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EFFECT OF S-ADENOSYLMETHIONINE ON HEPATIC AND METABOLIC DISORDERS IN GUINEA PIGS WITH NON-ALCOHOLIC STEATOHEPATITIS

NON-ALKOLİK STEATOHEPATİT OLUŞTURULAN KOBAYLARDA S-ADENOZİLMETIYONİNİN KARACİĞER VE METABOLİK BOZUKLUKLAR ÜZERİNE ETKİSİ

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ABSTRACT

Objective: S-adenosylmethionine (SAM) has antioxidant and anti-inflammatory actions and hepatoprotective potential. In this study, the therapeutic effectiveness of SAM was investigated in high-fat/cholesterol diet (HFCD)-induced non-alcoholic steatohepatitis (NASH).

Material and Methods: In this study, guinea pigs were fed a HFCD for ten weeks to induce NASH. SAM (50 mg/kg, i.p.) was administered to the animals during the last four weeks of the 10-week HFCD regimen. Hepatic damage markers, lipid levels (total cholesterol and triglyceride), inflammatory cytokines (tumour necrosis-α and interleukin-6) levels, and insulin resistance (HOMA-IR) were determined in the serum. Moreover, hepatic lipids, SAM and cytochrome p450-2E1 (CYP2E1) levels, prooxidant parameters (reactive oxygen species, lipid peroxides and protein carbonyls) and antioxidant parameters (glutathione levels and antioxidant activity) together with fibrosis indicators (α-smooth muscle actin and transforming growth factor-β1 protein expressions and hydroxyproline levels) were investigated in the liver. Steatosis, inflammation, and fibrosis scores were also detected histopathologically.

Result: SAM treatment diminished the increase in hepatic damage markers, inflammatory cytokine levels, and HOMA-IR levels

ÖZET

Amaç: S-adenozilmetiyonin (SAM), antioksidan ve anti-enflamatuar etkilere ve hepatoprotektif potansiyele sahiptir. Bu çalışmada, yüksek yağ/kolesterollü diyet (YYKD) ile indüklenen non-alkolik steatohepatit (NASH) üzerinde SAM'ın terapötik etkinliği araştırılmıştır.

Gereç ve Yöntem: Bu çalışmada, NASH oluşturmak için kobaylara 10 hafta boyunca YYKD verildi. Hayvanlara, 10 haftalık YYKD uygulamasının son dört haftasında SAM (50 mg/kg, i.p) uygulandı. Serumda hepatik hasar belirteçleri, lipitler (total kolesterol ve trigliserit), inflamatuar sitokin (tümör nekroz faktörü-α ve interlökin-6) düzeyleri ve insülin direnci (HOMA-IR) ölçüldü. Ayrıca, karaciğerde hepatik lipitler, SAM ve sitokrom p450-2E1 (CYP2E1) düzeyleri, prooksidan parametreler (reaktif oksijen türleri, lipid peroksidleri ve protein karbonil) ve antioksidan parametreler (glutatyon düzeyleri ve antioksidan aktivite) ile birlikte fibrotik parametreler (α-düz kas aktin ve transforme edici büyüme faktör-β1 protein ekspresyonları ve hidroksiprolin düzeyleri) belirlendi. Steatozis, inflamasyon ve fibrozis skorları da histopatolojik olarak tespit edildi.

Bulgular: SAM tedavisi, YYKD ile indüklenen NASH'lı kobayların serumunda hepatik hasar belirteçleri, enflamatuar sitokinler düzeyleri ve HOMA-IR düzeylerinde azalmaya neden oldu. Ayrıca,

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in the serum of guinea pigs with HFCD-induced NASH. Elevated levels of hepatic triglyceride and CYP2E1 and fibrosis indicators were also detected to decrease due to SAM treatment. This treatment reduced the decrease in SAM levels, disturbance in the prooxidant and antioxidant balance, and diminished the increases in steatosis, inflammation, and fibrosis scores in the liver of guinea pigs fed the HFCD diet.

