Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

EXAMINING THE EFFECT OF SELENIUM IN IMPROVING NONALCOHOLIC FATTY LIVER DISEASE IN RATS

Faezeh Sadeghian [1,2] , Mohsen Manochehri [3] , Hossein Kargar Jahromi [1,2*]

1. Research Center for Non-Communicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran.
2. Zoonoses Research center, Jahrom University of Medical Sciences, Jahrom, Iran.
3. Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran.

ARTICLE INFO

Received:
03th Jun 2017
Accepted:
29th Nov 2017
Available online:
14th Dec 2017

Keywords: *selenium, fatty liver, rat*

ABSTRACT

Introduction: Given the prevalence of risk factors for metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in society. In this study, the effect of selenium in improving nonalcoholic fatty liver disease was investigated in rats.
Methods: In this experimental study, 40 adult female Wistar rats were divided into 5 groups, each consisting of 8 rats. The five groups were control, high-fat diet and high-fat diet treated with 0.25, 0.5 and 1 mg/kg doses of selenium). Selenium was fed by gavage to the rats. At the end of the experiment, the rats were weighed. Blood samples were taken from heart of the rats (Blood samples were obtained by cardiac puncture). Finally, serum ALT, AST, ALK, LDL, HDL, TG and TC levels were measured. Five-micron tissue sections were prepared from liver tissue. The sections were stained with hematoxylin and eosin.
Findings: the results showed that mean serum concentration of TG, TC, LDL, ALT, AST and ALK significantly increased in the group receiving high-fat diet compared to control group. TC, LDL, ALT and ALK serum concentration significantly decreased in the groups receiving 0.5 and 1 mg/kg selenium compared to the group receiving high-fat diet. TG and AST serum concentrations significantly decreased in the group receiving 1 mg/kg selenium compared to the group receiving high-fat diet. All doses of selenium had no effect on mean serum levels of HDL. The best dose of treatment was 1 mg/kg. The results showed that selenium with antioxidant properties reduces and prevents damaging effects of fatty liver in rats.

To Cite This Article: Faezeh Sadeghian, Mohsen Manochehri , Hossein Kargar Jahromi (2017), "Examining the Effect of Selenium in Improving Nonalcoholic Fatty Liver Disease in Rats", ***Pharmacophore, 8(6S),*** *e-1173177.*

Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) was detected by Ludwig et al. in the people with no history of alcohol consumption for the first time in 1980. In fact, the patients suffered from a wide range of disorders including simple fat accumulation as large fat vesicles, accumulation of fat along with inflammation, damage to liver cells and cirrhosis (1). Nowadays, NAFLD is hepatic manifestation of metabolic syndrome. Clinical manifestations of metabolic syndrome include type 2 diabetes, obesity, dyslipidemia and hypertension (2). The prevalence of this disease was estimated from 20% to 30% in the general population. Given the rapid increase in prevalence of risk factors for metabolic syndrome, this disease is the most common cause of liver disease in Western society (3). Epidemiological studies reported prevalence of NAFLD as 2.8% in Iranian population (4). The cause of NAFLD is not known yet. Insulin resistance, obesity, oxidative stress and inflammatory cascade are involved in incidence and progression of the disease (5). So far, two-hit hypothesis is publicly acknowledged to explain pathogenesis of NAFLD. This hypothesis was proposed by Day and James for the first time. The hypothesis claims that insulin resistance leads to fat accumulation in the liver as the first hit. Consequently, liver becomes sensitive to oxidative stress caused by various factors as the second hit (6). Oxidative stress can increase lipid peroxidation in liver cellular membranes. Oxidation byproducts disturb nucleotide and protein synthesis. Moreover, these compounds

Corresponding Author: Hossein Kargar Jahromi, PhD, Research Center for Non-Communicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran, 2nd Zoonoses Research center, Jahrom University of Medical Sciences, Jahrom, Iran. Email: hossein.kargarjahromy@yahoo.com

increase secretion of inflammatory cytokines and activate hepatic stellate cells. These factors ultimately cause fibrosis, inflammation and apoptosis (8, 7). Antioxidants contain such nutrients as vitamins and minerals. Main antioxidants include beta-carotene (precursor of vitamin A in fresh and orange fruits, vitamin C found in fruits (especially citrus fruits), vitamin E found in vegetable oils (e.g. olive oil, canola), a variety of nuts (e.g. walnuts and almonds) and such minerals as zinc and selenium found in foods such as white meat (chicken, fish and a variety of seafood) and nuts). Selenium is a potent antioxidant mainly involved in enzymatic activity of glutathione peroxidase. Glutathione is the most abundant intracellular non-enzymatic defense mechanism against oxidants in a living organism. Glutathione peroxidase is the most important enzyme in glutathione metabolism pathway (13). Selenium is an essential element and a cofactor of glutathione peroxidase and thioredoxin reductase, which is the most important defense mechanism against oxidative stress (14, 15). Selenium binds to proteins and forms selenoproteins with antioxidant properties (16).

Yanjun et al. reported the most common test for liver disorder is increase in ALT and AST in China in 2001 (9, 10). Angelico et al. examined 282 patients with sonographic evidence of NAFLD in Italy in 2003. Hypertriglycemia and reduced HDL were reported as the main disorders in lipid profile of the patients with fatty liver disease (11). So far, no exclusive treatment is identified for these patients. Given the involvement of oxidative stress in pathogenesis of the disease and low levels of antioxidants in these patients, scholars have focused on use of antioxidants for treatment of this disease (12).

Kerstin E. Gelilinger et al. examined the effect of lower than normal selenium on liver metabolites in rats. They showed that reducing selenium lower than normal level significantly reduces activity of liver selenoenzyme with further increase in lipid peroxidation and glycogen and decrease in T3 hormone (17).

Farangis Ghasemi et al. examined protective effect of vitamin E and selenium on liver of rats with insulin resistance. They found out that vitamin E and selenium decrease activities of aspartate amino transferase (AST), alkaline phosphatase and glucose concentrations. No histological changes (e.g. hydropic cytoplasm and cell necrosis) in the groups treated with vitamin E and selenium (18).

Given that no study has examined the effect of selenium on nonalcoholic fatty liver disease, the present study sought to examine the effect of selenium in improving nonalcoholic fatty liver disease in rats.

Method

According to the articles published in this field, healthy adult female Wistar rats from 180g to 200g were used in this study. The rats were kept at animal breeding room in Jahrom University of Medical Sciences for a week to adapt to the environment. Light-dark cycle consisted of 12 hours of light and 12 hours of darkness. Humidity varied from 50% to 55%. The rats were weighted and kept in special cages (4 rats per cage). The rats were randomly divided into five control and experimental groups. Each group consisted of eight rats. Control received no treatment during the experiment (21 days). Sham group received high-fat diet for four successive weeks. The first, the second and the third experimental groups were given 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg intraperitoneal injection of selenium for 21 days with respect to body weight after induction of fatty liver (15). High-fat emulsion was used based on the method presented by Zhu et al. in 2006 to induce fatty liver and cause hepatic steatosis (Table 1) (19). In summary, the rats received 10 ml/kg high-fat emulsion at 8 am for 4 weeks via gavage in a daily manner. Blood samples were taken from head of the rats after the fourth week to ensure fatty liver induction. Then, tissue sections were prepared from the liver. The sections were stained with hematoxylin and eosin to ensure induction of fatty liver.

