ORIGINAL ARTICLE

Examining the Effect of Selenium in Improving Non-alcoholic Fatty Liver Disease in Rats

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Abstract

Introduction: Given the prevalence of risk factors for metabolic syndrome, non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in society. In this study, the effect of selenium in improving NAFLD was investigated in rats. **Methods:** In this experimental study, 40 adult female Wistar rats were divided into five groups, each consisting of eight rats. Fourty male Wistar rats were randomly assigned into 5 groups of 8: control, Sham (high-fat diet =HFD) and HFD 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 alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), and total cholesterol (TC) levels were measured. 5 µ 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 ALP significantly increased in the group receiving HFD compared to control group. TC, LDL, ALT, and ALP serum concentration significantly decreased in the groups receiving 0.5 and 1 mg/kg selenium compared to the group receiving HFD. TG and AST serum concentrations significantly decreased in the group receiving 1 mg/kg selenium compared to the group receiving HFD. 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.

Key words: Fatty liver, rat, selenium

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) was detected by Ludwig *et al.*The disease was first recognized 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 the 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.

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Oxidation by-products disturb nucleotide and protein synthesis. Moreover, these compounds increase secretion of inflammatory cytokines and activate hepatic stellate cells. These factors ultimately cause fibrosis, inflammation, and apoptosis.[7,8] 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 and 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.[9] Selenium is an essential element and a cofactor of glutathione peroxidase and thioredoxin reductase, which is the most important defense mechanism against oxidative stress.[10,11] Selenium binds to proteins and forms selenoproteins with antioxidant properties.[12]

Yanjun *et al.* reported the most common test for liver disorder is increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in China in 2001.[13,14] Angelico *et al.* examined 282 patients with sonographic evidence of NAFLD in Italy in 2003. Hypertriglycemia and reduced high-density lipoprotein (HDL) were reported as the main disorders in lipid profile of the patients with fatty liver disease.[15] 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 the use of antioxidants for treatment of this disease.[16]

Gelilinger *et al*. showed that reducing selenium lower than normal level significantly reduces the activity of liver selenoenzyme with further increase in lipid peroxidation and glycogen and decrease in T3 hormone.[17]

Ghasemi *et al.* examined the 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 AST, ALP, 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 NAFLD, the present study sought to examine the effect of selenium in improving NAFLD in rats.

METHODS

According to the articles published in this field, healthy adult female Wistar rats from 180 to 200 g 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 h of light and 12 h of darkness. Humidity varied from 50% to 55%. The rats were weighed 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 (HFD) for 4 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.[11] 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 through gavage in a daily manner. Blood samples were taken from the head of the rats after the 4th 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.

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), 5 cc blood samples were directly taken from rats using cardiac puncture under anesthesia by ether. Their serum was collected by centrifugation (3000 rpm for 15 min). The serums were kept at −20°C to measure ALT, AST, alkaline phosphatase (ALP), low-density lipoprotein (LDL), HDL, total cholesterol (TC), and triglyceride (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 tissues were processed for hematoxylin and eosin staining to semi-quantitatively assessment of the fatty degenerations using the

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

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

NAFLD activity score (NAS). The histological features were graded according to the 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%; and S3: >66%), lobular inflammation (score 0 to 3, I0: No foci; I1: <2 foci per 200× field; I2: 2-4 foci per 200× field; and I3: >4 foci per 200 × field), and ballooning (score 0 to 2, B0: None; B1: Few balloon cells; and B2: Many cells/prominent ballooning) were also summed to calculate the NAS score (ranging from 0 to 8).[20]

FINDINGS

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

Contents of Table 2 show a significant increase in concentration of triglycerides in the group with HFD and the groups treated with selenium compared to control group at 5% significance level. This shows the negative impact of HFD 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 HFD. This shows that selenium has positive effects in changes in triglyceride concentrations. These results are shown in Chart 1.