Conclusion: These results indicate that SAM may be effective in HFCD-induced NASH as a therapeutic agent by decreasing lipogenesis, oxidative stress, inflammation, and fibrosis.

Keywords: S-adenosylmethionine, nonalcoholic steatohepatitis, high fat/cholesterol diet, oxidative stress, inflammatory cytokines, guinea pigs

trigliserit ve CYP2E1 düzeyleri ile fibroz belirteçlerindeki yüksek seviyelerin de SAM tedavisine bağlı olarak azaldığı tespit edildi. Bu tedavi, YYKD diyeti ile beslenen kobayların karaciğerinde SAM düzeylerindeki azalmayı, prooksidan ve antioksidan dengesindeki bozukluğu iyileştirdi, steatozis, inflamasyon ve fibrozis skorlarındaki artışları azalttı.

Sonuç: Bu sonuçlar, SAM'ın lipojenez, oksidatif stres, enflamasyon ve fibrozisi azaltarak YYKD ile indüklenen NASH'ta terapotik bir ajan olarak etkili olabileceğini göstermektedir.

Anahtar Kelimeler: S-adenozilmetyonin, non-alkolik steatohepatit, yüksek yağlı/kolesterollü diyet, oksidatif stres, inflamatuvar sitokinler, kobay

INTRODUCTION

S-adenosylmethionine (SAM) is the main donor of the methyl group in the organism. It has direct antioxidant activity by scavenging reactive oxygen species (ROS) and is a precursor of glutathione (GSH), a major antioxidant in the cells. It inhibits the formation of proinflammatory molecules [tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6)], inhibits cytochrome P450-2E1 (CYP2E1) enzyme activity, and prevents mitochondrial dysfunction (1). Therefore, SAM is accepted as a hepatoprotective agent (1, 2). Indeed, SAM treatment has been found to have a protective potential against acetaminophen-(3), ischaemia-reperfusion, alcohol and cholestasis induced liver injuries and experimental alcohol plus carbon tetrachloride induced-fibrosis by inhibiting hepatic stellate cell activation (4-7).

The liver plays an important role in maintaining SAM homeostasis by regulating its synthesis and degradation. Approximately 85% of methylation reactions in the body are carried out by the liver. SAM is responsible for methylating various molecules, including phospholipids. Thus, phosphatidylcholine (PC) is formed by the methylation of phosphoethanolamine (PE). Since a low PC/PE ratio diminishes the secretion of very low density lipoproteins (VLDL) from the liver, the decrease in SAM levels impairs the export of VLDL from the liver and thus triglyceride accumulated in the liver. Furthermore, a low PC/PE ratio elevates membrane permeability and the sensitisation of liver to endotoxin-induced proinflammatory cytokines (8, 9).

Non-alcoholic fatty liver disease (NAFLD) is the most important cause of chronic liver disease. NAFLD encompasses nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NASH is the severe form of NAFLD and is characterised by steatosis, inflammation, and progressive fibrosis. According to the two-hit hypothesis, steatosis is the first hit and enhances the hepatic susceptibility to subsequent secondary hits such as oxidative stress, endotoxemia, and inflammatory cytokines, which contribute to the development of NASH and other advanced pathologies such as fibrosis/cirrhosis and hepatocellular carcinoma (8, 10). However, the pathophysiology of NASH and effective pharmacological treatment tools are not yet fully clarified (11). Low hepatic SAM levels have also been suggested to play a role in NASH development by serving as a second hit (8, 9, 12), and SAM treatment may be useful in the prevention of liver damage in NAFLD/NASH (13-16). However, there are few experimental and clinical studies on NASH, and the clinical benefit of SAM remains controversial (2, 8, 17).

