

EFFECT OF SEEDS EXTRACTION OF *Lepidium sativum* ON ZINC AND IRON ELEMENTS AND SOME BIOCHEMICAL PARAMETERS IN SERUM OF WHITE MALE RABBITS

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ABSTRACT:

The present study was aimed to evaluate the effect seeds extraction of *Lepidium sativum* on zinc , iron and some biochemical parameters of adult male rabbits . The results show that a significant decrease ($P < 0.05$) in both serum malondialdehyde (MDA) and total cholesterol concentration (TCC) of tocopherol and phenol extraction , but the terpen extraction caused insignificant decrease ($P > 0.05$) in both of MDA and TCC of serum of adult male rabbits compared to the control treatments . Also the results showed insignificant increase ($P > 0.05$) in serum total protein concentration (TPC) of tocopherol , phenol and terpen extraction compared to control groups . The extracts caused significant differences ($P > 0.05$) in zinc and iron level in treated serum animals compared to control treatments .

تأثير مستخلصات بذور نبات الرشاد *Lepidium sativum* على عنصري الخارصين والحديد وبعض المعايير الكيموحيوية في مصل الدم لذكور الأرانب البيض

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الخلاصة :

هدفت الدراسة الحالية تقييم تأثير مستخلصات بذور الرشاد *Lepidium sativum* على عنصري الزنك والخارصين وبعض المعايير الكيموحيوية في مصل ذكور الأرانب البيض البالغة ، وتأثير هذه المستخلصات كمضاد للأكسدة ومضاد للدهون . أظهرت النتائج انخفاض معنوي احصائيا ($P < 0.05$) في كل من تركيزي المالونداي الديهايد (MDA) والكولسترول الكلي total cholesterol concentration (TCC) للمستخلصات التوكوفيرول والفينول ، لكن سبب مستخلص التربين انخفاض غير معنوي احصائيا ($P > 0.05$) في كل من تركيزي MDA و TCC لمصل ذكور الارانب البالغة مقارنة بمعاملة السيطرة . أيضا أظهرت النتائج زيادة غير معنوية ($P > 0.05$) في تركيز البروتين الكلي المصلي لمستخلصات التوكوفيرول والفينول والتربين مقارنة بمجاميع السيطرة . سببت المستخلصات أعلاه اختلافات معنوية ($P > 0.05$) في مستويات الخارصين والحديد في مصل الحيوانات المعاملة مقارنة بمعاملة السيطرة .

Introduction:

Lepidium sativum (family Brassicaceae) , is a small herb with 30 to 50 cm in height and bears laciniate-pinnate entire leaves . Flowering of the plant has been observed in the

may to July months . It consumed mostly in salads and has been reported to have enormous biological activities such as cardiotoxic, Hypotensive , bronchodilator , antimicrobial , antiprotozoal , antibiotic and hypoglycemic (Agarwal and Verma, 2011; Sarikami and Yanmaz, 2011). Plants (fruits , vegetables , medicinal herbs , etc.) contain a wide variety of free radical scavenging molecules such as phenolic compounds (e.g. phenolic acids , flavonoids , anthocyanins and tannins) , nitrogen compounds (e.g. alkaloids , amines and betalains) , vitamins , terpenoids (including carotenoids) and some other endogenous metabolites , which are rich in antioxidant activity (Cai *et.al.* 2003; Muanda *et.al.*,2011) . Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress . A variety of free radical scavenging antioxidants are found in dietary sources like fruits , vegetables and tea (Souri *et.al.*,2008) .

Hypercholesterolemia has become the leading cause for the development of various diseases . It has drawn the attention of pharmaceutical companies to turn towards the herbal products and having the fewer side effects (Subasini *et.al.*, 2012) . The present study indicates that *Lepidium sativum* extract restrains scavenging activity of Reactive Oxygen Species . The reducing capacity of extracts of *Lepidium sativum* seeds may be used as a significant indicator of its potential antioxidant activity (Agarwal and Verma, 2011) . The results reveal that *Lepidium sativum* is a rich source for phytoconstituents like phenolic compounds , vitamin E and terpenoids, and can be used as a potent antioxidants, antilipidemic, and increase the total proteins content (Kousar *et.al.*, 2011). Metals play a vital role as structural and functional components of protein and enzymes in cells . Each mineral play a number of different functions in the body . The most important pathway of metals to transport into human is from soil to plant and from plant to human (Kirmani *et.al.*, 2011) . Some metals such as calcium , manganese and zinc have been reported to be essential for human health , whereas others such as phosphor , cadmium and aluminum have been identified as toxic , Rest of the elements are not toxic to human unless they are present in high concentrations (Nasli-Esfahani *et.al.*, 2011) . The present study is concerned with the determine whether plant extracts can influence the bioavailability of several elements important for the human healthy .

