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Effect of *Eruca sativa* leaves extract on liver enzymes and lipid profile in phosphoric acid induced liver damage in male rabbits

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Abstract
This study was conducted on the male rabbits to investigate the effect of *Eruca sativa* on the liver enzyme and lipid profile in damaged liver induced by phosphoric acid. Twenty healthy adult male rabbits were used during the period from 1 to 31 March 2017. Animals were randomly separated into four equal groups (5 rabbits / group) and treated for 30 days as the following: 1st group was a control group (C): rabbits were permitted to *ad libitum* provide of drinking water, 2nd group (T_1): were allowed to drinking water containing 1/10 of lethal dose1530 mg /kg b.w of phosphoric acid, 3rd group (T_2): were received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally and 1/10 of lethal dose1530 mg /kg b.w of phosphoric acid, 4th group (T_3): were received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally. Fasting samples were collected after 30 days of the experimental to estimate liver enzymes and liver profile. The results revealed that oral intubation of phosphoric acid for 30 days caused hepatic dysfunction manifested by a significant elevation($P<0.05$) in the serum AST(83.00 u/L), ALT(44.20 u/L), cholesterol(74.60 mg/dL), triglyceride(224.80mmol/L), LDL-C (77.90 mg/dL) and VLDL-C (44.96 mg/dL) in the T1.Also phosphoric acid caused a significant decreasing($P<0.05$) in HDL-C(31.96 mg/dL), while the animals received *Eruca Sativa* with phosphoric acid (T_2)for 30 days showed a considerable decreasing ($P<0.05$) in the levels of AST (30.60 u/L), ALT (24.60 u/L),triglyceride(124.80 mmol/L) and cholesterol (71.40 mg/dL). However the increasing in HDL-C (45.98 mg/dl) was not significant as compared with control but the increasing was significant ($P<0.05$) with the other groups. The histological section showed the pathological changes in the liver tissue in T_1 group while giving *Eruca Sativa* extract with and without phosphoric acid was effective in modified these changes into semi normal.

Keywords: Phosphoric acid, Eruca Sativa, ALT, AST, cholesterol, triglyceride HDL.C, LDL-C and VLDL-C

1. Introduction
In latest years, Rocket plant (*Eruca sativa*) has gotten more value as avegetable, and spice around the world, further it is considered to be an important chemoprotective plant. The rocket belongs to the family *Brassicaceae* [1] which is consist of *Eruca sativa* Mill., *Bunias* and *orientalis Diplotaxis*. They can be eaten at various ontogenic phases [2].The rocketis fast growing and grown in cold season. The plant can be cut twenty days later and is consecutively cultivated from re-growth [3]. The beneficial and positive usefulness of the phytochemicals existing in rocket on health have been notified by a numeral of scientific research studies. As well, the utilization of vegetables with green leaves has been related with the minimized hazard of cardiovascular diseases (CVD) [4]. These advantageous effects have been linked to the variety of phytochemicals they consist of, such as vitamins(C, A), glucosinolates and flavonoids, all of which are found in large quantities in *Brassicaceae* crops [5, 6].The rocket is believed to be an extremely good resource of antioxidants, as it includes phenolic compounds, glucosinolates carotenoids and degradation products like isothiocyanates [7]. Moreover, *Eruca sativa* Mill has cytoprotective, anti-inflammatory, anti-ulcer and anti-secretory action [8-10]. Phosphoric acid is a mineral inorganic acid having the chemical formula (H3PO4), it has many uses, including as rust inhibitor, food additive, electrolyte flux, disbanding agent, Part of the components of cleaning products, industrial etchant, dental and orthopedic etchant and fertilizer feedstock. Phosphates and Phosphoric acids are also essential in biology. About (90%) of phosphoric acid is used in the production of agricultural fertilizer [11]. Food-grade phosphoric acid (additive E338) [12] is used to make the beverages acidic and foods like different jams and colas. It gives a sour or tangy taste. Different salts of phosphoric acid, for

example monocalcium phosphate, are used as fermenting agents [11]. As a chemical oxidative agent for the production of activated carbon, as utilized in the Wentworth process [13]. There are no studies concerning the effect of phosphoric acid alone on liver. Therefore, the present study was conducted to investigate the oxidative effect of phosphoric acid on liver and tried to repair these damage with *Eruca sativa* extract in rabbits during the period from 1 to 31 March 2017.