In our previous study, it was determined by histopathological and metabolic markers that high fat/cholesterol diet (HFCD) feeding on for six weeks caused NASH in guinea pigs (18). In addition, when SAM was administered simultaneously with HFCD in this process, it was determined that SAM reduced the NASH formation process and this effect was achieved by reducing steatosis, inflammation, fibrosis and oxidative stress and had a preventive potential. Our aim in the current study was to investigate the therapeutic effect of SAM on NASH. For this purpose, in our experimental groups, which we planned independently from our previous study, we extended the feeding period with HFCD to 10 weeks and evaluated the therapeutic effect by giving SAM together in the last four weeks. Because steatosis, inflammation, fibrosis and oxidative stress play a fundamental role in the formation and progression of NASH, our investigations were performed within the framework of these parameters.

MATERIALS AND METHODS

Chemicals

The chemical, S-adenosyl-L methionine disulphate tosylate (SAM), was donated by Pure Encapsulations, Inc. (Sudbury, MA, USA). Cholesterol was purchased from Alfa Easer (Kandel, Germany), and other chemicals were obtained from Sigma-Aldrich (Darmstadt, Germany).

Animals and the experimental design

Dankin Hartley guinea pigs, weighing 600-650 g, were obtained from Aziz Sancar Institute of Experimental Medicine, İstanbul University. The animals were housed in a light- and temperature-controlled room on a 12 h:12 h light:dark cycles in stainless steel cages (two or three

per cage). The experimental procedures used in this study were approved by the İstanbul University Animal Care and Use Ethics Committee (Date: 02.03.2108, No: 2018/18).

Animals were divided into four experimental groups (each n=6) as follows: a) Control group: Animals were fed a normal guinea pig diet for 10 weeks and injected with 0.9% NaCl as a vehicle in the last four weeks. b) Control SAM group: They received a normal diet for 10 weeks and injected with SAM (50 mg/kg; five days per week; i.p.; freshly dissolved in 0.9%NaCl) in the last four weeks. c) HFCD-10w group: Guinea pigs were fed an HFCD diet (81% standard guinea pig chow diet, 1% cholesterol, 8% yolk powder and 10% beef tallow) for 10 weeks. d) SAM+HFCD-10w group: Guinea pigs were fed HFCD for 10 weeks as described above and were injected with SAM (50 mg/kg; freshly dissolved in 0.9% NaCl solution; five days per week; i.p) in the last 4 weeks.

The dose and duration of SAM used in our study are based on previous studies (15, 18, 19). Diets were prepared by the Barbaros Denizeri Company (Gebze) and kept at 4°C. There were no restrictions on water and food for animals, and food and drinking water intake were periodically monitored.

Samples

At the end of the experimental period, the guinea pigs were fasted overnight. They were then anaesthetised with ketamine (40 mg/kg, i.p., Pfizer, USA) and xylazine HCl (5 mg/kg, i.p., Bioveta, Czech Republic). Blood samples were collected via cardiac puncture into dry tubes and, then centrifuged at 1500xg for 10 min to separate the sera. The liver tissues from the animals were homogenised in ice-cold phosphate-buffered saline (PBS; 0.01M, pH: 7.4), then they were centrifuged at 600xg for 10 min at 4°C, and the supernatants were used for biochemical analyses in the liver. Both serum and liver tissue were stored at -80°C until analysed.

Hepatic damage markers in the serum

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in an autoanalyzer (Cobas Integra 800, Roche Diagnostics, Germany) to evaluate hepatic damage in the serum using enzymatic methods.

Determination of the glucose and insulin levels in the serum

Total cholesterol (TC), triglyceride (TG), and fasting glucose levels were determined using an autoanalyser (Cobas Integra 800, Roche Diagnostics, Germany). Serum insulin levels were estimated using guinea pig insulin ELISA kits (#KTE120010, Abbkine, Wuhan, China) in accordance with the manufacturer's instructions. Glucose and insulin levels were used to calculate the homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR), an index of insulin resistance, which is defined as follows: fasting insulin levels (pmol/L) x fasting glucose levels (mmol/L)/135 (20).

Determination of TNF-α and IL-6 levels in the serum

TNF-α (#KTE120004, Abbkine, China) and IL-6 (#KTE120003, Abbkine, China) levels were measured using ELISA kits according to the manufacturers' instructions. Results are expressed in nanograms per L.

