# Antihepatotoxic Effect of *Eruca Sativa* Extracts on Alcohol Induced Liver Injury in Rats

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**Abstract:** Food derived antioxidants have a strong potential for long term use as chemopreventive agents in disease states involving oxidative stress, such as hepatitis and alcoholic liver diseases. This study aimed to investigate the effect of different extracts of Eruca Sativa in ethanol induced liver injury in rats**.** Eighty eight male albino rats were divided into 3 main groups included control, prophylactic and treated groups using different extracts of *Eruca sativa*. Serum liver functions tests, lipid profile and oxidants/antioxidants profile were estimated. The results showed that Eruca Sativa extracts improved liver functions, Lipid profile and antioxidants parameters. We concluded that, *Eruca sativa* extracts may exert their prophylactic and treatment role against oxidative stress produced by ethanol by increasing/maintaining the levels of antioxidant molecules and antioxidant enzymes. [Journal of American Science 2010;6(11):381-389]. (ISSN: 1545-1003).

**Key words**: *Eruca sativa*, Ethanol, Liver, Ethanolic extract, Antioxidants, Oxidative stress.

## 1. Introduction

Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to many different diseases (Sakar et al., 2005). Alcohol administration is one of the most common causes of chronic liver disease in the world and it was found that alcohol affects the liver, through not only nutritional disturbances but also its direct toxicity, because its predominant metabolism in the liver is associated with oxidation- reduction changes and oxidative stress (Lieber, 2004). The body's natural defenses against free radicals (e.g. antioxidants) are inhibited by alcohol consumption resulting in the increasing of liver damage (Augustyniak et al., 2005).

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 . Several hundreds of plants have been examined for use in a wide variety of liver disorders including *Eruca sativa* (Family: *Cruciferae*) that modulate oxidative stress due to its antioxidant properties. 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 (Bennett et al., 2006) which have several biological activities including anticarcinogenic, antifungal, antibacterial and antioxidant effects (Kim et al., 2004).

Alam et al., (2007) 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.

Thus, the aim of this study is to evaluate the prophylactic and treatment effects of petroleum ether extract of *Eruca sativa* seeds (oil) and ethanolic extracts of both seeds and leaves on alcohol induced hepatotoxicity in rats.

## 2.Materials and Methods

Male albino rats weighting 160-180 g and mice of both sexes weighting 25-28 g were purchased from the animal house of National Research Center (NRC), Giza, Egypt .and Eruca sativa (seeds and leaves) was purchased from the local market.

Ethanolic and petroleum ether extracts were prepared according to the method of Harborne (Harborne, 1988).

$LD_{50}$ of ethanolic exracts of Eruca sativa leaves and seeds were determined according to Behrens & Karber (1970).

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of NRC.

Eighty eight healthy male albino rats were used in this study and randomly divided into 3 main groups , the 1st group is the control group and included 1-Normal control rats received saline 2-Ethanol group: normal rats received oral dose of 20% (v/v) ethanol 5ml/100g body weight daily for four weeks .Oil control group: normal rats received oral dose of *Eruca sativa* oil (0.06 ml / kg B.wt / day) for twelve weeks .Seed control group: normal rats received oral dose of *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt /day) for twelve weeks (according to $LD_{50}$).Leaf control group: normal rats received oral dose of *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt /day) for twelve weeks (according to $LD_{50}$).

The 2nd group is the Prophylactic groups and included 1- Oil prophylactic group: rats received oral dose of *Eruca sativa* oil (0.06 ml/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks 2- Seed prophylactic group: rats received oral dose of *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks. 3- Leaf prophylactic group: rats received daily oral dose of *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt / day) together with 20% ethanol (5ml/100g B.wt./day) for four weeks.

The 3rd group is the treated group ad included 1- Oil treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day) for four weeks followed by *Eruca sativa* oil (0.06ml/Kg B.wt / day) for twelve weeks.2-Seed treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day)for four weeks followed by *Eruca sativa* seeds ethanol extract (0.5 g/Kg B.wt / day) for twelve weeks.3-Leaf treated group: rats received oral dose of 20% ethanol (5ml/100g B.wt./day) for four weeks followed by *Eruca sativa* leaves ethanol extract (0.5 g/Kg B.wt / day) for twelve weeks.

At the end of the experimental period, animals were kept fasting, subjected to light ether anaesthesia, blood was collected from retro orbital venous plexus and sera were separated by centrifugation and kept at -20ºC until used.

Serum Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were assayed by using commercial kits purchased from BioMed Diagnostics, γ glutamile transferase (γGT) was estimated kinetically by using Linear Laboratories kit. Serum total protein (TP) and serum albumin (Alb) were estimated by Centronic GmbH-Germany kit . Lipid profile was determined by assaying serum total cholesterol (TC), triglycerides (TG) and High Density Lipoprotein Cholesterol (HDL-C) using commercial kits purchased from BioMed Diagnostics. Lipid according to the method of Uchimaya and Mihara (1978). Total antioxidants (TA) were measured kinetically using commercial kits purchased from Biodiagnostic. Nitric oxide (NO) was determined by the method of Miranda et al., (2001). Superoxid dismutase activity was determined according to Ming Sun and Zigman (1978).

