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Influence of Dietary Rocket Leaves on Diuresis and Urinary Electrolytes Excretion in High Fat Diet-Induced Obese Rats

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ABSTRACT
Green vegetables, as a dietary component are very important for health and constitute a group of the lowest calorie raw produce with a high nutritional value. It has been suggested that Eruca sativa leaves exert a beneficial hypolipidemic effect in rats. In present study, we evaluate the effect of methanolic extract of Eruca sativa leaves (MEESL) on serum cholesterol, urinary volume and electrolyte excretion in high fat diet (HFD)-induced obese rats. Rats were fed with high fat diet (normal diet + 1% cholesterol) for 4 weeks to induce obesity. Twenty four adult male rats were randomly divided into 4 groups (n=6). The 1st group was fed with normal pellet diet and served as control. Groups 2, 3 and 4 were feed with 1% cholesterol mixed pellet diet for 4 weeks.Group 3 and 4 were administered with MEESL in two doses of 250 and 500 mg/kg, for 4 weeks. Serum cholesterol, urine volume (ml), pH, conductivity and urinary electrolytes (sodium, potassium and chloride) excretion were evaluated in all rats. HFD resulted in significantly increased cholesterol level in serum and decreased in urine volume and urinary electrolytes excretion (Na, K and Cl) in obese rats. Orally administered MEESL significantly restore the altered parameters in HFD fed rats. The obtained results demonstrate theanti-hypercholesterolemic, diuretic, and saliuretic activity of dietary rocket leaves, in HFD induced obese rats.
***Keywords**: Cholesterol, Diuretic, Eruca sativa, Electrolytes,Obesity*

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INTRODUCTION

Hypercholesterolemia is a well-known risk factor in the development of atherosclerosis. There are strong evidences that hypercholesterolemia increases the production of reactive oxygen species [1, 2], which may play an important role in the pathogenesis and/or progression of several disorders. Diets rich in fat not only induce obesity in humans but also make animals obese. Hyperobese patients (BMI>60 kg/m^2) are at significant risk of acute renal dysfunction from fluid shifts and hypovolaemia. Urine output in these patients often falls well.

Since several years, plants have been used as a source of traditional medicine for the treatment of several diseases. The known lipid lowering drugs, such as fibrates, statins and bile acid binding resins have many side effects [3]. Thus, there is a considerable interest on development of lipid lowering drugs with diuretic activity from natural products for the treatment of obese people with water and sodium retention problem.

In previous literature, pharmacological activities of *Eruca sativa* (Brassicaceae) have been reported moderately related to their strong antioxidant properties [4, 5]. *Eruca sativa* is widely used in folklore medicine as a remedy of renal ailments. It's antihyperlipidemic, antihyperglycemic, and hepatoprotective activities have also been reported [6]. Sarwar *et al* reported that *Eruca sativa* produced potent antioxidant and renal protective activities in rats [7]. In previous research, we reported diuretic effect of rocket leaves in diabetic rats [8]. Therefore, the present study was designed to evaluate the diuretic effects of *Eruca sativa* leaves extracts in high fat diet induced obese rats.

MATERIALS AND METHODS

Plant Material and Extraction

The fresh leaves of *Eruca sativa* were purchased from local vegetable market, Saudi Arabia, and identified by expert taxonomist. Leaves were shade dried, coarsely powdered, and extracted with methanol at room temperature for 72 h (percolation method). The collected percolate was dried and stored for diuretic activity.

Cholesterol Supplemented Feed and Induction of Obesity

The normal pellet diet was crushed, cholesterol (1%w/w) powder thoroughly mixed; the pellets reconstituted with water and dried properly to avoid any fungal contamination. Dried cholesterol mixed pellet diet was feed to rats for 4 weeks to induced obesity.