Materials and Methods:

Preparation of Extracts:

The seeds of *Lepidium sativum* were cleaned and cut into small pieces with a blender . For phenol extraction , 1gram of sample was mixed with 25ml of (80% methanol : 20% distilled water) in water bath at 70°C for 4 hour , the extract was filtered and then filtrate dried in oven at 50°C for 24 hour (Harbone, 1973) . For terpen extraction , 60 of gram sample was extracted with chloroform for 24 hour by soxhlet method . For tocopherol extraction , 5gram sample was extracted with 25ml of (85% hexane : 15% ethyle acetate) for 24 hour by Soxhlet method . The terpen and tocopherol extracts

were filtered out and evaporated to dryness by oven at 45°C for 24 hour (Harbone, 1973 ; Brain and Turner ,1975) .

Experimental animals:

The study has been performed in animals house of science college / Babylon university . The animals involved 55 of adult male rabbits . The mean weight of animals average 1.513 gram , and the age of 4 months . All the animals put in cage under control of water , diet and light duration (12 hour light-12 hour dark respectively) . These animals were divided into 5 groups (5 animals for each group):

- First group (phenol control) treated orally with distilled water .
- Second group treated orally with 23 , 64 and 96 mg/kg body weight (B.W.). of phenol extraction .
- Third group (tocopherol and terpen controls) treated orally with corn oil .
- Four group treated orally with 23 , 64 and 96 mg/kg B.W. of tocopherol extraction .
- Five group treated orally with 23 , 64 and 96 mg/kg B.W. of terpen extraction .

The animals were treated for 50 days daily with above concentrations.

Experimental procedure:

After the end of the experiment rabbits were scarified under chloroform . Blood samples were collected in tubes (plain and coated with anticoagulant) . Plain tubes centrifuged for separation of serum at 3,000 rpm for 15 minutes , and sera were stored at -20 °C for determination of the biochemical measurements .

Assay of Biochemical Parameters:

Malondialdehyde was determined according to the method of (Esterbauer and Cheeseman,1990) . Total cholesterol was determined using Bio.Labo.S.A. kit (France) . Total protein was determined using Bio.Labo.S.A. kit (France) . zinc (Zn) and iron (Fe) were determined by Atomic Absorption Spectrophotometer (Jorhem and Engman, 2000).

Statistical Analysis:

The data is presented as mean \pm standard error (SE) , Student's F-test was applied to find statistically significance at $P < 0.05$.

Results:

Tables 1 , 2 and 3 revealed that phenol extraction caused a significant decreased ($P < 0.05$) and insignificant decreased ($P > 0.05$) in the iron concentration of 23 and 64mg/kg BW/day respectively , and also a significant increased ($P > 0.05$) in iron concentration of 96 mg/kg BW/day compared with control groups and a significant difference ($P < 0.05$) in serum concentration between treated groups . The results of

tocopherol extract showed insignificant differences ($P > 0.05$) in serum iron compared to the control groups and between treated groups . The significant increased ($P > 0.05$) in serum iron of 23 mg/kg BW/day of terpen extraction compared to the control groups and 64 , and 96 mg/kg BW/day of treated groups . There are a significant decreased ($P < 0.05$) in serum zinc in 96 mg/kg BW/day of terpen and phenol extraction , but a significant increased ($P < 0.05$) in serum zinc of 96 mg/kg BW/day of tocopherol extraction compared to the control groups . Also a significant differences ($P < 0.05$) in serum zinc between treated groups of tocopherol, phenol, and terpen extraction .

