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The Effects of Borage Oil on Hyperlipidemic Diet-induced Liver Damage in Male Golden Syrian Hamsters

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Abstract: This study was designed to examine the hepatoprotective effects of Borage seed oil rich in Gamma Linolenic acid (GLA) against hyperlipidemic diet-induced liver damage in the male golden Syrian hamster. In this experiment a total of 60 animals of male hamsters were used. This experiment was carried out in 2 periods. The first period lasted 4 weeks, in which hamsters were segregated into 4 groups. S1 served as control (given a commercial rodent diet only). Other groups, S2, S3 and S4 were fed with a diet containing of %80 commercial rodent diet + 20% fats (Hyperlipidemic Diet). The second period lasted 4 weeks, in which S2 only that continued fed with hyperlipidemic diet. S4 was orally gavaged with borage oil at 2 g/kg of the body weight daily. Hyperlipidemic diet intake caused lipid accumulation in hepatocytes and significantly ($P<0.05$) increased the activities of ALT, AST, ALP, LDH, GGT and CK enzymes in each of the S2, S3 and S4 groups at the end of the first period. These negative effects were removed only in S4 by the use of borage oil.

Keywords: Borage oil, Enzymes activities, Hamster, Hepatocytes.

Altın Suriye Hamsterlarında Hiperlipidemik Diyet ile Oluşturulan Karaciğer Hasarı Üzerine Hodan Yağının Etkileri

Öz: Bu çalışma, erkek altın Suriye hamsterinda hiperlipidemik diyet ile oluşturulan karaciğer hasarına karşı Gamma Linolenik Asit (GLA) açısından zengin olan hodan yağının hepatoprotektif etkilerini incelenmek için tasarlanmıştır. Bu deneyde, erkek hamsterden oluşan toplam 60 hayvan kullanılmıştır. Bu deney iki aşamada gerçekleştirilmiştir. İlk aşama 4 hafta sürdü, bu aşamada, hamsterler 4 gruba ayrılmıştır. S1 (kontrol), sadece ticari kemirgen diyeti ile beslenmiştir. Diğer gruplar S2, S3 ve S4 ise 80% ticari kemirgen diyeti +%20 yağ içeren diyet ile beslenmiştir (hiperlipidemik diyet). İkinci aşama da yine 4 hafta sürdü, sadece S2 hiperlipidemik diyetle beslenmiştir. S4'e hodan yağı günlük olarak 2g yağ/kg vücut ağırlığı gavaj yoluyla verilmiştir. Hiperlipidemik diyetin alımı, hepatositlerde lipid birikimine neden oldu ve anlamlı olarak ($P<0.05$) ilk aşama sonunda S2, S3 ve S4 gruplarının her birinde ALT, AST, ALP, LDH, GGT ve CK gibi enzimlerin aktivitesini arttırdı. Bu negatif etkiler sadece S4'te hodan yağı kullanımıyla ortadan kaldırılmıştır.

Anahtar Kelimeler: Enzim aktiviteleri, Hamster, Hepatositler, Hodan yağı.

