The Effect of Eurca Saliva Alcoholic Extract in Decreasing the Induced Toxicity of Liver and Kidney in Mice

Suhad A. Ahmed
University of Technology-Applied Science-Biotechnology Branch/Baghdad
Email:eve.daughter@yahoo.com
Dr.Abbas A. Mohammed
University of Technology-Applied Science-Biotechnology Branch/Baghdad
Ali H. Saadoon
University of Technology-Applied Science-Biotechnology Branch/Baghdad

Received on: 18/10/2012 & Accepted on: 10/1/2013

ABSTRACT

This study is aimed to investigate the effect of ethanolic extract of Eruca Sativa leafs in liver and kidney enzymes of mice. Thirty male albino mice average weight 25gm were divided into 5 groups included group1 (normal diet), group2 (injected with carbontetrachloride CCl_4), group3 (injected with CCl_4 + 0.5mg/ml Eruca sativa extract), group4 (injected with CCl_4 + 1mg/ml Eruca sativa extract), and group5 (injected with CCl_4 + 2mg/ml Eruca sativa extract). Serum liver and kidney functions tests were estimated. The results showed that Eruca Sativa extracts at concentration 2mg/ml improved liver and kidney functions. We concluded that, Eruca sativa extracts may exert their prophylactic and treatment role against oxidative stress produced by CCl_4 by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes.

Key words: Eruca Sativa, Liver, Enzymes

تأثير المستخلص الكحولي لـ Eurca sativa في تقليل السمية المستحثة بـ CCL_4 للكبد والكلية في الفئران

الخلاصة

هدفت هذه الدراسة الى التحري عن تأثير مستخلص الجرجير الكحولي في عمل انزيمات الكبد والكلى لدى الفئران المصابة بمادة رابع كلوريد الكاربون CCl_4، حيث تم استخدام مجموعة من الفئران (30) فأرا أبيض متوسط وزن الجسم 25غم. وقد قسمت هذه الفئران إلى خمسة مجاميع ليتم حقنها بمادة رابع كلوريد الكاربون CCl_4 المعروفة بتأثيرها السمي على الكبد والكلى.

المجموعة الأولى من الفئران تم تغذيتها على الغذاء العادي فقط في حين المجاميع الاربعة المتبقية تغذت على الغذاء العادي إضافة إلى 0.5 ، 1 و2ملغم/مل من المستخلص الكحولي لأوراق نبات الجرجير بمعدل حقنة واحدة يوميا وبمقدار 0.01مل لكل تركيز ولمدة 7 يوم.

https://doi.org/10.30684/etj.31.5B.1
2412-0758/University of Technology-Iraq, Baghdad, Iraq

تـم قيـاس التغيـرات فـي الانزيمـات GOT ، GPT و BloodUrea ، Creatinine لمصـل الفئـران. لـوحظ انخفـاض مسـتوى الانزيمـات GOT ، GPT و BloodUrea ، Creatinine للفئران المجرعة بالمستخلص عند تركيز 2 ملغم/مل مقارنة مع الفئران غير المجرعة بالمستخلص (مجموعة السيطرة).

فيما يتعلق بنتـائج هذه الدراسـة يمكن ان نخلص بـالقول بـأن مستخلص نبـات الجرجير لـه دور وقائي وعلاجي ضد إصابة الكبد المستحثة تجريبيا فـي الفئران، علـى الـرغم من أن الـدور الوقـائي لهذا المستخلص كان أكثر فعالية من القدرة على العلاج.

INTRODUCTION

There has been a great deal of interest in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases[1]. Several hundreds of plants have been examined for use in a wide variety of liver disorders including *Eruca sativa* (ES) Family: *Cruciferae* that modulate oxidative stress due to its antioxidant properties[2]. Fresh *Eruca sativa* has a characteristic pungent flavor that is thought to be related to the presence of glucosinolates and their breakbown products, e.g: isothiocyanates[3]. Eruca sativa is an annual plant approximately 20-50 cm high, with dull-green leaves which have a distinct spicy-pungent flavor[4]. It is an increasingly popular vegetable in Central Europe and is spreading to other areas. Eruca sativa is easily grown and is thus suitable as an experimental plant which have several biological activities including anticarcinogenic, antifungal, antibacterial and antioxidant effects[5, 6, 7 and 8]. [9] indicated that *Eruca sativa* seeds and leaves possessed a potent free radical scavenging antioxidants and protected against oxidative damage by increasing maintaining the levels of antioxidant molecules and antioxidant enzymes.

Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to many different diseases[10]. Coronary Heart Disease (CHD) is the leading cause of death in the UK [11]. According to the Medicine Health CHD in America kills as many people each year as the 'next 7 leading causes of deaths combined' Risk factors contributing to CHD range from uncontrollable factors such as age, heredity and gender through to areas of lifestyle and diet that can be influenced[12].

The kidneys are sophisticated reprocessing machines. Every day, a person's kidneys process about 200 quarts of blood to sift out about 2 quarts of waste products and extra water. The wastes and extra water become urine, which flows to the bladder through tubes called ureters[13].

The bladder stores urine until releasing it through urination. Most kidney diseases attack the nephrons, causing them to lose their filtering capacity. Damage to the nephrons can happen quickly, often as the result of injury or poisoning. But most kidney diseases destroy the nephrons slowly and silently. Only after years or even decades will the damage become apparent. Most kidney diseases attack both kidneys simultaneously[14].

Among the various mechanisms involved in the hepatotoxic effect of carbontetrachloride (CCl4), one is oxidative damage through free-radical generation [15] and antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of indigenous drugs.[16] The *Eruca sativa* has antioxidant

properties[17]. Hence, the objective of the study was to evaluate the effect of ethanolic extract of *Eruca sativa* on CCl_4-induced hepatotoxicity in mice.

MATERIALS AND METHODS

Plant extraction

The fresh leafs of *Eruca sativa* were collected from local market and air dried in the shade, grounded into a fine powder and then extracted with ethanol using sahxulate apparatus. The extracts of the leaf was evaporated to dryness in a rotary evaporator at 40ºC. The crude extracts were obtained by filtration through Whatman No.1 filter paper. The filtrate was reduced to 25 ml and then autoclaved at 121ºC and 15 lb pressure for 20 min. The extract was cooled and immediately use[18].

Animals

The animals used in this study were laboratory mice (males) Mus musculus Balb/C male adult mice (average weight of 25g and 8-12 weeks old), purchased from central health laboratory / Baghdad. There were 5 groups 6 mice in each group: group 1 served as negative control which received (normal diet), group 2 positive control (injected with CCl_4 100mg/kg), group 3 (injected with CCl_4 + 0.5mg/ml Eruca sativa extract), group 4 (injected with CCl_4 + 1mg/ml Eruca sativa extract), group 5 (injected with CCl_4 + 2mg/ml Eruca sativa extract) intraperitoneal injection of a single daily dose in mice [19]. During the experiment (7days) the animals were fed by pellet and drinking water. The cage floor was covered with wood chips and sawdust. Animal cages were cleaned and disinfected twice a week.

Blood sampling and biochemical analyses

After 7 days of treatment, the mice were kept overnight fasting and killed by cervical dislocation, blood samples were collected by direct cardiac puncture under ether anesthesia and the serum was used for the assay of marker enzymes viz., aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), Blood Urea and Creatinine. The enzyme levels were assayed using the standard kits from laboratories. The results were expressed as units/liter (U/l).

Statistical analysis

Data were expressed as means ± S.E. and statistical analysis was carried using computerized SPSS program. Significance was performed using the least significant difference and paired Student "t" test according to Klug and Cummings [20].

RESULTS AND DISSECTION

Injection of *Eruca sativa* extracts had no effect on groups 3 and 4 studied parameters compared to control group (injected with $CCl_{4)}$. Table (1).

Table(1) Effect of Eruca sativa extracts on the liver enzyme and kidney urea, Creatinine in albino mice treated with CCL_4.

Group	No. of Animals	GOT(U/I)	GPT(U/I)	Urea(mg/dl)	Creatinine (mg/dl)
Group-1(normal diet) M±SE	6	36.5±2.32	27.68±2.34	24.24±2.08	2.77±0.59
Group-2(injected with 0.01ml CCl_4) M±SE	6	66.59±3.18***	71.72±1.86***	61.29±1.01***	6.86±0.15**
Group-3(injected with 0.01ml CCl_4 + 0.5mg/ml Eruca sativa extract) M±SE	6	65.18±3.19***	70.99±1.12***	59.98±0.14***	5.73±0.32*
Group-4(injected with 0.01ml CCl_4 + 1mg/ml Eruca sativa extract) M±SE	6	48.61±0.80*	58.9±0.82***	53.42±1.23***	5.33±0.26*
Group-5(injected with 0.01ml CCl_4 + 2mg/ml Eruca sativa extract) M±SE	6	37.32±1.15 N.S	33.77±2.10 N.S	38.83±0.89*	3.12±0.12 N.S

