37.84±0.436^{d}	50.37±0.857^{b}	48.36±0.679^{c}	46.82±0.681^{c}
FFAs (μmol L^{-1})	59.78±0.686^{e}	181.50±0.151^{a}	102.90±0.275^{c}	91.25±0.669^{d}	131.53± 0.596^{b}
APO-B (ng mL^{-1})	38.94±0.948^{e}	91.25±0.564^{a}	67.50±0.233^{c}	73.80±0.265^{b}	65.38±0.402^{d}
APO-A1 (μg mL^{-1})	4.74±0.075^{a}	1.93±0.023^{d}	2.40±0.041^{c}	3.77±0.030^{b}	2.34±0.055^{c}

Values expressed as Mean±SE. There was no significant difference between means have the same superscript in the same row at p<0.05, TAGs: Triacylglycerols, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, FFAs: Free fatty acids, TC: Total cholesterol

Table 4: Effects of moringa, rosemary and purslane leaves water extracts' oral doses on adipokines and inflammatory markers in metabolic syndrome rats

Groups parameters	Normal	Metabolic syndrome	HF-HFD+moringa	HF-HFD+rosemary	HF-HFD+purslane
Leptin (ng mL^{-1})	0.702±0.005^{d}	3.23±0.042^{a}	1.05±0.007^{c}	1.02±0.006^{c}	1.40±0.024^{b}
Adiponectin (ng mL^{-1})	12.83±0.089^{a}	5.70±0.106^{e}	9.84±0.059^{c}	12.36±0.010^{b}	7.59±0.038^{d}
TNF-α (pg mL^{-1})	14.02±0.251^{e}	91.87±0.589^{a}	35.77±0.581^{c}	28.43±0.422^{d}	50.11±0.364^{b}
IL-6 (pg mL^{-1})	34.53±0.497^{e}	131.57±0.729^{a}	64.20±0.169^{c}	49.74±0.688^{d}	97.51±0.939^{b}

Values expressed as Mean±SE. There was no significant difference between means have the same superscript in the same row at p<0.05

It is obvious that oral administration of moringa, rosemary and purslane leaves water extracts had a hypolipidemic effect reflected by a significant (p<0.05) reduction the in levels of TAGs, TC, LDL-C, FFAs and APO-B level as compared with the metabolic syndrome group in addition to a significant (p<0.05) increase in the levels of HDL-C as well as APO-A1 when compared to metabolic syndrome group.

Rosemary leaves water extract markedly improved FFAs and apo-A1 levels, moringa leaves water extract had the best effect on increasing HDL-C level 50.37±0.857 mg dL^{-1}. In addition, purslane leaves water extract showed the greatest decrement in serum cholesterol and apo-B levels.

Effects of moringa, rosemary and purslane leave water extracts' oral doses on adipokines and inflammatory markers: Results in the Table 4 demonstrated that consumption of HF-HFD for 12 weeks induced an increment in adipose tissue and can cause tissue hypoxia, which could impair the production and release of obesity regulatory hormones, such as leptin, adiponectin and exacerbate inflammation, resulting in a significant (p<0.05) elevation of leptin and pro-inflammatory cytokines (TNF-α and IL-6) levels. Leptin level had increased in metabolic syndrome group to 3.23±0.042 ng mL^{-1} when compared to normal group (0.702±0.005 ng mL^{-1}) and a significant (p<0.05) decrease in adiponectin level (5.70±0.106 ng mL^{-1}) in metabolic syndrome group when compared to normal group (12.83±0.089 ng mL^{-1}). In addition, the TNF-α level was increased from 14.02±0.251 pg mL^{-1} in the normal group to 91.87±0.589 pg mL^{-1} in the metabolic syndrome group, similar results were found in the IL-6 level.

Oral treatment with moringa, rosemary and purslane leaves water extracts showed a significant reduction in leptin level, inflammatory markers and increase in adiponectin level. The best increment in adiponectin level was achieved by rosemary leaves water extract, reaching a value of 12.36±0.01 ng mL^{-1} and showed the maximum decrease in the values of leptin 1.02±0.006 ng mL^{-1}, TNF-α 28.43±0.422 pg mL^{-1} and IL-6.

