# *Eruca sativa* seed oil: Characterization for potential beneficial properties

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**Abstract**: *Eruca sativa* (ES) seed oil is used in food preparation and as source of natural medication. *Eruca sativa* (ES) seed oil was analysed for phenolic composition using high performance liquid chromatography with diode array detection (HPLC-DAD), pigment contents, quality characteristics. The oil was fed to rabbits for two weeks. Serum biochemistry, haematological and liver histological parameters were studied. Results showed that quercetin, caffeic acid and chlorogenic acids were the major phenolic compounds. Lycopene and other pigments were present in considerable amounts. Animal studies showed that the body weight of rabbits decreases with the increase of ES oil. The level of serum glucose, total cholesterols, triglycerides and LDL-cholesterol decrease significantly, while an increase was observed in the HDL-cholesterol. The level of white blood cells including lymphocytes and mean corpuscular haemoglobin concentration increases, while a significant increase occurred in platelets count with the increase of ES seed oil dose. In the present study microscopic observations in control and the treated groups showed similar cyto-architecture of the liver with no significant histological changes. It is concluded that *Eruca sativa* seed oil is a rich source of important phytochemicals with anti-obesity properties in selected animals.

**Keywords**: *Eruca sativa*, pigments, antioxidant activity, phenolic composition.

## INTRODUCTION

*Eruca sativa* belongs to the family Brassicaceae, which includes a large number of plant species and is used for different purposes such as food and medicine. The colour of the plant is dark green with a height of about 20-50 cm. It is an annual or perennial herb and its flowers appear mostly in February-April (Morales and Janick, 2002). All parts of the plant are in use for different purposes such as leaves and flowers for salad making. The leaves have a pungent taste due to the presence of specific substances such as erucic acid, while the roots are not exploited for consumption in humans. *Eruca sativa* plant is cultivated in different countries of the world but mostly in Mediterranean countries. In Pakistan, it is mostly grown for the isolation of seed oil known as *Jamba or Jamama oil*. The seeds constitute the most valuable and useful part of the plant. On the other hand seed oil carries its importance and applications. The seed oil of *Eruca sativa* is mixed with mustered oil to decrease the pungency. The seed oil is composed of important constituents like fatty acids i.e. palmitic, stearic, oleic, linoleic, linolenic, eicoseneic and erucic acids (Tonguc and Sabri, 2012).

*Eruca sativa* seed oil is used as hair tonic to prevent hair loss. The oil is also used for the treatment of burns, for eye infection as an ointment, and for the digestive problems (Garg and Sharma, 2014). It is also used as a future industrial oil source especially as biodiesel (Mumtaz *et al*., 2016), and an alternative fuel for transport and as a lubricant (Chakrabarti and Ahmad, 2009). In some areas of the world the plant is used as vegetable and spice and its seed oil is used for cooking. According to Melchini and Traka (2010), various types of cancer growth and development can be reduced by using Cruciferous vegetables. It was attributed to the presence of some active products, which offer resistance against cancer. In-vitro study of the compounds from the 70 % ethanolic extract showed cytotoxic effect on different human tumour cell lines (Michael *et al*., 2011).

In Pakistan, *Eruca sativa* seed and its oil are commonly used. In USDA germplasm collection, the largest number (144) of accession lines have suggested a uniform trait of Pakistani origin *Eruca sativa* (Morales and Janick, 2002). About 102 accessions of *Eruca sativa* for variations in seed storage proteins have been reported (Shinwari *et al*., 2013). The authors revealed a significant variation in the seed proteins of the selected samples. The *Eruca* seeds were evaluated for physico-chemical and antioxidant potential (Hamid *et al*., 2014). The aqueous extract of the seed was found to possess the higher antioxidant activity. The authors proposed that plant seed oil can be used as source of natural antioxidants and for medicinal purposes. *Eruca* leaves extracts were found effective as anti-ulcer agent (Khan and Khan, 2014). The study has validated scientifically the traditional use of *Eruca* for the treatment of certain ulcers. Similarly, *Eruca* seed oil was found to handover anti-microbial and anti-fungal activity (Ali *et al*., 2014). Several secondary metabolites and antioxidant composition of *Eruca* seed, stems, leaves and flowers were identified using RP-HPLC (Sadiq *et al*., 2014). These authors revealed important phenolic compounds in the selected parts of the *Eruca* plant grown in Pakistan.