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INTRODUCTION

The liver is the main organ of metabolic processes and it is a target for toxic effects. The liver is responsible for carbohydrate and lipid metabolism, apolipoprotein synthesis, and cholesterol remove through the biliary route. For the above reasons, the liver's health should be preserved as much as possible (1). Fatty Liver is described by excess increase of lipids into the liver cells and could be due to abnormal metabolic events such as hyperlipidemia, obesity (2,3). Diets rich in cholesterol and saturated fatty acids (SFA) are two major cause of hyperlipidemia, which mostly induce oxidative stress, which is now believed to be an essential component in the progress of Fatty Liver Disease (4). In fact, the searches for new drugs able to reduce Fatty Liver Disease have gained a great deal of importance in recent years. Lots of research interests have been focused on dietary supplements instead of conventional medicine for many reasons like: absence of side effects and they contain several antioxidants and rare biological compounds. Many traditional oils such as flaxseed and sesame oils were used to reduce blood lipids and improve fatty liver. Positive effects were attributed to the content of these oils from omega-3 fatty acid. Studies about the effect of oils rich in omega-6 fatty acid against fatty liver were poor. As a result of its therapeutic and nutritive capacity, Borage oil has obtained great interest among various research companies worldwide. Because there is high Gamma Linolenic Acid (GLA) in its product (5). The oil maintains antioxidant and free radical scavenging properties (6). In addition to GLA, Linoleic Acid (LA) is present in borage (7). GLA is regarded as omega-6 fatty acid. It is essential fatty acid because it can't be created in the body. It is a very rare fatty acid and founds in very few plants. The evening primrose and borage are major sources of GLA. It is necessary to clarify that most researches about borage oil were focused on the its role in preventing atherosclerosis (8), therapeutic efficacy against rheumatoid arthritis (9), obesity, asthma, cancer and acute respiratory distress syndrome (10,11). Unluckily, the hepatoprotective characteristics of borage oil rich in GLA are poorly described. Although GLA reduced the increase lipids in hepatocytes, it did not affect the liver histology in rats treated with ethanol (12). Borage oil improved the experimental ethanol-induced liver steatosis in rats (13). The purpose of this study was to examine the effect of borage oil rich in GLA against hyperlipidemic diet-induced liver damage in the male golden Syrian hamster.

MATERIALS and METHODS

Animals

60 of male golden Syrian hamsters, weighing between 45 and 50 g and 5-7 weeks age were obtained from experimental animals unit (Directorate of Animal Health, Syria, Damascus). One hamster was kept in a separate cage in an animal room at 22 ± 2 C with a 12/12 h light-dark cycle. The hamsters were fed with commercial rodent diet and water adlibitum for 2 weeks before the treatment to become acclimated. Then they were weighed again, and all the following parameters were measured in the serum; triglyceride, cholesterol, high density lipoprotein(HDL), low density lipoprotein(LDL), glucose, aspartateaminotransferase (AST), alanineaminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK).

Animals Treatment and Experimental Protocol

According to the results, the experiment was implemented on 60 healthy animals and passed through two periods. The first period: It lasted for 4 weeks in which the 60 hamsters were divided into 4 groups 15 ones in each group. Group1(S1) expressed negative control (fed with commercial rodent diet only). Liver damage was induced to the remaining three hamster groups by eating a diet consisting of 80% commercial rodent diet + 13.5% animal fat +

6.5% coconut oil (Hyperlipidemic Diet) for 4 weeks. At the end of the first period blood samples were taken from food-deprived hamsters (14 h). The samples were centrifuged, collected, and enzymes activities were measured. The considered hamsters with damaged liver were divided into three groups, Group2 (S2), Group3 (S3) and Group4 (S4). The second period: lasted 4 weeks in which S2 continued to be fed with hyperlipidemic diet and named as a fatty liver group. The hyperlipidemic diet was excluded from S3 and S4 only. S4 was orally gavaged with borage oil 2 g/kg of the bodyweight daily and named as borage oil group, whilst S3 named as a positive control group. To make the experience conditions equal in all groups, S1, S2 and S3 were orally gavaged with distilled water 2 g/kg of the body weight daily. S1, S3 and S4 were fed with rodent diet. To reduce the suffering of animals during the experiment, all necessary measures have been taken. All processes on animals were done strictly according to criteria accepted by the management of scientific research laboratories in the College of Veterinary Medicine, Albaath University and after obtaining a decision (date 24.04.2012 and number 1011-12) from the Animal Ethics Committee and the Council of Albaath University.

Oils and Fats

Borage oil was obtained from the cold compressing of borage seeds. Borage seeds used in this study were collected from the region of Hama (west of Syria). Coconut oil: production of an Indian company for Food Industries. Animal fat was collected from the carcasses of sheep**.**

Compositional Analysis of Fatty Acids

The composition of the fatty acids of borage seed oil was prepared according Garce and Mancha (14). Fatty acids were analysed by Gas Chromatography 3800 coupled with Mass Spectrometry 2000 (GC-MS) using an electron impact ionization (70 ev). Identification of the compounds was made by matching their recorded mass spectra with those stored in the library of the GC-MS (15).

