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WHO IS THE BEST AMONG THE THREE *ERUCA SATIVA*, *NEGELLA SATIVA* AND BEE POLLEN GRAINS: ROLE OF *ERUCA SATIVA*, *NEGELLA SATIVA* AND BEE POLLEN GRAINS IN IMPROVING LIVER, KIDNEY AND ANTIOXIDANT STATUS IN RATS

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ABSTRACT

Background: *Eruca sativa* oil, black cumin oil and pollen grains have antioxidant properties because it includes many active ingredients on the other hand these substances are used in public medicine for long time in addition the prophet Mohamad said that the black cumin is cure for every disease except aging. **Materials and methods**: four groups of animals were treated for one week with *Eruca sativa* oil, black cumin oil and pollen grains then liver enzymes, kidney urea, uric acid, createnine and some antioxidants were measured. **Results:** significant increase in GPT in *Eruca sativa* oil and pollen grains groups, significant increase in serum urea in *Eruca sativa* oil group, also there is a significant decrease in catalase in *Eruca sativa* oil group. **Conclusion:** black cumin oil maintain stability of liver enzymes and increase albumin in addition it maintains levels of most measured antioxidant enzymes.

Keywords: *Eruca sativa*- *Negella sativa*- Bee pollen grains-Liver -Kidney- Antioxidants

INTRODUCTION:

Eruca sativa Mill. is used as plant extract in food and herbal medicine. The water extract of *Eruca sativa* includes antioxidants these antioxidants were determined using different antioxidant tests the extract includes α- tocopherol butylated hydroxyanisole and butylatehydroxyltouelne. *Eruca sativa* Mill. Is source of natural antioxidants and source for active natural products.[1]

Antioxidants present in foods can used for long term use as chemopreventive

agents in diseases involving oxidative stress, such as hepatitis and alcoholic liver diseases. *Eruca sativa* extracts show improving liver functions, lipid profile and antioxidants it is concluded that *Eruca sativa* extracts may exerts their prophylactic and treatment role against oxidative stress produced by ethanol by increasing g/maintaining the antioxidant molecules levels and antioxidant enzymes. Liver is susceptible to many different diseases. *Eruca sativa* seeds and leaves possess free radical scavenging antioxidants which protect against oxidative damage by increasing and maintaining the level of antioxidant molecule and enzymes.[2]

Among the various treatments considered for the study, isothiocyanates combination (allyl isothiocyanate, phenylethylisothiocyanate and sulphoraphane; 1:1:1; 10 µM) exhibited optimum antioxidant activity, 51.95±1.14 µM glutathione per mg protein compared to seed oil 25.91±1.26 µM. Lipid peroxidation value was 9.97±1.72 µM malondialdehyde per mg wet weight for isothiocyanates combination against seed oil, 28.45±1.87 µM and rendered significant protection against oxidative stress induced by melanoma in liver tissue. Isothiocyanates combination significantly suppressed various parameters, such as tumor growth, isothiocyanates combination by 36.36% while the seed oil by 15.23%; tumor weight, isothiocyanates combination by 45.9% and seed oil by 19.6%; tumor volume, isothiocyanates combination by 41.7% while the seed oil by 32.3%, measured for antimelanoma activity at a concentration of 10 µM. Isothiocyanates combination has been found to be more cytotoxic bioagent against B16F10 melanoma cells induced in C57BL/6 mice compared to naturally occurring *Eruca sativa* seed oil.[3]

Oxytetracycline OTC-treated animals revealed significant alterations in serum biochemical hepato-renal injury markers, and showed a markedly increase in hepato-renal lipid peroxidation and inhibition in tissue antioxidant biomarkers. Nigella sativa oil NSO and ascorbic acid AA protect against OTC-induced serum and tissue biochemical alterations when each of them is used alone or in combination along with OTC treatment. Furthermore, both NSO and AA produced synergetic hepatoprotective and antioxidant properties.[4]

Nigella sativa oil protects kidney from renal dysfunction and morphological abnormalities resulted from oxygen free radicals .[5]The cytotoxic activity of N. sativa was tested on the human hepatoma Hep G_2 cell line by [6]and 88% inhibitory

effect on Hep G_2 was found after 24-hr incubation with different concentrations (0-50 mg/ml) of the *Negella sativa* extract.

Nagi and Almakki (2009) recorded that oral administration of thymoquinone present in Nigella sativa is effective in increasing the activities of quinone reductase and glutathione transferase and makes thymoquinonea promising prophylactic agent against chemical carcinogenesis and toxicity in hepatic cancer.