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However, there is lack of information about the phenolic composition and potential of oil in experimental animals. The present study was therefore aimed to provide an insight in to the chemical and biological potential of the seed oil obtained from the *Eruca sativa* grown in Pakistan.

## MATERIAL AND METHODS

### *Materials*

*Eruca sativa* seed oil was purchased from the local extraction plant, with brand name "Jamama oil". All chemicals were of HPLC grade and were obtained from Sigma-Aldrich (Germany) or otherwise mentioned.

### *Spectrophotometric analyses*

Peroxide value (PV) of oil was determined using AOCS method (AOCS, 1998). Total phenolic contents (TPC) were determined using Folin-Ciocalteu reagent. *Chlorophyll a & b*, β-carotene and lycopene were determined using the method reported previously, with the help of Pharmaspec 1700 spectrophotometer (Shimadzu, Japan) (Barros *et al*., 2010). The oil samples were dissolved in hexane and the absorbance was measured as $OD_{453}$, $OD_{505}$, $OD_{645}$ and $OD_{663}$ nm. The values were expressed as mg/100mL.

### *HPLC-DAD analyses of phenolic compounds*

Phenolic compounds were extracted from the oil using the modified method reported previously (Pirisi *et al*., 2000). Briefly 1g of the oil was dissolved in 10mL methanol-water (60:40) mixture. After 30min of vigorous shaking, 10mL of n-hexane was added and stirred for 20 min. The mixture was separated and the methanol fraction was collected with three replications of the above method. The methanol fraction was air dried to 10mL. The samples were then filtered and injected in to the HPLC system.

The phenolic compounds were determined using Agilent 1260 HPLC system consists of quaternary pump, degasser, auto-sampler and diode array detector (DAD). The binary gradient system consists of solvent A (methanol: acetic acid: deionized water, 10: 2: 88, v/v) and solvent B (methanol: acetic acid: deionized water, 90: 2: 8, v/v). The gradient program was started with 100% A at 0 min, 85% A at 5 min, 50% A at 20 min, 30% A at 25 min, and 100% B from 30 to 50 min (Zeb, 2015). The separation was achieved with help of Agilent Zorbax Eclipse C18 (4.6 × 100mm, 3.5µm) column. The DAD was set to 280 nm for analysis of phenolic compounds. The spectra were recorded from 190 to 500nm. The identification was carried out using available standards, retention times, and UV spectra. The quantification of identified compounds was based on the peak area and expressed as mg/100g.

### *Animal feeding*

The animal studies were performed under the approved guidelines for the proper care of animals of the Department of Biotechnology, University of Malakand. The acclimatized rabbits (Himalayan strain) were classified into four groups containing three replicates (n=3). The rabbits were fed with *Eruca sativa* seed oil as: Group A: Selected as control and were fed on normal food, Group B: Fed on 1mL/kg body weight *Eruca sativa* seed oil, Group C: Fed on 2mL/kg body weight *Eruca sativa* seed oil, and Group D: Fed on 3mL/kg body weight *Eruca sativa* seed oil.

### *Biochemical parameters*

After feeding the rabbits for seven days and fourteen days, the blood samples were collected for haematological and serum biochemical tests. The blood samples for haematological study were collected in evacuated blood collection tube and for serum analysis the blood samples were added to gel and clot activator tubes or centrifuged directly at 2250 × *g* for five minutes to obtain serum. The serum was added to labelled Eppendorf tubes and kept in refrigerator at -20ºC or used for direct analyses. The rabbits were slaughtered at fourteenth day of feeding and the internal organs i.e. heart, kidney, brain and liver were weighed. The liver samples were preserved in 10 % formalin for histological study. The serum biochemical indices such as triglycerides, total cholesterols, HDL-cholesterol, LDL-cholesterol, ALT and serum glucose were analysed using standard diagnostic kits (HUMAN, Germany).

### *Haematology*

For haematology, two millilitre blood was taken after each treatment of 1st and 2nd week treatments, from the rabbits jugular vein in EDTA tubes. Automatic Haema analyser (Abbott Diagnostic Division, Canada) was used for the determination of the following parameters: White blood cells, red blood cells, lymphocytes count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelets.

### *Histopathology of liver*

The microscopic slides of the liver specimens were prepared according to the protocols recently reported (Zeb and Ullah, 2015). The prepared slides were analysed by microscope (Swift Instruments, Inc Japan) and the image was documented by the built in digital camera with 1.3 MP resolution.