M=mean SE=standard error

*** $P < 0.01$ ** $P < 0.025$ * $P < 0.05$

The mean activities of serum liver enzymes GOT, GPT, Blood Urea and Creatinine were significantly increased in Group-2 (injected with CCl_4) compared to group-1(Normal diet), These results were in agreement with[21] who indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Also, [22] reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzyme into the blood. While the mean values of GOT, GPT, Blood Urea and Creatinine were significantly decreased in group-5(injected with CCl_4+ 2mg/ml Eruca sativa extract) compared to group-2(injected with CCl_4). In addition, urea decreased in the tested meals compared to that of the control-fed group. The activities of GOT and GPT enzymes tended to decrease indicating improved liver function tests, Such reduction of liver enzyme activities with no effect on relative weight of liver and also reduction of urea concentration exhibit healthy, non-pathological and nontoxic effect of dietary rocket diet. These results were in agreement with [23, 24], They reported that GOT and GPT activities were significantly decreased as the result of the studied treatments. As to rocket, this decrease may be due to their antioxidant status as reported by [25]. Similar results were obtained by [21] who indicated that

administration of rocket caused improving in GOT, GPT activities in male rabbits, which may be due to the high content of sulfur in Eruca sativa that works as a cleansing of body wastes, clearing congestion like sinusitis and assisting liver and immune function. Several studies on phytochemical analysis of Eruca sativa leafs has shown the presence of many compounds to which antioxidant activity may be ascribed, these include glucosinolate, flavonoids (Quercetin,Kaempherol and isohamnetin),Carotenoids, Vitamine C [18].

In case of rocket inclusion, the decrease in blood urea may be due to the effective role of rocket isothiocyanates volatile oils as diuretic,[26] found that the ethanolic extract and volatile oil of Eruca sativa (rocket) seeds have been shown to act as diuretics in dogs and the oil significantly increased Na+, K+ and Cl- extraction in urine.

Regarding the present study it could be concluded that Eruca sativa extracts possessed both prophylactic and therapeutic effects against experimentally induced liver injury in mice. However, the prophylactic role of these extracts was more potent than their treatment capacity.

REFERENCES

[1].Ahmad, S.S. 2007. Medicinal wild plants from Lahore-Islamabad Motorway (M-2). Pak. J. Bot., 39(2): 355-375.

[2].M. Khoobchandani., B.K. Ojeswi, N.Ganesh, M. Srivastava, S. Gabbanini, R. Matera, R Iori, L.Valgimigli. (2010). Antimicrobial properties and analytical profile of traditional Eruca sativa seed oil: Comparison with various aerial and root plant extracts. Food Chemistry 120 :217–224.

[3].Bennett RN, Rosa EAS, Mellon FA, Kroon PA. (2006). Ontogenic Profiling of Glucosinalates, Flavonoids and other Secondary Metabolite in Eruca sativa (Salad Rocket), Diplotaxis erucoides (Wall Rocket), Diplotaxis tenuifolia (Wild Rocket), and Bunias orientalis (Turkish Rocket). J. Agric. Food Chem., 54: 4005-4015.

[4].Heimler D, Isolani L, Vignolini P, Tombelli S, Romani A. (2007). Polyphenol content and antioxidative activity in some species of freshly consumed salads. J. Agric. Food Chem., 55: 1724–1729.

[5].Helana Naguib Michael1*, Reham Ezzat Shafik1, George Emad Rasmy.(2011). Studies on the chemical constituents of fresh leaf of Eruca sativa extract and its biological activity as anticancer agent in vitro. Journal of Medicinal Plants Research Vol. 5(7), pp. 1184-1191.

[6].Hanfi, M.; Eman, M.; Rowida, M. and Amer, H.A.(2010). Bio-protective effect of Eruca Sativa seed oil against the Hazardus effect of aflatoxin B1 in male rabbits. International Journal of academic research Vol.2. No. 2.

[7].Yehuda H, Khatib S, Sussan I, Musa R, Vaya J, Tamir S. (2009). Potential skin antiinflammatory effects of 4- methylthiobutylisothiocyanate (MTBI) isolated from rocket (Eruc sativa) seeds. Biofactors, 35(3): 295-305.

[8].Lamy E, Schröder J, Paulus S, Brenk P, Stahl T, Mersch-Sundermann V (2008). Antigenotoxic properties of Eruca sativa (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo(a)pyrene and their mode of action. Food Chem. Toxicol., 46 (7):2415-21.

[9]. Bennett, R.N.; Rosa, E.A.; Mellon, F.A.; Kroon, P.A.(2006). Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in Eruca sativa (salad rocket), Diplotaxis erucoides (wall rocket), Diplotaxis tenuifolia (wild rocket), and Bunias orientalis (Turkish rocket). J. Agric. Food Chem., 54, 4005–4015.