Data were analyzed by one way analysis of variance (ANOVA) followed by LSD test. Results were expressed as mean ±S.E, p-values <0.05 were regarded as statistically significant.

## 3. Results

Administration of *Eruca sativa* extracts had no effect on the all studied parameters compared to control group indicating its safe administration (Table 1).

The mean values of serum liver enzymes ALT, AST and γGT were significantly increased, while the mean values of TP, Alb and A/G ratio were significantly decreased in ethanol group compared to control group. Oil, seeds and leaves prophylactic groups and seeds treated group showed a significant decrease in the mean values of serum ALT, AST and γGT and a significant increase in TP, Alb and A/G ratio compared to ethanol group (Table 2).

The mean values of serum lipid profile TC, TG and Low Density Lipoprotein Cholesterol (LDL-C) were significantly increased, while the mean values of HDL-C and HDL-C/LDL-C ratio were significantly decreased in ethanol group compared to control group. Oil, seeds and leaves prophylactic groups and seeds treated group showed a significant decrease in the mean values of serum TC, TG and LDL-C and a significant increase in HDL-C and HDL-C /LDL-C ratio compared to ethanol group (Table 3).

The mean values of serum (TBARS) and NO were significantly increased, while the mean values of serum SOD and TA were significantly decreased in ethanol group compared to control group. All prophylactic groups showed a significant decrease in the mean values of serum TBARS and NO and a significant increase in SOD and TA compared to ethanol group but seeds treated group showed a significant decrease in NO only (Table 4).

**Table (1): Effect of *Eruca sativa* extracts in all studied parameters**

| Groups | *Liver function tests* AST (U/l) | ALT (U/l) | γ GT (U/l) | T.P (g/dl) | Alb (g/dl) | Glob .(g/dl) | A/G ratio |
|---|---|---|---|---|---|---|---|
| Control | | | | | | | |
| Mean | 57.50 | 34.75 | 2.88 | 6.75 | 3.75 | 3.00 | 1.27 |
| ±S.E | 0.87 | 1.44 | 0.40 | 0.11 | 0.06 | 0.14 | 0.07 |
| Oil control | | | | | | | |
| Mean | 55.63 | 33.88 | 2.63 | 6.88 | 3.88 | 3.00 | 1.31 |
| ±S.E. | 0.98 | 1.33 | 0.46 | 0.06 | 0.07 | 0.10 | 0.06 |
| %Change from control | -3.25 | -2.50 | -8.68 | 1.93 | 3.47 | 0.00 | 3.15 |
| Seeds control | | | | | | | |
| Mean | 60.13 | 34.75 | 2.88 | 6.76 | 3.88 | 2.88 | 1.35 |
| ±S.E. | 0.79 | 0.67 | 0.44 | 0.06 | 0.06 | 0.09 | 0.06 |
| %Change from control | 4.57 | 0.00 | 0.00 | 0.15 | 3.47 | -3.67 | 6.30 |
| Leaves control | | | | | | | |
| Mean | 56.38 | 34.13 | 2.75 | 6.63 | 3.76 | 2.86 | 1.32 |
| ±S.E. | 0.56 | 0.69 | 0.49 | 0.07 | 0.05 | 0.06 | 0.04 |
| %Change from control | -1.95 | -1.78 | -4.51 | -1.78 | 0.27 | -4.67 | 3.94 |

| Groups | *Lipid profile* T.C (mg/dl) | TG (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | HDL-C/ LDL-C ratio |
|---|---|---|---|---|---|
| Control | | | | | |
| Mean | 112.38 | 94.88 | 41.75 | 51.65 | 0.81 |
| ±S.E. | 0.68 | 0.74 | 0.99 | 1.17 | 0.04 |
| Oil control | | | | | |
| Mean | 114.13 | 94.13 | 43.13 | 52.18 | 0.83 |
| ±S.E. | 0.44 | 0.52 | 0.81 | 0.59 | 0.02 |
| %Change from control | 1.56 | -0.79 | 3.31 | 1.03 | 2.47 |
| Seeds control | | | | | |
| Mean | 113.37 | 96.50 | 41.00 | 53.08 | 0.77 |
| ±S.E. | 1.07 | 0.78 | 0.46 | 0.90 | 0.01 |
| %Change from control | 0.89 | 1.71 | -1.80 | 2.77 | -4.94 |
| Leaves control | | | | | |
| Mean | 111.25 | 93.50 | 42.38 | 50.18 | 0.85 |
| ±S.E. | 0.41 | 0.68 | 0.98 | 1.33 | 0.04 |
| %Change from control | -1.01 | -1.98 | 1.51 | -2.91 | 4.94 |