2. Material and Methods

Twenty healthy adult male rabbits, weighted (1000-1500 g) were used during the period from 1 to 31 March 2017. Rabbits were housed in iron cages and kept for ten days for acclimation. The room temperature was maintained at (22-25 ^{o}C). Animals were permitted freely access to water and pellets along the experimental period. Rabbits were housed in an animal's house /Department of Physiology and Pharmacology/College of Veterinary Medicine/ University of Baghdad. Animals were randomly divided into four equal groups(5 rabbits/group) which treated for 30 days as the following: control group (C): rabbits in this group were allowed to ad libitum provide of drinking water, treated group (T_1): rabbits in this group were allowed to drinking water containing 1/10 of lethal dose 1530 mg /kg b.w[14]of phosphoric acid, treated group (T_2): rabbits in this group received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally and 1/10 of lethal dose 1530 mg /kg b.w of phosphoric acid, treated group (T_3): animals in this received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally[15]. Blood samples were collected after 30 days of the experiment. The blood was collected directly from heart, blood specimens were preserved in tubes and centrifuged at 3500 rpm for 10 minutes, and then serum samples were a liquated and frosted at -20 ^{o}C till analysis, serum samples were used to measure the following parameters:- serum aspartate aminotransferase (AST), alanin aminotransferase (ALT), serum cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL.C) by using particular kits from LINEAR company. LDL-C concentrations were calculated as: Total cholesterol - HDL.C- VLDL.C [16] while, VLDL-Cholesterol was calculated as: Triglyceride /5 [17]. For histological studies, rabbits were anesthetized, sacrificed via drag the blood from the heart, after that liver was excised, opened longitudinally and conserved in 10% neutral formalin for preparing the histological sections [18].

2.1. Preparation of plant extracts

Plant was purchased from a home market place. The leaves were dried in the air and powdered. Fifty grams of the powder have been extracted for 3 hours in 250 ml of the distilled water by using soxhlet equipment. A warm water bath(45°C) was used for heating, after that the leaves extract solution was vaporized at 45°C using a rotary evaporator, and the eventual crude extract was iced up at -20°C till use to set up the wanted dose [19].

2.2. Statistical analysis

Data are shown as the Mean± SE. Data were analyzed by using one way analysis of variance (ANOVA) within SPSS program. Means were tested by LSD at a probability level of ($p<0.05$) [20].

3. Results

The effect of *Eruca sativa* and phosphoric acid on liver function enzymes and lipid profil in male rabbits for 30 days are represented in Table 1.The results revealed that there were a significant increase in serum AST activity ($P<0.05$) in T_1 group (83.00±26.99) in compared to control and T_2 groups (24.75±2.78), **(**30.60±2.76), while T_3 group (50.40±14.82) shows no differences with the control, $T_1$and T_2 groups (24.75±2.78), (83.00±26.99), (30.60±2.76). Also, there was a significant change ($P<0.05$) in serum ALT activity in T_1 group (44.20±6.82) as compared with the T_2 group (24.60±6.80). Though the control and T_3groups did not differ (37.25±4.83), (40.60±4.67) on group $T_1$and T_2 (44.20±6.82), (24.60±6.80). On the other hand the differences in triglyceride among control, $T_1$and T_3 groups were significant ($P< 0.05$). The highest estimate was detected in T_1 group (224.80±13.42) which differed significantly ($P<0.05$) as compared with T_2 group (124.80±22.74). Data showed that the differences in cholesterol were significant ($P< 0.05$) in all groups. The highest approximate was detected inT_1 group (74.60±12.29). Aswell, this study revealed that serum HDL-C was significantly decreased ($P<0.05$) in T_1 group (31.96±2.12) as compared to control, T_2 and T_3 groups (42.40±0.66), (45.98±1.33), (41.64±0.50), whereas serum HDL-C was significantly increased ($P<0.05$) in T_2 group (45.98±1.33)as compared to $T_1$and T_3 groups (31.96±2.12),(41.64±0.50). Furthermore the serum VLDL-C was increased significantly ($P<0.05$) in T_1, T_2 and T_3 groups (44.96±2.68), (37.60±5.21), (37.68±3.35) as compared to control group (24.96±4.54). The highest estimation was detected in T_1 group (44.96±2.68). In addition in this experiment the serum LDL-C was significantly increased in all experimental groups ($P<0.05$) particularly in T_1group (77.90± 14.18).

