



**AMELIORATIVE EFFECT OF *ERUCA SATIVA* EXTRACTS ON GLUCOSE AND
URINARY VOLUME IN STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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ABSTRACT

Diabetic nephropathy is one of the major complications of diabetes. In recent years, several plant extracts and herbal formulations are used as antidiabetic formulations. It has been suggested that *Eruca sativa* exert a beneficial antidiabetic effect in rats by reducing oxidative stress. The present research work was designed to evaluate the effect of methanolic and aqueous extracts of *Eruca sativa* on blood glucose and urinary volume in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced in rats after intraperitoneal injection of streptozotocin (STZ; 55 mg/kg). Thirty six adult male rats were randomly distributed into 6 equal groups, each of 6 animals. The 1st and 2nd groups were served as control and diabetic control respectively and other four groups of diabetic rats were treated with aqueous and methanolic extracts of *Eruca sativa* in doses of 250 and 500 mg/kg, for 15 consecutive days. Blood glucose (mg/dl), urine volume (ml), urinary electrolytes (sodium, potassium and chloride) and diuretic index were evaluated in control, diabetic and drug treated diabetic rats. Blood glucose, and urine volume were significantly increased while urinary electrolytes excretion (Na, K and Cl) significantly decreased in diabetic control rats. Orally administered *Eruca sativa* extracts significantly restore the altered parameters in diabetic animals. The obtained results demonstrate the antihyperglycemic and diuretic activity of *Eruca sativa* extracts, in the treatment of STZ-induced diabetic rats. Thus, a better characterization of the medicinal potential of this plant will be able to provide a better understanding of its mechanisms of action in these pathological processes.

Keywords: *Eruca sativa*, Diuretic, Urinary Sodium, Diabetes, Streptozotocin

INTRODUCTION

Diabetes is the leading cause diabetic nephropathy, and approximately 30% of diabetic patients experience nephropathy which gradually develops to final renal failure [1]. Although the complete mechanism of hyperglycemia that causes diabetic complications is not clear, several biochemical pathways are involved in the pathogenesis, including increased formation of glucose-derived glycosylated end products, increased formation of reactive oxygen species, activation of aldose reductase pathway, and glucose-induced activation of protein kinase C [2-4]. Oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the increased incidence of vascular disease in diabetes [5, 6]. It was shown that the activity of the antioxidant systems is decreased in people with diabetes [6, 7].

Plants have been used for years as a source of traditional medicine to treat various diseases. Many of these medicinal plants are also excellent sources for phytochemicals, many of which contain potent antioxidant activities [8]. Several medicinal plants have been used in traditional medicine for the treatment of diabetic patients in different ethnic societies of Africa, Asia, and South America [9].

In previous studies, pharmacological activities of rocket plants (Brassicaceae)

have been moderately related to their strong antioxidant properties [10, 11]. Rocket and other Cruciferous vegetables contain a group of anticancer compounds known as glucosinolates, these compounds exert antioxidant activity, and are potent stimulator of natural detoxifying enzymes in the body, such compound exert anti-secretory, anti-ulcer and cytoprotective properties in the ethanolic extract of the plant in rats [12, 13]. Weckerle *et al.*, [14] isolated and identified three new quercetins from *Eruca sativa* leaves. A number of health-promoting properties of *Eruca* have been reported, such as antiphlogistic, depurative, diuretic, digestive, aphrodisiac and rubefacient [13, 15]. Antidiabetic effect of *Eruca sativa* seeds has been reported in chemically induced diabetic rats by reducing oxidative stress [16]. It has also antihyperlipidemic, antihyperglycemic, antiephrolethiatic and hepatoprotective activity [17]. *Eruca sativa* also known as salad rocket is widely used in folklore medicine as a remedy of renal ailments. Sarwar *et al.*, [18] reported that *Eruca sativa* produced potent antioxidant and renal protective activities and precluded oxidative damage inflicted to the kidney by mercuric chloride in rats. The present study was carried out to investigate the antidiabetic, and diuretic effects of *Eruca sativa* extracts

by evaluating blood glucose and urinary analysis in Wistar albino rats.

MATERIALS AND METHODS

Plants Collection and Extract Preparation

The fresh *Eruca sativa* leaves were purchased in October, 2013, from Al-Kharj local vegetable market, Saudi Arabia, and identified by expert taxonomist Mr. Osman Ali Elmakki. Leaves were shade dried, coarsely pulverized, and placed in glass percolator with methanol and water at room temperature for 72 h (percolation method). The collected percolate was dried under reduced pressure *in vacuo*. The obtained aqueous and methanolic extracts of *Eruca sativa* were later used after suspending in the vehicle (3% v/v Tween 80 in distilled water) for further diuretic activity.

Animals

Thirty six male Wistar albino rats, approximately of same age (8-10 weeks), weighing 180-200 g were used in this study. The rats were procured from the Laboratory Animal Care Unit, College of Pharmacy, Salman bin Abdulaziz University, Saudi Arabia. Animals were acclimatized for 7 days, under hygienic conditions at a constant temperature of $22\pm 2^{\circ}\text{C}$ with relative humidity of $55 \pm 1\%$ and on 12 h light/12 h dark cycles in the Research Laboratory of Department of Pharmacology.