Contents of Table 2 with regard to cholesterol showed a significant increase in cholesterol in the group with HFD compared to control at 5% significance level. This shows the negative impact of HFD 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 HFD [Chart 2].

The results of LDL measurement showed a significant increase in LDL concentration in the group with HFD 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 HFD. This shows that selenium has positive effects in improving LDL serum concentration [Chart 3].

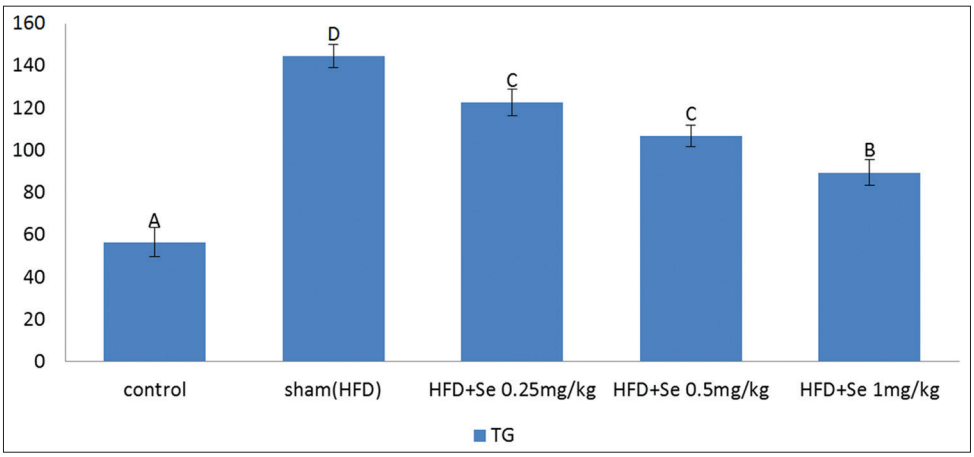

Chart 1: Mean serum triglyceride levels in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test

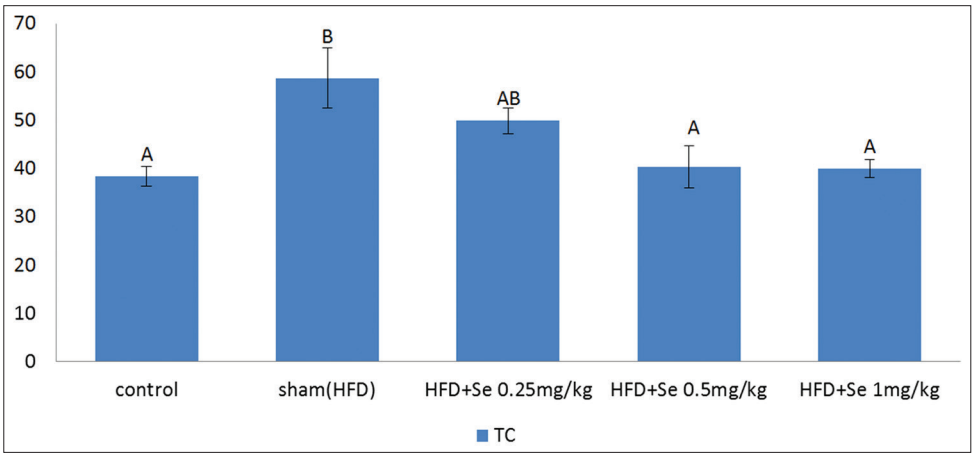

Chart 2: Mean serum total cholesterol concentration in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test

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 HFD [Chart 4].

The results of liver enzyme measurement showed a significant increase in AST concentration in the experimental group receiving HFD 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 HFD. This shows positive effect of selenium in improving AST factor ($P < 0.05$) [Chart 5].