Biochemical Examination

Two blood samples were taken (once at the end of each period) from food-deprived hamsters (14 h) and collected via the retro-orbital sinus into capillary tubes under anesthesia (16). Taken blood samples which were allowed to clot at normal temperature of the room and centrifuged at 3000 rpm for 30 min. The serums which were not hemolysed were removed at once and kept at -20°C. The activities of the serum enzymes such as, AST, ALT, gamma glutamiltransferaz (GGT), alkalenfosfataz (ALP), LDH and CK were measured with test kits from BioSystems by using Spectrophotometer/ BioSystems-Model BTS-310 EU/Spain.

Tissue Processing

The histological examination was evaluated by conventional methods. Livers were immediately excised, immersed in 10% neutral formalin solution for stabilization. After that, 28 samples (liver) were taken randomly at the rate of seven samples from each group. Biopsies were taken from different regions of each sample and embedded in paraffin. For measuring the histological changes in the liver, sections were stained with Hematoxylin and Eosin (H&E).

Statistical Analysis

Data were expressed as mean$\pm$Standard Deviation (SD). Data were tested using a one way analysis of variance (ANOVA) by a computerized statistics program (USA). $P<0.05$ was considered to be the least limit of significance.

RESULTS and DISCUSSION

Animal Model Used

Hamsters are frequently used for assessing the effect of hyperlipidemic diet on the lipids metabolism (17,18) Golden Syrian hamsters were used in the

present study, hamster proved to be a useful model in the evaluation of changes after treatment with a high-fat diet (HFD), as well as its small size and ease of handling.

Borage Seed Oil Analysis

In this study, LA (18:2 cis-9,12) and GLA (18:3 cis-6,9,12) were the main fatty acids of the oil, representing 69.89%, 13.21% of the oil respectively (Table 1). Borage seed oil contained a very high proportion of unsaturated fatty acids (UFA) to SFA (87.28% to 12,67%), these results in agreement with the data reported previously (19). The percentage of GLA in this study was 13.21%, while the percentage of GLA was ranged from 17 to 25% (20) and 21 to 23% (21) based on total fatty acids (TFA). The proportion of LA in this study increased in comparison with previous studies (22,23). It may be due to the soil of the region and the time of seed harvesting. This is a positive factor in the Syrian borage oil, especially LA will be converted into GLA in the body. Fatty acid change as a result of growth. LA is in the highest rate while the amounts of GLA and oleic acid start to increase during seed formation stage (7).

Table 1. Fatty acids composition of borage seed oil.
Tablo 1. Hodan tohum yağının yağ asitleri bileşimi.

Fatty acids	% of total fatty acids
Palmitic (C16:0)	11.65
Stearic (C18:0)	0.61
Oleic (C18:1)	2.71
Linoleic (C18:2)	69.89
G-Linolenic (C18:3)	13.21
Arachidic (C20:0)	0.41
Eicosanoic (C20:1)	1.05
Erucic acid (C22:1)	0.42
SFA	12.67
UFA	87.28

SFA, saturated fatty acids; UFA, unsaturated fatty acids.