Table 1 - High-fat emulsion composition to feed the rats by gavage

Composition	Dose
Corn oil	400g
Sucrose	150g
Milk powder	80g
Cholesterol	100g
Sodium dioxide collate	10g
Tween	36.4g
Propylene glycol	31.1g
Multivitamin	2.5g
Salt	10g
Mineral Mix	1.5g
Distilled water	300ml

After induction of fatty liver, selenium was injected intraperitoneally each day at 10 am to rats by insulin syringe based on body weight. The course of treatment with selenium lasted for 21 days. At the end of the project (22 days after full induction of fatty liver and selenium treatment), 5cc blood samples were directly taken from rats using cardiac puncture under

anesthesia by ether. Their serum was collected by centrifugation (3000 rpm for 15 minutes). The serums were kept at (-20)ºC to measure ALT, AST, ALP, LDL, HDL, TC and TG levels.

After blood sampling, the livers were removed immediately and washed with physiological saline; fragments of liver tissue were cut and being kept in solution of 10% buffered formaldehyde. Formalin-fixed and paraffin-embedded tissue were processed for hematoxylin and eosin staining, in order to semi-quantitatively assessment of the fatty degenerations using the NAFLD activity score (NAS). The histological features were graded according to percentage of distributions while pathologists were blinded regarding experimental groups. Scores for steatosis (score 0 to 3, S0: <5%; S1: 5%-33%; S2: 33%-66%; S3: >66%), lobular inflammation (score 0 to 3, I0: No foci; I1: <2 foci per 200× field; I2: 2-4 foci per 200× field; I3: >4 foci per 200 × field), and ballooning (score 0 to 2, B0: None; B1: few balloon cells; B2: many cells/prominent ballooning), were also summed to calculate the NAS score (ranging from 0 to 8)(20,21).

Findings

Results of mean comparison in different groups in terms of studied parameters are shown in Table 2.

Table 2- Mean comparison in different groups in terms of studied parameters

Group	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	56.5±16.70 a	38.33±5.04 a	27.16±2.40 a	26.83±8.90 a	70.0±15.08 a	26.83±4.83 a	174.83±15.79 a
Sham (HFD)	144.66±13.50 d	58.66±15.31 b	118.16±10.81 d	22.66±6.34 a	113.5±14.85 c	51.5±9.39 b	404.83±73.57 b
HFD+Se 0.25mg/kg	122.66±15.12 c	49.83±6.67 ab	109.66±12.12 d	28.5±3.72 a	110.16±17.78 c	46.83±7.73 b	385.83±156.54 b
HFD+Se 0.5mg/kg	106.83±12.36 c	40.33±10.76 a	88.16±10.04 c	29.16±4.44 a	98.66±12.25 bc	35.5±7.09 a	253.5±74.32 a
HFD+Se 1mg/kg	89.5±14.51 b	40.0±4.56 a	69.16±8.49 b	30.33±5.35 a	82.33±13.82 ab	32.16±7.98 a	204.0±19.79 a

Abbreviations: ALT, Alanine Transferase; ALP, Alkaline phosphatase; AST, Aspartate Transferase; g/dL, miligram per deciliter; IU/L, Internation Unit Per Liter;

P value below 0.05 was considered as significant. There was no significant statistical difference between the levels.

Contents of Table 2 show a significant increase in concentration of triglycerides in the group with high-fat diet and the groups treated with selenium compared to control group at 5% significance level. This shows the negative impact of high-fat diet in changes in serum triglyceride levels in rats. On the other hand, a significant decrease was observed in concentration of triglycerides in the groups treated with selenium at minimum, average and maximum doses compared to the group with high-fat diet. This shows that selenium has positive effects in changes in triglyceride concentrations. These results are shown in Chart 1.

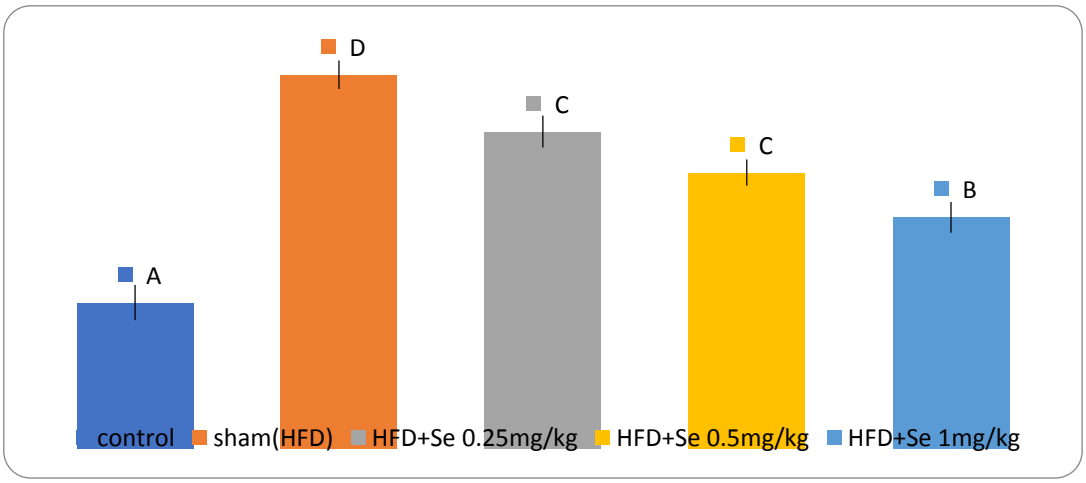

Chart 1: mean serum triglyceride levels in the studied groups

-The means in each column with at least one common letter are not significantly different based on Duncan's test

Contents of Table 2 with regard to cholesterol showed a significant increase in cholesterol in the group with high-fat diet compared to control at 5% significance level. This shows the negative impact of high-fat diet on serum concentration of cholesterol in rats. A significant decrease was found in serum cholesterol concentration in the groups treated with average and maximum doses of selenium compared to the group with high-fat diet (Chart 2).