Diuretic Activity

Twenty four adult male rats (180-200g) were randomly divided into four groups (n=6). The rats were procured from the Animal Care Unit, College of Pharmacy, Prince Sattam bin Abdulaziz University, Kingdom of Saudi Arabia and acclimatized for 7 days, under standard hygienic conditions. Standard rodent pellet diet and water were provided *ad libitum.* Group 1 was served as normal control. Group 2 was fed with HFD for 4 weeks and served as HFD only. Rats in groups 3 and 4 were administered with HFD and methanolic extract of *Eruca sativ a*leaves (MEESL) at doses of250 and 500 mg/kg for 4 weeks, respectively. After the last dose, blood was withdrawn from retro-orbital plexus for cholesterol estimation in serum, then, rats were placed in metabolic cages for 24 h. Urine samples were collected at two time points (5h and 24h).In fresh samples, urinary pH, conductivity and urinary electrolytes (sodium, potassium and chloride) levels were estimated.

Estimation of Urine Parameters

Conductivity and pH of urine samples were analyzed by conductivity meter (420, Jenway, UK) and pH meter (HI 110 series, Bench model, Hanna Instruments, Lynnfield, MA), respectively. Urinary electrolyte levels of sodium, potassium and chloride were estimated using Professional Ion Chromatography (Metrohm, Switzerland).

Saluretic Activity, Natriuretic Activity and Carbonic Anhydrase Inhibition

Sum of Na^+ and K^+ was calculated as parameter for saluretic activity. The ratio of Na^+/K^+ was calculated for natriuretic activity. To estimate carbonic anhydrase inhibition activity, the ratio of $Cl^-/(Na^+ + K^+)$ was computed [9].

Statistical Analysis

All values are expressed as means ± SEM. Data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P<0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS program (version 8) software package (SPSS_ Inc., USA).

RESULTS

Effects on Urine Output and Diuretic Activity

Table 1 showed urine volume analysis. Obesity resulted in decreased urine volume by 39.28%and 50.52% at 5h and 24h interval of sample collection, as compared to normal control rats. MEESL treatment showed significant increase in urine volume at both doses of 250 and 500 mg/kg, compared to the obese rats.

Effects on Serum Total Cholesterol Level

HFD administration in normal rats significantly increased cholesterol level in serum. Treatment with MEESL in HFD treated obese rats resulted in significant decreased in cholesterol level (Figure 1).

Effects on Urine pH and conductivity

The urinary pH of obese control rats were decreased as compared to control rats and MEESL treatment increased the pH as compared to obese control groups but changes were not significant. Significantly decreased urine conductivity of obese rats was found to be significantly increased in MEESL treated groups (Table 2).

Effects on Urinary Electrolyte Excretion

Table 3depicted the urinary electrolyte content following the administration of the extracts. Urinary electrolyte (Na^+, K^+ and Cl^-) excretion was significantly decreased in obese rats. The MEESL at both dose (250 and 500 mg/kg) showed a significant increase in the Na^+, K^+ and Cl^- excretion, compared with HFD only group ($p< 0.01$).

Effects on Natriuretic, Saliuretic and Carbonic Anhydrase Inhibition

Saluretic, natriuretic and CAI activity results were presented in Table 4. Oral administration of MEESL showed a significant saluretic activity but no carbonic anhydrase inhibition was observed.

Table 1.Effect of methanolic extract of *Eruca sativa* leaves on urine volume and diuretic index in normal and obese rats

Groups	After 5h		After 24h	
	Urine volume (mL)	Diuretic index	Urine volume (mL)	Diuretic index
Normal Control	1.12±0.09	1.00	7.64±0.44	1.00
HFD only	0.68±0.06[a]	0.61	3.78±0.30[a]	0.49
MEESL (250 mg/kg) + HFD	1.96±0.13[b]	1.75	12.32±0.53[b]	1.61
MEESL (500 mg/kg) + HFD	2.38±0.17[b]	2.12	16.52±1.23[b]	2.16

- The values are presented as means ± SEM, (n = 6).
- MEESL, methanolic extracts of *Eruca sativa* leaves; HFD, high fat diet; CAI, carbonic anhydrase inhibition
- Index=Excretion in test group/Excretion in control group
- [a] Significant differences as compared with normal control group (P < 0.05).
- [b] Significant differences as compared with diabetic control group at (P < 0.05).