Effects of moringa, rosemary and purslane leave water extracts' oral doses on the gene expression of eNOS in liver tissue: Results in Table 5 showed that the high fat-high fructose-fed group had a significant (p<0.05) down regulation of liver eNOS mRNA expression by 0.41 fold as compared to the normal diet-fed group. Whereas oral administration of

Table 5: Effects of moringa, rosemary and purslane leaves water extracts' oral doses on the gene expression of endothelial nitric oxide synthase (eNOS) in the liver in metabolic syndrome rats

Parameter groups	Relative gene expression of (eNOS)/GAPDH)
Normal	1.00±0.00^{d}
Metabolic syndrome	0.41±0.020^{e}
HF-HFD+moringa	2.81±0.277^{b}
HF-HFD+rosemary	2.06±0.242^{c}
HF-HFD+purslane	3.78±0.245^{a}

Values expressed as Mean±SE. There was no significant difference between means have the same superscript at p<0.05

Table 6: Effects of moringa, rosemary and purslane leaves water extracts' oral doses on oxidative stress and antioxidant biomarkers in metabolic syndrome rats

Groups parameters	Normal Group (1)	Metabolic syndrome Group (2)	HF-HFD+moringa Group (3)	HF-HFD+rosemary Group (4)	HF-HFD+purslane Group (4)
PCC (ng mL^{-1})	25.76±0.460^{e}	116.21±0.689^{a}	69.30± 0.677^{c}	46.39±0.439^{d}	87.17±0.614^{b}
NO (µmol g^{-1})	11.99±0.410^{d}	47.82±0.793^{a}	30.61±0.442^{bc}	30.10± 0.325^{c}	31.93±0.483^{b}
MDA (nmol g^{-1})	2.86±0.043^{e}	5.79±0.082^{a}	3.76±0.131^{d}	4.63± 0.114^{b}	4.16±0.101^{c}
GSH (mg g^{-1})	10.81±0.375^{a}	2.76±0.108^{d}	6.08±0.249^{c}	8.35±0.276^{b}	8.11±0.171^{b}
CAT (U g^{-1})	0.912±0.022^{a}	0.348±0.009^{d}	0.751±0.007^{c}	0.842±0.006^{b}	0.753±0.007^{c}
TAOC (mmol g^{-1})	2.43±0.031^{a}	1.08±0.015^{c}	1.78±0.029^{b}	1.75±0.019^{b}	1.73±0.030^{b}

Values expressed as Mean±SE. There was no significant difference between means have the same superscript in the same row at p<0.05

moringa, rosemary and purslane leave water extracts showed a significant (p<0.05) up regulation of liver eNOS mRNA expression when compared to the metabolic syndrome group. Purslane leaves water extract increase liver eNOS mRNA expression by 3.78 fold, followed by moringa leaves water extract by 2.81 fold then rosemary leaves water extract by 2.06 fold as compared to the normal group.

Effects of moringa, rosemary and purslane leave water extracts' oral doses on oxidative stress and antioxidant biomarkers in metabolic syndrome rats: The current results in Table 6 showed that high fat-high fructose diet-fed rats for 12 weeks increased oxidative stress biomarkers such as in protein carbonyl content, nitric oxide and malondialdehyde levels when compared to the normal group. The values of PCC and MDA were increased to (116.21±0.689 ng mL^{-1} and 5.79±0.082 nmol g^{-1}, respectively) from the normal group values (25.76±0.460 ng mL^{-1} and 2.86±0.043 nmol g^{-1}, respectively). On the other hand, the anti-oxidant biomarkers GSH level, CAT activity and TAOC level were reduced in the metabolic syndrome group as compared to the normal group. The value of TAOC was decreased to 1.08±0.015 mmol g^{-1} from the value of (2.43±0.031 mmol g^{-1}) in the normal group.

Oral administration of moringa, rosemary and purslane leaves water extracts concurrently with HF-HFD resulted in a significant reduction in oxidative stress parameters and amelioration in antioxidant biomarkers. It is concluded from the results that rosemary leaves water extract had the best effect on lowering protein carbonyl content value 46.39±0.439 ng mL^{-1} and elevating catalase activity. In addition, rosemary showed the maximum effect in reducing glutathione levels and reducing NO levels. While moringa leaves water extract markedly decreased MDA level 3.76±0.131 nmol g^{-1}.

DISCUSSION

This study demonstrates that oral administration of moringa, rosemary and purslane leaves water extracts at the tested dose to adult male rats fed a diet rich in saturated fat and fructose for 12 weeks has a protective effect against the development of metabolic syndrome characteristics, glucose intolerance, insulin resistance, visceral fat accumulation, dyslipidemia, inflammation and oxidative stress.