Body Liver and Visceral Fat Weights

At the end of the first period, bodyweights of S2, S3 and S4 were higher ($P<0.05$) than those of S1 (Table 2). After scarifying hamsters, visceral fat and liver were collected and weighed (Fig.1 and Fig.2). The treatment of hyperlipidemic diet after 8 weeks showed a significant increase ($P<0.05$) of visceral fat weights for S2 in comparison with S1 and S3. No changes ($P>0.05$) were observed in liver weights amoung S1, S2 and S3. HFD increased body weight, adipose tissue in comparison with control mice (24). There was a hug amount of visceral fat and livers in the coconut oil and butter groups compared to the control and flaxseed oil groups (25). In this study the significant increase of the visceral fat after treatment with hyperlipidemic diet, can be attributed to the increase of the triglycerides synthesis in the peripheral tissues, with the aim of lowering the high level of blood lipids, especially that triglycerides are synthesized mainly in the liver and excessed fat is sent from the liver to the periphery. Nowadays fatness is considered as a dangerous problem in Western Countries due to the increasing fat intaking. Increasing fat intake is considered as a major reason to induce fatness, hyperlipidemia and fatty liver. The intragastric administration of borage oil for 4 weeks showed a significant reduction ($P<0.05$) in body and visceral fat weights for S4 in comparison with S2 and S3 (Table 2) and (Fig.1). Intake of GLA can prevent weight regain after weight loss (26). In the present study, the size of visceral fat was decreased upon treatment with borage oil (Fig.3). GLA is metabolized into dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eventually into various eicosanoids (27). Lower levels of DGLA had increased the body fat (28,29). Most likely, the decrease of body, liver and visceral fat weights in the S4 may be due to the role of GLA in increasing the DGLA, βoxidation and inhibition of fatty acids synthesis.

Table 2. Effect of hyperlipidemic diet and borage oil on body weight which is estimated in gram.
Tablo 2. Hiperlipidemik diyet ve hodan yağının gram olarak tahmin edilen vücut ağırlığına etkisi.

Time / Group	Body weight (4 weeks)	Body weight (8 weeks)
S I	a 48.76±3.32 B	a 52.92±4.15 C
S II	a 69.53±4.87 A	a 74.23±4.58 A
S III	a 69.38±3.37 A	a 72.46±3.01 A
S IV	a 67.23±4.49 A	a 61.38±3.92 B

Values are expressed as mean±SD; n=15. Different small letters indicate significant differences in the same row (P<0.05), Different large letters indicate significant differences in the same column (P<0.05). S1 (negative control group), S2 (fatty liver group), S3 (positive control group) and S4 (borage oil group).

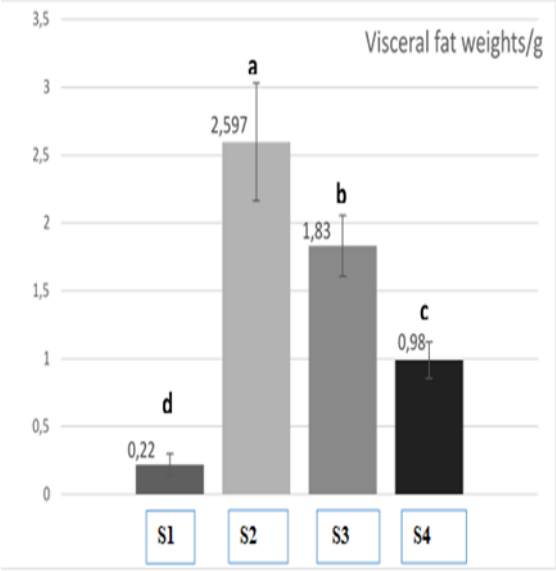

Figure 1. Visceral fat weights of the experimental hamsters in the end of second period.
Şekil 1. İkinci aşama sonunda deneysel hamsterlerin iç yağ ağırlıkları.
The data are given as mean±SD; n=15. Mean values with different letters indicate a significant difference (P<0.05). S1 (negative control group), S2 (fatty liver group), S3 (positive control group) and S4 (borage oil group).

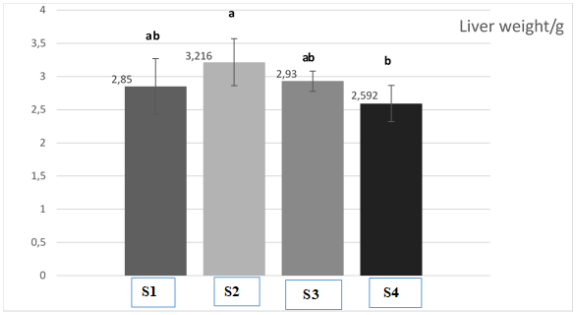

Figure 2. Liver weights of the experimental hamsters in the end of second period.
Şekil 2. İkinci aşama sonunda deneysel hamsterlerin karaciğer ağırlıkları.
The data are given as mean±SD; n=15. Mean values with different letters indicate a significant difference (P<0.05). S1 (negative control group), S2 (fatty liver group), S3 (positive control group) and S4 (borage oil group).