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

Histological results in different groups

No abnormal morphology was found in the 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 micro-vesicular 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 was observed in the rats with HFD. Fatty change in hepatocytes, hyperemia, and lymphocytic infiltration in the liver parenchyma was significantly inhibited in the rats with HFD + selenium powder [Figures 3-10]. The greatest improvement was observed in the group receiving 1 mg/kg selenium [Figures 9 and 10, Table 3].

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 0.25 mg/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 0.5 mg/kg	106.83±12.36^{c}	40.33±10.76^{a}	88.16±10.04^{c}	29.16±4.44^{a}	98.66±12.25b^{c}	35.5±7.09^{a}	253.5±74.32^{a}
HFD+Se 1 mg/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}

TG: Triglyceride, TC: Total cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, HFD: High-fat diet, g/dL: Milligram per deciliter, IU/L: Internation unit per liter. *P* value below 0.05 was considered as statistically significant. There was no significant statistical difference between the levels

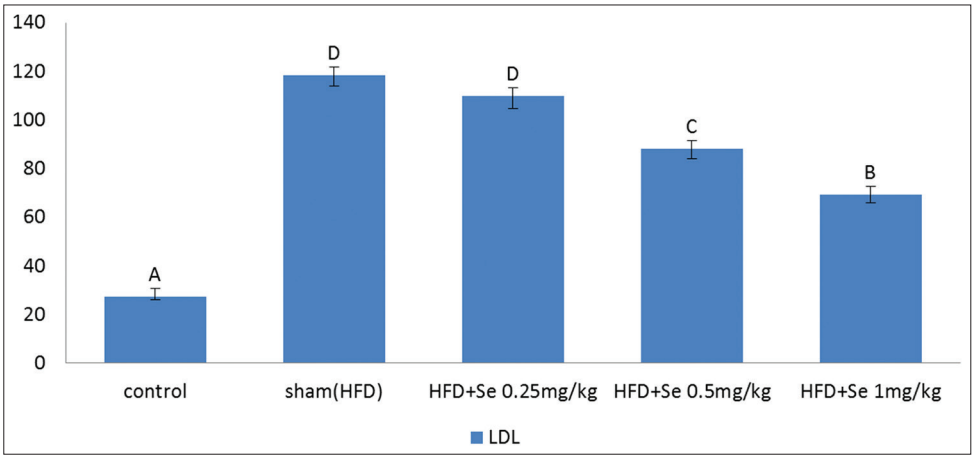

Chart 3: Mean low-density lipoprotein serum concentration in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test.