Determination of lipids in the liver

Hepatic TC (#87356, Biolabo Biochemistry and Coagulation, France) and TG (#87319, Biolabo Biochemistry and Coagulation, France) levels were assayed using commercial colorimetric kits in lipid extracts obtained from the tissues. Results were expressed as µmol per g liver.

Determination of SAM, hydroxyproline (Hyp), and CYP2E1 levels in the liver

Hepatic SAM (#201-01-1072, Sunred Bio, Shanghai, China), Hyp (#E0148Gp, Bioassay Technology Laboratory, Shanghai, China) and CYP2E1 (#KTE120024, Abbkine, China) levels were measured in liver homogenates using ELISA kits according to the manufacturers' instructions.

Assessment of oxidative stress parameters in the liver

The level of reactive oxygen species (ROS) in the liver homogenates was assayed using a fluorescent compound (2',7'-dichlorodihydrofluorescein diacetate) that is sensitive to oxidation (21). Fluorescence intensities were detected at Ex 485/Em 538 using a Fluoroskan Ascent microplate fluorometer from Thermo Scientific Inc., USA. Results were given as relative fluorescence units per mg protein.

Hepatic lipid peroxidation was assessed by determining the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugate (DC) levels. TBARS was determined using the spectrophotometric method developed by Buege and Aust (22). The liver homogenate and Buege-Aust reagent (consisting of 26 mM thiobarbituric acid and 0.92 M trichloroacetic acid in 0.25 M HCl), the mixture was heated in boiling water for 15 min. Following cooling and centrifugation at 1000g, the absorbances of the resulting supernatants were read at 532 nm. The results were computed using a molar extinction coefficient of $1.56 \times 10^{-5} M^{-1} cm^{-1}$. Results of TBARS were expressed in pmol per mg protein. For this assay, the levels of DC in the hepatic lipid extracts were also measured spectrophotometrically at 233 nm. For this assay, tissue lipids were extracted with a chloroform/methanol (2:1, v:v) mixture. The extracted lipids were evaporated and dissolved in cyclohexane. The absorbances were read at 233 nm, and the results were computed using a molar extinction coefficient of $2.52 \times 10^{4} M^{-1} cm^{-1}$ (22). Results of DC were expressed in nmol per mg protein.

The level of oxidative protein damage in the liver homogenates was assessed by measuring the protein carbonyl (PC) groups using a method developed by Reznick and Packer (23). This involved calculating the absorbance of the protein hydrazone formed by reacting the protein carbonyls with 2,4-dinitrophenylhydrazine, and the absorbances were read at 360 nm. The results were then calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}. Results were expressed in nmol per mg protein.

Glutathione (GSH) levels were measured spectrophotometrically using 5,5-dithiobis (2-nitrobenzoic acid) as an indicator, following the method described by Beutler et al. (24). The ferric reducing antioxidant power (FRAP) assay was used for the spectrophotometric determination of the antioxidant power of the liver homogenates, based on the method outlined by Benzie and Strain (25). In this assay, the ferric-tripyridyltriazine complex was reduced to the ferrous form, which is blue coloured and the absorbances were monitored at 593 nm. Results were expressed in nmol per mg protein.

Protein levels in the liver homogenates were determined using the bicinchoninic acid assay, with serum albumin serving as the standard (26).

Histopathological analysis

Liver tissues were fixed in a 10% formalin buffer solution for 24 h before embedding in paraffin. After that, 5 µm slides were obtained from each paraffin block, and all paraffin was removed from the slides and stained with haematoxylin and eosin (H&E) for histological examinations. Reticulin staining was also performed to show reticulin fibres of fibrotic areas. Steatosis, liver damage, and fibrosis scores were calculated according to the protocol proposed by Goodman, which was previously reported by us (20, 27).