The antioxidant capacity related to the phenolic composition of monospecific honey bee- collected pollen extract from the mesquite tree (*prosopisjuliflora*) from Durango, Mexico, was evaluated in an vitro-biological system (as inhibitor of lipid peroxidation on mouse hepatic microsomal preparations)and in an invivo system (on homogenized liver of bromobenzene-intoxicated mice) by quantification of thiobarbituric acid- reactive substances (TBARS). The comparison of results obtained from these two different systems was also made. The obtained results suggest that pollen of *P. julifora* is an important source of flavonoids which considered as natural antioxidants. Mesqite pollen extracts showed antioxidant activity related to the flavonol concentration in both the in vitro-biological system and the in vivo system with a lower activity in the latter of these system .[8]

MATERIALS AND METHODS

Twenty female albino rats (weighing about 120-170 g weight obtained from National agricultural research center – Dokki - Egypt) are divided into four groups *Eruca sativa*oil group, black seed oil group, pollen grains group all groups are housed in cages five rats in each , food and water was ad libitum the accommodation period was seven days then, rats , rats in *Eruca sativa* oil group were administered 1 ml *Eruca sativa* oil for one week one time daily and rats in black seed oil group were administrated 1ml of black seed oil one time daily for one week ; where pollen grains group was administrated 1 gm of Egyptian pollen grains for one week one time daily the administration method was using stomach tube. after one week of administration rats were sacrificed and blood was collected then centrifuged for 10 min at 3000 rpm[9]then serum were kept in ependorphs for analysis. After biochemical analysis data statistically analyzed using t-test by excel of Microsoft office.

Biochemical parameters:

1-Determination of serum aspartate amino transferase (ASAT)/(GOT) and serum alanine aminotransferase (ALAT)/(GPT) activity : The recommended method for the measurement of

aminotransferases (ASAT and ALAT) activity In serum is based on the principles outlined by**.** Modifications include optimization of Substrate concentration using Randox kits.[10]

2-Determination of serum total protein concentration:

Principle:

Colorimetric determination of serum total protein was calculated by the method of**.**[11]

3-Assessment of serum albumin level:

It determined according to using Randox kits.[12]

4-Measurement of serum urea level (Urease-Berthelot Method):

Serum urea level is determined according to the methodof[13]**using Bio-Adwic kit.**

Determination of serum uric acid concentration

Colorimetric determination of serum Uric Acid was determined by the method of**.**[14]

5-Measurement of serum creatinine concentration

The color intensity was measured in Kinetic colorimetric assay, using kits of SCICO, into intervals at wavelength492nm.[15]

Detection of Antioxidants:

1. **Determination of Thiobarbituric Acid Reactive Substances (TBARS) Level**

Principle

The level of TBARS was determined according to the method of [16]**.**The method is based on the determination of malondialdehyde (MDA) an end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink colored complex exhibiting a maximum absorption at 532nm.

2. **Determination of Catalase (CAT) activity**

Principle

The CAT activity was assayed according to the procedure described by[17].The dichromate/acetic acid reagent can be thought of as a stop bath for catalase activity. As soon as enzyme reaction mixture hits the acetic acid, its activity was destroyed; any hydrogen peroxide which hasn't been split by the catalase will react with the dichromate to give a blue precipitate of perchromic acid. This unstable perciptate was then decomposed by heating to give the green solution. This green color was measured spectrophoto-meterically at 570 nm.

3. **Determination of Reduced Glutathione:**

Principle

DTNB is a disulfide chromogen which is readily reduced by SH groups to an intensely yellow color. The absorbance of the reduced chromogen is measured at 412 nm. This is directly proportional to

GSH concentration in the sample. Reduced glutathione (GSH) was determined by the method of [18].

Glutathione Peroxidase (GPx)Activity

Principle:

Glutathione peroxidase (GPx) activity was assayed according to the method of[19]. The method relies on the following reaction:

$$2GSH+H_2O \xrightarrow{GPx} GSSG+2H_2O$$

The statistical analysis were done according to Excell of micro soft office program And it was significant at probability ≤ 0.5.

RESULTS

Table (1) Role of *Eruca sativa* oil, black seed oil and pollen grains on liver functions tests

parameter		control	*Eruca sativa* oil	Black seed oil	Pollen grains
GOT U/ml	Mean±SE	94.33±1.33	74.5±13.20	93.00±2.30	111.33±30.02
GPT U/ml	Mean±SE	60±8	83.25±7.44*	77.33±10.98	169±30.55*
Total protein g/dl	Mean±SE	11.09±1.09	8.73±1.01	10.20±0.65	9.64±0.70
Albumin g/dl	Mean±SE	2.87±0.05	3.45±0.92	4.27±0.10*	3.10±0.20