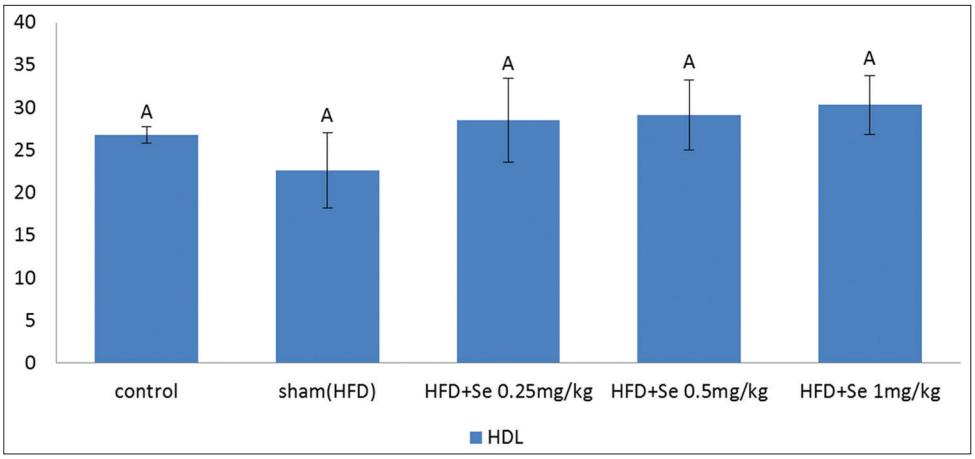

Chart 4: Mean serum high-density lipoprotein concentration in the studied groups

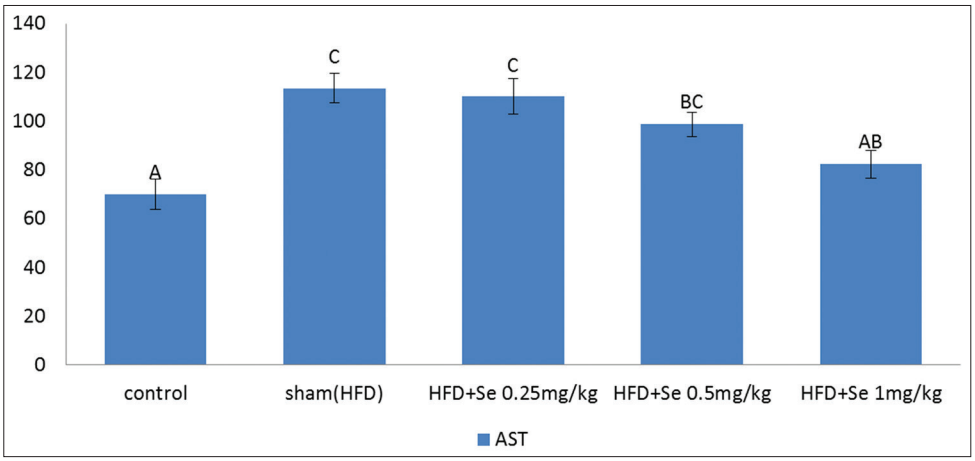

Chart 5: Aspartate aminotransferase mean serum concentration in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test

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 causes NAFLD.[21-24] Fatty liver disease along with viral hepatitis increases liver damage and accelerates liver fibrosis, which result in liver failure.[21,25] 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 the liver echogenicity depends on severity of fat infiltration in the liver.[26] More than 5% of liver is composed of fats in fatty liver disease.[27] Measurement of triglyceride and cholesterol levels and liver enzymes are the best parameters to evaluate the severity of fatty liver.[28,29] 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.[30] A significant increase was observed in LDL, triglyceride, and cholesterol levels in the group receiving HFD compared to control in this study. This shows negative effects of HFD 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.[31] The results of another study showed that the risk of fatty liver disease increases in the people with high cholesterol, triglycerides, and LDL levels.[31] 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.[32] Other studies have shown that mean activity of AST enzyme increases since this enzyme leaks into the blood serum.[33] ALP 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.[34] The results of the present study showed a significant increase in liver enzymes in the groups with HFD compared to control. This indicates liver tissue destruction in the group with HFD. Histological studies

confirm this finding. Changes in liver enzyme are associated with liver tissue damage caused by HFD.

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 leads to membrane lipid peroxidation.[35] 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, the 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 ALP) 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 HFD. 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.[36] 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 reduces liver damage and leakage of liver enzymes. The results of the present study confirm 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 the use of selenium and Vitamin E can improve AST, ALT, and ALP levels in treatment groups compared to control. Histological studies showed that hydropic cytoplasm and cell necrosis in the treated group