The present study indicated the high phenolic content of the tested plant leaves water extract. The analysis of the moringa leaves water extract was supported by the results reported by Divi *et al.*[27] and Khan *et al.*[28]. Afonso *et al.*[29] reported that the major phenolic compound identified in rosemary leaves water extract is rosmarinic acid and Ramadan *et al.*[13] announced the presence of phenolic compounds in purslane leaves water extract nearly similar to our results.

Rats fed on the HF-HF diet for 12 weeks developed the signs of metabolic syndrome including abdominal obesity, hyperglycemia and insulin resistance. These results go hand in hand with Wong *et al.*[30]. Rats fed on HF-HF diet had higher daily energy intake resulting in increased body weight and higher fat mass reflected in increased anthropometric measurements[31]. This can be explained by the fact that fructose favours the buildup of triacylglycerols and cholesterol in the liver because of its lipogenic properties, consequently

leading to diminished insulin sensitivity and insulin resistance and glucose intolerance will result[32]. Also, beef tallow is very effective in stimulating hyperglycemia, insulin resistance and dyslipidemia by increasing the free fatty acids concentration in the blood[33]. Moringa, rosemary and purslane leave water extracts dramatically improved anthropometric measurements as well as glucose hemostasis parameters. Similar results were obtained by Othman *et al.*[34], Shatla *et al.*[35] and El-Dreny[36]. Moringa reduces the uptake of glucose by the intestines and the skeletal muscle. Phenolic compounds found in moringa leaves water extract was reported to improve insulin activity[37].

Rosmarinic Acid (RA) found in rosemary leaves water extract dramatically lessened gluconeogenesis in the liver. Additionally, elevated GLUT4 expression, both mechanisms decrease glucose output by the liver and increase glucose uptake by skeletal muscles reducing blood glucose, serum insulin levels and HOMA-IR[38]. Purslane mechanism of action is multifaceted including increased insulin secretion from pancreatic β-cells or could be attributable to the free radical scavenging properties to protect β- cells from destruction[39].

Rats fed on HF-HF diet for 12 weeks developed dyslipidemia, the data were in parallel with Altiner *et al.*[40] and Jensen *et al.*[41] studies. HF-HF diet causes dyslipidemia as fructose induces hepatic lipogenesis as it provides substrates for lipid synthesis and fatty acid oxidation inhibition, which in turn raises the production and secretion of Very-Low-Density Lipoproteins (VLDL). This VLDL is high in TAGs that can be hydrolyzed by the lipoprotein lipase (LPL) and stored by the adipose tissue, resulting in obesity[42]. The current study revealed that moringa, rosemary and purslane leave water extracts had improved the serum lipids profile in rats fed HD-HFD. These findings are in line with those reported by Bais *et al.*[43], Quirarte-Báez *et al.*[8] and El-Dreny[36]. Moringa leaves include various types of antioxidant compounds such as ascorbic acid, carotenoids, flavonoids and phenolics. Therefore, moringa could inhibit the oxidization of LDL-C with the consequent rise in HDL-C level[44]. Moringa reduces intracellular cholesterol and HMG CoA reductase activity in liver cells as it can diminish intracellular cholesterol[45]. Hypolipidemic effect of rosemary leaves water extract might be due to inhibition of pancreatic lipase activity and gastric lipase activity, therefore, reducing digestion and absorption of fat[46]. Besides, the anti-oxidant effects of rosemary extract impeded oxidative stress induced by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase activity, thus reducing cholesterol synthesis. In addition, polyphenols were demonstrated to promote faecal excretion of total cholesterol and bile acids[47]. Oral treatment with purslane leaves water extract produces a marked improvement of lipids profile possibly due to its contents of flavonoids, which have been shown to possess hypolipidemic and anti-oxidant properties[48].