The effect of plant extracts on malondialdehyde concentration in serum of tocopherol and phenol extraction are shown in Fig. 1 , 2 . The MDA concentration was decreased significantly ($P < 0.05$) in treated animals with 23 , 64 and 96 mg/kg BW/day for tocopherol and phenol extraction compared with control groups , while the comparison between treatment groups with tocopherol and phenol extraction showed insignificant decreased ($P > 0.05$) in serum MDA . Also insignificant decreased ($P > 0.05$) in serum MDA of animals treated with 23 , 64 and 96 mg/kg BW/day of terpen extraction compared to the control group Fig. 3. Fig. 4 and 5 showed a significant decrease ($P < 0.05$) in serum total cholesterol concentration of 23 , 64 and 96 mg/kg BW/day of tocopherol extraction and in the 96 mg/kg BW/day of phenol extraction compared with control treatment , and a significant decrease ($P < 0.05$) of group 96 mg/kg BW/day of phenol extraction compared to the groups 23 and 64 mg/kg BW/day of phenol extraction , while there are insignificant decreased ($P > 0.05$) in serum TCC between treatment groups of tocopherol extraction , and insignificant decreased ($P > 0.05$) between control group with 23 and 64 mg/kg BW/day of phenol extraction . Also insignificant decreased ($P > 0.05$) in serum total cholesterol concentration of animals treated with 23 , 64 and 96 mg/kg BW/day of terpen extraction compared with control group Fig. 6 . The insignificant increased ($P > 0.05$) in serum Total protein concentration in treated animals with 23 , 64 and 96 mg/kg BW/day of tocopherol , phenol , and terpen extraction compared to the control groups and between treated groups were shown in Fig. 6 , 7 and 9.

Table 1. The level mean values of zinc and iron in serum sample of phenol groups .

Groups (mg/kg)	Level of mental in serum sample (mean±SE)	
	Zinc	Iron
Control group	4.928±0.426 ^a	12.173±1.751 ^a
32	5.138±0.682 ^a	5.913±1.205 ^b
64	4.019±0.458 ^a	10.898±0.418 ^a
96	2.377±0.355 ^b	16.000±0.438 ^c

Different superscripts are significantly different at($p < 0.05$) .

Table 2. The mean values of zinc and iron in serum sample of terpen groups .

Groups (mg/kg)	Level of mental in serum sample (mean±SE)	
	Zinc	Iron
Control group	4.928±0.426 ^a	12.173±1.751 ^a
32	4.089±0.060 ^{ab}	23.651±1.448 ^b
64	3.180±0.865 ^{ab}	16.115±1.636 ^a
96	2.377±0.493 ^b	12.521±1.004 ^a

Different superscripts are significantly different at($p < 0.05$) .

Table 3. The mean values of zinc and iron in serum sample of tocopherol groups .

Groups (mg/kg)	Level of mental in serum sample (mean±SE)	
	Zinc	Iron
Control group	4.928±0.426 ^a	12.173±1.751 ^a
32	4.603±0.179 ^a	20.869±3.213 ^a
64	6.641±0.636 ^{ab}	17.333±4.089 ^a
96	7.081±0.829 ^b	9.738±1.391 ^a

Different superscripts are significantly different at($p < 0.05$) .

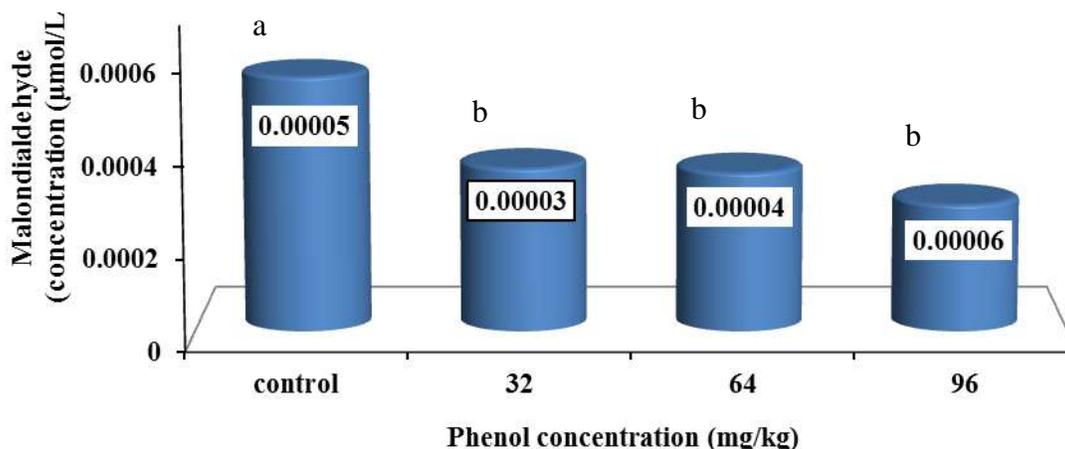


Fig. 1. Effect of different doses of phenol extraction for *lepidium sativum* seeds on Malondialdehyde concentration in serum of adult male rabbits.