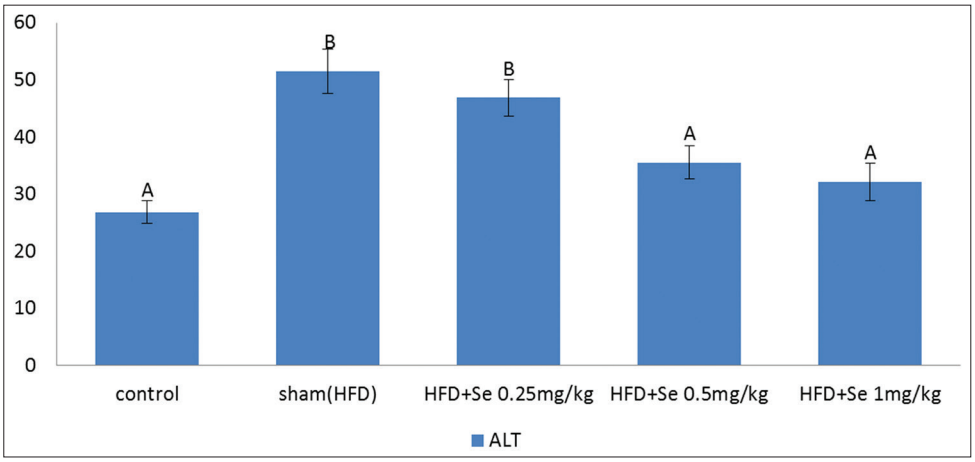

Chart 6: Mean serum alanine aminotransferase concentration in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test.

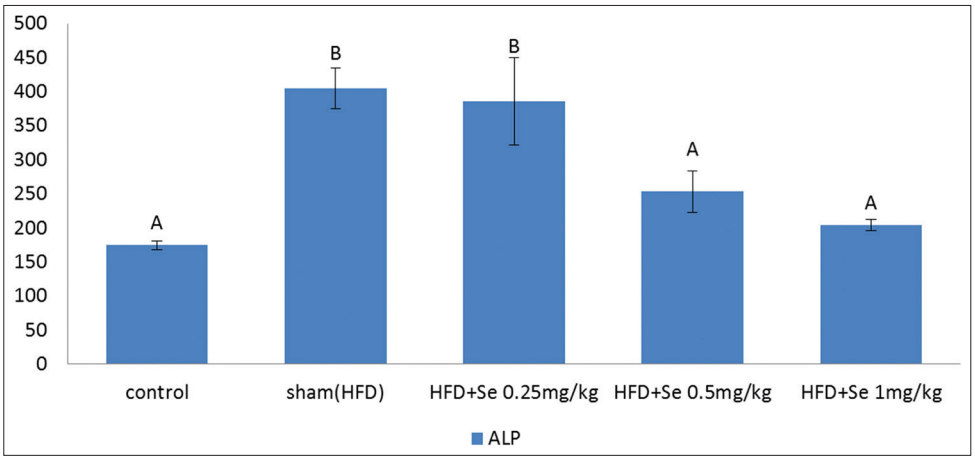

Chart 7: Mean serum alkaline phosphatase concentration in the studied groups. The means in each column with at least one common letter are not significantly different based on Duncan's test.

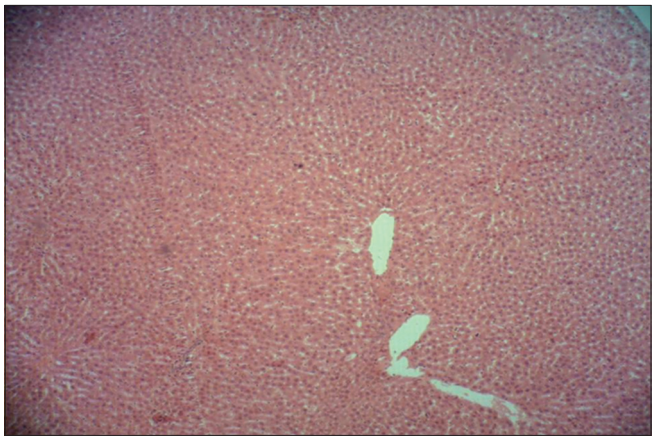

Figure 1: Control. With a natural structure (H and E, 40×)