Steatosis was scored as 0 = <5% (none), 1 = 5%–33% (mild), 2 = 34%–66% (moderate), 3 = ≥67% (severe). Fibrosis was classified using Ishak's staging (27). 0 = no fibrosis, 1 = fibrous expansion of some portal areas, with or without short fibrous septa, 2 = fibrous expansion of most portal areas, with or without short fibrous septa, 3 = fibrous expansion of most portal areas with occasional portal to portal bridging, 4 = fibrous expansion of portal areas with marked bridging, 5 = marked bridging with occasional nodules, 6 = cirrhosis, probable or definite.

Liver damage parameters were scored as follows: 0 = no visible cell damage, 1 = focal damage on <25% of the tissue (mild), 2 = focal damage on between 26 and 50% of the tissue (moderate), 3 = extensive lesions in >51% of the tissue (severe), 4 = global lesion (global) (27).

Protein expressions of α-smooth muscle actin (α-SMA) and transforming growth factor-β1 (TGF-β1)

The immunochemistry analysis was used to measure the expressions of α-SMA and TGF-β1 in the liver, as described previously (18, 20). Briefly, liver sections were incubated with α-SMA (dilution 1:100, #ABP52852, rabbit polyclonal, Abbkine, Wuhan, China) and TGF-β-1 (dilution 1:100, #APB52598, rabbit polyclonal, Abbkine, Wuhan, China) as primary antibodies for 1 h at room temperature. Negative control sections treated with phosphate-buffered antibodies were confirmed to be unstained. In addition, positive control studies were conducted in sections of healthy human liver. The secondary antibody reacted with the sections for 25 min. AEC (ScyTek Laboratories, Inc.205 South 600 West Logan, UT 84321, USA) chromogen was used to visualise the reaction, and the sections were then washed in distilled water. The presence or absence of brown staining was considered indicative of a positive or negative result for each antibody, respectively. The sections were evaluated under the light microscope and the score was made up of 0-5% positive cells as (-), 5- 30% positive cells as (+), 30-60% positive cells as (++) and 60% and over positive cells as (+++). Digital photographs were assessed using the Olympus AnalySIS five image analysis programme.

Statistical analysis

Results are presented as mean±standard deviation (SD). The normality of the results was tested using the Kolmogorov-Smirnov test. Parametric data was analysed using a one-way ANOVA test with post-hoc Tukey's test, while non-parametric data was compared using the Kruskal-Wallis test with post-hoc Mann Whitney-U test. A P-value <0.05 was considered statistically significant. The analyses were performed using SPSS for Windows, version 21.0 (IBM SPSS Corp., Armonk, NY, USA).

RESULTS

SAM treatment did not alter the increases in liver weight and liver index values in the HFCD group

Body weight did not alter in the HFCD and SAM+HFCD groups compared with the controls. However, the liver weight and liver index values remained unchanged in the HFCD group due to SAM treatment (Table 1).

SAM treatment decreased the high levels of ALT and AST activities in the serum of the HFHC group

Serum ALT and AST activities were significantly elevated in guinea pigs fed HFCD. SAM treatment decreased these enzyme activities in the HFCD group (Table 1).

SAM treatment reduced the high levels of TC and HOMA-IR in the serum of the HFCD group

In the HFCD group, the serum TC increased, but not the TG levels. Additionally, serum glucose and insulin levels and HOMA-IR values also shown an increase. However, among these parameters, the serum TC and glucose levels, as well as the HOMA-IR values, diminished due to SAM treatment in the HFCD group (Table 2).

Table 1: The effect of SAM treatment on body weight, liver weight and liver index values and ALT and AST activities in guinea pigs fed on HFCD (mean±SD; n=6, each)

	Control	SAM	HFCD	SAM+HFCD
Body weight (g)	746.7±40.8	730.0±28.3	689.0±98.1	715.4±37.2
Liver weight (g)	34.8±2.86	34.3±2.71	69.8±8.11[a]	68.5±12.3[a]
Liver index* (%)	4.68±0.54	4.71±0.39	10.2±0.54[a]	9.51±1.28[a]
ALT (U/L)	51.2±6.27	53.0±6.16	127.1±22.4[a]	65.8±7.01[a,b]
AST (U/L)	95.5±15.0	99.0±16.7	757.5±68.9[a]	379.5±71.9[a,b]

[a]: p<0.05 as compared to control, [b]: p<0.05 as compared to HFCD group, *Liver weight x100/body weight, SAM: S-adenosylmethionine, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HFCD: High-fat/cholesterol diet.

