



ISSN: 0067-2904

The Effects of the Aqueous Extract of the Leaves of the Local Plant *Eruca sativa* on Lipid Profile and Some Minerals in the Blood of Male White Mice

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Received: 22/5/2020

Accepted: 24/7/2020

ABSTRACT

Eruca sativa, commonly known as rocket salad, is a popular vegetable to which a wide range of health benefits are attributed. This study aimed to examine the effects of the aqueous extract of *E. sativa* leaves on lipid profile and some minerals, such as calcium and magnesium, in blood of male albino mice under normal physiological condition. Two experiments were separately conducted, each with eighteen male albino mice divided into 3 equal groups, which included control, treated group (1), and treated group (2) which were orally administrated with 0, 0.2 and 0.4 mg/kg body weight, respectively, of the leaves extract for 28 days. In the first experiment, serum lipid profile, including cholesterol, triglyceride, HDL, LDL and VLDL, were estimated. In the second experiment, serum calcium and magnesium were estimated. The results showed that *E. sativa* have effects on lipid profile by decreasing cholesterol, triglyceride, and LDL levels, but increasing that of HDL. In addition, *E. sativa* increased the levels of both calcium and magnesium. This study concludes that *E. sativa* leaves exerts possibly beneficial effect on lipid profile and calcium and magnesium levels in albino male mice under normal conditions.

KEYWORDS: *E. sativa*, lipid profile, calcium, magnesium.

تأثير المستخلص المائي لاوراق النبات المحلي اروكا ساتيفا (*Eruca sativa*) على الملف الدهني

وبعض المعادن في دم الفئران البيض الذكور

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الخلاصة

Eruca sativa المعروفة باسم سلطة الصواريخ، هي خضروات شائعة ويعزى إليها مجموعة واسعة من الفوائد الصحية. هدفت هذه الدراسة إلى دراسة آثار المستخلص المائي من *E. sativa* على مرتسم الدهون وبعض المعادن مثل الكالسيوم والمغنيسيوم في دم الفئران البيض الذكور في حالة طبيعية فسيولوجية. في التجربة الأولى ، تم تقسيم ثمانية عشر من الفئران البيض إلى 3 مجموعات متساوية شملت المجموعة الضابطة والمجموعة المعالجة (1) والمجموعة المعالجة (2) باستخدام تركيز مختلف (0 ، 0.2 و 0.4 ملغم / كغم من وزن الجسم على التوالي) من خلاصة *E. sativa*. وقدرت الملف الشخصي للدهون في الدم بما في ذلك الكوليسترول ، والدهون الثلاثية ، HDL ، LDL و VLDL. في التجربة الثانية ، تم تقسيم ثمانية عشر من الفئران البيض إلى 3 مجموعات متساوية شملت المجموعة الضابطة والمعاملة (1) والمجموعة المعالجة (2) باستخدام تركيز مختلف (0 ، 0.2 و 0.4 ملغم / كغم من وزن الجسم على التوالي) من خلاصة *E. sativa*. تم تقدير الكالسيوم والمغنيسيوم في الدم. أوضحت النتائج أن *E. sativa* لها تأثيرات على خصائص الدهون عن طريق خفض الكوليسترول والدهون الثلاثية و LDL ولكنها تزيد من مستوى HDL. بالإضافة إلى ذلك ، زادت *E. sativa* مستوى كل من الكالسيوم والمغنيسيوم. وخلصت هذه الدراسة إلى أن *E. sativa* تمارس التأثيرات المفيدة على مرتسم الدهون. والمعادن مثل الكالسيوم والمغنيسيوم في ذكور الفئران البيض في حالة طبيعية.