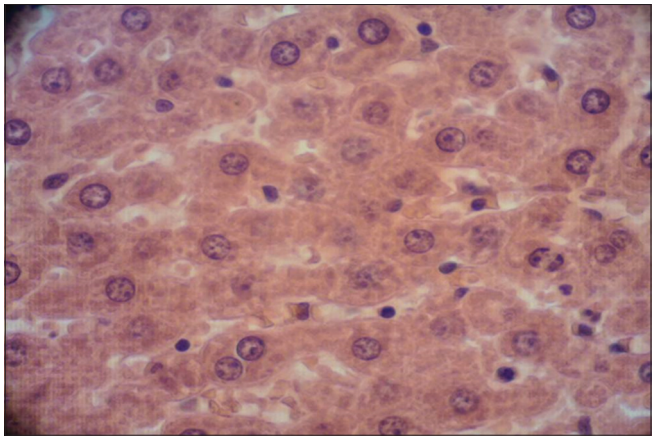

Figure 2: Control. With a natural structure (H and E, 400×)

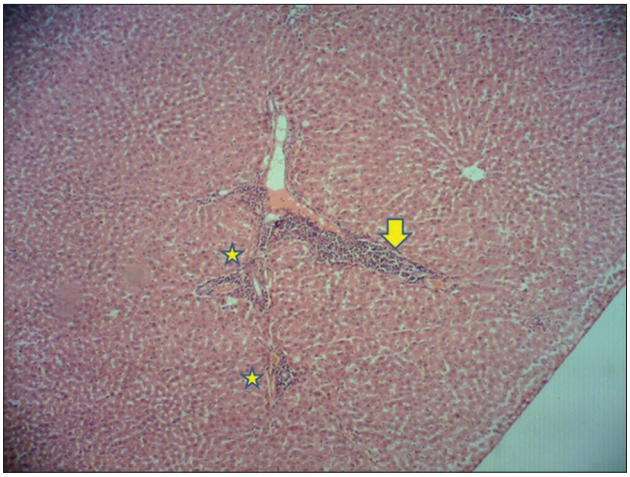

Figure 3: High fat diet. Severe infiltration of inflammatory cells in the tissue parenchyma (arrow), severe infiltration of inflammatory cells around the portal space (asterisk) (H and E, 40×)

were less than the control. This reflects the positive and synergic effects of these two substances on the liver.[37] 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

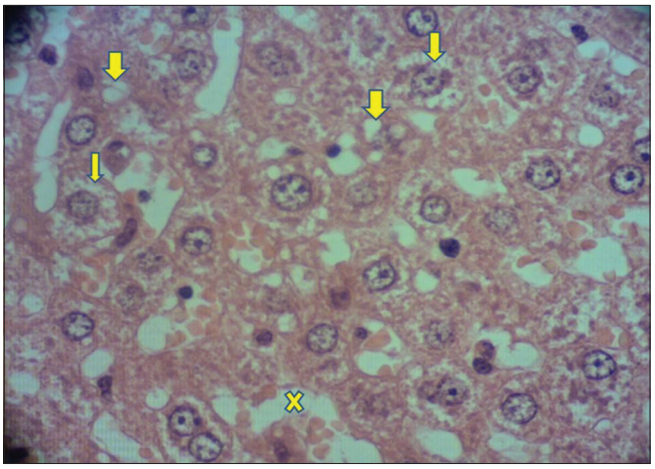

Figure 4: High fat diet. Sever cellular ballooning (thick arrow), hypertrophy and cellular ballooning (thin asterisk), enlargement of sinusoids space (multiplication sign) (H and E, 400×)