### *Data analysis*

All samples were measured in triplicate. Data were analysed for variation by one way analysis of variance (ANOVA) and student t-test for paired data at $\alpha = 0.05$ using Graph Pad Prism 5 for windows version 5.03 (Graph Pad Software Inc, 2009).

## RESULTS

The peroxide value of ES oil was 5.8±0.4meq/kg (mean ± SD). TPC was 4.43±1.6 mg of GAE /g (443mg of GAE

/100g) chlorophyll a was 15.6±1.1mg/100mL, chlorophyll b was 42.8±3.2 mg/100mL, β-carotene was 17.6±2.1 mg/100 mL and lycopene was 5.9±0.5 mg/100mL (mean ± SD).

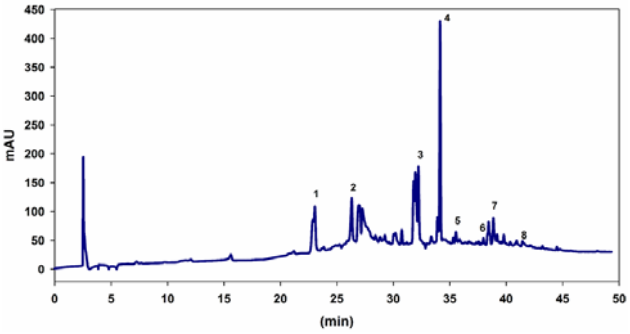


**Fig. 1**: Typical HPLC-DAD chromatogram of the *Eruca sativa* seed oil at 280 nm. The details of the identification of compounds are given in table 1.

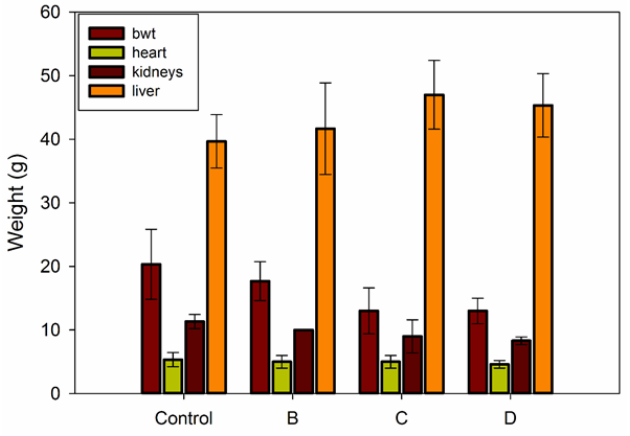


**Fig. 2**: Effect of *Eruca sativa* seed oil on the changes in the body weight of whole rabbit, heart, kidneys and liver.

Fig. 1 shows the separation of different phenolic compounds in the *Eruca* oil. The detailed description and identification of phenolic compounds are given in table 1. The identification was based on the retention time of standard compounds (3-O-Caffeoyl-quinic acid, caffeic acid, quercetin) and compared with the adsorption maxima reported in the literature. Total of eight compounds were tentatively identified. Compound 1 was identified as 3-O-caffeoyl-quinic acid which was found to elute at the retention time of 23 min. Compound 2 was caffeic acid, which was identified by comparing the retention time and absorption maxima of standard compound. Compound 3 was identified as chlorogenic acid, compound 4 as Quercetin, compound as 5 myricetin hexoside, compound 6 as kaempferol, compound 7 as kaempferol hexoside and compound 8 as 5-O-p-coumaroylquinic acid. In the identified compounds caffeic acid and quercetin were present in highest amounts.

The results showed gain in the body weight of control group, while in treated groups there was loss in body weights at the end of study duration as compared with the initial weights. The net gain in the body weight of control group was 20.33±5.5 (mean ± SD). The weights of treated groups were found to decrease during feeding with *Eruca sativa* seed oil at different doses, as compared with the control group. But the results showed a little decrease in weights of the treated groups with respect to initial weights. The decrease was statistically non-significant ($P < 0.05$) as compared with control group (fig. 2). Organ of the rabbits were weighted, such as liver, kidneys and heart. The control group liver weight was 39.67±14.2 (Mean ± SD) and kidneys weight were 11.33±1.1 (Mean ± SD). The heart weight of control group was calculated as 5.33±1.1 (Mean ± SD). The results showed that with the increase of dose the weight of liver increases while the weight of kidneys decreases as compared with the control group. This increase in liver weight and decrease in kidneys weights were non-significant ($P<0.05$) as compared with control group. The heart weight of treated groups was slightly lower than the control group, but the decrease was non-significant as shown in fig. 2.