INTRODUCTION

E. sativa is also known as rocket plant, Arugula, rocket salad, white pepper, and Roquette. It is documented in the old literature in Palestine, with a Greek name of “Euzomon” that means good broth. This herb has the name of Gar-Gir or Jarjeer in the dialects of the Arabic lands, including Iraq [1, 2, 3]. It is a spontaneously grown plants that natively originates from the Mediterranean area and other parts of the world, but it is more popular in the United States [4]. *E. sativa* consumption, in addition to its nutritional values, has been shown to lead to a wide range of beneficial effects on health. These effects are due to its constituents of phytochemicals, including flavonoids, glucosinolates, and vitamins such as vitamin C [5, 6]. *E. sativa* was proved to be used for the treatment of type-2 diabetes, due to its antidiabetic activities in insulin-responsive cell lines [7]. In addition, a study conducted by Alam concluded that *E. sativa* seeds possess potent antioxidant and renal protective activities and prevent oxidative damage imposed by HgCl₂ to the kidney [8]. Moreover, the vasodilatory and cardiogenic effects of the crude extract of *E. sativa* were confirmed [9], due to the presence of flavonoid and isothiocyanate, as determined by HPLC analysis. Several other health benefits of *E. sativa*, such as its positive effects on Leydig’s cell number and hormonal profile in rats exposed to cadmium [10], improving skin barrier function as a skin therapeutic agent for atopic dermatitis, due to its suppression of inflammatory cytokines [11], and anticancer potential against melanoma cells induced in mice [12]. Seeds and leaves extracts of *Eruca sativa* were reported to exert inhibitory effects on the activity of enterotoxins produced by local isolates of *Yersinia enterocolitica* [13]. On the other hand, many studies notified the effects of *Eruca sativa* on lipid profile. The aqueous extract of *E. sativa* leaves improved liver enzymes function, liver tissues , and lipid profile in male rabbits exposed and damaged by phosphoric acid [14]. Another study confirmed the prophylactic and therapeutic roles of *E. sativa* extract against oxidative stress and liver injury produced by ethanol in rats, by improving liver functions, lipid profile and antioxidants parameters [15]. Moreover, *E. sativa* contains a high number of bioactive constituents, specifically minerals such as calcium (Ca) [16, 17] and magnesium (Mg) [18]. Calcium is the most abundant mineral in the body. Between 96 and 99% of the total Ca in the body is stored in bone tissues [19]. Ingested calcium comes from food sources and dietary supplements. Calcium serves two primary functions in the body; first, promoting structural integrity of bones and teeth, and second, acting as a regulatory ion that maintains homeostasis [20]. Magnesium is required for many of the major organs and plays a crucial role in mammalian physiology. Magnesium is essential for the structure of bones and teeth and acts as a cofactor for more than 300 enzymes in the body, including binding to ATP for kinase reactions [21]. In the literature, there are no studies concerning the prophylactic effects of *E. sativa* on lipid profile

and calcium and magnesium levels in blood in normal conditions. Therefore, the present study was designed to examine the role of the leaves aqueous extract of *E. sativa* local plant on lipid profile and calcium and magnesium levels in the blood of mice in normal conditions.

MATERIALS AND METHODS

Preparation of aqueous *Eruca sativa* extract

The leaves of *E. sativa* vegetables were bought from a local market in Sulaimaniya province, Kurdistan region, Iraq and scientifically approved by a botanist specialist. The plant was dried in a shaded place for 14 days and then grinded by an electric blender to obtain powder. An amount of 200 mg/Kg body weight of the powder was given to each animal. The dose was prepared by dissolving the powder in 1 ml of distilled water and allowing it to stand at room temperature for one hour before using. The experiments, i.e. the collection and preparation of the plant, animals breeding, and blood sample collection and analysis, were conducted from the first of May to the first of August, 2019.

Animals

Thirty six adult male mice, weighted between 25-35 grams, were used during the experiments. Mice were housed in the animal's house of the University of Sulaimani/ College of Science in plastic cages and kept until the time of blood sample collection. Each cage containing only three mice. The room temperature was maintained at 22-25 °C. The animals were kept for at least 2 weeks for adaptation before beginning the experiments and were permitted free access to water and pellets along the experiment period.