Table 2: Effect of methanolic extract of *Eruca sativa* leaves on urinary pH and conductivity in normal and obese rats

Groups	pH	Conductivity
Normal Control	7.30 ± 0.07	12.90 ± 0.92
HFD only	6.26 ± 0.08	7.58 ± 0.65[a]
MEESL (250 mg/kg) + HFD	8.08 ± 0.11	14.41 ± 0.81[b]
MEESL (500 mg/kg) + HFD	8.15 ± 0.13	15.84 ± 0.84[b]

- The values are presented as means ± SEM, (n = 6).
- MEESL, methanolic extracts of *Eruca sativa* leaves; HFD, high fat diet;
- [a] Significant differences as compared with normal control group (P < 0.05).
- [b] Significant differences as compared with diabetic control group at (P < 0.05).

Table 3.Effect of methanolic extract of *Eruca sativa* leaves on urinary electrolytic excretion in 24 h of sample collection of control and obese rats

Parameters	Normal control	HFD only	HFD + MEESL	
			250 mg/ kg	500 mg/ kg
Urinary Na^+ (mmol/L)	95.4±6.11	52.4±2.75[a]	109.8±5.79[b]	119.4±6.27[b]
Urinary K^+ (mmol/L)	67.4±4.02	33.8±2.55[a]	76.4±2.82[b]	85.4±3.48[b]
Urinary Cl^- (mmol/L)	72.4±5.74	40.4±3.37[a]	77.8±3.07[b]	85.8±3.84[b]
Na^+ Index	1.00	0.55	1.15	1.25
K^+ Index	1.00	0.50	1.13	1.27
Cl^- Index	1.00	0.56	1.07	1.18

- The values are presented as means ± SEM, (n = 6).
- MEESL, methanolic extracts of *Eruca sativa* leaves; HFD, high fat diet;
- Index=Excretion in test group/Excretion in control group
- [a] Significant differences as compared with normal control group (P < 0.05).
- [b] Significant differences as compared with diabetic control group at (P < 0.05).

Table 4: Effect of methanolic extract of *Eruca sativa* leaves on saluretic, natriuretic and Carbonic Anhydrase Inhibition (CAI) activity in 24 h of urine collection of control and obese rats

Parameters	Normal control	HFD only	HFD + MEESL	
			250 mg/ kg	500 mg/ kg
Saluretic (Na+ Cl)	167.8±8.05	92.8±4.31[a]	187.6±7.33[b]	205.2±7.90[b]
Natriuretic (Na/K)	1.44±0.12	1.59±0.12	1.45±0.10	1.40±0.04
CAI{Cl/(Na+K)}	0.45±0.05	0.48±0.06	0.42±0.02	0.42±0.02
Saluretic Index	1.00	0.55	1.12	1.22
Natriuretic Index	1.00	1.10	1.01	0.97
CAI Index	1.00	1.06	0.93	0.93

- The values are presented as means ± SEM, (n = 6).

- MEESL, methanolic extracts of *Eruca sativa* leaves; HFD, high fat diet; CAI= Carbonic Anhydrase Inhibition;
- Index=Excretion in test group/Excretion in control group
- [a] Significant differences as compared with normal control group ($P < 0.05$).
- [b] Significant differences as compared with diabetic control group at ($P < 0.05$).