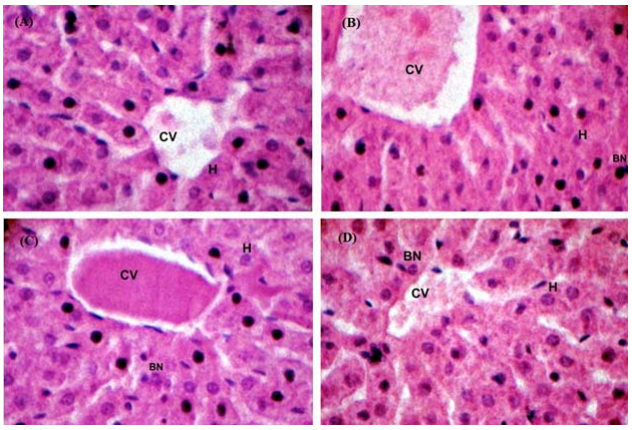


**Fig. 3**: Representative histological slides of the rabbit liver. Haematoxylin & Eosin stained, 5 µm thick section of rabbit Liver, treated for two weeks with 1-3 mL *Eruca sativa* seed oil showing, Central vein (CV), Bi-nucleated cells (BN) & Hepatocytes (H) arranged in radiating hepatic cords. (Photomicrograph 40X).

The control group serum triglycerides level of rabbits was 68.46±2.0 mg/dL (mean ± SD) and 65.76±3.6mg/dl (mean ± SD) corresponds to one week and two weeks study respectively. The results of study after one week showed a decrease in the level of serum triglycerides in a specific manner as shown in the table 2. But there were no significance differences between the treated groups as compared with control group. Although after the completion of two weeks treatment there was a decrease in the triglycerides level of the treated groups as compared with control group. Moreover, the statistical analysis of results after two weeks showed significant difference ($P < 0.05$) of group C and D as compared with control group.

The serum total cholesterol level of control group after one week and two weeks were 115.00±3.8 mg/dL (mean ± SD) and 105.7±2.2 mg/dL (mean ± SD) respectively. The level of total cholesterol decreases with increase of the ES oil. There was a gradual decrease in the serum total cholesterol level of groups in both weeks as compared with control group such as shown in the table 2. The statistical analysis showed significant difference ($P< 0.05$) between the groups as compared to control group. The results showed a significant difference ($P<0.05$) in group B, C and D as compared with control group after one week treatment of rabbits with *Eruca sativa* seed oil. In the end of study duration and analysis, it was found that C and D groups showed significant difference ($P< 0.05$) as compared with control group.

The serum HDL-cholesterols level of rabbits was increased with the increase of dose of *Eruca sativa* seed oil per kg body weight of rabbits. The level of HDL-cholesterols for the control group was 78.75±2.7 mg/dL (mean ± SD) and 74.52±0.8 mg/dL (mean ± SD) after one week and two weeks respectively. Although there was gradual increase in the treated groups as compared with the control group in both weeks results. In first week there was significant difference ($P<0.05$) between C and D groups as compared with the control group. Moreover, the statistical analysis after two weeks study showed significant difference ($P<0.05$) between groups D as compared to control group as shown in table 2.

The serum LDL-cholesterols levels of control group were 49.96±5.6 mg/dL (mean ± SD) and 44.37±3.1 mg/dL (mean ± SD) after one week and two weeks respectively. The level of LDL-cholesterol was decreases with the increase of *Eruca sativa* seed oil as compared with control group. There was a significant difference ($P < 0.05$) among B, C and D as compared with control group in the first week treatment. On the other hand LDL-cholesterol level after two weeks study also showed a decrease with increase of ES oil as compared with control group. From the results of two weeks statistical analysis it was clear that there was found statistical difference ($P < 0.05$) between the two treated groups C and D as compared with control group (table 2).