Protocol of the study

- Two experiments were separately conducted, each with eighteen male albino mice divided into 3 equal groups, which included control, treated group (1), and treated group (2) which were orally administrated with 0, 0.2 and 0.4 mg/kg body weight, respectively, of the leaves extract for 28 days. In the first experiment, serum lipid profile, including cholesterol, triglyceride, HDL, LDL and VLDL, were estimated. In the second experiment, serum calcium and magnesium were measured.

Blood sample collection

At the end of the 4 weeks treatment, animals were anesthetized by intramuscular injection of 0.1 ml of a general anesthetic, namely Ketamine/Xylazine) and blood was collected directly via heart puncture using disposable insulin syringes. Blood specimens were centrifuged at 2500 rpm for 10 minutes, and then serum samples were stored in freezer at -20 °C till analysis.

Blood sample analysis

Serum samples were used to measure the levels of cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Ca, and Mg, using COBAS INTEGRA 400 plus analyze, Roche, Germany, which is a fully automated, software controlled system. All tests were determined by a single system and a single type of reagent cassette using a particular kit for each test.

Statistical analysis

Data are shown as Mean± SD. Data were analyzed using one way analysis of variance (ANOVA) within SPSS program. Differences were considered significant at a probability level of $p < 0.05$.

RESULTS

The results of experiment (1) of the current study, which is related to the effects of the treatment for 28 days with the aqueous extract of *E. sativa* on lipid profile in the serum of male albino mice, are presented in Table-1. The results showed a decrease in the serum mean values of all lipid profile parameters, both in the treatment 1 (T1; 0.2 mg/kg body wt.) and treatment 2 (T2; 0.4 mg/kg body wt.) groups, as compared to the control.

Table 1-The potential effects of 28 days oral treatment with two different concentrations of aqueous extract of *Eruca sativa* on lipid profile parameters in the serum of albino male mice

Treatments	Dose (mg/kg body wt.)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	0	122.5±7.4Aa	112.17±11.86Aa	78.33±5.32Aa	30.17±4.92Aa	15.5±6.09Aa
Treatment 1	0.2	102.5±6.19Aab	81.83±6.55Bb	89.83±14.8Aa	14.23±2.25Bb	12±3.35Aa

Treatment 2	0.4	80.5±8.29Bb	59.67±12.94Cb	61.83±14.46Aa	13.2±4.43Cb	15.75±1.83Aa
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- Values are presented as means ±SD (n=6 mice/group).
- Different capital letters in the same column denote significant differences within groups (p<0.05).
- Different small letters in the same column denote significant differences between groups (p<0.05).

The results revealed that there were decreases in the serum cholesterol level in T1 group compared to the control group but the difference was statistically not significant. In T2 group, there was a statistically significant decrease (p<0.05) in serum cholesterol level as compared to the control group. However, no differences were found between (T1) and (T2) groups. In addition, the level of serum triglyceride in both treatment groups was decreased significantly (p<0.05) as compared to the control group. However, there were no significant differences between (T1) and (T2) groups. Moreover, HDL level was increased in the T1 group as compared to the control group, but the change was statistically insignificant. In the T2 group, HDL level was decreased as compared to the control group, but also without statistical significance. On the other hands, LDL level decreased significantly in both T1 group and T2 groups as compared to the control group. However, no significant difference was found between T1 and T2 groups. The mean values of serum VLDL were decreased in the T1 group and slightly increased in the T2 as compared to the control group, but the changes were statistically insignificant.

The results of experiment (2), which dealt with the effects of the 28 days treatment with the aqueous extract of *E. sativa* on calcium and magnesium levels in the serum of male albino mice are presented in Table-2.

Table 2-The potential effects of 28 days oral treatment with two different concentrations of aqueous extract of *Eruca sativa* on the calcium and magnesium serum level

Treatments	Dose (mg/kg body wt.)	Calcium (mg/dl)	Magnesium (mg/dl)
Control	0.0	9.10±0.13Aa	3.57±0.10Aa
Treatment 1	0.2	9.48±0.21Aab	3.40±0.18Aa
Treatment 2	0.4	9.88±0.21Bb	3.98±0.15Bb

- Values are presented as means ±SD (n=6 mice/group).
- Different capital letters in the same column denote significant differences within groups (p<0.05).
- Different small letters in the same column denote significant differences between groups (p<0.05).