| Groups | *Oxidants/antioxidants profile* TBARS (µmol/l) | SOD (U/ml) | TA (mmol/l) | NO (µmol/l) |
|---|---|---|---|---|
| Control | | | | |
| Mean | 0.33 | 0.66 | 1.30 | 18.25 |
| ±S.E | 0.04 | 0.05 | 0.05 | 1.10 |
| Oil control | | | | |
| Mean | 0.35 | 0.71 | 1.41 | 18.13 |
| ±S.E. | 0.03 | 0.04 | 0.05 | 0.58 |
| %Change from control | 6.06 | 7.58 | 8.46 | -0.66 |
| Seeds control | | | | |
| Mean | 0.36 | 0.70 | 1.38 | 18.13 |
| ±S.E. | 0.03 | 0.05 | 0.06 | 0.58 |
| %Change from control | 9.09 | 6.06 | 6.15 | -0.66 |
| Leaves control | | | | |
| Mean | 0.30 | 0.73 | 1.54 | 18.00 |
| ±S.E. | 0.05 | 0.04 | 0.05 | 0.53 |
| %Change from control | -9.09 | 10.61 | 18.46 | -1.37 |

Values are given as mean ± S.E. for 8 rats in each group.

**Table (2): Liver function tests in prophylactic and treated groups**

| Groups | Control | Ethanol | Prophylactic | | | Treated | | |
|---|---|---|---|---|---|---|---|---|
| | | | Oil | Seeds | Leaves | Oil | Seeds | Leaves |
| AST (U/l) | | | | | | | | |
| Mean | 57.50 | 83.13 [a] | 75.00 [a,b#] | 72.38 [a,b♦ #] | 68.50 [a,b*#] | 83.00 [a] | 79.0 [a,b] | 82.00 [a] |
| ±S.E. | 0.87 | 1.02 | 0.71 | 0.62 | 0.87 | 0.57 | 0.46 | 0.50 |
| %Change from control | 0.00 | 44.56 | 30.43 | 25.88 | 19.13 | 44.35 | 37.39 | 42.61 |
| %Change from ethanol | -- | 0.00 | -9.78 | -12.93 | -17.60 | -0.16 | -4.97 | -1.36 |
| ALT (U/l) | | | | | | | | |
| Mean | 34.75 | 51.38 [a] | 47.25 [a,b] | 44.75 [a,b#] | 41.38 [a,b*#] | 50.00 [a] | 47.63 [a,b] | 51.00 [a] |
| ±S.E. | 1.44 | 1.19 | 0.65 | 0.59 | 1.41 | 0.46 | 0.46 | 0.33 |
| %Change from control | 0.00 | 47.84 | 35.97 | 28.78 | 19.08 | 43.88 | 37.06 | 46.76 |
| %Change from ethanol | -- | 0.00 | -8.04 | -12.90 | -19.46 | -2.69 | -7.30 | -0.74 |
| γ GT (U/l) | | | | | | | | |
| Mean | 2.88 | 11.63 [a] | 8.88 [a,b] | 8.00 [a,b] | 6.38 [a,b*]# | 11.13 [a] | 9.50 [a,b] | 11.63 [a] |
| ±S.E | 0.40 | 0.50 | 0.52 | 0.46 | 0.37 | 0.40 | 0.57 | 0.50 |
| %Change from control | 0.00 | 303.82 | 208.33 | 178.78 | 121.53 | 286.46 | 229.86 | 303.82 |
| %Change from ethanol | -- | 0.00 | -23.65 | -31.21 | -45.14 | -4.30 | -18.31 | 0.00 |
| T.P (g/dl) | | | | | | | | |
| Mean | 6.75 | 5.63 [a] | 5.90 [a,b] | 6.00 [a,b#] | 6.30 [a,b*#] | 5.50 [a] | 5.80 [a] | 5.60 [a] |
| ±S.E. | 0.11 | 0.06 | 0.06 | 0.06 | 0.03 | 0.05 | 0.05 | 0.05 |
| %Change from control | 0.00 | -16.59 | -12.59 | -11.11 | -6.67 | -18.52 | -14.07 | -17.04 |
| %Change from ethanol | -- | 0.00 | 4.80 | 6.67 | 11.90 | -2.31 | 3.02 | -0.53 |
| Alb (g/dl) | | | | | | | | |
| Mean | 3.75 | 2.50 [a] | 2.93 [a,b] | 3.13 [a,b♦#] | 3.40 [a,b*#] | 2.50 [a] | 2.80 [a,b] | 2.38 [a] |
| ±S.E. | 0.06 | 0.05 | 0.06 | 0.06 | 0.07 | 0.05 | 0.05 | 0.04 |
| %Change from control | 0.00 | -33.33 | -21.87 | -16.35 | -9.33 | -33.33 | -25.33 | -36.53 |
| %Change from ethanol | -- | 0.00 | 17.20 | 25.20 | 36.00 | 0.00 | 12.00 | -4.80 |
| Glob. (g/dl) | | | | | | | | |
| Mean | 3.00 | 3.13 | 2.98 | 2.88 | 2.90 | 3.00 | 3.00 | 3.23 |
| ±S.E. | 0.14 | 0.09 | 0.07 | 0.08 | 0.07 | 0.07 | 0.06 | 0.05 |
| %Change from control | 0.00 | 4.33 | -0.67 | -4.00 | -3.33 | 0.00 | 0.00 | 7.67 |
| %Change from ethanol | -- | 0.00 | -4.79 | -7.99 | -7.35 | -4.15 | -4.15 | 3.19 |
| A/G ratio | | | | | | | | |
| Mean | 1.27 | 0.81 [a] | 1.00 [a,b] | 1.10 [b#] | 1.18 [b♦#] | 0.84 [a] | 0.94 [a,b] | 0.74 [a] |
| ±S.E. | 0.07 | 0.04 | 0.04 | 0.05 | 0.05 | 0.03 | 0.03 | 0.02 |
| %Change from control | 0.00 | -36.22 | -22.05 | -13.39 | -7.09 | -33.36 | -25.98 | -41.73 |
| %Change from ethanol | -- | 0.00 | 23.46 | 35.80 | 45.68 | 3.70 | 16.05 | -8.64 |