Table 2: The effect of SAM treatment on serum TC, TG, glucose, insulin, HOMA-IR levels, as well as TNF-α and IL-6 levels in guinea pigs fed on HFCD (mean±SD; n=6, each)

	Control	SAM	HFCD	SAM+HFCD
TC (mmol/L)	1.55±0.47	1.53±0.30	8.93±1.16[a]	5.78±0.75[a,b]
TG (mmol/L)	0.83±0.14	0.80±0.11	0.90±0.12	0.79±0.08
Glucose (mmol/L)	8.24±1.38	8.14±1.37	11.0±1.01[a]	8.72±0.78[b]
Insulin (pmol/L)	27.6±3.04	25.1±2.26	29.6±1.02[a]	27.8±0.86
HOMA-IR	1.61±0.38	1.53±0.36	2.34±0.09[a]	1.80±0.16[b]
TNF-α (ng/L)	19.0±2.74	17.5±2.09	27.4±2.34[a]	20.3±2.11[b]
IL-6 (ng/L)	23.9±2.19	22.3±2.32	32.6±5.03[a]	22.3±2.33[b]

[a]: p<0.05 as compared to control, [b]: p<0.05 as compared to HFCD group, SAM: S-adenosylmethionine, TC: Total cholesterol, TG: Triglyceride, HOMA-IR: Homeostasis model assessment for insulin resistance, TNF-α: Tumour necrosis factor-alpha, IL-6: Interleukin-6, HFCD: High-fat/cholesterol diet.

SAM treatment reduced the high levels of TNF-α and IL-6 in the serum of the HFCD group

Both TNF-α and IL-6 levels were detected to increase in the serum of the HFCD group. The SAM treatment diminished the levels of these cytokines in the HFCD group (Table 2).

Changes in the liver histology

The control and SAM-control groups showed normal hepatic architecture. The HFCD group exhibited severe macrovesicular steatosis, along with fibrous bands and mild chronic infiltration between the central and portal veins. Sinusoids could not be observed. Increased reticulin fibres, which form bundles in fibrous bands, were detected by reticulin staining. Less steatosis was observed in guinea pigs with HFCD due to SAM treatment. Furthermore, the fibrous bands were thinner and had a shorter course. The reticulin stain demonstrated that reticular fibres reduced, and the bundles became thinner and had a shorter course due to SAM treatment (Figure 1).

The reticulin stain revealed an increase in reticulin fibres forming bundles in the fibrous bands. In guinea pigs with HFCD, less steatosis was observed due to SAM treatment. Additionally, the fibrous bands were thinner and had a shorter course. The reticulin stain demonstrated a reduction in reticular fibres, with the bundles becoming thinner and having a shorter course due to SAM treatment (Figure 1).

According to the histopathological scorings, SAM treatment significantly diminished the increased hepatic steatosis, inflammation, and fibrosis scores in the HFCD group (Figure 2).

SAM treatment increased hepatic SAM levels and decreased lipid, Hyp, and CYP2E1 levels in the HFCD group

The hepatic SAM levels significantly decreased in the HFCD group compared to the controls. However, TC and TG as well as Hyp and CYP2E1 levels increased significantly in the liver of the HFCD group. SAM treatment caused increases in SAM levels and decreased hepatic TC, TG, Hyp and CYP2E1 levels in the HFCD group (Figure 3).