The serum alanine aminotransferase (ALT) test were done for the functional performance of liver and kidneys. The ALT levels of the control group were 32.67 ± 2.0 IU/L (mean ± SD) and 35.00 ± 4.5 IU/L (mean ± SD) after one week and two weeks studies respectively. The serum ALT level in the treated groups was decreasing with the increase of dose of ES oil as compared with the control group in the first week. But the decrease in ALT level showed no significant difference ($P<0.05$) as compared with control group in the first week. In the second week study the results followed the same pattern of changes like that of first week. From these results it was observed that the treated groups showed no significant differences ($P<0.05$) in both weeks studies, as compared with the control group.

The serum glucose of the control groups was 117.3 and 105.0 mg/dL corresponds to one week 1st and 2nd week studies respectively. The results for both weeks showed decrease in the amount of glucose as compared to the control group. From the data it is clear that with the increase of ES oil the level of glucose decrease in the serum. There was significant difference ($P<0.05$) between C and D groups as compared with the control group after first week analysis. On the other hand after two weeks study the data also showed statistical significance as well as decrease in the level of glucose in the serum with respect to the increase of oil dose. From the results it was clear that C and D groups showed significance difference ($P<0.05$) as compared to control group.

The WBCs concentration of control group after one week and two weeks studies were 5.87±0.5 × $10^3$/µL (mean ± SD) and 9.13±4.0 × $10^3$/µL (mean ± SD) respectively. The WBCs concentrations showed a little increase in the first week study with the increase of dose of *Eruca sativa* seed oil, but the treated groups represent no significant difference ($P<0.05$) as compared to the control. The results of second week showed increase in WBCs count with respect to the first week study. In the second week it was observed that the ES oil treated groups showed increase in WBCs count as compared with the control group. But the increase in WBCs count with the increase of ES oil dose showed non-significant difference ($P< 0.05$) as compared with the control group (table 3).

The RBC of the control rabbits were 7.92 × $10^6$ cells per µL (mean) and 4.84 × $10^6$ cells per µL (mean) corresponds to 1st and 2nd weeks studies respectively. Also The RBCs counts of groups were B (7.93±0.1, 3.47±1.4), C (7.85±0.2, 3.43±3.3) and D (7.70±0.1, 2.73±1.7) respectively in the first and second weeks. All treated groups in the first week show a little decrease in the RBCs count with the increase of dose of ES oil, when compared with control group. But the decrease in first week study was found non-significant ($P<0.05$) as compared with control group. These results also showed a decrease in the RBCs count in the second week as compared with the first week. The treated groups also showed a decrease in RBCs count in the second week study as compared with control group, when the ES oil dose increases. The treated groups in the second week showed no significant difference ($P<0.05$) in RBCs count, in comparison with control group (table 3).

The lymphocytes concentration of control group after one week and two weeks study were 78.97±7.7 (mean ± SD) and 58.77±1.7 (mean ± SD) respectively. The lymphocytes concentrations of treated groups were B

**Table 1**: Identification of phenolic compounds in *Eruca sativa* oil. The identification was based on comparison with standard compounds and comparing with the absorption maxima of the reported literature.

| Peak | Retention time (min) | Identity | HPLC-DAD $\lambda_{max}$ (nm) | Amount (mg/100 g) |
|---|---|---|---|---|
| 1 | 23.0 | 3-O-Caffeoyl-quinic acid | 240, 303, 324 | 2.70 |
| 2 | 26.3 | Caffeic acid | 238, 302, 305 | 5.12 |
| 3 | 32.2 | 5-O-Caffeoyl-quinic acid | 240, 326 | 5.10 |
| 4 | 34.0 | Quercetin | 253, 366 | 21.3 |
| 5 | 35.6 | Myricetin hexoside | 267, 360 | 0.82 |
| 6 | 37.9 | Kaempferol | 260, 370 | 0.95 |
| 7 | 38.9 | Kaempferol hexoside | 264, 290, 360 | 2.24 |
| 8 | 41.4 | 5-O-Coumaroyl-quinic acid | 231, 310 | 1.32 |