Values are given as mean ± S.E. for 8 rats in each group.
a: Significant difference at $P<0.05$ compared to control group.
b: Significant difference at $P<0.05$ compared to ethanol group.
*:Significant difference at $p<0.05$ compared to seed and oil prophylactic groups.
♦:Significant difference at $p<0.05$compred to oil prophylactic group.
#:Significant difference at $p<0.05$compred to seed treated group.

**Table (3): Lipid profile in prophylactic and treated groups**

| Groups | Control | Ethanol | Prophylactic | | | Treated | | |
|---|---|---|---|---|---|---|---|---|
| | | | Oil | Seeds | Leaves | Oil | Seeds | Leaes |
| TC (mg/dl) | | | | | | | | |
| Mean | 112.38 | 144.87 [a] | 130.75[a,b#] | 126.00[a,b♦#] | 122.00[a,b*#] | 144.00[a] | 135.00[a,b] | 143.00[a] |
| ±S.E. | 0.68 | 0.69 | 0.53 | 0.71 | 0.60 | 0.46 | 0.71 | 0.71 |
| %Change from control | 0.00 | 28.92 | 16.35 | 12.12 | 8.56 | 28.14 | 20.13 | 27.25 |
| %Change from ethanol | -- | 0.00 | -9.75 | -13.03 | -15.79 | -0.61 | -6.82 | -1.30 |
| TG (mg/dl | | | | | | | | |
| Mean | 94.88 | 131.5[a] | 126.00[a,b#] | 122.0[a,b♦#] | 117.63[a,b*#] | 130.0[a] | 128.00[a,b] | 131.00[a] |
| ±S.E. | 0.74 | 0.80 | 0.46 | 0.53 | 0.94 | 0.46 | 0.46 | 0.46 |
| %Change from control | 0.00 | 38.60 | 31.75 | 28.58 | 23.98 | 37.02 | 34.91 | 38.07 |
| %Change from ethanol | -- | 0.00 | -4.94 | -7.22 | -10.55 | -1.14 | -2.66 | -0.38 |
| HDL -C (mg/dl) | | | | | | | | |
| Mean | 41.75 | 32.63 [a] | 36.25[a,b] | 37.00[a,b] | 38.00[a,b] | 32.13[a] | 36.00[a,b] | 32.00[a] |
| ±S.E | 0.99 | 0.86 | 0.59 | 0.60 | 0.89 | 0.91 | 0.46 | 0.91 |
| %Change from control | 0.00 | -21.84 | -13.17 | -11.38 | -8.98 | -23.05 | -13.77 | -23.35 |
| %Change from ethanol | -- | 0.00 | 11.09 | 13.39 | 16.46 | -1.55 | 10.33 | -1.93 |
| LDL -C (mg/dl) | | | | | | | | |
| Mean | 51.65 | 85.95 [a] | 69.50[a,b#] | 64.77[a,b♦#] | 60.48[a,b*#] | 85.88[a] | 73.40[a,b] | 84.80[a] |
| ±S.E. | 1.17 | 0.73 | 0.79 | 0.97 | 0.49 | 1.03 | 0.62 | 1.37 |
| %Change from control | 0.00 | 66.41 | 34.56 | 25.07 | 17.10 | 66.34 | 46.91 | 64.18 |
| %Change from ethanol | -- | 0.00 | -19.14 | -24.84 | -29.63 | 0.00 | -11.68 | -1.34 |
| HDL-C/LDL-C ratio | | | | | | | | |
| Mean | 0.81 | 0.38 [a] | 0.52[a,b] | 0.57[a,b♦#] | 0.63[a,b*#] | 0.38 [a] | 0.49[a,b] | 0.38[a] |
| ±S.E. | 0.04 | 0.01 | 0.01 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 |
| %Change from control | 0.00 | -53.09 | -35.80 | -29.63 | -22.22 | -53.09 | -40.74 | -53.09 |
| %Change from ethanol | --- | 0.00 | 36.84 | 50.00 | 65.79 | 0.00 | 26.32 | 0.00 |