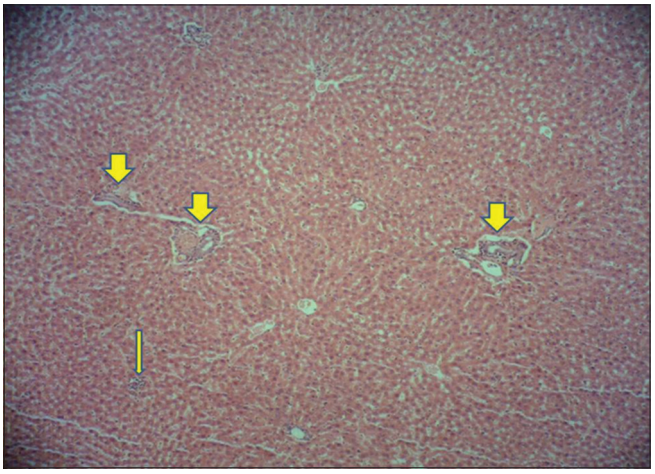

Figure 5: High fat diet + Se 0.25 mg/kg. Hard infiltration of inflammatory cells around the portal space (thick arrow), Hard infiltration of inflammatory cells in the tissue parenchyma (thin asterisk) (H and E, 40×)

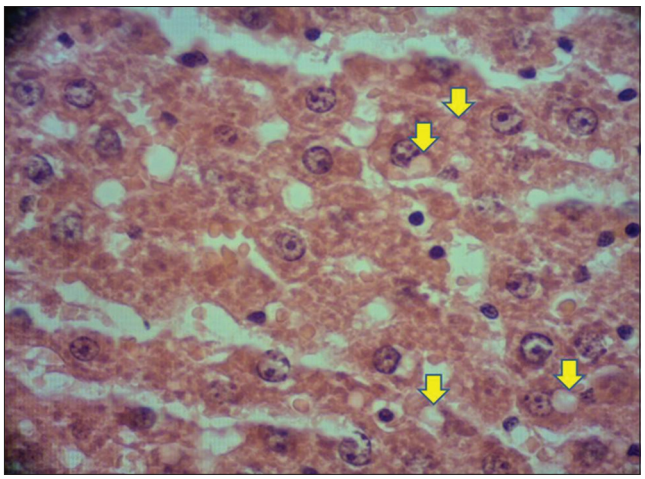

Figure 6: High fat diet + Se 0.25 mg/kg. Hard cellular ballooning (arrow) (H and E, 400×)

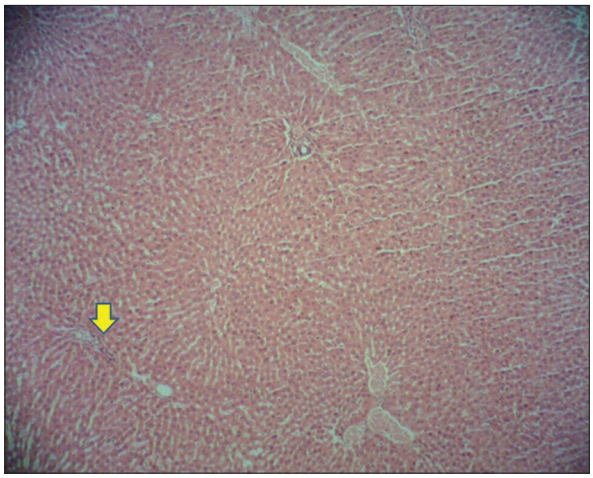

Figure 7: High fat diet + Se 0.5 mg/kg. Moderate infiltration of inflammatory cells in the tissue parenchyma (thick arrow) (H and E, 40×)

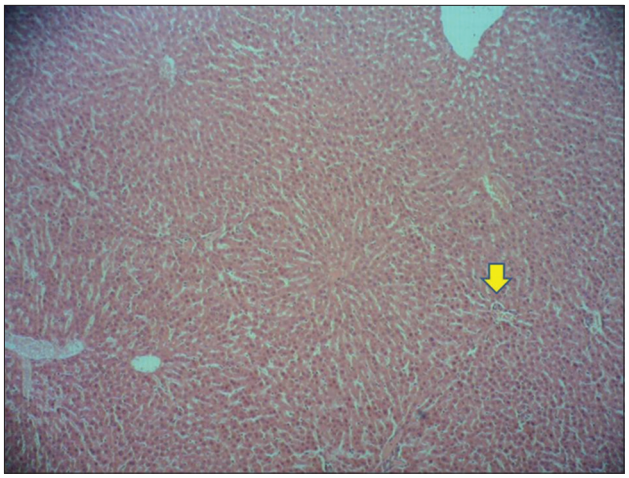

Figure 9: High fat diet + Se 1 mg/kg. Very mild infiltration of inflammatory cells in the tissue parenchyma (thick arrow) (H and E, 40×)