**Table 2**: Effects of *Eruca sativa* oil on the serum biochemical parameters of rabbits.

| Group | Triglycerides (mg/dL) | | Total cholesterols (mg/dL) | | HDL-cholesterols (mg/dL) | | LDL-cholesterols (mg/dL) | | ALT (IU/L) | | Glucose (mg/dL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 |
| Control | 68.4 ±2.0 | 65.7 ±3.6 | 115.0 ±3.8 | 105.7 ±2.2 | 78.7 ±2.7 | 74.5 ±0.8 | 49.9 ±5.6 | 44.3 ±3.1 | 32.6 ±2.0 | 35.0 ±4.5 | 117.3 ±7.4 | 105.0 ±4.6 |
| B | 63.1 ±3.9 | 60.9 ±1.8 | 102.6[a] ±1.7 | 103.8 ±1.4 | 83.4 ±9.2 | 75.97 ±3.9 | 31.6[a] ±8.2 | 40.0 ±4.3 | 30.0 ±1.7 | 32.3 ±4.7 | 104.3 ±6.5 | 95.1 ±7.3 |
| C | 61.2 ±3.6 | 58.3[a] ±1.8 | 103.2[a] ±3.3 | 97.5[a] ±1.6 | 97.7[a] ±1.6 | 77.4 ±1.7 | 17.6[a] ±4.1 | 31.8 ±1.4 | 28.3 ±3.2 | 32.0 ±3.0 | 93.8[a] ±7.4 | 88.9[a] ±3.1 |
| D | 64.6 ±2.8 | 57.1[a] ±2.1 | 98.7[a] ±3.3 | 96.5[a] ±4.8 | 97.8[a] ±3.4 | 84.4[a] ±4.5 | 13.9[a] ±2.4 | 23.5[a] ±7.9 | 27.0 ±2.6 | 27.3 ±5.0 | 97.4[a] ±4.2 | 86.7[a] ±5.1 |

*Values are Mean and SD of triplicate readings. Superscript letter a represent significant difference from the control at $p< 0.05$ (Tukey test) in a column

**Table 3**: Effects of *Eruca sativa* oil on the haematological parameters of rabbits.

| Group | WBC (□$10^3$/μL) | | RBC (□$10^6$/μL) | | Lym (%) | | HB (g/dL) | | HCT (%) | | Platelets (□$10^3$/μL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 | Week 1 | Week 2 |
| Control | 5.87 ± 0.5 | 9.13 ±4.0 | 7.92 ±0.3 | 4.84. ±1.8 | 78.97 ±7.7 | 58.77 ±1.7 | 11.10 ±1.0 | 10.80 ±0.7 | 50.90 ±4.0 | 31.37 ±10.7 | 513.0± 205.3 | 442.0± 117.5 |
| B | 5.67 ±1.6 | 11.90 ±2.3 | 7.93 ±0.1 | 3.47 ±1.4 | 80.67 ±3.3 | 61.93 ±1.9 | 10.93 ±0.4 | 10.50 ±0.5 | 47.73 ±1.3 | 22.23 ±8.8 | 625.7± 153.3 | 699.0[a]± 336.8 |
| C | 5.97 ±2.0 | 12.80 ±7.9 | 7.85 ±0.2 | 3.43 ±3.3 | 68.73 ±15.7 | 66.50 ±9.8 | 10.90 ±0.3 | 10.47 ±0.2 | 47.83 ±2.3 | 21.03 ±18.7 | 1006[a]± 148.8 | 543.0± 92.4 |
| D | 6.27 ±4.1 | 10.07 ±7.9 | 7.70 ±0.1 | 2.73 ±1.7 | 71.20 ±12.7 | 72.43 ±15.5 | 10.43 ±0.8 | 10.40 ±1.3 | 44.97 ±3.6 | 17.80 ±10.3 | 868.3[a]± 124.4 | 906.5[a]± 945.4 |

*Values are Mean and SD of triplicate readings. Superscript letter a represent significant difference from the control at $p< 0.05$ (Tukey test) in a column.

(80.67±3.3, 61.93±1.9), C (68.73±15.7, 66.50±9.8) and D (71.20±12.7, 72.43±15.5) respectively in the first and second weeks studies. The lymphocytes concentrations showed an increase in B group as compared with the control group in the first week. But C and D groups showed a little decrease in lymphocytes count with the comparison to control group. Also in the first week the treated groups showed no significant difference ($P<0.05$) as compared with the normal group. On the other hand, the lymphocytes count in the second week study in the treated groups was found to increase as compared with the control group, when the doses become high. The increase in lymphocytes count showed no significance difference ($P<0.05$) between treated groups and control group (table 3).