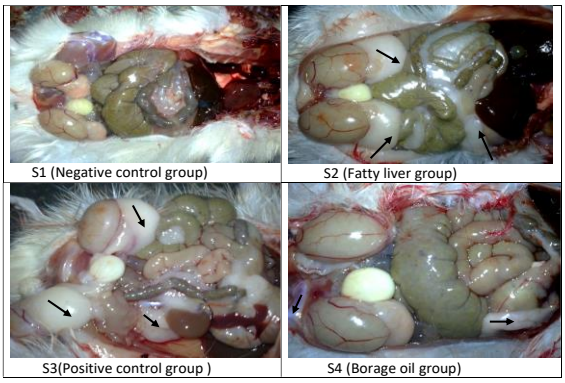

Figure 3. Size of visceral fat of the experimental hamsters in the end of second period.
Şekil 3. İkinci aşama sonunda deneysel hamsterın iç organ yağının büyüklüğü.

Serum Enzymes Activities and Liver Histopathology

Diet composition and fatty liver development are closely related. Accumulation of fat in liver maybe caused by diet. The liver is mostly affected by high blood lipids, followed by an increase in lipid peroxidation (LPO) and free radicals and cell damage (30). Five enzymes were examined to determine the effect of hyperlipidemic diet on the liver. ALT is found in higher activity in cytoplasm. It is primarily localized in hepatocytes (31). AST is a mitochondrial enzyme. The highest activity of AST was observed in the heart muscle. This enzyme is used together with the ALT in assessing liver function (32). ALP is localized in bones.

The main part of the serum ALP activity is influenced by the damage to the liver and bones (33). GGT is existed in hepatocytes and biliary cells. Elevated GGT levels can be noticed in a diversity of nonhepatic diseases, including renal failure and acute myocardial infarction (34). Although LDH is not specific to any organ, but high activity of this enzyme has been observed in cases of myocardial infarction and liver damage in humans (35). Hyperlipidemic diet intake in this study caused a significant increase ($P<0.05$) in the ALT, AST, ALP, GGT and LDH activities approximately (163%, 163%, 23%, 60%, 206%) in each of S2, S3 and S4 groups in comparison with S1 after 4 weeks of feeding (first period) (Tables 3-1, 3-2, 3-3, 3-4 and 3-5). These significant differences increased to reach approximately (214%, 242%, 35%, 103%, 237%) respectively for S2 only in the second period. There was no significant difference ($P>0.05$) in the activities of the enzymes for S3 between the first and second periods (Tables 3-1, 3-2, 3-3, 3-4 and 3-5). Elevated ALT, AST and ALP activities in this study indicate that lipid accumulation was harmful to the liver. The high activity of AST indicates that the damage reached to the mitochondria of hepatic cells.