The results revealed that there were increases in serum calcium level in T1 group as compared to the control group, but without statistical significance, while in T2 group, there was a statistically significant increase (p<0.05) in serum calcium level as compared to the control group. However, no differences were found between T1 and T2 groups.

Furthermore, the level of serum magnesium in T1 group was slightly decreased, while it was increased significantly (p<0.05) in T2 group as compared to the control group. In addition, a significant difference (p<0.05) was also found between T1 and T2 groups.

DISCUSSION

In experiment (1), cholesterol levels were decreased in the treated groups with *E. sativa* compared to controls. This indicates that the aqueous extraction of *E. sativa* has the potential effects by decreasing the level of cholesterol in normal mice. This might be due to the effects of *E. sativa* on the functions of enzyme in the liver. According to an earlier report [14], the aqueous extract of *E. sativa* leaves can improve liver enzymes function and lipid profile against phosphoric acid treatment. The present result is in agreement with those of a previous study [22], which confirmed that total cholesterol level was decreased significantly by feeding rabbits for 2 weeks with *E. sativa* oil. In addition, the current results reveal that the triglyceride levels were decreased in both treated groups. These results are in consistence with those of an earlier investigation [15] which indicated that, in prophylactic groups (oral dose of *E. sativa* together with ethanol), *E. sativa* significantly decreased serum triglyceride level. In addition, it was reported that *E. sativa* induced a marked decrease in different lipid parameters including triglyceride [23]. On the other hand, this study has found that *E. sativa* insignificantly increased HDL and significantly decreased LDL levels in the blood of treated mice. These effects of *E. sativa* on HDL level might be due to the effects of vitamin C, one of the *E. sativa* components that inhibit lipid peroxidation in human HDL [24]. The decrease in the LDL serum

level is in agreement with the findings of a previous work [15], which studied different extracts of *E. sativa* plant parts on alcoholic hepatotoxic rats and observed that the serum level of LDL decreases by introducing *E. sativa* oil seeds and leaves extracts. Regarding VLDL, the current study determined that VLDL was not significantly changed by *E. sativa*. This result is in disagreement with that of an earlier work [25], which indicated that very low-density lipoproteins decreased significantly in the serum of male quail after *E. sativa* seeds supplementation.

In experiment (2), calcium levels were increased in the treated groups as compared to the control group. This indicates that the administration of *E. sativa* extract led to an enhancement of calcium level in blood. This might be due to the content of the nutrient elements in *E. sativa* extract. *E. sativa* was reported to have 2.95% calcium content [26], while it was also confirmed that the seeds and leaves of *E. sativa* contain 1805 and 655 µg/g calcium, respectively [16]. In addition, the increase in calcium level in the *E. sativa* treated group is in agreement with a previous study [27], which confirmed the beneficial effects of *E. sativa* in treating osteoporosis induced by phosphoric acid in male rabbits. The authors explained that osteoporosis takes place when bones lose minerals, like calcium, and that the body cannot replace these minerals, leading to a loss of bone thickness (bone mass). In addition, magnesium levels were decreased in the treated groups with *E. sativa* as compared to the control. The explanation for this effect can be that *E. sativa* contains a large amount of magnesium. These results are in consistence with a previous study [28], which found that *E. sativa* reduces free oxalate in the intestine and urine, due to its high content of magnesium.

CONCLUSIONS

The aqueous extract of *E. sativa* has a potential role in normal male mice following 28 days of treatment. *E. sativa* extract can decrease cholesterol, triglyceride, and LDL and improve lipid profile. *E. sativa* has a potential role in maintaining and increasing calcium and magnesium levels in the serum of male mice.

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