Standard rodent diet and water were allowed *ad libitum*.

Induction of Diabetes

Overnight-fasted Wistar albino rats were rendered diabetic by a single intraperitoneal injection of STZ (55 mg/kg) in citrate buffer (0.1 M, pH 4.5) [19]. Diabetes was confirmed by measuring the fasting blood glucose level after 72 h of STZ injection. Rats with a blood glucose level above 300 mg/dl were considered diabetic, and included in the experiment.

Experiment and Grouping of Rats

Thirty six adult male rats were randomly distributed into six equal groups, each of 6 animals. Group 1 was administered with normal saline (1.0 mL/day) and kept as normal control. Group 2 was served as diabetic control. Diabetic rats of groups 3, 4, 5 and 6 were given orally, methanolic and aqueous extracts of *Eruca sativa* (250 and 500 mg/kg), respectively. After the last administration, animals were placed in separate metabolic cages for 24 h and total urinary volume, pH and conductivity were measured immediately after collection. The urinary electrolytes (sodium, potassium and chloride) levels were analyzed within 24h of collection and expressed as mmole/L. Glucose was estimated in blood.

Urine Analysis

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. Urine levels of sodium, potassium and chloride were determined using Professional Ion Chromatography (Metrohm, Switzerland). 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 $\text{Cl}^-/(\text{Na}^+ + \text{K}^+)$ was computed [20].

Statistical Analysis

Data are expressed as means \pm SEM. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test for comparisons in different treatment groups. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS program (version 8) software package (SPSS_Inc., USA).

RESULTS

Blood Glucose

The results presented in **Table 1** showed blood glucose level in control, diabetic control and treated groups. Blood glucose level was found to be significantly ($p < 0.01$) higher in diabetic rats than those in the normal control group. The glucose level in blood was significantly ($p < 0.01$) declined following *Eruca sativa* treatment as

compared with those of diabetic control rats.

Urine Volume, pH and Conductivity

Table 1 shows that diabetes, increased urine volume by 213.51% as compared to normal control rats. The methanolic extracts showed significant decrease in urine volume by 47.78% ($p < 0.01$) and 56.03% ($p < 0.001$) at dose of 250 and 500 mg/kg respectively, compared to the diabetic control group, while for the aqueous extract at 250 mg/kg and 500 mg/kg showed 37.36% ($p < 0.01$) and 46.55% ($p < 0.001$), decrease in urine volume respectively. The pH were decreased in diabetic control rats as compared to control rats and increased in *Eruca sativa* treated groups as compared to diabetic control groups but changes were not significant. Urine conductivity of diabetic rats was significantly decreased as compared to control rats and found to be significantly increased in *Eruca sativa* treated groups as compared to diabetic control groups.

Urinary Electrolytes Excretion

Table 2 shows the urinary electrolyte content following the administration of the extracts. Urinary electrolyte (Na^+ , K^+ and Cl^-) excretion was significantly decreased in diabetic rats. The methanolic and aqueous extracts at dose of 250 and 500 mg/kg showed a significant increase in the Na^+ , K^+ and Cl^- excretion, compared with the diabetic control group ($p < 0.01$).

Saluretic, Natriuretic and Carbonic Anhydrase Inhibition (CAI) Activity

Table 3 shows the saluretic, natriuretic and CAI activity of normal control, diabetic control and extracts treated groups. Oral administration of methanolic and aqueous extracts of *Eruca sativa* at doses of 250 and 500 mg/kg, showed a significant saluretic activity than diabetic control group.

DISCUSSION

In the present study, diuretic and antidiabetic activities of *Eruca sativa* extracts against streptozotocin (STZ) - induced diabetic rats were investigated. Oral route was chosen to meet the way used by people in traditional medicine. STZ-induced diabetes in rodents results in development of nephropathy, similar to early stage clinical nephropathy [21]. Therefore, this animal model was selected for the study. The obtained results revealed that intraperitoneal injection of STZ (55 mg/kg) to rats caused signs of diabetes manifested by significant increase in blood glucose level and urine volume. Further, urine analysis showed significant decreases of urinary excretion of sodium (Na^+), potassium (K^+) and chloride (Cl^-) ion in STZ-treated rats.

Diabetes has been shown to be responsible for a number of patho-physiological alterations in renal function, including modifications in glomerular hemodynamics [22] and in urinary acidification [23]. The

decrease in urinary sodium level in diabetic rats compared to normal control rats corroborates with the previous reports, which shows that pathologically sustained hyperglycemia results in hypo-perfusion, tubular necrosis and structural and functional decrease of glomerular filtration. This may lead to high extra cellular fluid concentration of sodium which due to concentration gradient, enter the internal environment of cell and may have deleterious effects on number of enzyme system [24].

In the present work, diabetic control rats developed severe hyperglycemia with polyuria as a result of osmotic diuresis. However, the diabetic rats that were treated with oral administration of *Eruca sativa* extracts (methanolic and aqueous) at doses of 250 and 500 mg/kg for 15 days, produced antidiabetic and diuretic effects as evident by significant decrease in urinary volume, probably as a result of the normalization of plasma glucose level or synergistic effect with insulin as shown in other studies [25, 26].