4. Dissection

The results showed that phosphoric acid caused a significant increase in the serum activity in both of ALT and AST enzymes, this increase may be due to chronic exposure to phosphoric acid which result in hepatocellular injury. At the same time In T_2 group, liver enzymes (AST, ALT) were significantly decreased compared to the phosphoric acid group. These results were in agreement with El-Nattatand El-Kady [21] who mentioned that administration of rocket resulted in promoting in ALT and AST activities in male rabbits, which may possibly because of the high sulfur content in *Eruca sativa* that works as a removing of body wastes, clearing congestion such as sinusitis and supporting the liver and immune function. *Eruca sativa* leaves and seeds have a strong free radical scavenging antioxidants and protected from damage caused by oxidation through maintaining or rising the levels of antioxidant molecules and antioxidant enzymes [22].On the other hand the current experiment illustrates that phosphoric acid caused a considerable increase ($p<0.05$) in serum triglyceride concentration, an increased acid load may increase hepatic desaturases actions, stimulate the creation of monounsaturated fatty acids and triglycerides in the liver and raise the serum triglyceride concentration [23]. Also this study demonstrated a significant increase in cholesterol levels in all experimental groups mainly in T_1group. At the same time the phosphoric acid group showed a significant decrease in HDL-C, while there was a significant increase in LDL-C and V-LDL, whereas a considerable increase was observed in serum HDL-C and a significant decrease in both LDL-C and V-LDL in T_2 group. The hypolipidimic effect of *Eruca Sativa* leaves extract possibly due to the activation of the enzyme 7 alpha-hydroxylase by vitamin C one of the *Eruca Sativa* leaves component which enhances the conversion of plasma cholesterol into bile acid, thus resulting in a decrease in serum levels of cholesterol,

furthermore the ability of the vitamin C to inhibit the oxidation of HDL [24]. The light microscopic examination of liver tissues sections of control group showed normal histological structure **(**Fig. 1),whereas in the group that received phosphoric acid (Fig 2 and 3)revealed the negative and harmful effects of phosphoric acid represented in congestion of central venous with disappearance of lobular sinusoid and the parenchyma showed acute degenerative changes that characterized by cellular swelling of hepatocytes that lead to compress the sinusoids, this damaged effect may be caused by the long time of exposure to phosphoric acid [25]. Liver tissue section of rabbits received *Eruca Sativa* extract and phosphoric acid showed general central venous congestion and portal venous congestion with mild secondary amyloidosis at the portal area (Fig.4).The histological section of treated group that received *Eruca Sativa* extract showed moderate central and portal venous congestion (Fig. 5 and 6), the effect of *Eruca Sativa* extract in T_2 and T_3 groups was effective in modified these changes into semi normal, this effect may be due to its potent antioxidant activity in rabbits [26].

5. Conclusion

Aqueous extract of *Eruca Sativa* leaves can improve liver enzymes function and lipid profile against phosphoric acid through 30 days of treatment.