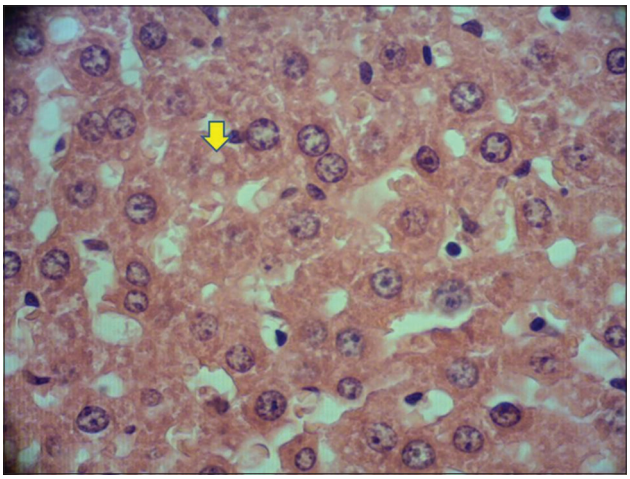

Figure 8: High fat diet + Se 0.5 mg/kg. Moderate cellular ballooning (arrow) (H and E, 400×)

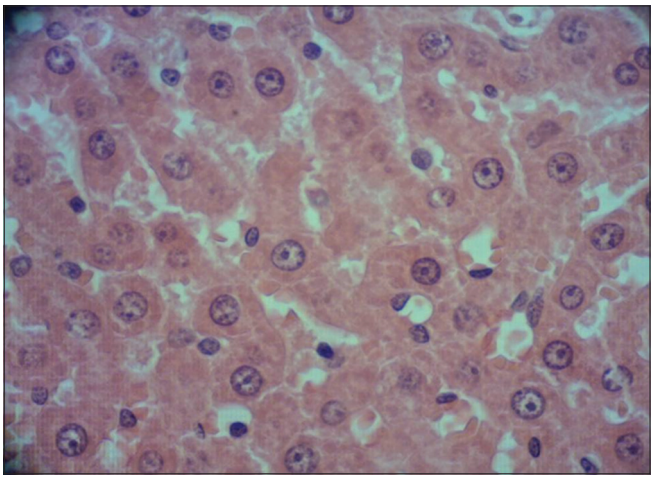

Figure 10: High fat diet + Se 1 mg/kg. With a natural structure (H and E, ×400)

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 0.25 mg/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 1 mg/kg	1.16±0.16^{b}	1.16±0.17^{b}	1.00±0.26^{b}	2.33±0.21^{b}

HFD: High-fat diet, NAFLD: Non-alcoholic fatty liver disease

selenium prevents many diseases. Selenium is a key component of several functional selenoproteins, which are positively involved in normal function of various organs.[38] This element is embedded in the structure of antioxidant enzymes such as glutathione peroxidase, which removes free radicals and reactive oxygen species.[39] Selenium as a cofactor of antioxidants reduces free radicals and protects DNA and other cellular components against oxidative damage.[40] Various studies have shown that selenium nanoparticle as an antioxidant can reduce the risk of toxicity.[41,42] Several mechanisms of selenium include restoration of damaged DNA, modulation of oxidative stress, reduced inflammation, detoxification, and improved immune function.[43] Various studies have shown that selenium with antioxidant properties decreases ALT, AST, and

ALP levels and prevents histological changes in the liver.[44] 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 HFD.

Liver tissue in the group receiving selenium was less damaged compared to the group with HFD. 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 ALP). 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 HFD. The results of this study can be generalized to humans. Thus, the 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.

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