Table (2) Role of*Eruca sativa* oil, black seed oil and pollen grains on kidney function tests

parameter		control	*Eruca sativa* oil	Black seed oil	Pollen grains
Urea mg/dl	Mean±SE	39.28±6.54	59.87±2.23*	45.36±2.24	30.13±2.13
Uric acid mg/dl	Mean±SE	1.69±0.73	2.16±55	5.78±2.61	0.84±0.18
Createnine mg/dl	Mean±SE	6.4±2.80	4.94±2.14	1.68±0.82	1.92±0.40

Table (3) Role of *Eruca sativa* oil, black seed oil and pollen grains on some antioxidants

parameter		control	*Eruca sativa* oil	Black seed oil	Pollen grains
MDA nmol/ml	Mean±SE	120.65±18.58	110.48±1.22	119.86±22.63	34.65±6.33*
Glutathion U/ml	Mean±SE	3.78±2.00	7.08±0.25	5.84±0.67	4.90±0.55
Catalase U/ml	Mean±SE	6.21±0.31	4.72±0.12*	5.81±0.47	3.07±0.04*
GPX µg/ml	Mean±SE	2.98±0.57	6.36± 1.3*	2.53±0.58	3.94±0.02

Data in table (1) fig (2) show significant increase in GPT in *Eruca sativa* oil and pollen grains groups where mean ±SE were 83.25±7.44 and 169±30.55 respectively.

Data in table (2) fig (5) show significant increase in serum urea in *Eruca sativa* oil group comparing with control where Mean ± SE was 59.87 ± 2.23.

Data in table (3) fig (8,10) show significant decrease in MDA level in pollen grains group comparing with control where Mean ±SE was 34.65±6.33 also, there is a significant decrease in catalase in*Eruca sativa* oil and pollen grains in comparing with control where mean ± SE were 4.72 ± 0.12 and 3.07 ±0.04 respectively.

On the other hand GPX table (3), Fig (11) increased significantly *Eruca sativa* oilcomparing with control. Where mean ± SE was 6.36 ± 1.3.

In another direction there are no significant changes in GOT level in all treated groups also, black seed oil treatment did not change GPT level in the serum.

Total protein did not show any significant changes in all treated groups.

Also albumin level did not change in *Eruca sativa* oil group and pollen grains group did not show any significant changes

Uric acid and createnine did not show any significant changes between all groups comparing with the control.

MDA, glutathione, catalase and GPX level did not change in black seed oil also, MDA and glutathione did not change in *Eruca sativa* oil group, where glutathione and GPX level did not change in pollen grains group.

Fig (1): Effect of *Eruca sativa* oil, black seed oil and pollen grains on liver GOT in rats

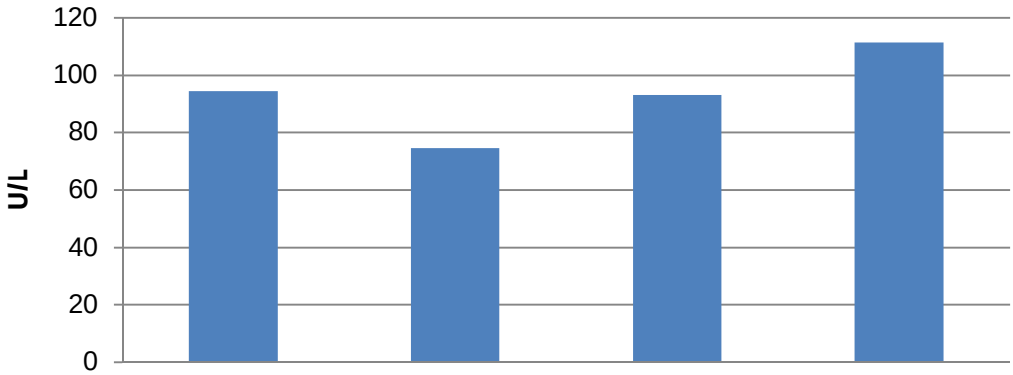


Fig (3): Effect of *Eruca sativa* oil, black seed oil and pollen grains on liver total protein in rats

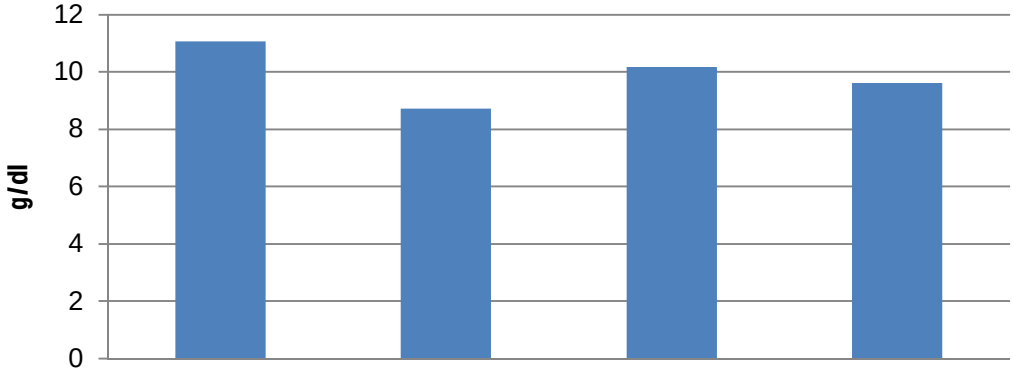


Fig (2): Effect of *Eruca sativa* oil, black seed oil and pollen grains on GPT level in rats