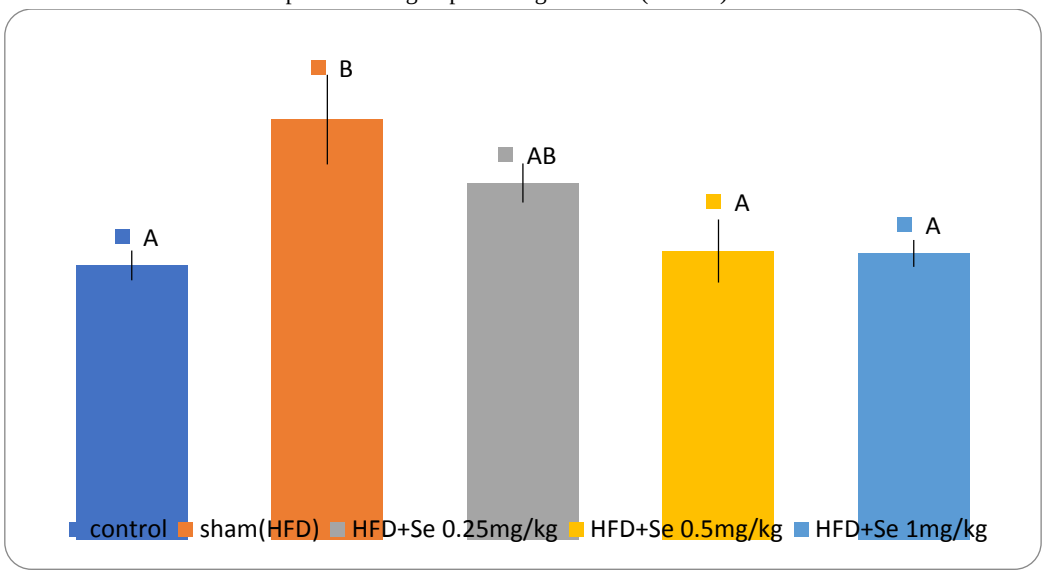

Chart 2 - Mean serum Total cholesterol concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

The results of LDL measurement showed a significant increase in LDL concentration in the group with high-fat diet and the groups treated with selenium compared to control at 5% significance level. A significant decrease was found in LDL serum concentration in the groups treated with average and maximum doses of selenium compared to the group with high-fat diet. This shows that selenium has positive effects in improving LDL serum concentration (Chart 3).

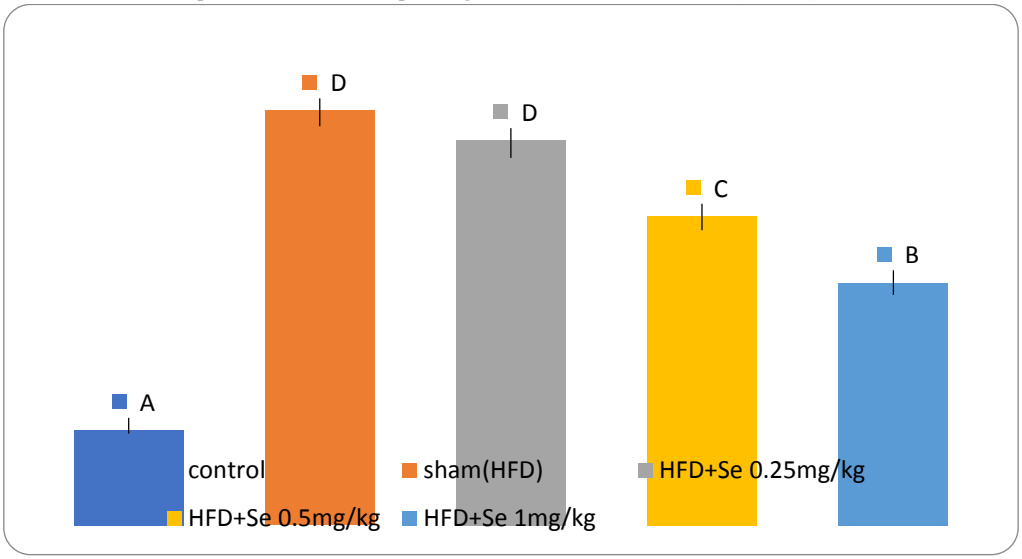

Chart 3 - Mean LDL serum concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

Results of HDL measurement showed no significant changes in HDL concentrations in different groups at 5% significance level based on statistical tests. This showed improvement in HDL concentration in the group receiving selenium compared to the group receiving high-fat diet (Chart 4).

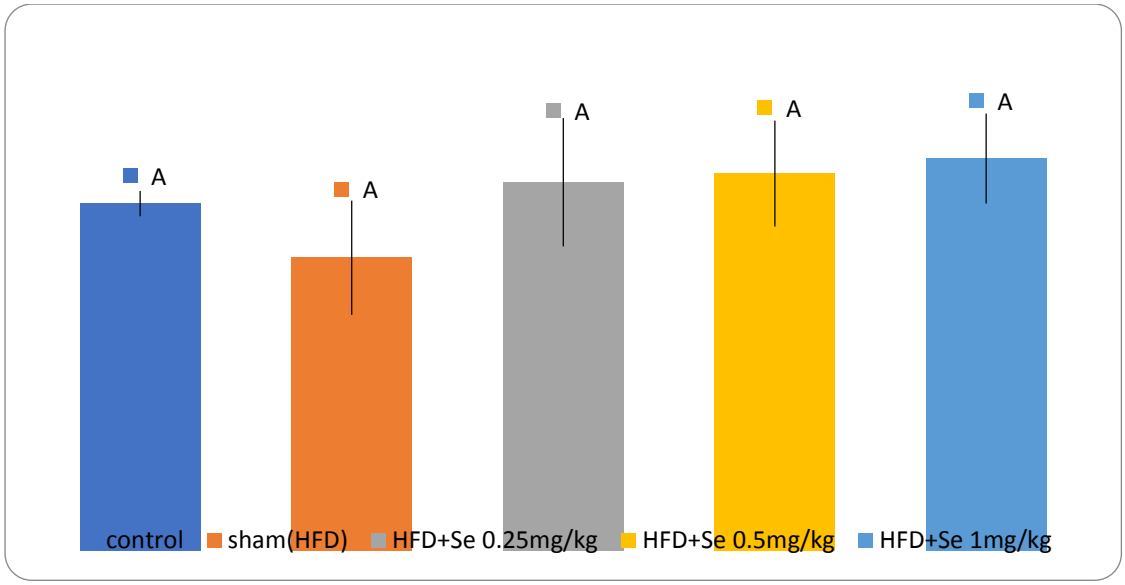

Chart 4 - mean serum HDL concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

The results of liver enzyme measurement showed a significant increase in AST concentration in the experimental group receiving high-fat diet and the group receiving minimum and average doses of selenium compared to control. A significant decrease was also found in AST concentration in the group receiving maximum dose of selenium compared to the group receiving high-fat diet. This shows positive effect of selenium in improving AST factor ($p < 0.05$) (Chart 5).