Values are given as mean ± S.E. for 8 rats in each group.
a: Significant difference at $P<0.05$ compared to control group.
b: Significant difference at $P<0.05$ compared to ethanol group.
*:Significant difference at $p<0.05$ compared to seed and oil prophylactic groups.
♦:Significant difference at $p<0.05$compred to oil prophylactic group.
[#]:Significant difference at $p<0.05$compred to seed treated group.

**Table (4): Serum Oxidants/antioxidants profile in prophylactic and treated groups**

| Groups | Control | Ethanol | Prophylactic | | | Treated | | |
|---|---|---|---|---|---|---|---|---|
| | | | Oil | Seeds | Leaves | Oil | Seeds | Leaves |
| TBARS (μmol/l) | | | | | | | | |
| Mean | 0.33 | 0.90 [a] | 0.70[a,b] | 0.60[a,b#] | 0.44 [b*#] | 0.90 [a] | 0.80 [a] | 0.90 [a] |
| ±S.E. | 0.04 | 0.06 | 0.05 | 0.04 | 0.04 | 0.05 | 0.05 | 0.03 |
| %Change from control | 0.00 | 172.73 | 112.12 | 81.82 | 33.33 | 172.73 | 142.42 | 172.73 |
| %Change from ethanol | -- | 0.00 | -22.22 | -33.33 | -51.11 | 0.00 | -11.11 | 0.00 |
| SOD(U/ml) | | | | | | | | |
| Mean | 0.66 | 0.30 [a] | 0.46 [a,b] | 0.50[a,b] | 0.55 [b]# | 0.35 [a] | 0.40 [a] | 0.30 [a] |
| ±S.E | 0.05 | 0.03 | 0.04 | 0.05 | 0.04 | 0.03 | 0.03 | 0.05 |
| %Change from control | 0.00 | -54.55 | -30.30 | -24.24 | -16.67 | -46.97 | -39.39 | -54.55 |
| %Change from ethanol | -- | 0.00 | 53.33 | 66.67 | 83.33 | 16.67 | 33.33 | 0.00 |
| TA (mmol/l) | | | | | | | | |
| Mean | 1.30 | 0.40 [a] | 0.60 [a,b] | 0.80[a,b♦#] | 1.10[a,b*]# | 0.40 [a] | 0.50 [a] | 0.40 [a] |
| ±S.E. | 0.05 | 0.03 | 0.04 | 0.05 | 0.05 | 0.03 | 0.03 | 0.03 |
| %Change from control | 0.00 | -69.23 | -53.85 | -38.46 | -15.38 | -69.23 | -61.54 | -69.23 |
| %Change from ethanol | -- | 0.00 | 50.00 | 100.00 | 175.00 | 0.00 | 25.00 | 0.00 |

| NO(µmol/l) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mean | 18.25 | 46.00 [a] | 34.00 [a,b#] | 30.00 [a,b♦#] | 25.00 [a,b*#] | 46.00 [a] | 39.00 [a,b] | 45.00 [a] |
| ±S.E. | 1.10 | 0.46 | 0.46 | 0.46 | 0.63 | 0.53 | 0.57 | 0.38 |
| %Change from control | 0.00 | 152.05 | 86.30 | 64.38 | 36.99 | 152.05 | 113.70 | 146.58 |
| %Change from ethanol | -- | 0.00 | -26.09 | -34.78 | -45.65 | 0.00 | -15.22 | -2.17 |

Values are given as mean ± S.E. for 8 rats in each group.
a: Significant difference at $P<0.05$ compared to control group.
b: Significant difference at $P<0.05$ compared to ethanol group.
*:Significant difference at $p<0.05$ compared to seed and oil prophylactic groups.
♦:Significant difference at $p<0.05$compred to oil prophylactic group.
#:Significant difference at $p<0.05$compred to seed treated group.

## 4. Discussion

In this study, alcohol intake increased the mean values of liver enzymes (ALT, AST and γ GT). These results were in agreement with Rajakrishnan and Menon (2001) who indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation. Also, Das et al., (2005) reported that excess alcohol consumption has been linked with altered liver metabolism and liver damage, with leakage of cytoplasmic liver enzyme γGT into blood.