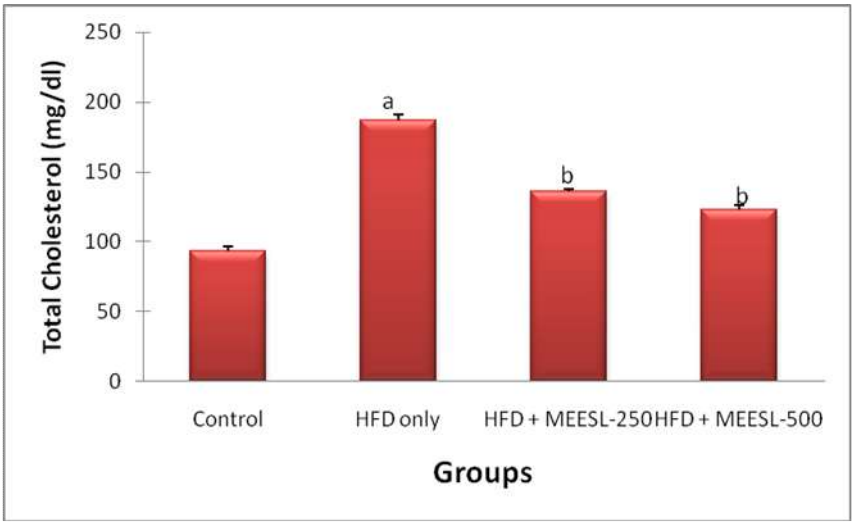

Figure 1. Effect of methanolic extract of *Eruca sativa* leaves on serum total cholesterol level. All values are means ± SEM. [a]*P*<0.05, significant against the control group; [b]*P*<0.05, significant against the HFD group

DISCUSSION

Hyperlipidemia continues to be a major health problem in developing countries, which lead to important risk factors like atherosclerosis, stroke etc. An increased lipid level in blood evokes the damages in various tissues, which in turn, leads to various pathological conditions as a result of dysregulation in cellular functions [10].

The present study was performed to assess the diuretic and anti-hypercholesterolemic effect of dietary rocket leaves extract HFD-induced obese rats. Rocket leaves are used by people in many countries, as a salad. The results from present study revealed that HFD (normal pellet diet + 1% cholesterol) fed to normal rats caused signs of obesity that is manifested by significant increase in serum cholesterol level. Further, urine analysis showed significant decrease of urinary volume and excretion of electrolytes namely sodium (Na^+), potassium (K^+) and chloride (Cl^-) ion. It is previously reported, that obesity is strongly related to bladder dysfunction [11] and without disturbed glucose metabolism alters bladder function by reducing urodynamic pressure and volume in rats [12].

Dietary rocket leaves widely used in traditional medicine as a remedy of various diseases. In our study, the obese rats after treatment with oral administration of MEESL at both doses for 4 weeks, produced anti-obesity and diuretic effects which is clearly evidenced by significant decrease in serum cholesterol level and increased urinary volume in all rats. Rocket seed oil administered to rat's fed on high fat diet reduced all plasma lipid profile, that's may be because of higher ratio of unsaturated fatty acids, and high amount of plant sterols [13].

It was reported that *Eruca sativa* produced potent antioxidant and renal protective activities and precluded oxidative damage inflicted to the kidney by mercuric chloride in rats [7]. Moreover, recently Alqasoumi reported hepatoprotective effect of *Eruca sativa* L. extract through its potent antioxidant activity in rats[14]. It also showed statistically significant high excretion of urinary sodium, potassium and chloride ions in obese rats treated with *Eruca sativa* leaves extracts with alkalinization of urine.

The urinary pH was not significantly changed by HFD as well as extracts treatment. The conductivity, which is an indirect measure of the ionic concentration of the urine, was significantly decreased in obese rats after administration of HFD and significantly increased in MEESL extract treated groups.

From the observed results, we can conclude and confirm the folkloric medicinal use of rocket leaves as anti-obesity and diuretic, as their oral administration produced significant reduction in serum cholesterol level which was increased by HFD administration. Therefore, the study recommends that dietary intake of rocket leaves may be beneficial for patients who suffer from obesity with problem of water and sodium retention.

Conflict of interest statement

We declare that we have no conflict of interest.

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