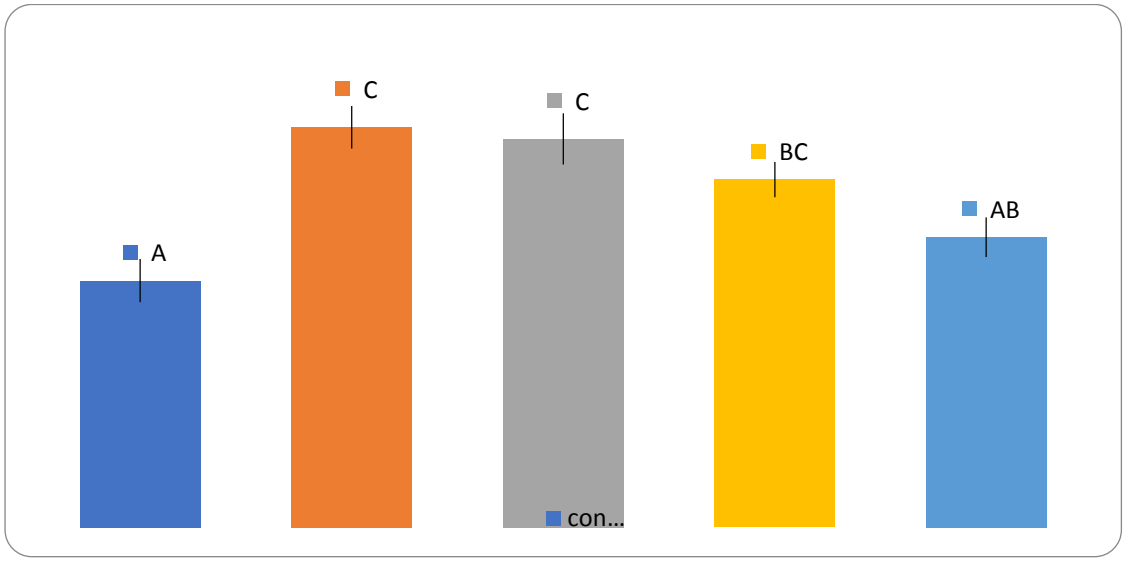

Chart 5 - AST mean serum concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

Results of ALT and ALK measurements showed a significant increase in ALT and ALK concentrations in the group receiving high-fat diet and the groups receiving minimum dose of selenium compared to control at 5% significance level. A significant decrease was found in ALT and ALK concentrations in the groups receiving average and maximum doses of selenium compared to the group receiving high-fat diet. This shows positive effect of selenium in improving ALT and ALK factors ($p < 0.05$) (Charts 6 and 7).

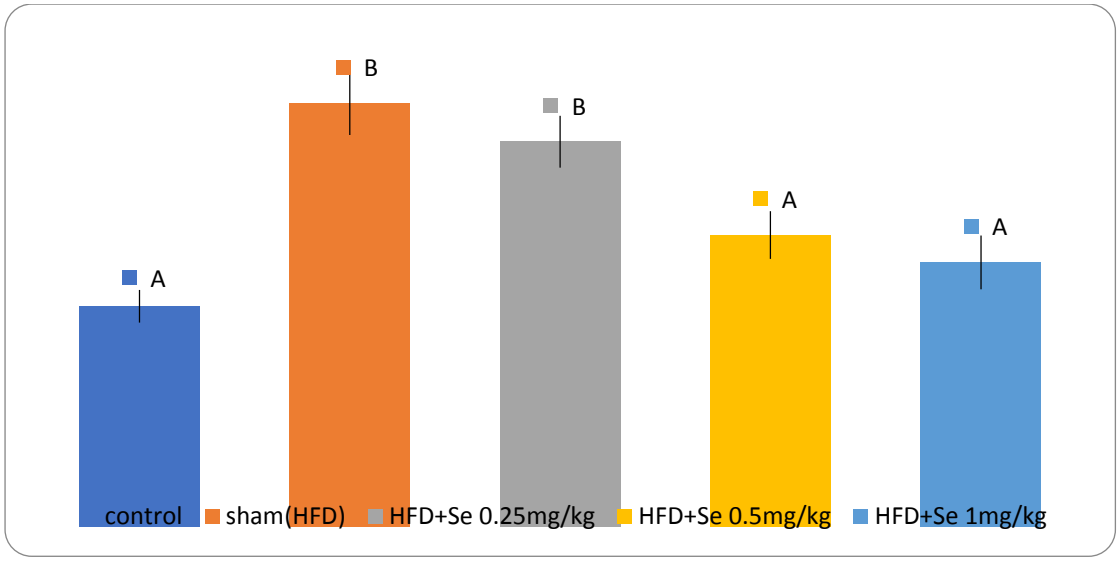

Chart 6 - Mean serum ALT concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

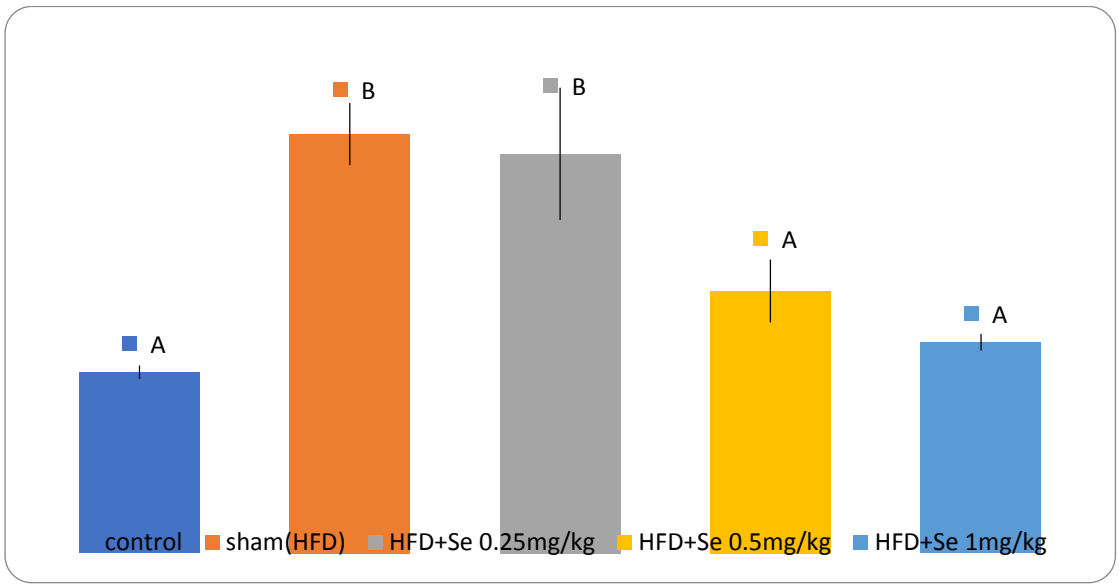

Chart 7 - Mean serum ALK concentration in the studied groups
-The means in each column with at least one common letter are not significantly different based on Duncan's test

Histological results in different groups:
No abnormal morphology was found in liver of the rats in control in microscopic studies. Liver tissues were normal in control (Figures 1 and 2). However, severe liver steatosis as macro- and microvesicular steatosis (vacuolation) along with swelling of hepatocytes, infiltration of inflammatory cells around the portal space and dispersed in sinusoid space, transparency of cellular cytoplasm and mild necrosis of hepatocytes were observed in the rats with high-fat diet. Fatty change in hepatocytes, hyperemia and lymphocytic infiltration in the liver parenchyma were significantly inhibited in the rats with high-fat diet + selenium powder (Figures 3 to 10). The greatest improvement was observed in the group receiving 1 mg/kg selenium (Figures 9 and 10).