The haemoglobin concentrations of control group were 11.10±1.0 g/dL (mean ± SD) and 10.80±0.7 g/dL (mean ± SD) after one week and two weeks studies respectively. In the first week analysis there was found a little decrease in Hb concentration in the treated groups as compared with the control group, with the increase of ES oil dose. The results after one week study showed no significant difference ($P<0.05$) among the treated groups and control group. The results obtained after two weeks showed decrease in Hb concentration in all groups as compared

with first week data. The Hb concentration in the treated groups also decreases in the second week study as compared with control group, when the dose of ES oil increases. The ES oil treated groups showed no significant difference ($P<0.05$) in the second week study as compared with the control group (table 3).

The haematocrits of the control group after one week and two week studies were 50.90±4.0 (mean ± SD) and 31.37 ±10.7 (mean ± SD) respectively. These results showed a decrease in the haematocrits concentration with the increase of dose of ES oil as compared with the control. There were no significant changes ($P<0.05$) among the treated groups and the control group in the first week study. On the other hand the results obtained after two weeks study also showed a decrease in the HCT concentration in the treated groups with the increase of dose of ES oil, as compared with the control group. Similarly, the decreases in haematocrits of treated groups were non-significant at $P<0.05$ as compared with control group (table 3).

The blood platelets level of control group was 513.0± 205.3 × $10^3$ cells per µL (mean) and 442.0±117.5× $10^3$ cells per µL (mean) corresponds to one and two weeks studies respectively. There was found a small decrease in the control group's platelets in the second week as compared with the first week. The numbers of platelets increase with the increase of ES oil doses in both weeks as compared with the control group. The statistical analysis of first week showed a significant difference of D group as compared with the control group at $P < 0.05$. Other groups showed a non-significant difference in the first week as compared with the normal group. The second week results showed an increase in the platelet count with the increase of dose of ES oil as compared with the non-treated group. But there was no significant difference among the treated group with respect to control group (table 3).

The effects of *Eruca sativa* oil was further confirmed by a comparative histological examination of the liver sections from rabbits. The Haematoxylin (H) and Eosin (E) stained sections in control group showed normal and intact architecture of liver. The Central vein appears normal. Hepatocytes were arranged well, radiating from the central vein. Nuclei were rounded in shape and centrally located. The portal triads showed normal shape. There were few mononuclear cells in the area of portal triad, which contained small branches of portal vein, hepatic artery and bile duct (fig. 3 A). Supplementation of *Eruca* oil had no significant effects on the histology of liver as shown in fig. B-D.

## DISCUSSION

The peroxide value of *Eruca* seed oil determined by Muuse *et al*. (1992) was 4.0 meq per kg which is comparable with the present study. The higher value (609 mg of GAE/100 g) of TPC reported by Serap *et al*. (2010) may be due to the variation in variety, extraction and methods of analyses. Pigments contents were enough in *Eruca* oil, is a good indicator for the stability of this oil. Caffeic acid, its derivatives and quercetin were among the major phenolic compounds. Quercetin and its derivatives were identified as major compounds in the *Eruca sativa* leaves (Weckerle *et al*., 2001). Thus these phytochemicals may be considered as marker in authentication of oils and have been found to possess chemoprotective properties (Jin *et al*., 2009).

The present results showed that the weight of liver increased with the increase of *Eruca sativa* seed oil non-significantly with respect to control group. The increase in liver weight may be due to the presence of trace elements in the *Eruca sativa* plant or glycogen accumulation in the hepatocytes. The present result is in agreement with El-Tohamy and El-Kady (2007), who studied the rocket plant seed meal on New Zealand white rabbits and observed a non-significant increase in the liver weight. Similarly, the result was also in accordance to findings of Bajilan and Al-Naqeeb (2011), who observed significant increase in the liver weight of mice fed with *Eruca sativa* leaves extract (different doses) as compared with the control group.

Gradual and significant decreases in the serum triacylglycerol level of ES oil treated groups were observed. The present study was in accordance to El-Missiry & El-Gindy (2000), who observed the triacylglycerol level in the Alloxan induced diabetes mellitus rates supplemented with *Eruca sativa* seeds. He reported that the triacylglycerol level decreases in the ES oil treated group significantly as compared with control group. According to Hussain *et al*. (2010) the level of triacylglycerol decreases with the administration of *Eruca sativa* seeds, oil and leaves extracts to the alcoholic injured liver rabbits. Serum cholesterol level was found to decrease significantly as compared with the control group in the rabbits feed with ES oil for two weeks. The present study was in agreement with Hussain *et al*. (2010), who showed that some extracts of *Eruca sativa* plant parts act as anti-hepatotoxic. The authors reported that the extracts were also effective on the cholesterol level that is the level of cholesterol decreases by feeding rats with ES oil, seeds and leaves extracts. Serum HDL-cholesterol levels in the ES oil treated groups were found to increase significantly as compared with control group. The increase in HDL-cholesterol showed a gradual increase when the dose of ESS oil increases. According to Hussain (2013), the concentration of HDL-cholesterol in the *Eruca sativa* treated group increases significantly as compared with control group. It is in accordance to the present study results. The level of serum LDL-cholesterol in the ES oil treated groups showed a significant decrease in the