Results from this study indicated that the HF-HF diet significantly raised leptin level while reduced adiponectin level, eliciting a local inflammatory response in male rats[49,50]. The consumption of the HF-HF diet increased fat mass and subsequently a positive trigger for more leptin production by fat tissues. Mechanistically, the impairment in leptin transportation, reduction in leptin signalling and chronic inflammatory state are the common causes of obesity-associated leptin resistance[51]. The decline in adiponectin level in the animals fed on HF-HF diet might be one of the contributing factors for the imbalance in blood glucose and energy profile leading to MetS[52]. Oral treatment with moringa, rosemary and purslane leaves water extracts elicited a significant improvement in some appetite-related hormones and inflammatory markers, these findings were in parallel with Metwally *et al.*[53], Bai *et al.*[54] and Rahimi *et al.*[10]. Moringa leaves act as a good source of anti-oxidants which could scavenge free radicals with consequent inhibition of leptin level as there is a significant positive correlation between leptin level and Reactive Oxygen Species (ROS) generation[44]. Moringa produced a marked increase in adiponectin levels it has an anti-inflammatory capacity and it can inhibit the TNF-α level. This may be due to the presence of the anti-inflammatory compounds which may elicit an improvement in the adiponectin level in rats via inhibition of TNF-α level[55]. Coumaric acid in rosemary leaves water extract down regulated the expression of adipogenic transcription factors, C/EBPα, PPARγ and leptin and then up-regulated expression of adiponectin[56]. The RA was able to diminish the systemic release of pro-inflammatory cytokines and lower organ dysfunction marks by controlling NF-κB and metalloproteinase-9[57]. Caffeic acid and ferulic acid present in purslane leave water extract have shown the capacity to secrete adiponectin in 3T3-L1 adipocytes. It can adjust adiponectin secretion via inhibition of nuclear factor-kappa during the inflammatory process[58].

Rats fed HF-HF diet develop hyperinsulinemia, hypertriacylglyceridemia and endothelial dysfunction, this finding was consistent with Yoo *et al.*[59]. HF-HF feeding induces endothelial dysfunction by increased NO inactivation, secondary to enhanced formation of superoxide and diminished vascular relaxation via impaired eNOS activity caused by relative insufficiency of tetrahydrobiopterin (a cofactor of NO synthase) in endothelial cells[60]. Oral administration of moringa, rosemary and purslane leaves

water extracts had a role in the upregulation of eNOS mRNA expression in HF-HF fed rats, these findings were in line with Sierra-Campos *et al*.[61], Karthik *et al*.[62] and Amin *et al*.[63] studies. Flavonoids such as rutin and apigenin which are abundant in the tested plants have multiple cardiovascular protective effects. The studies showed that in oxidative stress, rutin successfully induced cells' NO production, promoted NO production by inducing eNOS gene expression and eNOS protein synthesis[64]. The RA (a major phenolic acid in rosemary leaves water extract) has a cardioprotective effect and blood pressure-lowering effect by stimulating NO production and down regulating vasoconstrictor endothelin (ET)-1[62].

A significant increase in protein carbonylation, nitric oxide and lipid peroxidation levels and decreased levels of anti-oxidants could be found in long term HF-HF feeding[65]. Excess intake of fat and/or fructose could influence the *in vivo* antioxidant status, which might be one of the key contributing factors of metabolic disorders[66]. The findings of the current study revealed that moringa, rosemary and purslane leave water extracts displayed protection against HF-HFD induced oxidative stress and maintained remarkable levels of anti-oxidants in the liver and blood. This coincides with the study of Othman *et al*.[34], Goncalves *et al*.[67] and Djellouli *et al*.[68]. Moringa leaves water extract to possess strong radical scavenging and antioxidant activities. They inhibit lipid peroxidation by acting as chain-breaking peroxyl radical scavengers[69]. Rosemary leaves water extract has been reported to possess antioxidant activity, exhibited significant radical-scavenging activity probably due to its high content of RA[18]. Purslane leaves water extract is rich in phenolic compounds which act against oxidative stress[70].

CONCLUSION

In conclusion, feeding male rats a high fat-high fructose diet for 12 weeks noticeably induced the features of metabolic syndrome. Moringa, rosemary and purslane leave water extracts produced different physiological responses to alleviate the symptoms of metabolic syndrome at the same dosage. Rosemary leaves water extract showed the highest reduction of metabolic syndrome different features owing to its high content of rosmarinic acid, which is a potent anti-oxidant, followed by moringa leaves water extract which increased HDL-C and decreased lipid peroxidation as well as leptin level. On the other hand, purslane leaves water extract showed pronounced hypocholesterolemic and vasodilating effects. Regular dietary intake of these plants could represent a potential strategy to reduce the risk of metabolic syndrome.

SIGNIFICANCE STATEMENT

This study discovers the beneficial impact of moringa, rosemary and purslane leave water extracts administration against MetS and its related conditions of adiposity, insulin resistance, hyperlipidemia, inflammation and oxidative stress. This might have a significant impact as protective means against the development of MetS, type 2 diabetes mellitus, CVD and hypertension. This study will help the researchers to uncover the critical areas of herbal and medicinal plants that many researchers were not able to explore. As a consequence, this study will open a modern approach for the researchers to discover safer and more efficient protective ways against metabolic syndrome and its related diseases.

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