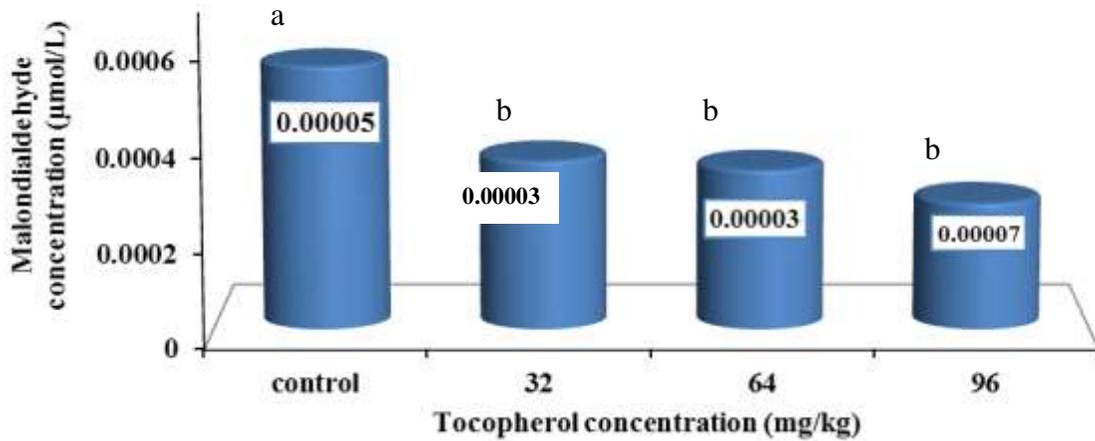


Fig. 2. Effect of different doses of tocopherol extraction for *lepidium sativum* seeds on Malondialdehyde concentration in serum of adult male rabbits.

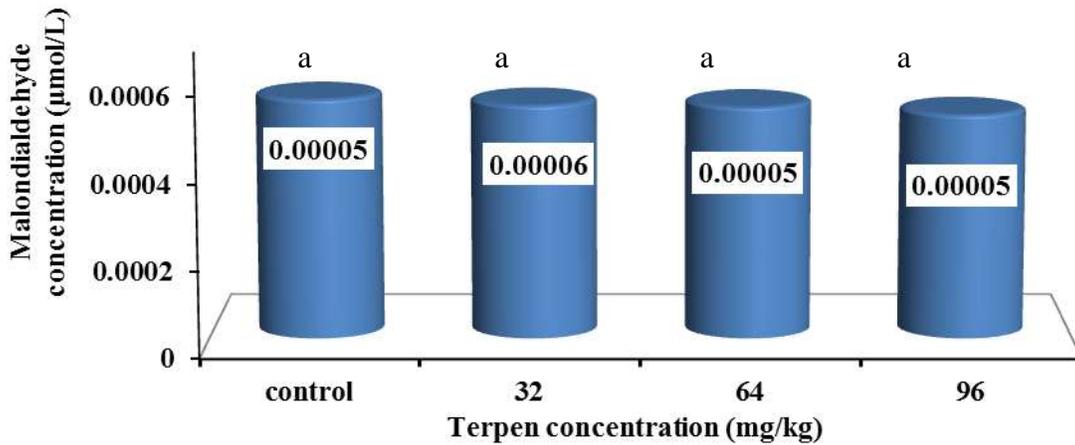


Fig. 3. Effect of different doses of terpen extraction for *lepidium sativum* seeds on Malondialdehyde concentration in serum of adult male rabbits.