[10]. Azza Salah 2, Fatma Oraby 1, Amany Nour El-Deen 2 and Zakarya El-Khayat Antihepatotoxic.(2010). Effect of Eruca Sativa Extracts on Alcohol Induced Liver Injury in Rats Jihan Hussein1 , 1journal of American Science;6(11)

[11].Martinez-Sanchez A, Gil-Izquierdo A, Gil MI, Ferreres F. (2008). A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. J. Agric. Food Chem., 56: 2330–2340.

[12].Melchini, A.; Costa, C.; Traka, M.; Miceli, N.; Mithen, R.; De Pasquale, R.; Trovato, A. Erucin (2009). a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells. Food Chem. Toxicol., 47, 1430–1436.

[13] Yassin MM, Ashour AR and Elyazji NR (2004).Alternations in body weight, protein profile, non protein nitrogen constituents and kidney structure in diabetic rats under glibenclamid treatment. Journal of the Islamic University of Gaza (Natural Science Series).

[14] Atang-who IJ, Ebong PE, Eteng MU, Eyong EV and Obi AU (2007). Effect of Vernomia Amygdalina Del leaf on kidney function of diabetic rats. International Journal of Pharmacology, 3 (2): 143 – 148.

[15].Alqasoumi S (2010). Carbon tetrachloride-induced hepatotoxicity: Protective effect of 'Rocket' Eruca sativa L. in rats. Am. J. Chin. Med., 38(1):75-88.

[16].Alqasoumi S, Al-Sohaibani M, Al-Howiriny T, Al-Yahya M, Rafatullah S (2009). Rocket "Eruca sativa": a salad herb with potential gastric anti-ulcer activity. World J. Gastroenterol., 15(16):1958-1965.

[17].Kin SJ, Ishii G (2006). Glucosinalate profiles in the seeds, leaves and roots of rocket salad (Eruca sativa Mill.) and anti-oxidative activities of intact plant powder and purified 4-methoxyglucobrassicin. Soil Sci. Plant Nutr., 52: 394-400

[18].Jin J, Koroleva OA, Gibson T, Swanston J, Magan J, Zhang Y, Rowland IR, Wagstaff C (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (Eruca sativa and Diplotaxis tenuifolia) leafy salad following cultivation in different environments. J. Agric. Food Chem., 57(12): 5227-5234.

[19].Sharma N and Shukla S. 2011. Hepatoprotective potential of aqueous extract of Butea monosperma against CCl4 induced damage in rats. Exp Toxicol Pathol, 63, 671-6.

[20].Klug, W.S. and Cummings, M.R. (2005). Essentials of genetics. Fifth Ed. Pearson, prentice Hall.P.120-121.

[21].Ibrahim, S.A.M., 2005. Effect of some medicinal plants as feed additives on growth and some metabolic changes in rabbits. Egypt J. Nutr. Feeds, 8: 207–19

[22].Lamy, E.; Schroder, J.; Paulus, S.; Brenk, P.; Stahl, T.; Mersch-Sundermann, V. (2008). Antigenotoxic properties of Eruca sativa (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo(a)pyrene and their mode of action. Food Chem. Toxicol., 46, 2415–2421

[23]. Kuzu, N., K. Metin, A.F. Dagli, F.A. Akdemir, C. Orhan, M. Yalniz, I.H. Ozercan, K. Sahin and I.H. Bahcecioglu.(2007). Protective role of genistein in acute liver damage induced by carbon tetrachloride. Mediators Inflamm.: 36381.

[24]. Alam, M.S. G. Kaur, Z. Jabbar, K. Javed and M.Athar. (2007). Eruca sativa seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. Food and Chemical. Toxicology,45 : 910–920

[25]. Hsieh, P.C., G.J. Huang, Y.L. Ho, Y.H. Lin, S.S. Huang, Y.C. Chiang, M.C. Tseng and Y.S. Chang.(2010). Activities of antioxidants, glucosidase inhibitors and aldose reductase inhibitors of the aqueous extracts of four Flemingia species in Taiwan. Bot. Stud. 51(3): 293–302.

[26].Yang, G.; Gao, Y.T.; Shu, X.O.; Cai, Q.; Li, G.L.; Li, H.L.; Ji, B.T.; Rothman, N.; Dyba, M.; Xiang, Y.B.; Chung, F.L.; Chow, W.H.; Zheng, W. (2009). Isothiocyanate exposure, glutathione S-transferase polymorphisms, and colorectal cancer risk. Am. J. Clin. Nutr. 91