In all prophylactic groups and Eruca sativa seeds ethanolic extracts treated group, liver enzymes (AST, ALT and γGT) were significantly decreased compared to ethanol group. These results were in agreement with El-Nattat and El-Kady (2007) who indicated that administration of rocket caused improving in AST, ALT and γGT 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.

In the present study, there was a significant decrease in serum total proteins, albumin and A/G ratio in ethanol group. These results were in agreement with Ahmed et al (2002) who found a decrease in serum total proteins and albumin in ethanol-administered rats and he suggested that was due to the decrease in the functional ability of liver in ethanol-administered rats. Also, the decrease in A/G ratio is a predictor of a bad out come and poor health.

In the current study, serum total proteins, albumin and A/G ratio were significantly increased in all prophylactic groups and also in treated group of Eruca sativa seeds ethanolic extracts compared to ethanol group. In the same line, El-Missiry and El-Gindy (2000) indicated the ability of Eruca sativa oil to stimulate the regeneration of hepatic tissue which increased protein synthesis in damaged liver and improved the functional statues of the liver cells.

Several studies demonstrated that alcohol intake is associated with changes in serum lipid concentrations and whole–body lipid balance (Siler et al., 1999). In the present study, there was a significant increase in the mean values of serum TC, TG and LDL-cholesterol and a significant decrease in the mean values of serum HDL-C and HDL-C / LDL-C ratio in ethanol group. These results were in agreement with kumar et al. (2002) who reported that ethanol blocks fat oxidation and favors fat accumulation. The accumulation of fat in liver acts as a stimulus for the secretion of lipoproteins into the blood stream and the development of hyperlipidemia.

In prophylactic groups, Eruca sativa significantly decreased serum cholesterol, triglycerides and low density lipoprotein cholesterol levels while the mean values of high density lipoprotein cholesterol and HDL-C/LDL-C ratio were significantly increased. These results were in agreement with El-Gengaihi et al., (2004) who reported that Eruca sativa induced a marked decrease in different lipid parameters values.

It was found that the inflammatory reactions and oxidative stress play a major role in alcohol hepatotoxicity (Albano et al., 2002). In this investigation, there was a significant increase in serum MDA concentration in ethanol treated rats; these results were in agreement with Saravanan et al., ( 2006) who observed a significant increase in MDA concentration in ethanol-treated rats and he suggested that reactive oxygen intermediates, generated during the metabolism of ethanol, these free radicals attack the polyunsaturated fatty acids in membranes and organelles to produce lipid peroxides leading to decrease in the membrane permeability, and ultimately cellular necrosis and death.

Free radicals are involved in various human diseases that can possibly be prevented by antioxidants (Chatterjee et al., 2005). Exposure of living organisms to a constant generation of reactive oxygen species (ROS) resulting in the development of antioxidative defense systems which protect cells and tissues against their harmful effects. The

efficiency of enzymatic and non-enzymatic antioxidative systems could be detected by the determination of single components of this system or by so-called total antioxidant capacity (TAC) (Kankofer et al., 2005) In the present study, a significant decrease in serum total antioxidants in ethanol treated rats was observed. In agreement, Masalkar et al., (2005) found a decrease in antioxidant statue in alcoholic patients and showed that increased generation of free radicals and deficiencies of dietary antioxidants can be important etiological factor in alcoholic liver disease.

In this study, NO level was significantly elevated in alcohol -treated rats. In agreement, Li et al., (2004) showed an elevation of NO level with the increased volume of alcohol infusion.

In the present study, the activity of superoxide dismutase was significantly decreased in ethanol -treated rats. In agreement, Puntarula et al., (1999) reported that superoxide dismutase and other antioxidative enzymes may be inactivated by ethanol.

All prophylactic groups showed a significant decrease in malondialdehyde (MDA) and nitric oxide (NO) levels and a significant increase in total antioxidants levels and superoxide dismutase activity. El-Gindy & El-missiry (2000) indicated that oil of Eruca sativa seed extract (ESS) induced an increase in hepatic GSH content which might enhance the GSH/GSSG ratio and decrease hepatic lipid peroxidation and hence aldehydic concentration. Parallel to these events, hepatic SOD activity was increased in rats supplemented with ESS. That is may be due to the fact that Eruca sativa seeds possess a potent free radical scavenging, antioxidants activities.