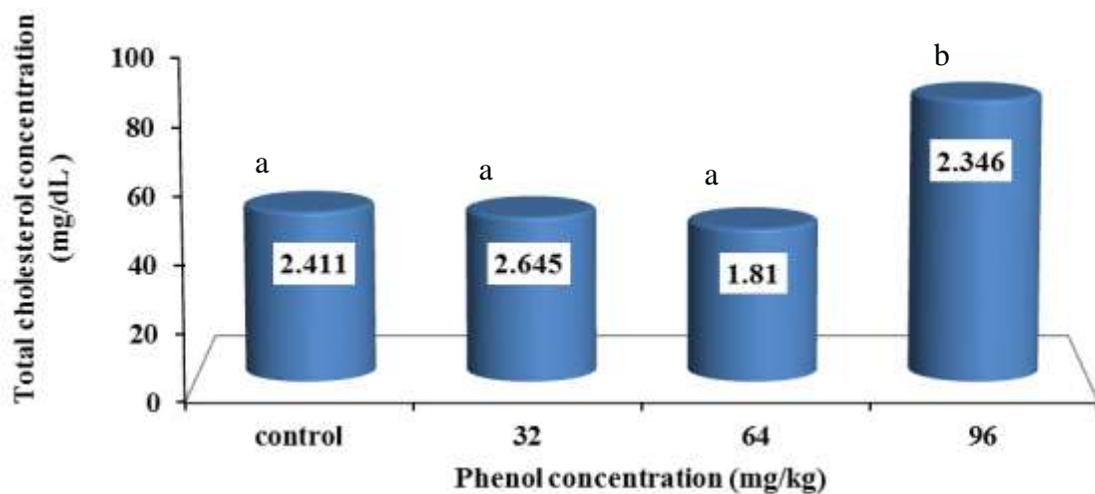


Fig. 4. Effect of different doses of phenol extraction for *lepidium sativum* seeds on Total cholesterol concentration in serum of adult male rabbits.

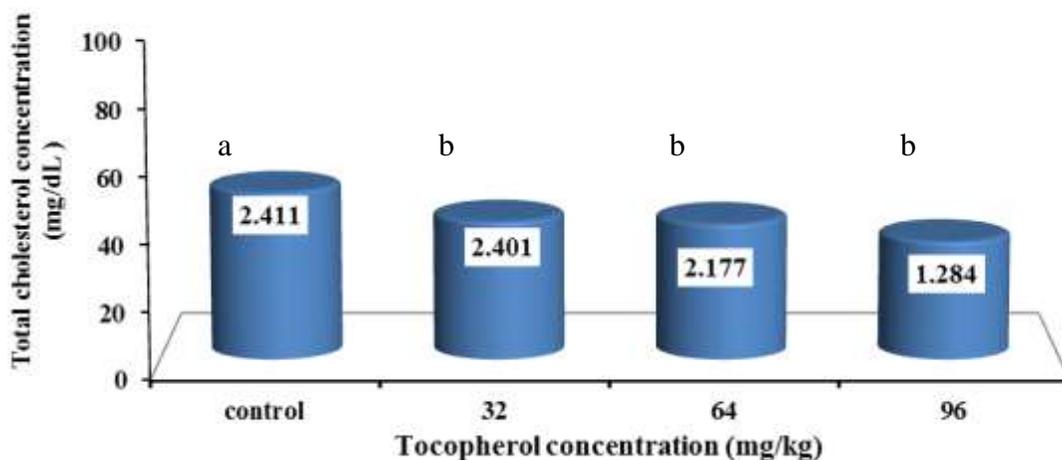


Fig.5. Effect of different doses of tocopherol extraction for *lepidium sativum* seeds on Total cholesterol concentration in serum of adult male rabbits.