Table 3. Histopathological assessment in study groups.

Group	Liver fat score	Ballooning score	Lobular inflammation score	NAFLD activity score (NAS)
Control	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Sham (HFD)	2.83±0.17 d	2.00±0.01 d	2.83±0.17 d	6.83±0.31 e
HFD+Se 0.25mg/kg	2.50±0.22 d	1.66±0.21 cd	2.50±0.23 d	4.33±0.33 d
HFD+Se 0.5mg/kg	1.83±0.16 c	1.33±0.22 bc	1.66±0.21 c	3.50±0.34 c
HFD+Se 1mg/kg	1.16±0.16 b	1.16±0.17 b	1.00±0.26 b	2.33±0.21 b

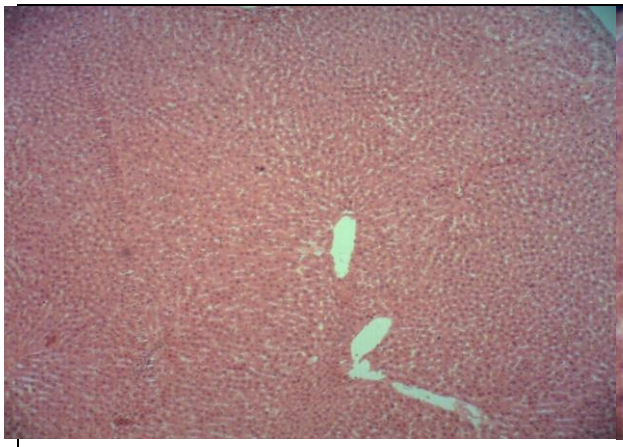

Figure 1. Control With a natural structure (Hematoxylin-eosin, 40X magnification).

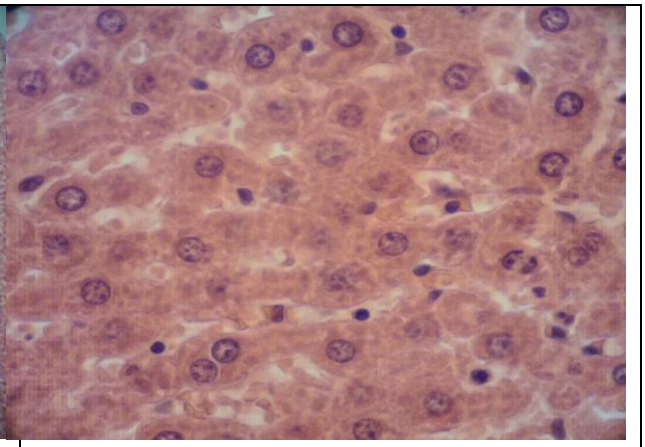

Figure 2. Control With a natural structure (Hematoxylin-eosin, 400X magnification).

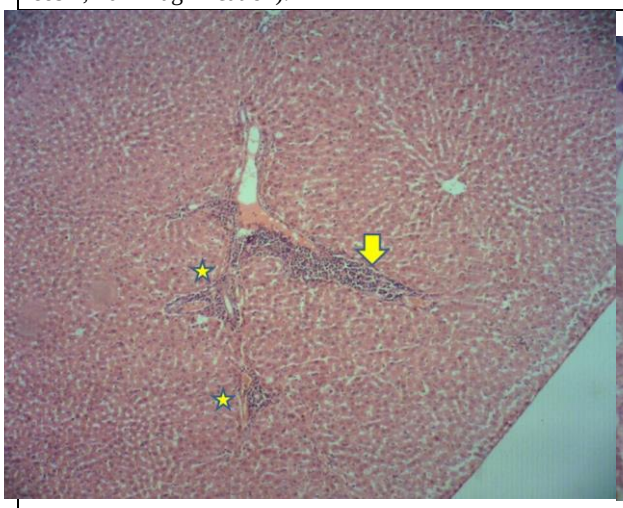

Figure 3. High Fat DietSevere infiltration of inflammatory cells in the tissue parenchyma (arrow), Severe infiltration of inflammatory cells around the portal space (asterisk) (Hematoxylin-eosin, 40X magnification).

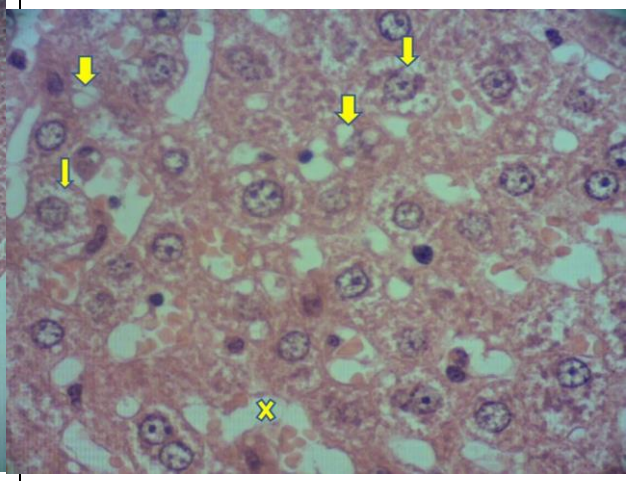

Figure 4. High Fat DietSever cellular ballooning (thick arrow), Hypertrophy and cellular ballooning (thin asterisk), Enlargement of sinusoids space (multiplication sign) (Hematoxylin-eosin, 400X magnification).

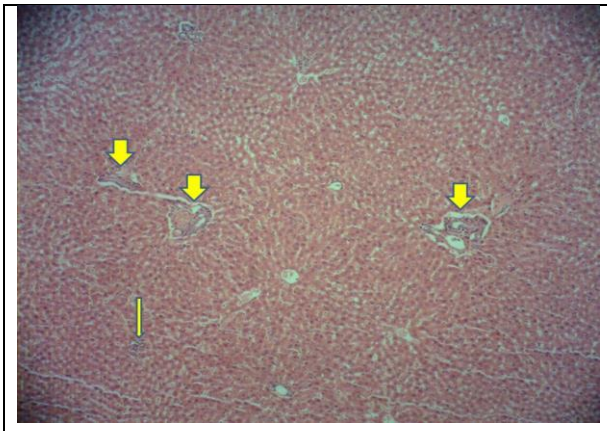

Figure 5. High Fat Diet + Se 0.25 mg/kg Hard infiltration of inflammatory cells around the portal space (thick arrow), Hard infiltration of inflammatory cells in the tissue parenchyma (thin asterisk) (Hematoxylin-eosin, 40X magnification).