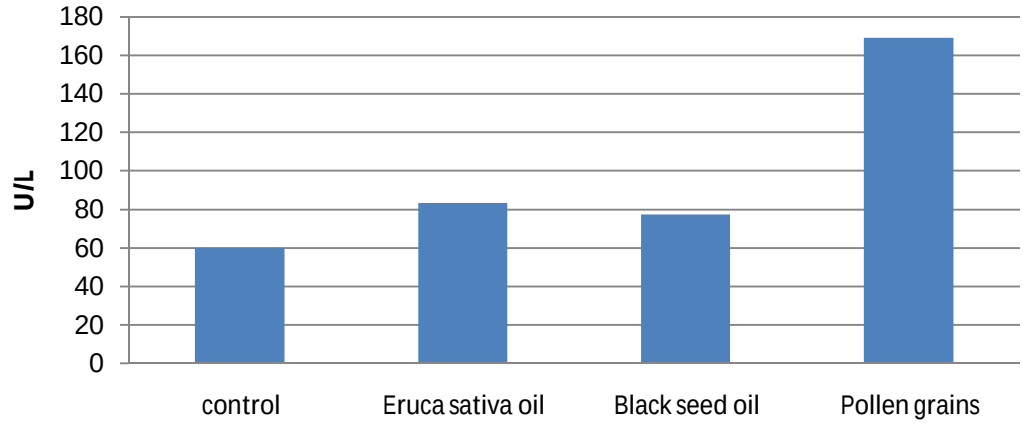


Fig (4): Effect of *Eruca sativa* oil ,black seed oil and pollen grains on liver albumin in rats

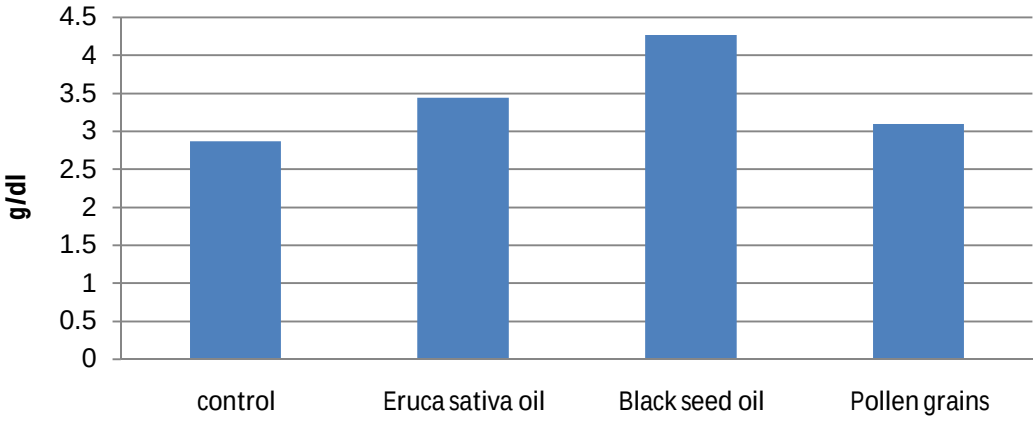
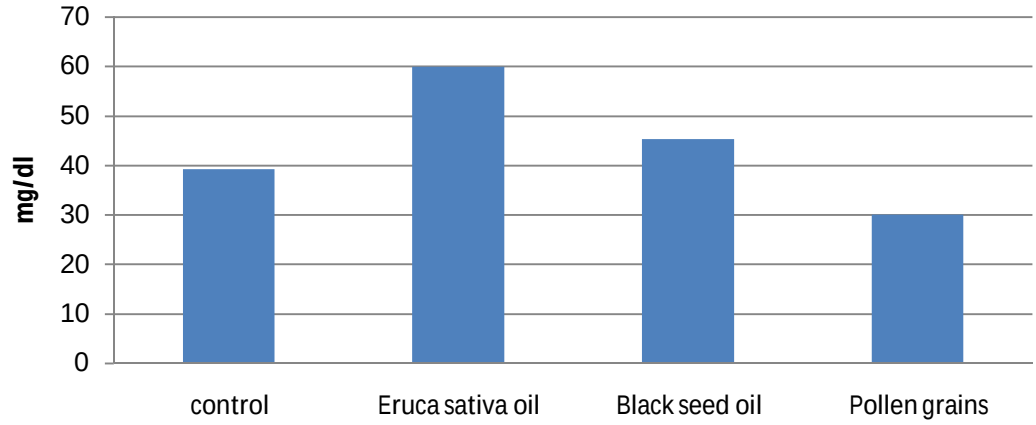