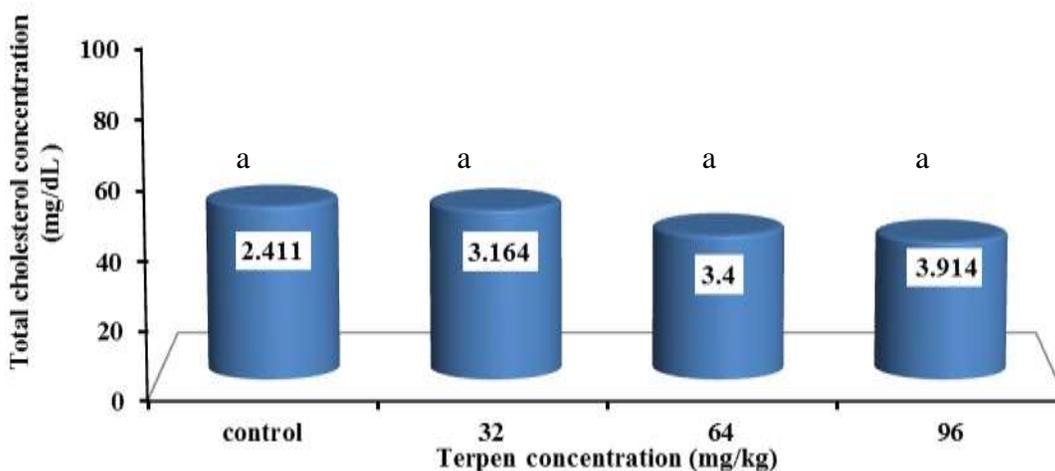


Fig. 6 Effect of different doses of terpen extraction for *Lepidium sativum* seeds on Total cholesterol concentration in serum of adult male rabbits.

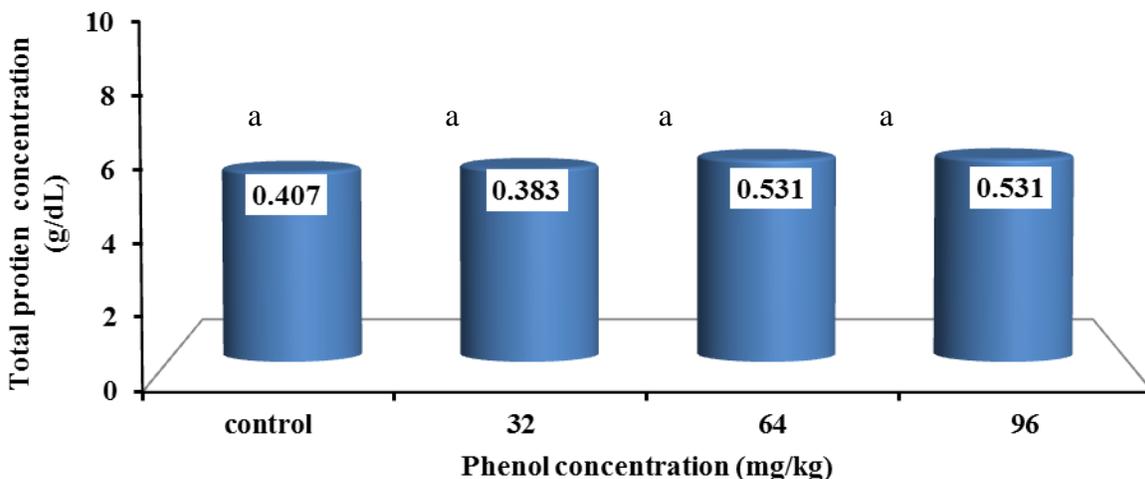


Fig. 7. Effect of different doses of phenol extraction for *lepidium sativum* seeds on Total protien concentration in serum of adult male rabbits.

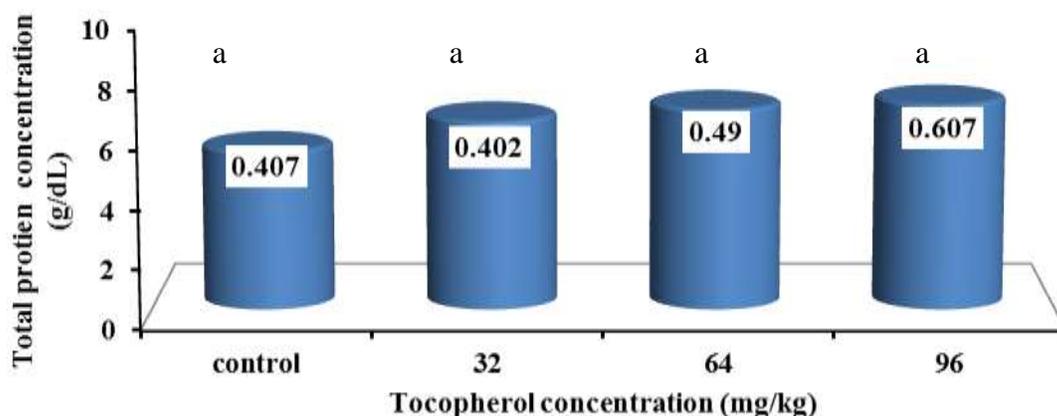


Fig. 8. Effect of different doses of tocopherol extraction for *lepidium sativum* seeds on Total protien concentration in serum of adult male rabbits.