In the liver, mitochondrial dysfunction, reduces the β-oxidation of fatty acids, because this oxidation occurs in the mitochondria. Consequently, lipid accumulated in the liver. These results in agreement with the data reported previously (36,37,38). As expected, hyperlipidemic diet increased the blood lipids concentrations (This result was not mentioned in the article) and presented lipid accumulation in the liver tissue as shown by H&E staining. This can be viewed as vesicular steatosis, represented by small white vesicles in S3 and large white vesicles in S2 (Fig.4). At the same time no changes had been observed in the liver tissue for S1, where liver cells appeared normal, and the cytoplasm was dark red because it was rich in glycogen (Fig.4). We suppose that elevated levels of SFA, triglyceride and cholesterol exceeded the liver's ability to metabolize them and thus, lipids increased in the blood. As a result, lipids accumulated into the liver and caused damage to hepatic cells, thus led to the flow of large quantities of enzymes into the blood. In this study, the AST/ALT ratio was (1.2) for S2, S3 and S4 groups in comparison with S1 after 4 weeks of hyperlipidemic diet intake. When the AST/ALT ratio is greater than (1) it indicates the progress of muscle injury (39). This ratio was (1.47) after feeding the hamster on a diet rich in SFA for (15) weeks, and it was associated with damage in the arteries of the heart (40). We hypothesize that this ratio was associated with muscle damage that is likely to be cardiac. This belief was reinforced by the increase of CK activity which was approximately (18%) in each of S2, S3, and S4 groups in comparison with S1 after 4 weeks of feeding a hyperlipidemic diet. This a significant difference had increased to reach approximately (40%) for S2 only in the second period Table 3-6. High CK activity in serum leads to muscle damage (41). Several studies suggested that omega-3 fatty fatty acids may be effective in the treatment of Fatty Liver Disease (42,43). Unfortunately, the effect of omega-6 fatty fatty on Fatty Liver Disease is poorly described. In this study, we figured out the impact of dosed borage oil rich in GLA for a period of 4 weeks on coconut oil and sheep fat-induced liver damage in a male golden Syrian hamster. The hepatoprotective effect of borage oil in this study was evaluated by the using activity of serum enzymes like ALT, AST, ALP, LDH, GGT in addition to hepatic tissue examination. The hepatoprotective effect of Borage oil is clearly seen in normalization of ALT activity, the square of the sudanophylic area and the contents of triglycerides in the liver (13). The intragastric administration of borage oil led to a significant decrease ($P<0.05$) of the ALT, AST, ALP, GGT, LDH and CK activities approximately (53%, 57%, 26%, 30%, 78%, 10%) respectively for S4 in comparison with S3 in the second period Tables 3-1, 3-2, 3-3, 3-4, 3-5 and 3-6. This decrease was accompanied by a reduction in fatty changes for S4 in comparison with S3, where hepatocytes returned to their semi-normal state (Fig.4). The aim of depriving the hyperlipidemic diet in S3 was to observe the decline of negative changes on its own or not. In this study negative effects on serum and liver tissue did not reverse when the hyperlipidemic diet was

deprived. Our results in agreement with the data reported previously (13) which demonstrated that borage oil decreased experimental ethanol-induced liver steatosis and normalized serum marker enzyme activities in rats. Botanical oils enriched in omega-6 fatty acids contributed to the prevention of atherosclerosis and fatty liver in mice (8). The hepatoprotection by borage oil in alcoholic steatosis is interrelated with its antioxidant characteristics.

Borage oil which contains GLA cancelled ethanol-stimulated reactive oxygen species (ROS) production and LPO (13). In the human body, GLA is a product of the conversion of LA. This conversion is organized by a Δ6-desaturase enzyme. This enzyme determines the speed of LA conversion to GLA (27,28,44). DGLA leads to the production of Prostaglandin E1 (PGE1) which has anti-atherosclerotic effects (8,28). Earlier we found that gastric intubation of borage oil for 4 weeks in hyperlipidemic hamsters caused a significant decrease of serum triglyceride, cholesterol and LDL concentrations. On the other side, it caused significant increasing of HDL concentration (45). That was the positive factor in minimizing the amount of liver lipids in this research. We suppose that the hepatoprotective effect of borage seed oil in this study is associated with the role of GLA and its eicosanoids such as AA in lowering blood lipids and improving the liver tissue and serum enzymes activities.

Table 3. Effect of hyperlipidemic diet and borage oil on activities of some enzymes.
Tablo 3. Hiperlipidemik diyet ve hodan yağının bazı enzimlerin aktiviteleri üzerine etkisi.