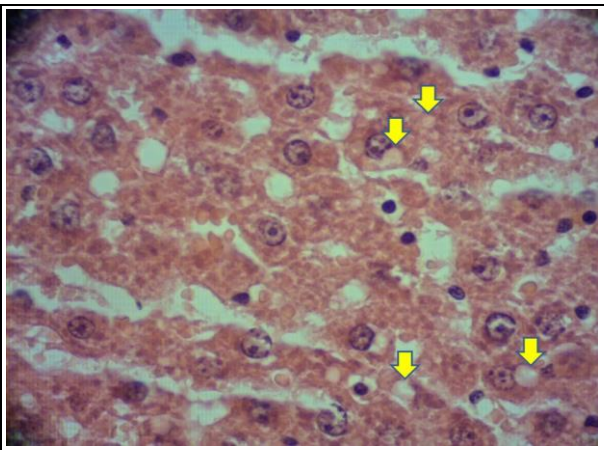

Figure 6. High Fat Diet + Se 0.25 mg/kg Hard cellular ballooning (arrow) (Hematoxylin-eosin, 400X magnification).

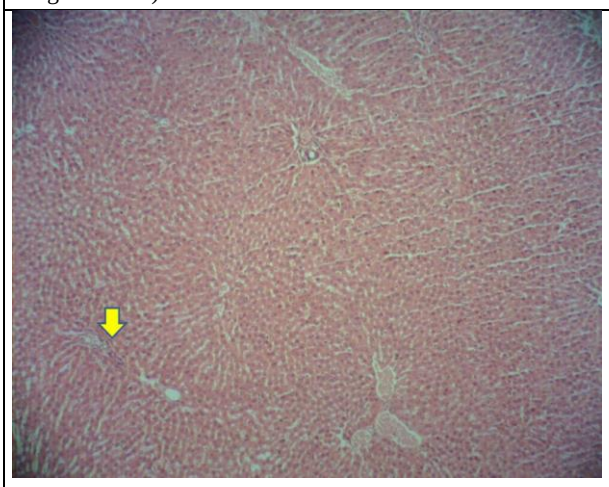

Figure 7. High Fat Diet + Se 0.5 mg/kg Moderate infiltration of inflammatory cells in the tissue parenchyma (thick arrow) (Hematoxylin-eosin, 40X magnification).

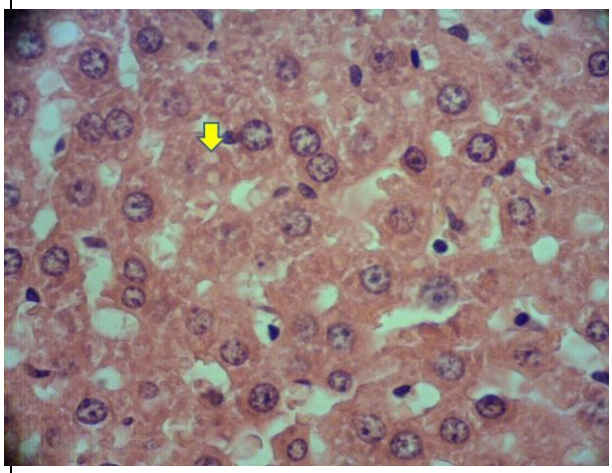

Figure 8. High Fat Diet + Se 0.5 mg/kg Moderate cellular ballooning (arrow) (Hematoxylin-eosin, 400X magnification).

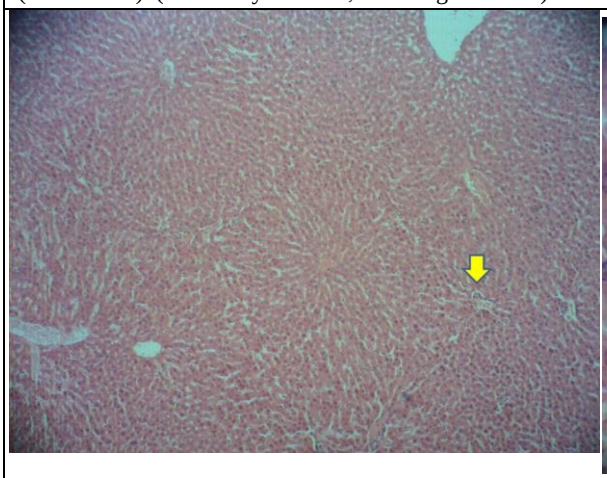

Figure 9. High Fat Diet + Se 1mg/kg Very mild infiltration of inflammatory cells in the tissue parenchyma (thick arrow) (Hematoxylin-eosin, 40X magnification).

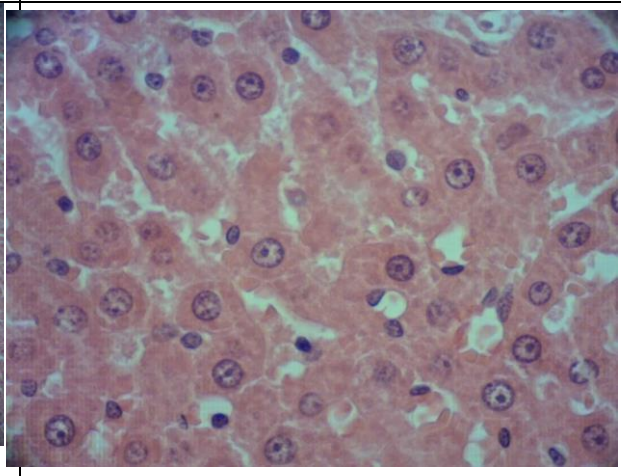

Figure 10. High Fat Diet + Se 1mg/kg With a natural structure (Hematoxylin-eosin, 400X magnification).

Discussion

Fatty liver is an important cause of chronic liver disease in people. Normally, fats consumed in food chain are metabolized in the liver. Fatty liver syndrome occurs when liver cells start to accumulate fat droplets (which are mainly triglycerides). Consecutive fat accumulation in liver cells cause nonalcoholic fatty liver disease (22-25). Fatty liver disease along with viral hepatitis increase liver damage and accelerate liver fibrosis, which result in liver failure (22, 26). Histological results showed liver failure and fibrosis in high-fat group compared to control. This represents tissue rupture and liver damage due to accumulation of fat in the liver.

Infiltration of fat in the liver is associated with increased liver echogenicity. Severity of liver echogenicity depends on severity of fat infiltration in the liver (27). More than 5% of liver is composed of fats in fatty liver disease (28). Measurement of triglyceride and cholesterol levels and liver enzymes are the best parameters to evaluate the severity of fatty liver (29, 30). Various studies have shown that increased serum LDL, cholesterol and triglycerides and reduced HDL are involved in pathogenesis of many diseases such as fatty liver disease (31). A significant increase was observed in LDL, triglyceride and cholesterol levels in the group receiving high-fat diet compared to control in this study. This shows negative effects of high-fat diet on changes in LDL, triglyceride and cholesterol levels and progression of fatty liver disease. Various studies have shown that serum cholesterol, triglyceride and LDL levels increase in patients with fatty liver, which is associated with prevalence of the disease (32). The results of another study showed that the risk of fatty liver disease increases in the people with high cholesterol, triglycerides and LDL levels (32). In addition, an elevation was observed in liver enzymes in case of liver tissue destruction and fat accumulation in the fat, which usually indicates fatty liver disease (33). Other studies have shown that mean activity of AST enzyme increases since this enzyme leaks into the blood serum (34). ALK and AST enzymes leak into blood serum from different tissues in case of muscular and liver damage. Alanine aminotransferase is known as specific marker of liver damage since there is high level of this enzyme in cytoplasm of liver cells, which leaks into the blood serum through cellular membrane in case of liver damage (35). The results of the present study showed a significant increase in liver enzymes in the groups with high-fat diet compared to control. This indicates liver tissue destruction in the group with high-fat diet. Histological studies confirm this finding. Changes in liver enzyme are associated with liver tissue damage caused by high-fat diet.