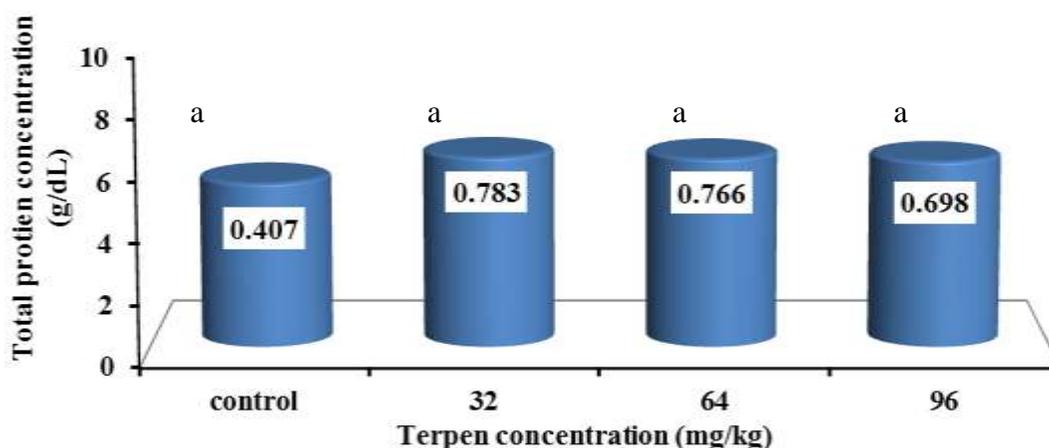


Fig. 9. Effect of different doses of terpen extraction for *lepidium sativum* seeds on Total protien concentration in serum of adult male rabbits.

Discussion

Result of the present study provides vital data on the availability of some essential minerals (zinc , iron) , which can be useful to provide healthy information for designing value . Mineral elements play important roles in health and disease states of humans and domestic animals (Soetan *et.al.*, 2012) . Iron is necessary for the formation of hemoglobin and also plays an important role in oxygen and electron transfer in human body (Bhowmik *et.al.*, 2012). Zinc , an essential trace element , has antioxidant functions , stabilizes membranes , and plays a role in the activity of a host of Zinc metalloenzymes . Erythrocytes and leukocytes carry considerable zinc, most of it rather tightly bound (Agte *et.al.*, 2005;Bhuvaneshwari *et.al.*, 2012) . On the other hand , the results of investigations into the effect of tocopherol , phenol , and terpen compounds applied in different doses on the bioavailability of serum elements , using various mechanisms including the use of antioxidants to remove free radicals and improved levels of serum elements . Therefore it can be supposed that the applied dose of tocopherols, and phenols had a positive effect as

it reduced the amount of Iron ; and the terpen and phenols had a positive effect as it reduced the amount of Zinc in the body without causing any toxic effect on human health . The phenolic compounds are released from the foods and beverage during digestion process and can complex with iron and making it unavailable for absorption. Adversely with heme iron, this form have a low absorption and markedly affected by gastro intestinal acidity , tannins , polyphenols , phytates , calcium and phosphate. The presence of absorption inhibitors such as phytic acid or polyphenol compounds in the plant foods is a major cause of iron deficiency . Inhibited absorption of iron by phenolic compounds or the saponins in lepidium extract is the possible reason (Abdulkarimi and Daneshyar, 2012) . Magnesium , copper , selenium , zinc , iron , manganese and molybdenum are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways process and can complex with iron and making it unavailable for absorption There is a co-operative action between enzymatic and non-enzymatic antioxidants have been shown to scavenge free radicals and ROS . Non enzymatic antioxidants include vitamins A , C and E , GSH and trace elements like zinc and selenium (Ali , 2012).