Fig (5): Effect of *Eruca sativa* oil,black seed oil and pollen grains on kidney urea in rats



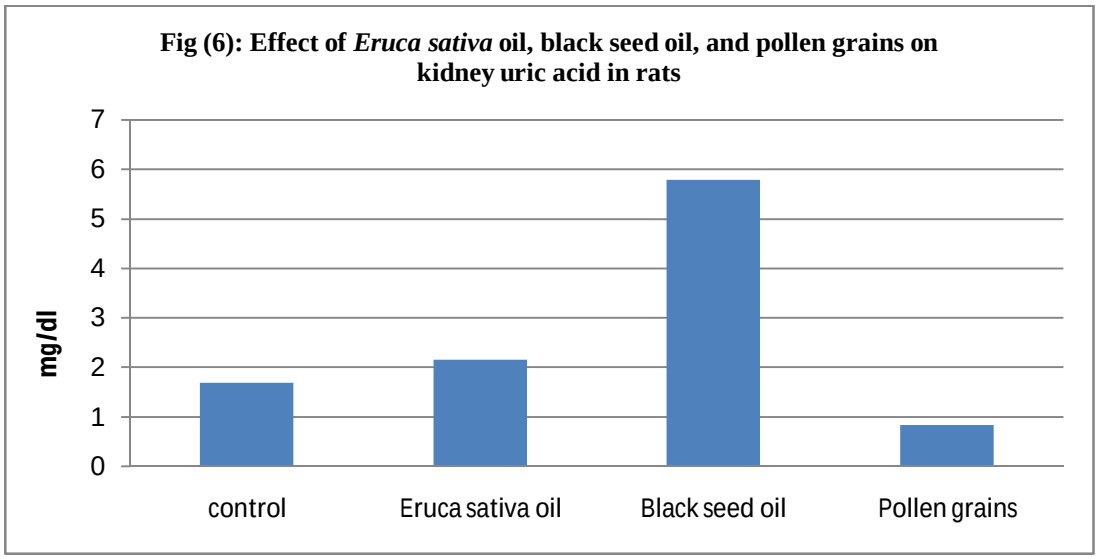


Fig (6): Effect of Eruca sativa oil, black seed oil, and pollen grains on kidney uric acid in rats
mg/dl
7
6
5
4
3
2
1
0
control
Eruca sativa oil
Black seed oil
Pollen grains

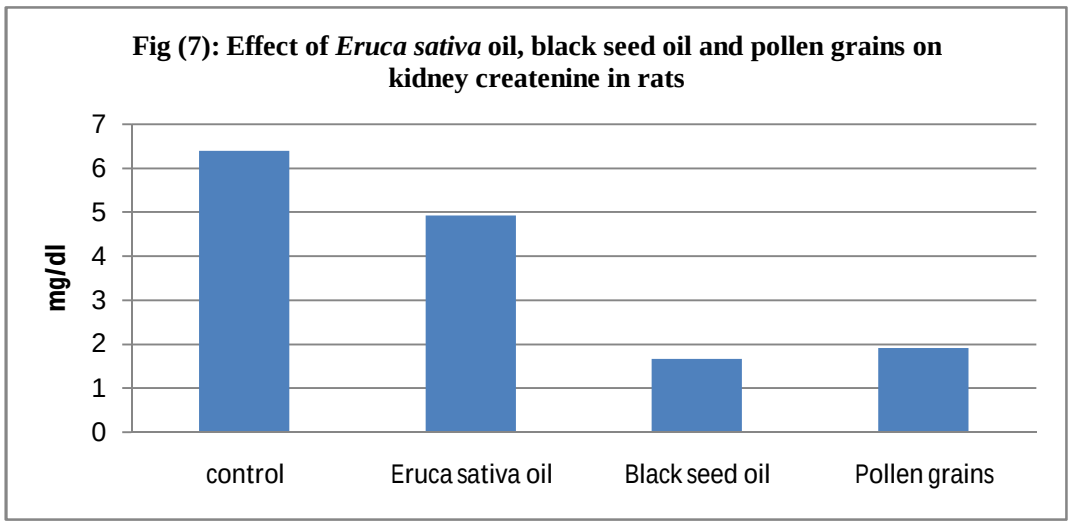


Fig (7): Effect of Eruca sativa oil, black seed oil and pollen grains on kidney createnine in rats
mg/dl
7
6
5
4
3
2
1
0
control
Eruca sativa oil
Black seed oil
Pollen grains