Various studies have shown that the most important hypothesis in etiology of fatty liver disease claims oxidative damage, which leads to inflammation and progression of the disease (2). In normal conditions, aerobic metabolism of the liver produces peroxidants (e.g. reactive oxygen species) at a constant rate, which is balanced with constant production of antioxidants. Peroxidant/antioxidant imbalance for peroxidant substitution (peroxidation) raises the hypothesis of oxidative stress in the liver (these conditions cause pathological changes in the liver). Reactive oxygen species with toxic effects lead to membrane lipid peroxidation (36). Therefore, fat accumulation leads to membrane lipid peroxidation, oxidative stress and consequently leakage of liver enzymes in the patients with fatty liver disease. Various studies have shown that reducing these risk factors (blood lipids and liver enzymes) can improve the patients. Since oxidative stress is an important mechanism of fatty liver; use of such antioxidants as selenium can reduce the risk of fatty liver disease. The results of this study showed decrease in concentrations of cholesterol, triglyceride, LDL and liver enzymes (ALT, AST and ALK) in the group receiving selenium. Greater increase was observed in these factors at higher doses of selenium. This shows positive effect of selenium in improving fatty liver disease in the groups with high-fat diet. The results of the study also indicated that reduced blood fat is an important mechanism for treatment of fatty liver disease. Selenium concentration in the blood is associated with HDL concentration. Accordingly, HDL concentration decreases by reducing selenium concentration (37). Thereby, serum LDL and triglyceride concentrations increase by decreasing HDL. Thereby, HDL, LDL and triglyceride are risk factors for liver damage. As expected, selenium improves blood lipids and consequently reduce liver damage and leakage of liver enzymes. The results of the present study confirms this issue.

On the other hand, various studies on the effect of selenium and vitamin E in changes and improvement in liver fatty disease in rats showed that use of selenium and vitamin E can improve AST, ALK and ALK levels in treatment groups compared to control. Histological studies showed that hydropic cytoplasm and cell necrosis in the treated group were less than the control. This reflects the positive and synergic effects of these two substances on the liver (38). These results are consistent with the results of this study.

Selenium is an essential trace element involved in many metabolic functions. Several studies have confirmed that selenium prevents many diseases. Selenium is a key component of several functional selenoproteins, which are positively involved in normal function of various organs (39). This element is embedded in the structure of antioxidant enzymes such as glutathione peroxidase, which removes free radicals and reactive oxygen species (40). Selenium as a cofactor of antioxidants reduces free radicals and protect DNA and other cellular components against oxidative damage (41). Various studies have shown that selenium nanoparticle as an antioxidant can reduce the risk of toxicity (42, 43). Several mechanisms of selenium include restoration of damaged DNA, modulation of oxidative stress, reduced inflammation, detoxification and improved immune function (44). Various studies have shown that selenium with antioxidant properties decrease ALT, AST and ALK levels and prevent histological changes in the liver (45). These results are consistent with the results of the present study.

In the present study, selenium with antioxidant properties reduced inflammation and affected reactive oxygen species as risk factors for liver tissue damage in liver fatty disease, which reduced lipid peroxidation in liver tissue that ultimately reduced liver enzyme concentrations in the groups treated with selenium compared to the group with high-fat diet.
Liver tissue in the group receiving selenium was less damaged compared to the group with high-fat diet. Thus, changes in liver enzymes and lipid in the present study were consistent with histological changes in the liver in the studied groups.

Conclusion

According to the above cases, increased fat (cholesterol, triglycerides and LDL) and decreased HDL lead to liver tissue damage and destruction, which cause oxidative stress and inflammation. As a result, an increase was observed in concentrations of liver enzymes (ALT, AST and ALK). Selenium as a potent antioxidant modulates oxidative stress, reduces inflammation and repairs damaged tissues, which consequently improves liver tissue and prevents leakage of enzymes in the liver. These factors ultimately improved fatty liver disease in the rats with high-fat diet. The results of this study can be generalized to humans. Thus, use of selenium as an antioxidant can improve fatty liver disease in these patients. Thereby, it is recommended to use selenium to improve fatty liver disease.

Acknowledgments

This article was extracted from projects approved by Jahrom University of Medical Sciences and Health Services and Medical Doctoral thesis (MD) by Mr. Mohsen Manochehri. Hereby I appreciate the cooperation of Research and Technology in Jahrom University of Medical Sciences for their help in conducting the research.

Conflict of interest:

The authors declare no conflict of interest with respect to the compilation and / or publication of this article.