Table-3.1

Group \ Time	ALT (4 weeks)	ALT (8 weeks)
	a	a
S1	**62**.84±7.51	65.76±7.70
	B	C
	b	a
S2	162±12.51	206.69±9.05
	A	A
	a	a
S3	159.69±10.01	163.15±13.23
	A	B
	a	b
S4	165±10.18	75.76±9.09
	A	C

Table-3.2

Group \ Time	AST (4 weeks)	AST (8 weeks)
	a	a
S1	**77**.38±13.54	72.5±14.38
	B	C
	b	a
S2	203.53±20.14	248.42±13.46
	A	A
	a	a
S3	201.53±18.28	203.61±17.97
	A	B
	a	b
S4	199.92±30.12	86.78±18.80
	A	C

Table-3.3

Group \ Time	ALP (4 weeks)	ALP (8 weeks)
	a	a
S1	227.61±17	220.92±7.06
	B	B
	a	a
S2	278.76±22.78	299±24.96
	A	A
	a	a
S3	279.92±22.91	283.38±22.10
	A	A
	a	b
S4	269.15±21.25	208.15±13.25
	A	B

Table-3.4

Group \ Time	GGT (4 weeks)	GGT (8 weeks)
	a	a
S1	55.42±5.35	53.97±4.25
	B	B
	b	a
S2	81.13±10.45	110±9.41
	A	D
	a	a
S3	75.46±6.18	77±8.77
	A	C
	b	a
S4	88.75±8.44	53.48±5.21
	A	A

Table 3. Effect of hyperlipidemic diet and borage oil on activities of some enzymes. (Continued)
Tablo 3. Hiperlipidemik diyet ve hodan yağının bazı enzimlerin aktiviteleri üzerine etkisi. (Devamı)

Table-3.5		
Time Group	LDH (4 weeks)	LDH (8 weeks)
S1	a 504.15±89.69 B	a 477.92±68.50 B
S2	a 1545.6±205.4 A	a 1612.2±128.4 A
S3	a 1521.61±193.88 A	a 1550.30±159.93 A
S4	a 1430.8±225.4 A	b 332.38±34.31 C

Table-3.6		
Time Group	CK (4 weeks)	CK (8 weeks)
S1	a 170.38±11.66 B	a 171.92±10.73 C
S2	b 202.38±11.89 A	a 241.53±12.19 A
S3	a 198.23±7.56 A	a 202.23±7.04 B
S4	a 201.38±8.96 A	b 180.69±6.27 C

3-1:Serum ALT(U/L), 3-2:AST(U/L), 3-3:ALP(U/L), 3-4: GGT(U/L), 3-5:LDH(U/L) and 3-6:CK(U/L) of the experimental hamsters. Values are expressed as mean±SD; n=15. Different small letters indicate significant differences in the same row (P<0.05), Different large letters indicate significant differences in the same column (P<0.05). S1 (negative control group), S2 (fatty liver group), S3 (positive control group) and S4 (borage oil group).

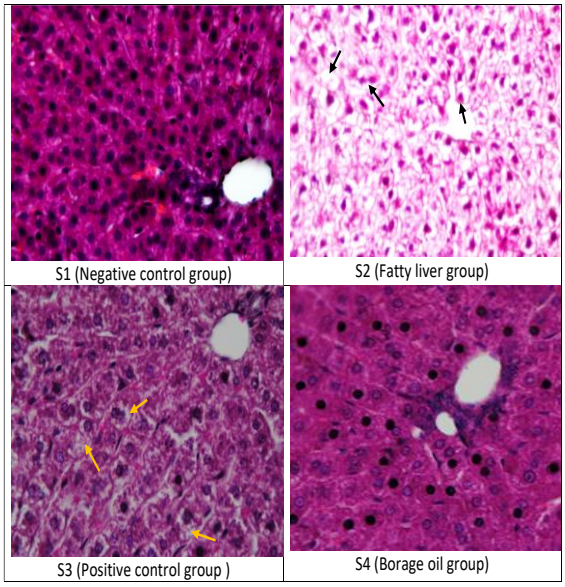

Figure 4. Photomicrography of liver section, stained with H&E (400X).
Şekil 4. Karaciğer bölümünün fotomikrografisi, H&E ile boyandı (400X).

In conclusion, our findings suggest that borage oil supplement can attenuate hyperlipidemic diet-induced liver damage in male golden Syrian hamsters.

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Conflict of interest

The authors declare that they have no conflict of interest.

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