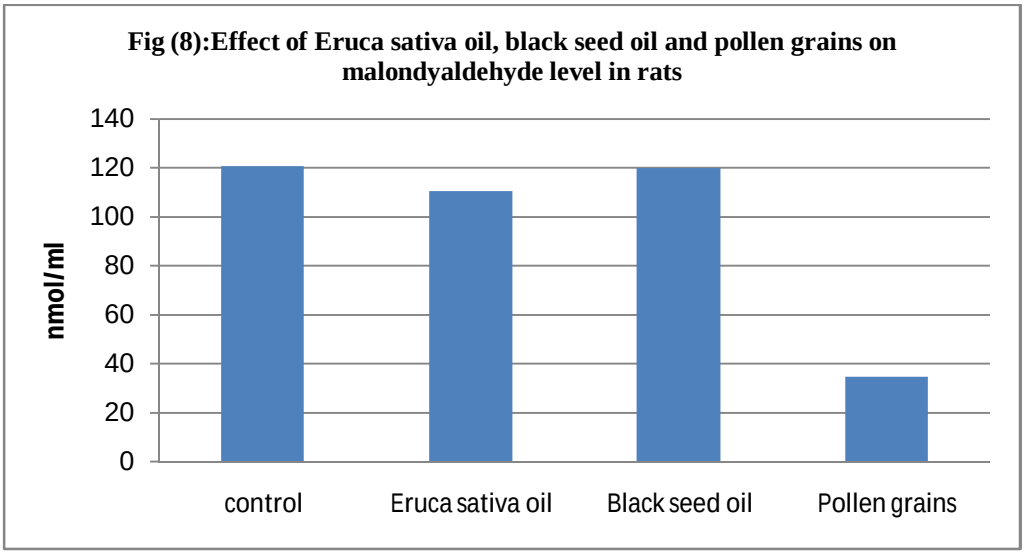


Fig (8):Effect of Eruca sativa oil, black seed oil and pollen grains on malondyaldehyde level in rats
nmol/ml
140
120
100
80
60
40
20
0
control
Eruca sativa oil
Black seed oil
Pollen grains

Fig (9):Effect of *Eruca sativa oil*, black seed oil and pollen grains on antioxidant glutathion in rats

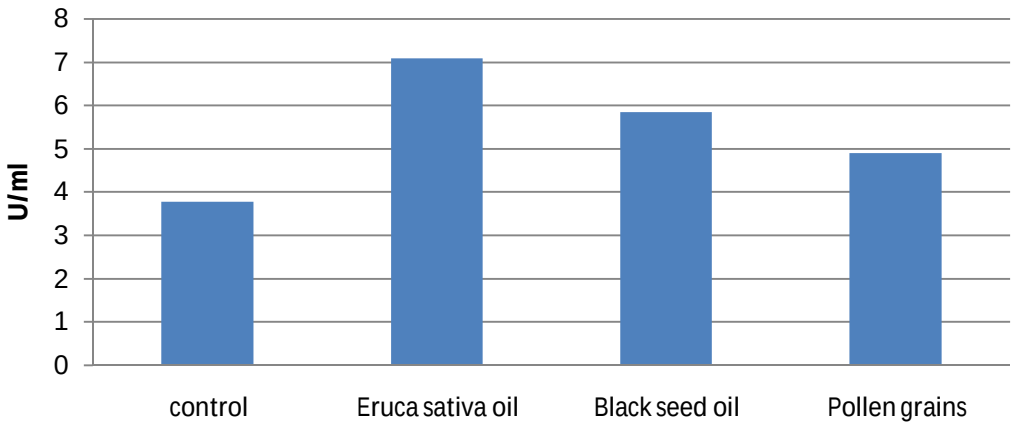


Fig (10): Effect of *Eruca sativa* oil, black seed oil and pollen grains on antioxidant catalase in rats