6. Acknowledgement

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Table 1: Assessment of the effect of *Eruca sativa* leafs extract and phosphoricacid on AST, ALT, Triglyceride, Cholesterol, HDL, VLDL and LDL

Parameters	Control	T_1	T_2	T_3
AST (u/L)	24.75±2.78b	83.00±26.99a	30.60±2.76b	50.40±14.82ab
ALT (u/L)	37.25±4.83ab	44.20±6.82a	24.60±6.80b	40.60±4.67ab
Triglyceride mmol/L	188.00±26.09 a	224.80±13.42 a	124.80±22.74 b	190.20±15.83 a
Cholesterol (mg/dl)	73.40±1.77 a	74.60±12.29 a	71.40±17.40 a	72.00±6.63 a
HDL-C (mg/dl)	42.40±0.66 ab	31.96±2.12 c	45.98±1.33 a	41.64±0.50 b
VLDL-C (mg/dl)	24.96±4.54 b	44.96±2.68 a	37.60±5.21 a	37.68±3.35 a
LDL-C (mg/dl)	66.22 ± 16.93 a	77.90± 14.18 a	68.18±2.90 a	75.32 ±7.94 a

Means with different lettersin the same row significantly different ($p<0.05$)

C: control group

- T_1: Animals received drinking water contain 1530 mg /kg b.w of phosphoric acid.
- T_2: Animals received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally and 1530 mg/kg b.w of phosphoric acid.
- T_3: Animals received 250 mg/ kg b.w aqueous extract of *Eruca Sativa* orally.

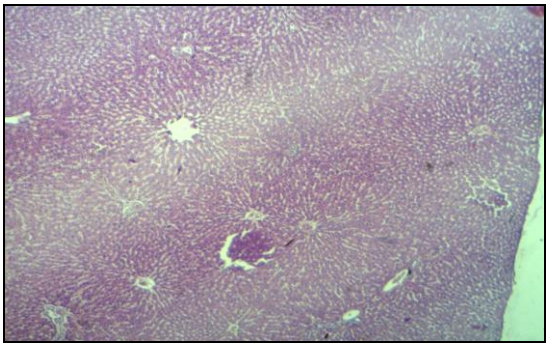

Fig 1: liver (Control group) showed normal appearance of liver H&E 40X

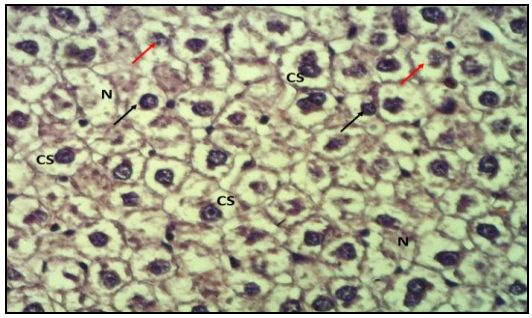

Fig 3: magnified section of liver (phosphoric acid group) shows: acute cellular swelling (CS), necrosis (.N), pyknosis (black arrows) and Karyorrhxes (Red arrows) H&E 400X

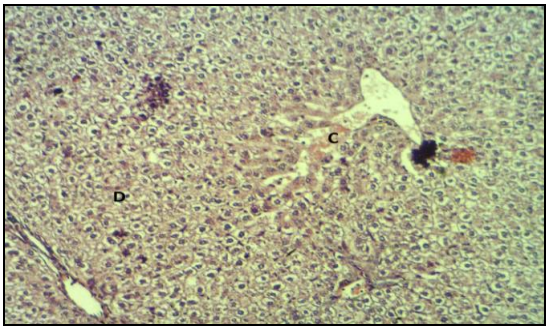

Fig 2: section of liver (phosphoric acid group) Shows: congestion (C) and acute degenerative changes (D). H&E 100X