From the current study we noticed that, Eruca sativa leaves extract is consider the best hepatoprotective extract in prophylactic groups and Eruca sativa seeds extract is consider the best treated extract in treated groups. That's may be related to the fact that, Eruca sativa seeds ethanolic extract have a potent antioxidant activity and protect against ethanol induced hepatotoxicity. Several studies on phytochemical analysis of Eruca sativa seeds 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 (Barillari et al., 2005). The main compound that exerts antioxidant activity in Eruca sativa seeds extract is glucoerucin, unlike other glucosinolates (GLS) (e.g. glucoraphanin, the bio-precursor of sulforaphane), glucoerucin (GER) possesses good direct as well as indirect antioxidant activity (Alam et al., 2007). The antioxidant activity of glucoerucin, the bio-precursor of erucin (ERN) implicates free radical scavenging activity and an ability to induce phase II metabolizing enzymes (e.g. glutathione transferases, GSTs; NAD-(P) H: Quinone reductase (QR),epoxide hydrolase and heme oxygenase)which are important in the detoxification of electrophiles. Reactions of glucoerucin and erucin with free radicals (hydroperoxides) produce glucoraphanin (GRP) and sulphoraphane, respectively (Barillari et al., 2005).

In contrast, Perocco et al., ( 2006) reported that glucoraphanin only slightly affects glutathione-S-trasferase, which was the selected marker of phase II detoxifying enzymes, and also found that, GRP powerfully induces phase –I bioactivating enzymes(e.g.CYP1A which activates polycyclic aromatic hydrocarbons,CYP3A1 that activates aflatoxin and CYP2E1 activates ethanol). CYP2E1 metabolizes and activates many toxicological substrates, including ethanol, to more toxic products and it generates superoxide anion radical (O2·) and hydrogen peroxide ($H_2O_2$) .This effect on the redox status of the liver can cause activation of Kupffer cells and subsequently, hepatic cells, and thus contributing to the generation of alcoholic liver disease (Kessova and Cederbaum, 2003).

So, this extract (ethanolic extract of seeds) induced the beneficial effect in treatment study and not in prophylactic one. On the other hand, Bennett et al. (2002) indicated that leaves of Eruca sativa contain 4-mercaptobutyl GL as the major GL among nine, while GER is present only in small amounts. In addition Kim et al., (2004) isolated 4-(B-D-Glucopyranosyldisulfanyl) butyl a new glucosinolate from leaves of rocket and reported that this new glucosinolate has antioxidant activity in vitro..Rocket seeds and sprouts contain glucoerucin(GER) as main glucosinolates ,in large amounts in comparison to leaves (mature plants which contain 4-mercaptobutyl GL in large amount beside 4-(B-D-Glucopyranosyldisulfanyl) butyl and a small amount of GER ( Weckerle et al., 2001).From these results we concluded that, Eruca sativa ethanolic extract of leaves was better than ethanolic extract of seeds and petroleum ether extract of seed (oil) in the prophylactic study. Since in prophylactic study glucoraphanine(produced by glucoerucin) which found in Eruca sativa seeds activates phase 1 enzyme (CYP2E1) which in turn activates ethanol metabolism to produce free radicals and more toxic products, despite giving a beneficial effect in treatment study. Regarding the present study it could be concluded that Eruca sativa extracts possessed both prophylactic and therapeutic effects against experimentally induced liver injury in rats. However, the prophylactic role of these extracts was more potent than their treatment capacity.