In the present study, we used oral doses of tocopherol , phenol and terpen at doses of 32 , 64 and 96 mg/kg /day . The used dose of tocopherol in this study give best results in decreased in MDA and TCC concentration and insignificant increased in TPC concentration as agreed with (Akinloye *et.al.*, 2011; Soliman and Bahagt, 2012) ,which indicates that vitamin E specifically targeted the mechanisms of dyslipidemia as evidenced by the strong inverse relationship between vitamin E and total cholesterol observed in treated group . Vitamin E protects low density lipoproteins (LDL) particles from oxidative attack and a potent antioxidant that reduces Reactive Oxygen Species formed during fat oxidation , it functions through the glutathione pe-oxidase pathway protecting cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Tzanetakou *et.al.*,2012).

Oxidative stress is a putative factor in the pathogenesis of many human disorders of the central nervous system . Therefore , antioxidants such as vitamin E have become attractive as therapeutic agents in the treatment of several diseases . In addition, vitamin E seems to play a specific role in the nervous system . As a result, vitamin E has been used in pharmacologic doses in the treatment of disorders such as Parkinson disease , Alzheimer disease , and tardive dyskinesia (Vatassery *et.al.*,1999) . The results of the present study showed a significant decreased in serum MDA , and in TCC concentration and insignificant increased in TPC concentration of treated male rabbits with phenol extraction . This results were agree with other studies , which indicated that Some phenolic compounds in herbs have the capacities to quench lipid peroxidation , prevent DNA oxidative damage , and scavenge reactive oxygen species , such as superoxide , hydrogen peroxide and hydroxyl radicals (Yoo *et.al.*, 2008) . Hesam, *et.al.*, (2012) , showed the correlation between total phenolic content of Metanolic extracts of three potato cultivars and Diphenyl-2-picrylhydroxy radical scavenging activity indicates that

phenolic compounds are responsible for antiradical activity. Phenolic compounds can suppress free radical-induced oxidative stress , and the antioxidant activity of plant materials was traditionally attributed to well-known phytochemicals such as Phenolic compounds , alpha-tocopherol , ascorbic acid and beta-carotene . Other study showed that daily intake of cocoa powder decreased the susceptibility of LDL to oxidation and increased HDL-cholesterol concentrations in plasma in humans . It is possible that increases in HDL-cholesterol concentrations may contribute to suppression of LDL oxidation . Because polyphenolic substances derived from cocoa powder contribute to the elevation of HDL cholesterol , it would be anticipated that intake of polyphenol-rich foods , such as cocoa , tea , wine , fruit and vegetables , should lead to a decrease in the incidence of arteriosclerotic disease . Moreover , it is irrefutable that a balanced daily diet is important for the promotion of human health (Baba *et.al.*, 2007). Alsaif,*et.al.* (2007) , showed that consuming more Flavonoids may have positive effect on lowering blood lipids . The ethanolic extract of fruit of *Moringa oleifera* showed highest phenolic content , strong reducing power and free radical scavenging capacity , and was able to increase the GSH and reduce MDA level in a concentration-dependent manner (Luqman *et.al.*, 2012) .

The insignificant decreased in MDA , and TCC concentration and insignificant increased in TPC concentration of terpen extraction may be refer to the presence of some active ingredients such as Alkaloids , Tannins , Sponginess , Phenols , glycosides , steroids , terpenoids and flavonoids (Rao *et.al.*, 2012) , this could be due to its antioxidative of phenols (Ahmed *et.al.*, 2012) , terpenoids (Chih-Chun *et.al.*, 2007; Panigrahi *et.al.*, 2012). Francis,*et.al.*,(2002), showed the nature of the interaction between the particular saponin and cholesterol , and the nature of the cholesterol moieties and other ligands in the diet are essential to arrive at an effective dietary dose of that particular saponin that could have a significant hypocholesterolaemic effect , and the saponin rich extract of *Achyranthes aspera* could prevent the changes induced by high fat diet and may act as effective antiobese agent and the Reductions in LDL , VLDL , and TC levels in serum could be due to the inhibition of lipid absorption in the gastrointestinal tract in presence of saponins (Lathaa *et.al.*,2011).

According to the results, it is observed that *Lepidium sativum* seeds possess a considerable scavenging antioxidant , antiradical capacity and antilipidemic therefore these antioxidant properties might increase the therapeutic value of this medicinal plant.

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