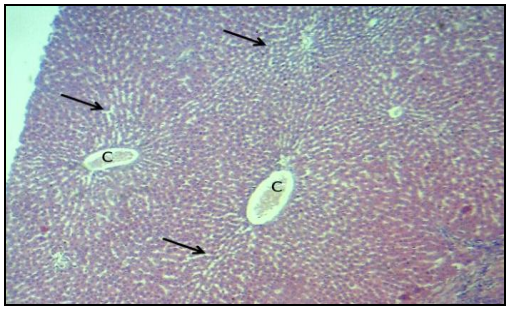

Fig 4: liver (phosphoric acid and *Eruca Sativa* group) shows: moderates venous congestion (Arrows) and dilatation of sinusoid. H&E 100X

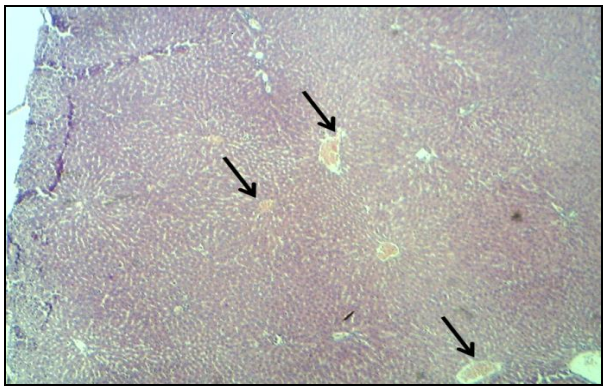

Fig 5: liver (*Eruca Sativa* group) shows: moderates venous congestion (Arrows). H&E 100X

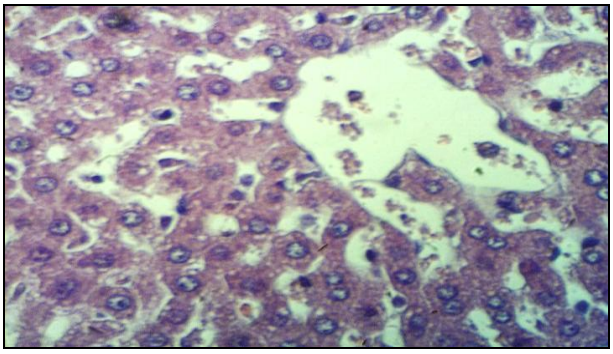

Fig 6: magnified section of liver (*Eruca Sativa* group) shows: normal hepatocytes within