References

1. McCullough AJ. The clinical features, diagnosis and nutural history of nonalcoholic fatty liver disease.clinics in liver disease.2004; 8:521-34
2. Marchesini G. Bugianesi E, Forlani G, Cerrelli F, Lenzi M. Manimi R. Nonalcoholoic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003;37(4):917-23.
3. Bedogni G, Migliolo L, Masutti F, et al. prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrution and liver study Hepatology. 2005;42(1):44-52
4. Jamali R, Khonsari M, Merat S,et al. Persistent alanine aminotransferase elevation among the general Iranian population: prevalence and causes. World journal of gastrorntrology: WjG. 2008; 14(18):2867
5. Jou J, Choi SS, Diehl AM.Mechanisms of disease progression in nonalcoholic fatty liver disease. 2008 Nov;28(4):370-9. doi: 10.1055/s-0028-1091981
6. Day CP, James OFW. Steatohepatitis: a tale of two "hits"?Gastroenterology. 1998;14(18):2867.
7. Edmison J, Mc Cullough AJ.Pathogenesis of nonalcoholic steatohepatitis: juman data. Clinics in liver disease.2007;11(1):75-104
8. Madan K, Bhardwaj P, et al.Oxidant stress and antioxidant status among patient with nonalcoholic fatty liver disease(NAFLD). Journal of clinical gastroenterology.2006
9. Jimba S, Nakagamit T, Takahashi M, et al. Prevalence of non- alcoholic fatty liver disease and association with impaired glucose metabolism in Japanese adults. UK Diabetic medicine. 2005; 22:1141-1145.
10. Diehl AM, Brunt EM , Ludwig J, et al. Clinico pathological analysis of non- alcoholic steatohepatitis, Chinese J Digest Dis. 2001; 2:184-187.
11. Matteoni CA, Younssi ZM, Gramlich T, et al. Non alcoholic fatty liver syndrome: A hepatic consequence of common metabolic disease, J Gastroenterol Hepatol. 2003; 18: 588-594.
12. Younossi Z. Review article: curent management of nonalcoholic fatty liver disease.alimentary pharmacology & therapeutics. 2008 Jul;28(1):2-12. doi: 10.1111/j.1365-2036
13. Liang LP1, Patel M. Seizure-induced changes in mitochondrial redox status. Free Radic Biol Med. 2006 Jan 15;40(2):316-22.
14. Harrison SA1, Brunt EM, Goodman ZD and et al. Diabetic hepatosclerosis: diabetic microangiopathy of the liver. Arch Pathol Lab Med. 2006 Jan;130(1):27-32.
15. Cerri RLA, Rutigliano HM, Lima FS, Araujo DB, Santos JEP. 2009. Effect of source of supplemental selenium on uterine health and embryo quality in high-producing dairy cow. Theriogenology, 71: 1127-1137.
16. G.V. Kryukov, S. Castellano, S.V. Novoselov, A.V. Lobanov, O. Zehtab, R. Guigo, et al. Characterization of mammalian selenoproteomes Science, 300 (2003), pp. 1439–1443
17. Kerstin E. Geillingera, , ,Daniel Rathmannb,et al. Hepatic metabolite profiles in mice with a suboptimal selenium status. 2014 Sep;25(9):914-22. doi: 10.1016/j.jnutbio.2014.04.003
18. Ghassemi F, Yosefi Nasab A, Kargar H. The protective effect of vitamin E and selenium on liver tissue in fructose- induced diabetic rat. Par J Med Sci 2014;12(2):47-55.

19. Zou Y1, Li J, Lu C, Wang ,et al. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sci. 2006 Aug 8;79(11):1100-7
20. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313-21. doi:10.1002/hep.20701
21. Bedossa P, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, Tordjman J, Clement K. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology. 2012;56(5):1751-9. doi:10.1002/hep.25889
22. Bronald AD, Principal of internal medicine. Translated to Persian by: Arjmand M, Gouran O. Tehran: Arjmand Pub; 2005. P: 474.
23. Robert A, Scat A, Robert S. fundamental principal of sport physiology. Translated to Persian by: Gaeini A, Dabidi V. Tehran: Samt Pub; 2005. P: 127.
24. Aargerius M. Sport and metabolism. Translated to Persian by: Gaeini A, Nazem F. Tehran: Samt Pub; 2005. P: 311.
25. Aiger E, Datz Ch. Iran perturbation in human Non-Alcholic fatty liver disease. Internal medicine department. Salzburg, Austria. 2008; 8(3): 213-20.
26. Sotude M. Relationship between surface of serum enzyme of ALT and AST with change degree of edema and fibrosis in nonalcoholic fatty liver disease. J Nash. 2005.
27. Sutton D. Textbook of radiology and imaging. 7th edition. London: Churchil Livingstone; 2003. 724, 756- 65.
28. Mc Gahan JP, Barry BG. Diagnostic ultrasound: A logical approach. 2nd edition. London: Lippincott-Raven Publisher; 1998.1046.
29. Kakkos SK, Yarmenitis SD, et al. Fatty liver in obesity: Relation to Doppler Perfusion Index measurement of liver. J Gastroentrol 2000; 35: 976 -80.
30. Liu TY, Lu SN, Su WP, et al. Prediction of fatty liver from serum TG levels and Body Weight Index. Gaoxing Yi Xue Ke Xue Za Zhi 1990; 6: 289-94.
31. Rahim Golmohammadi , Gholam Reza Dashti, Parviz Vahedi. Studying the effect of vitamin C on blood serum lipoproteins and liver tissue in high cholesterol-fed rabbits. FEYZ 2009; 13 (1): 20-24.
32. Savad Kohi F, Tabatabaei SMT, Shabinezhad S. Fatty liver ultrasound frequency in people with no history of liver disease and its association with cholesterol and triglyceride. ZUMS 2003; 5 (3): 9-15.
33. Mohsen Davoodi , Hamed Moosavi, Masoud Nikbakht. The effect of eight weeks selected aerobic exercise on liver parenchyma and liver enzymes (AST, ALT) of fat liver patients. The journal of shahrekord university of Medical Sciences 2012; 14(1); 84-90.
34. Hau DK, Gambari R, Wong RS, et al. Phyllanthus urinaria extract attenuates acetaminophen induced hepatotoxicity, Involvement of cytochrome P450 CYP2E1. Phytomed 2009; 16(8): 751-760.
35. Tohidi M , Harati H, Hadaegh F, et al. Association of liver enzymes with incident type 2 diabetes: A nested case control study in an Iranian population. BMC Endocr Disord 2008; 8: 5.
36. Videla LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quinones L, et al. Oxidative stress–related parameters in the liver of non-alcoholic fatty liver disease patients. Clin Sci (Lond) 2004; 106: 261-8.
37. Lee O, Moon Jg Y. The relationship between serum selenium levels and lipid profiles in adult women. J Nutr Sci Vitaminol (Tokyo). 2003;49 (6): 397- 404.
38. Orangi E, Ostad Rahimi A, Mahdavi R, Somi M, Tarzemani M. Oxidative Stress-related Parameters and Antioxidant Status in Non-alcoholic Fatty Liver Disease Patients. Iranian Journal of Endocrinology and Metabolism 2011; 12: 5 (5); 493-499.
39. Rayman MP. The importance of selenium to human health. Lancet. 2000;356(15):233–234.
40. Behne D, Hofer T, Von Berswardt- Wallrabe R, Elger W. Selenium in the testis of the rat: studies on its regulation and its importance for the organism. J Nutr. 1982;102(11):1682-1687.
41. Burk RF, Hill KE, Motley AK. Seleno protein metabolism and function: evidence for more than one function for seleno protein P. J Nutr. 2003;133(5):151-178.
42. Zhang JS, Wang H, Yan X, Zhang LD. Comparison of short-term toxicity between Nano-Se and Selenite in mice. Life Sci. 2004;75(2):447–459.
43. Zhang, J, Gao X, Zhang L, Bao Y. Biological effects of a nano red elemental selenium. Bio Factors. 2001;15(1):27–38.
44. Almondes K, Ieal G, Cozzolino S, Philippi S, Rondó P. The role of selenoproteins in cancer. Rev Assoc Med Bras. 2010 ;56 (4): 484- 488.PMID: 20835649.
45. Jamileh Abedi, Amir Tukmechi, Vahid Nejati, Rahim Hobbenaghi. Preventive Impacts of Selenium-Enriched Yeast on Liver and Enzyme Activity after Inducing Colorectal Cancer in Rat. Journal of Isfahan Medical School; 2014; 32 (291): 991-1003.