Concerning *Eruca sativa*, it is widely used in folklore medicine and has a good reputation as a remedy of renal ailments. 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 [18]. Moreover, Alqasoumi [27] reported that *Eruca sativa* L. extract protected the liver against CCl₄-induced hepatic injury through its potent antioxidant activity in rats.

The conductivity, which is an indirect measure of the ionic content of the urine, was increased in a dose-dependent manner in all the extracts-treated groups. The pH values are not significantly influenced by diabetes as well as extracts treatment. It also showed, statistically significant high urinary sodium excretion, potassium excretion, chloride excretion and saluretic activity in diabetic rats treated with *Eruca sativa* extracts with slight alkalization of urine.

CONCLUSION

In conclusion, our results apparently validate the folk medicinal use of *Eruca sativa* extracts as an antidiabetic and diuretic remedy in Saudi Arabian population, as their oral administration produced significant reduction in biochemical alterations caused by STZ. Therefore, the study recommends that intake of *Eruca sativa* may be beneficial for patients who suffer from kidney diseases and diabetes.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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Table 1: Effect of Oral Administration of *Eruca sativa* Extracts on Blood Glucose, Urine Volume, Diuretic Index, pH, and Conductivity and of Control and Diabetic Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES		Diabetic + AEES	
			250 mg/ kg	500 mg/ kg	250 mg/ kg	500 mg/ kg
Blood Glucose (mg/dl)	115.7±12.35	546.6±17.47 ^a	378.8±20.17 ^b	353.5±22.06 ^b	387.1±16.76 ^b	368.9±19.93 ^b
Urine Volume (ml/day)	22.2±1.70	69.6±3.70 ^a	36.2±2.57 ^b	30.6±2.33 ^b	43.6±1.60 ^b	37.2±2.40 ^b
Diuretic index	1.00	3.13	2.15	1.90	1.96	1.67
Urinary pH value	7.37±0.05	6.66±0.07	8.41±0.05	8.24±0.03	7.06±0.06	6.91±0.07
Conductivity (mS)	13.90±0.34	5.58±0.65 ^a	15.01±0.41 ^b	16.24±0.24 ^b	10.26±0.41 ^b	11.51±0.31 ^b

Table 2: Effect of Oral Administration of *Eruca sativa* Extracts on the Electrolytic Excretion Index in 24 h of Urine Collection of Control and Diabetic Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES		Diabetic + AEES	
			250 mg/ kg	500 mg/ kg	250 mg/ kg	500 mg/ kg
Urinary Na ⁺ (mmol/L)	123.4±6.11	48.2±3.70 ^a	93.8±3.89 ^b	97.4±4.17 ^b	85.6±5.73 ^b	91.4±6.43 ^b
Urinary K ⁺ (mmol/L)	163.4±8.56	57.8±3.57 ^a	120.4±6.93 ^b	131.8±5.79 ^b	114.2±5.19 ^b	126.2±4.98 ^b
Urinary Cl ⁻ (mmol/L)	61.2±8.89	25.8±1.99 ^a	47.8±3.88 ^b	55.8±4.59 ^b	44.4±4.61 ^b	49.8±3.51 ^b
Na ⁺ Index	1.00	0.39	0.76	0.79	0.69	0.74
K ⁺ Index	1.00	0.35	0.74	0.81	0.70	0.77
Cl ⁻ Index	1.00	0.42	0.78	0.91	0.73	0.81

NOTE: The values are presented as means ± SEM, (n = 6); MEES, methanolic extracts of *Eruca sativa*; AEES, aqueous extracts of *Eruca sativa*; Diuretic index, urine volume of test group/urine volume of 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 3: Effect of Oral Administration of *Eruca sativa* Extracts on Saluretic, Natriuretic and Carbonic Anhydrase Inhibition (CAI) Activity in 24 h of Urine Collection of Control and Diabetic Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES		Diabetic + AEES	
			250 mg/ kg	500 mg/ kg	250 mg/ kg	500 mg/ kg
Saluretic (Na + Cl)	184.6±10.18	74.0±3.39 ^a	141.6±6.67 ^b	153.2±5.32 ^b	130.0±7.93 ^b	141.2±7.46 ^b
Natriuretic (Na/K)	0.77±0.06	0.83±0.01	0.79±0.05	0.74±0.01	0.77±0.07	0.74±0.07
CAI{Cl/(Na+K)}	0.21±0.03	0.25±0.03	0.23±0.02	0.25±0.02	0.22±0.03	0.23±0.01
Saluretic Index	1.00	0.40	0.77	0.83	0.70	0.76
Natriuretic Index	1.00	1.08	1.03	0.96	1.00	0.96
CAI Index	1.00	1.19	1.09	1.19	1.05	1.09

NOTE: The values are presented as means ± SEM, (n = 6); MEES, methanolic extracts of *Eruca sativa*; AEES, aqueous extracts of *Eruca sativa*; Diuretic index, urine volume of test group/urine volume of 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)