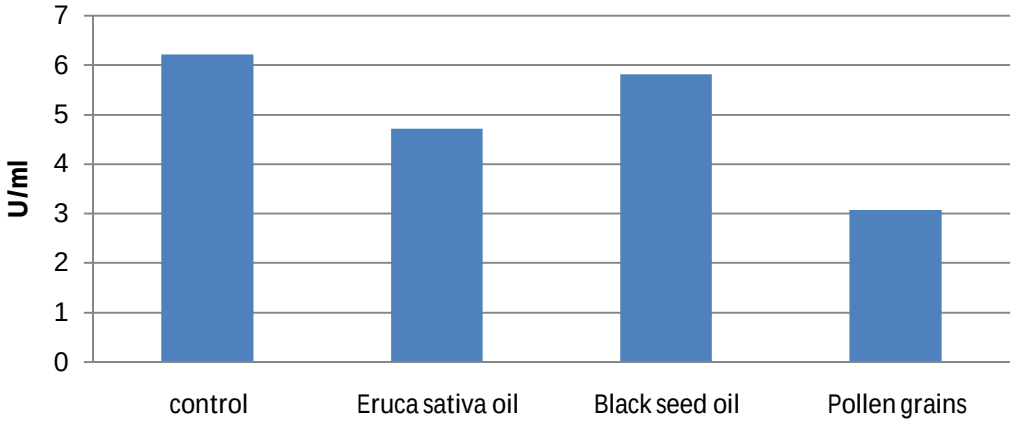
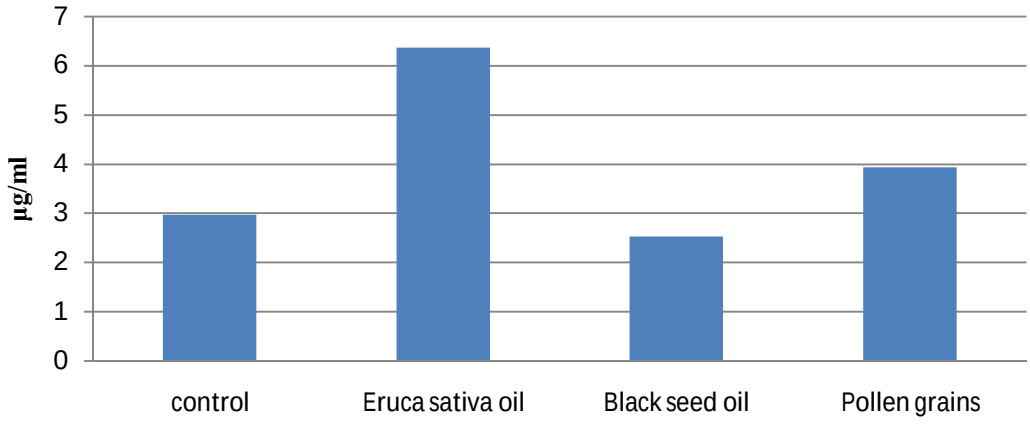


Fig (11):Effect of *Eruca sativa* oil, black seed oil and pollen grains on antioxidant GPX in rats



DISCUSSION

The resulted data show significant increase in GPT level in *Eruca sativa* oil and pollen grains groups in another direction there are no significant changes in Got level in all treated groups also, black seed oil treatment did not change GPT level in the serum. Total protein did not show any significant changes in all treated groups. Also albumin level did not change in Eruca sativa oil group and pollen grains group. My results are in agreement with Asaduzzaman et al., 2011 who reported that Thymoquinone (TQ) and the crude oil from seeds of Nigella sativa are effective against cancer and many diseases like diabetes,, Asthma , kidney disease etc. it is effective against cancer in blood system, lung , kidney, prostate, breast, cervix skin with much safety, some studies showed that TQ has antioxidant role and improves bodies defense system, induces apoptosis.

Where Luterdot*et al.*, 2010 stated that black cumin (Nigella sativa L.) belonging to family ranunculacae is a spice that has been used for decades for both culinary and medical purposes. It is also used as a natural remedy for, hypertension, asthma, diabetes, in inflammation caugh, bronchitis, eczema, headache, fever, dizziness and influenza.

The seeds are known to be carminative,stimulant and diuretic (Shah and Ray 2003. Black seed oil contains high concentrations of thymoquinone and its related compounds such as thymol and dithymoquinone, which have been implicated in the prevention of inflammation [22] ,antioxidant activities[23] as quenching reactive oxygen species, antimicrobial activity [24] and anticarcinogenic and antiulcers activity .[21]The major component in black cumin oil was thymoquinone (3706%) followed by P-cymene (31.4%), α- thujene (5.6%), thymohydroquinone (3-4%), longifolene (2.0%) and Carvacrol (1.4%). Burits and Bucar2000, characterized many components in black cumin essential oil as P- Cymone (71-15.5%), thymoquinone (27.8-57.0%), trans-anethole (0.25-2.3%), Carvacol (5.8-11.6%), (1.0-8.0%) longifolene and 4-terpeneol (2.0-6.6%).

On the other hand there are stability in urea in black seed oil group and also uric acid and createnine is the same this are in agreement with Singh et al., 2014 who stated that essential oil and oleoresins of black cumin exhibited higher antioxidant activity than synthetic antioxidants so, black cumin can used in pharmaceutical applications and its usage as dietary source of antioxidant should be considered largely for alleviating and ameliorating diseases.