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**Refrences**

1. Sarkar M, Sarkar K, Bhattacharjee R, Chatterjee M and Sil P. Curative role of the aqueous extract of the herb, phyllanthus niruri, against nimesulide induced oxidative stress in murine liver. Biomed. Res 2005; 16 (3):171-176.
2. Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to infla-mmation and fibrosis Alcohol 2004; 34 (1):9-19.
3. Augustyniak A, Waszkiewicz E and Skrzydlewska E. Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol .Nutrition 2005 ; 21: 925-932.
4. Bennett RN, Rosa EA, Mellon FA and Kroon P. Ontogenic profiling of glucosino-lates, 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 .2006; 54(11):4005-4015.
5. Kim S, JIN Sh and Ishii G. Isolation and structural elucidation of 4-(β-D-Gluco-pyranosyldis-ulfanyl) butyl glucosinolate from leaves of rocket salad (Eruca sativa L.) and its antioxidative stress. Bio Sci. Biotechnol. Biochem 2004; 68 (12): 2444-2450.
6. El-Missiry M and El-Gindy A. Amelioration of alloxan induced diabetes mellitus and oxidative stress on rate by oil of Eruca sativa seeds. Ann. Nutr. Metab 2000; 44: 97-100.
7. Alam MS, Kaur G, Jabbar Z, Javed K and Athar M. Eruca sativa seeds possess antioxidant activity and exerta protective effect on mercuric chloride induced renal toxicity. Food Chem. Toxicol. 2007; 45(6):910-920.
8. Harborne JB. In "Phytochemical Methods: A Guide to Modern Technique of Plant Analysis" (ed.) 3rd ed. Chapman and hall, London 1988; p: 1-138.
9. Behrens B and Karber G. Chemotherapy of Neoplastic Diseases. Selli C, Ckhardt S and Nmeth L (eds.). Budapest, The publishing House of the Hungarian Academy 1970; P: 37.
10. Uchiyama M and Mihara M. Determination of malondialdehyde in tissue by thiobarbituric acid test. Anal. Biochem 1978; 86: 271-278.
11. Miranda KM, Espey MG and Winl DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrae and nitrite. Nitric Oxide Boil. Chem 2001; 5(1): 62-71.
12. Ming Sun and Zigman. Determination of superoxide dismutase in erythrocytes using the method of adrenatin autooxidation. Anal. Bioche m 1978; 81-89.
13. Rajakrishnan V and Menon VP. Potential role of antioxidants during ethanol induced changes in the fatty acid compostion and arachidonic acid metabolites in male Wistar rats. Cell Biol. Toxicol 2001; 17:11-22.
14. Das D, Mukherjee MM, Das AS and Mitra C. Aqueous extract of black tea (Camellia sinensis) prevents chronic etha-nol toxicity. Curr. Sci 2005; 88 (6): 952-961.
15. El-Nattat WS and El-Kady RI. Effect of different medicinal plant seeds residues on the nutritional and reproductive perfor-mance of adult male rabbits. Int.J. Agric. Bio 2007; 9 (3):479-485.
16. Ahmed B, Alam T, Varshney M and Khan AS. Hepato- protective activity of two plants belonging to the A piaceae and the Euphorbiaceae family.J.Ethnopharmacol 2002; 79 (3): 313-316.
17. Siler SQ, Neese RA and Hellerstein MK. De Novo lipogenesis lipid kinetics and whole-body lipid balances in humans after acute alcohol consumption. Am. J. Clin. Nutr 1999; 70 (5): 928-936.
18. Kumar RS, Ponmozhi M, Viswanathan P and Nalini N . Effect of cassia auriculata leaf extract on lipids in rats with alcoholic liver injury. Asia Pac. J. Clin. Nutr 2002; 11( 2) : 157-163.
19. El-Gengaihi SE, Salem A, Rashandi SA, Ibrahim NA and Abd El-Hamid SR. Hypo-lipidemic effect of some vegetable oils in rats. Food, Agriculture and Environment 2004; 2 (2): 88-93.
20. Albano E. Free radicals and alcohol-induced liver injury. In "Ethanol and the Liver". Sherman CDIN, Preedy VR and Watson RR (eds.).London: Taylor and Francis 2002; P: 153-190.
21. Saravanan R, Viswana than P and Pugalendi KVC. Protective effect of urosolic acid on ethanol-mediated experimental liver damage in rats. Life Sci 2006; 78: 713-718.
22. Chatterjee S, Poduval TB, Tilak JC and Thomas PA. A modified economic, sensitive method for

measuring total antioxidant capacities of human plasma and natural compound using Indian Saffron (Corcus sativus). Clinica. Chimica. Acta 2005; 352: 155-163.
23. Kankofer M, Lipko J and Zdunczyk S. Total antioxidant capacity of bovine spontaneously released and retained placenta. Pathophysiology 2005; 11(4): 215-219.
24. Masalkar PD and Abhang SA. Oxidative stress and antioxidant status in patients with alcoholic liver disease. Clin. Chim. Acta 2005; 355: 61-65.
25. Li YM, Chen SH, Yu CH, Zhang Y and Xu GY. Effect of acute alcoholism on hepatic enzymes and oxidation/ antioxi-dation in rats. HBPD INTl 2004; 3(2):241-244.
26. Barillari J, Canistro D, Paolini M, Ferroni F, Pedulli GF, Iori R and Valgimigli L. Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (Eruca sativa Mill.) seeds and sprouts. J. Agric. Food Chem 2005; 53 (7): 2475-2482.
27. Perocco P, Bronzetti G, Canistro D, Luea V, Sapone A, Affatato A, Pedulli GF, Pozzetti L, Broccoli M, Iori R Barillari J, Sblendorio V, Legator MS, Paolini M and Abdel-Rohman Sh Z. Gulcoraphanin, the bio precursor of the widely extolled chemo preventive agent sulforaphane found in broccoli, induces phase-I Xenobiotic metabolizing enzymes and increases free radical generation in rat liver. Mutat. Res. 2006; 595:125-136.
28. Kessova I and Cederbaum AI. CYP2E1: biochem- istry, toxicology, regulating and function in ethanol-induced liver injury. Curr. Mol. Med 2003; 3 (6): 509-518.
29. Bennett RN, Mellon FA, Botting NP, Eagles J, Rosa EA and Williamson G. Identification of the major glucosinolate (4-mercaptobutyl glucosinolate) in leaves of Eruca sativa L. (rocket salad). Phytochemistry 2002; 61: 25-30.
30. Weckerle B, Michel K, Balazs B, Schreier and Toth G. Quercetin 3,3,4-tri-O-β-D-glucopyranosides from leaves of Eruca sativa Mill. Phytochemistry 2001 ; 57: 547-551.

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