present study as compared with control group. The decrease in the LDL-cholesterol was found in a specific manner when the dose of ES oil increases. The present study was in agreement with Hussain *et al*. (2010), who studied different extracts of *Eruca sativa* plant parts on alcoholic hepatotoxic rates. He observed that the level of LDL-cholesterol decreases in the serum of rates by introducing *Eruca sativa* oil seeds and leaves extracts to them.

The present study was in agreement with Ahmed *et al*. (2013), who studied the effect of *Eruca sativa* ethanolic leaves extract on mice treated with carbon tetra chloride ($CCl_4$) and concluded that the level of ALT decreases significantly as compared with control group. This decrease in ALT shows the improvement of liver function. Similarly, the present study was in accordance to Hussain *et al*. (2010), who observed the effect of *Eruca sativa* seeds, oil and leaves as anti-hepatotoxic in rates. They reported that the oil, seeds and leaves of *Eruca sativa* significantly decrease the ALT level. From the discussion it can be concluded that ES oil shows anti-hepatotoxic effect as well as enhance liver function. And it may be due to the fact that *Eruca sativa* possesses several beneficial antioxidants. A significant decrease was found in serum glucose of rabbit's blood feed with ES oil for two weeks as shown in table 3. The results showed that the level of serum glucose decreases significantly as compared with the control group, when the dose of ES oil increases. The decline in the serum glucose may be due to the upsurge production of insulin in the liver. The present study was in accordance to El-Missiry & El-Gindy (2000), who studied the effect of *Eruca sativa* seed oil on alloxan treated rates and observed a significant decrease in the serum glucose level as compared with control group.

Blood circulatory system is the most important system of all living organisms. Any alteration in the haematological parameters is the indication of the presence of toxic chemicals, change in the metabolic reactions or any other stimulus. In the present study there was increase in WBC & PLT, decrease in HCT, and no significant changes in platelets count. The present study was in disagreement with the study of El-Nattat & El-Kady (2007), who studied the effect of *Eruca sativa* seed residues diet on rabbits and reported similar beneficial observations in rabbits.

The pathogenesis can occur through two basic ways. In the First step biochemical changes occur, followed by morphological and functional alterations in the cell, tissue or organs. These changes are the responses of cells to stimuli. There are four types of cellular responses hypertrophy, hyperplasia, atrophy and metaplasia. In the present study microscopic observations in control and treated groups showed similar cyto-architecture of the liver with no significant histological changes. This was due to the antioxidant activity of *Eruca sativa*. This was in agreement with the study conducted by Sajeeth *et al*. (2010), who observed no pathological alteration in the liver of diabetic and non-diabetic rates treated with *Eruca sativa* at the dose of 5000 mg/kg body weight for twenty-one days. Bajilan and Al-Naqeeb (2011) also reported cellular hypertrophy and vaculation in hepatocytes of mice liver, which were administered extract of *Eruca sativa* leaves at the dose of 250 & 500 mg/ kg body weight for 30 days. These results are in contrast to the present study, due to the high dose of *Eruca* leaves extract.

## CONCLUSION

In conclusion, the ES oil contains high amounts of phenolic compounds such as quercetin, caffeoylquinic acid, caffeic acid and kaempferol glycoside, important pigments and significant amount of total phenolic contents. The effects of *Eruca sativa* seed oil on lipid profiles, haematology and histology of rabbit liver were observed. The serum HDL-cholesterol increased, while LDL-cholesterol declined with the supplementation of *Eruca sativa* seed oil, which shows a beneficial and protective effect. Moreover, the level of serum ALT and triglycerides also remain with in normal limit, which may be attributed to the antioxidant property of ES oil. No significant alteration in the haematology and histology of rabbit liver was observed. It is concluded that *Eruca sativa* seed oil from Pakistan has protective effects on the administered dose and duration and can be used as supplement for nutritional purposes.

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