In another direction MDA, glutathione, catalase and GPX level did not

change by another mean there are stability in its level and this is in agreement with Hamed*et al.,* 2013 who reported that treatment with black cumin oil alleviated the elevation of GSH, serum protein, G-6-Pase, No, phospholipids levels, Na^+-K-ATPase and attenuated MDA, SOD, AST, ALT and ALP diminution of collage content and improvement in liver and kidney architectures were observed. Black cumin oil enhanced the hepato-renal protection mechanism, reduced disease complications and delayed it progression.

Khan and sultana (2005) reported the chemopreventive effect of N. sativa against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, renal carcinogenesis and hyper- proliferative response. Treatment of rats orally with *N.sativa* (50-100 mg/kg body weight) resulted in significant decrease in H_2O_2 generation, incidence of tumors and DNA synthesis.

My results show no significant change in glutathione and GPX in pollen grains group and this are in dis agreement with[28]and [29]who recorded that bee pollen is rich in carotenoids, phytosterols and flavonoids, while the exact profile of bee pollen content varies depending on the plant sources and growth conditions. The antioxidants presents in honey bee- pollen have free radical scavenger and as lipid peroxidation inhibitor. This activity has been associated with the phenolic pollen content. [30]Furthermore, Turkey bee pollen has inhibitory effects against mycelia growth and several pharmacological activities. [31]

Propolis and bee pollen are apicultural products includes considerable amounts of polyphenol substances which may act as potent antioxidants. [32] In addition, phenolic compounds are known to counteract oxidative stress in the human body by helping maintaining a balance between oxidant and antioxidant substance. [33]

It is reported that flavonoids and phenolic acids are major classes of polyphenolic compounds, whose structure-antioxidant activity relationships in aqueous or lipophilic systems have been extensively reported. Also many phenolic compounds exert anticarcinogenic or anti-mutagenic activity to a great or lesser extent. [34]

Mechanisms of antioxidant action of these compounds (flavonoids and phenolic acids) may include suppression of oxygen reactive species (ROS) formation, upregulation or protection of antioxidant defense and removal or inactivation of oxygen reactive species. [35] In this context one of the most important intracellular antioxidant systems is the glutathione redox cycle. Glutathione is one of the essential compounds for maintaining cell integrity

because of its reducing properties and participation in the metabolism [36]on the other hand, catalase has the ability to convert hydrogen peroxide to water. [37]

Bee pollen is rich in amino acids, enzymes proteins, minerals, carbohydrates, fats, a considerable amount of vitamins, phenolic substances, phytochemicals and significant quantities of antioxidant agents. [38, 39]

Bogdanov 2004 and Eraslan*et al.,* 2009 recorded that pollen contains about 1-5% total phenolic substances, which includes different subtypes such as phenolic acid flavonoids, anthocyanins and tannins they exhibit a wide range of biological activities including antioxidant, antimicrobial, anti- inflammatory, antiatherogenic, anticarcinogenic and antithrombotic activities.

Phenolic compounds are beneficial for human health since they decrease the risk of degenerative diseases caused by oxidative stress. Many researchers have demonstrated that the phenolic compounds within the pollens inhibit the occurrence and development of numerous degenerative disorders. [40]

Bogdanove 2004, Hegazi 2012 and D'Anderea et al., 2005 suggested that the bee pollen, which contains many phenolic substances has similar effect as silibinin in terms of hepatoprotection for this purpose many natural extracts and honey bee products such as honey and pollen were used to treat hepatic disorders in laboratory animals.[40,30,42,43]

Oktay*et al.,* 2013 dtermined the therapeutic effects of chestnut bee pollen on the CCl_4 induced liver damage in the rat model. Also he determined that pollen supplementation recovered the body weight, ALT and AST enzymes levels, malondialdehyde (MDA) and superoxide dismutase levels as well as decreased the histological damage and apoptosis at the hepatocytes following the CCl_4 treatment.

CONCLUSION

1- Black seed oil maintains stability of liver enzymes and increase albumin where pollen grains and *Eruca sativa* oil increase GPT activity
2- *Eruca sativa* oil increases serum urea where black cumin oil and pollen grains maintains on the levels of urea uric acid and createnine.
3- The black cumin oil maintains on the measured antioxidants in serum especially catalase where *Eruca sativa* oil increases catalase;and pollen grains decreases it
4- *Eruca sativa* oil increases levels of GPX in serum
5- Black seed oil maintains stability of liver enzymes and antioxidant enzymes in rat bodies.

6- The black cumin oil plays an important role in liver protection.

RECOMMENDATIONS

Black cumin oil important in treating liver and kidney diseases.

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