7. References

1. Silvarajan VV. Introduction to the Principle of Taxonomy. 1st ed. Oxford and IBH Publishing Co. PVT. Ltd.
2. Bennett RN, Rosa EA, Mellon FA, Kroon PA. 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). Journal of Agriculture Food Chemistry. 2006; 54:4005-4015.
3. Jakse M, Hacin J, Kacjan N. Production of rocket (*Eruca sativa* Mill.) on plug trays and on a floating system in relation to reduced nitrate content. Acta Agriculture Slovenica. 2013; 101:59-68.
4. Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE *et al.* The effect of fruit and vegetable intake on risk for coronary heart disease. Annual Internal Medicin. 2001; 134:1106-1114.
5. Jin J, Koroleva OA, Gibson T, Swanston J, Magan J, Zhang Y, *et al*. Analysis of phytochemical composition and chemoprotective capacity of rocket (*Eruca sativa* and *Diplotaxis tenuifolia*) leafy salad following cultivation in different environments. Journal of Agriculture. Food Chemistry. 2009; 57:5227-5234.
6. BellL, Wagstaff C. Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (*Eruca sativa* and *Diplotaxis tenuifolia*). Journal of Agriculture. Food Chemistry. 2014; 62:4481-4492.
7. Villatoro-Pulido M, Font R, Saha S, Obregon-Cano S, Anter J, Munoz-Serrano A, *et al*. *In vivo* biological activity of rocket extracts (*Eruca vesicaria* subsp. *sativa* (Miller) Thell) and sulforaphane. Food and Chemical Toxicology. *Nutrients*, *6* 5851, 2012; 50:1384-1392.
8. Alqasoumi S, Al-Sohaibani M, Al-Howiriny T, Al-Yahya M, Rafatullah S. Rocket "*Eruca sativa*".A salad herb with potential gastric anti-ulcer activity. World Journal of Gastroenterology. 2009; 15:1958-1965.
9. Khan H, Khan MA. Antiulcer Effect of Extract/Fractions of *Eruca sativa*: Attenuation of Urease Activity. Journal of. Evidaned Based Complement. Alternative Medicine. 2014; 19:176-180.
10. Heimler D, Isolani L, Vignolini P, Tombelli S, Romani A. Polyphenol content and antioxidative activity in some species of freshly consumed salads. Journal of Agriculture Food Chemistry. 2007; 55:1724-1729.
11. Schrödter K, Bettermann G, Staffel Klein T, Hofmann T. Phosphoric Acid and Phosphates in Ullmann & apos; s Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim, 2008
12. Current EU. Approved additives and their E Numbers. Foods Standards agency. https://www.food.gov.uk/science/additives/enumberlist, 2012.
13. Toles C, Rimmer S, Hower JC. Production of activated carbons from a Washington lignite using phosphoric acid activation. Carbon. 1996; 34(11):1419.
14. Biofax. Datasheet 19-4 /70.Northbrook, IL: Biofax industrial Bio-test Laboratories, Inc.1970.
15. Mahdy SS. The antigenotoxicity of *Eruca Sativa* Mill extract on bone marrow cells of male albino mice treated with Vincristine. Ibin Al-Haitham Journal For pure and Applied. 2012; 2(25):26-32.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifugation. Clinical Chemistry. 1972; 18:499-502.
17. DeLong DM, DeLong ER, Wood PD, Lippel K, Rifkund BM. A comparison of methods for the estimation of plasma low- and very low-density lipoproteun cholesterol: the Lipid Research Clinics Prevalence Study. Journal of America Medical Association. 1986; 256:2372-7
18. Lee G, Luna LG. Manual of histological staining methods of armed Forces Iinstitutes of pathology. 3rdEd. MC Grow-Hill book company. New York. 1968, 12-31.
19. Harbone JB. Phytochemical Methods Science Paperable Active, chemopmanan Hall pup. M Sc. Thesis, college of Science, AL-Nahrain University, 1973.
20. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.1. SAS Institute, Inc., Cary, N.C., USA, 2010.
21. El-Nattat WS, El-Kady RI. Effect of different medicinal plant seeds residues on the nutritional and reproductive performance of adult male rabbits. International Journal of Agriculture and Biology. 2007; 9(3):479-485.
22. 22. Alam MS, Kaur G, Jabbar Z, Javed K, Athar M. Eruca sativa seeds possess antioxidant activity and exerta protective effect on mercuric chloride induced renal toxicity. Food and Chemical Toxicology. 2007; 45(6):910-920.
23. Høstmark AT, Lunde MS, Eilertsen E. Does an acid load promote liver desaturases and increase serum lipids? Clinical Reviews and Opinions. 2010; 2(1):008-016
24. Hillstrom RJ, Yacapin-Ammons AK, Lynch SM. Vitamin C inhibits lipid peroxidation in human HDL. Journal of Nutrition. 2003; 133(10):3047-3051.
25. Aquila l, Pepe F, Di Nunzio C, Ausania F, Serra A, Ricci P. Suicide case due to phosphoric acid ingestion: case report and review of literature. Journal of Forensic

Science. 2014; 59(6):1665-7.
26. Alqasoumi S. Carbon tetrachloride-induced hepatotoxicity: Protective effect of Rocket *Eruca sativa* L. in rats. The American Journal of Chinese Medecine. 2010; 38(1):75-88.