SAM treatment reduced hepatic oxidative stress parameters in the HFCD group

The levels of oxidant parameters [ROS, lipid peroxides (TBARS and DC) and protein oxidation products (PC)] significantly increased, while the levels of antioxidant

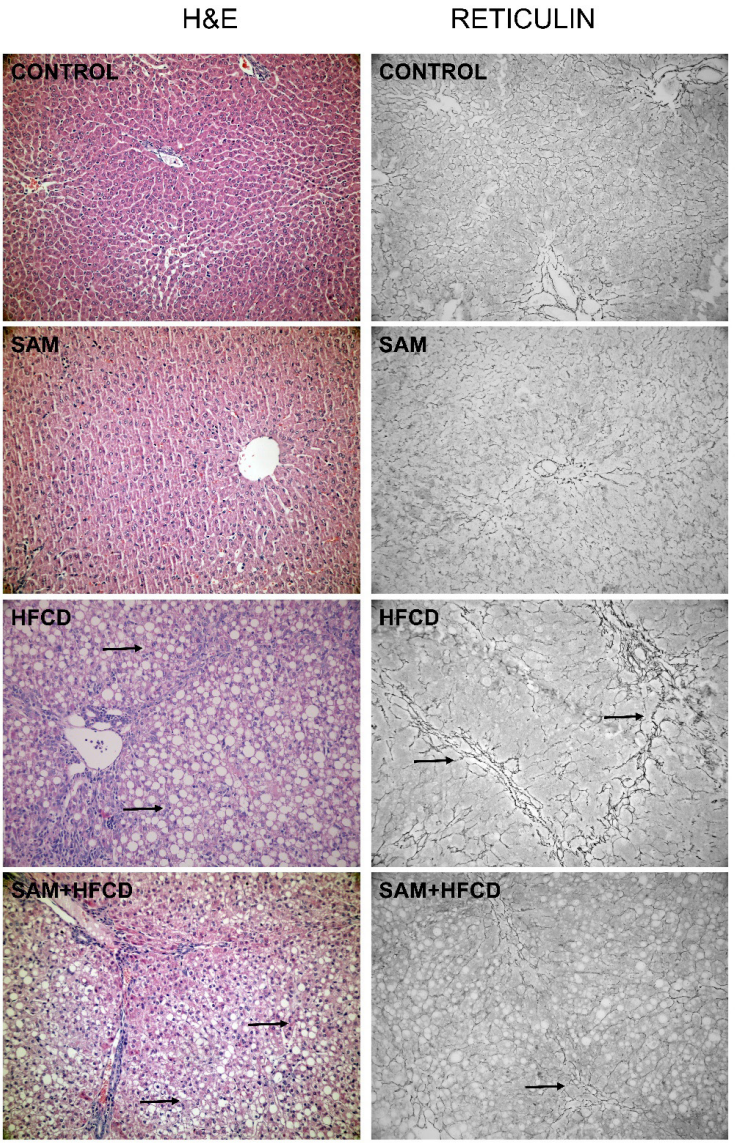

Figure 1: The effect of S-adenosylmethionine treatment on the hepatic and histopathology of guinea pigs fed a high-fat/cholesterol diet. (Haematoxylin and eosin x200 and Reticulin x200). Arrows in haematoxylin and eosin staining of the liver (first column) indicate macrovesicular steatosis, arrows in reticulin staining (second column) also show reticular fibres. Groups: Control; SAM; HFCD; SAM + HFCD. H&E: Haematoxylin and eosin, HFCD: High-fat/cholesterol diet, SAM: S-adenosylmethionine

parameters (GSH and FRAP levels) decreased in the HFCD group. SAM treatment diminished oxidant parameters and increased antioxidant parameters in the HFCD group (Figure 4).

Changes in the hepatic α-SMA and TGF-β1 protein expressions

Significant increases in α-SMA and TGF-β1 protein expressions were observed in guinea pigs fed the HFCD. However, these expressions decreased in the HFCD group due to SAM treatment (Figure 5).

DISCUSSION

Diets rich in fat, fructose or cholesterol or their combinations and the methionine-choline deficiency (MCD) diet are dietary experimental models used to understand the pathogenesis of NAFLD/NASH and to test treatment possibilities (28). The development of fibrosis was the primary feature that distinguished NASH from NAFLD. It has been suggested that fibrosis development is probably influenced not only by dietary cholesterol but also by the interaction between dietary

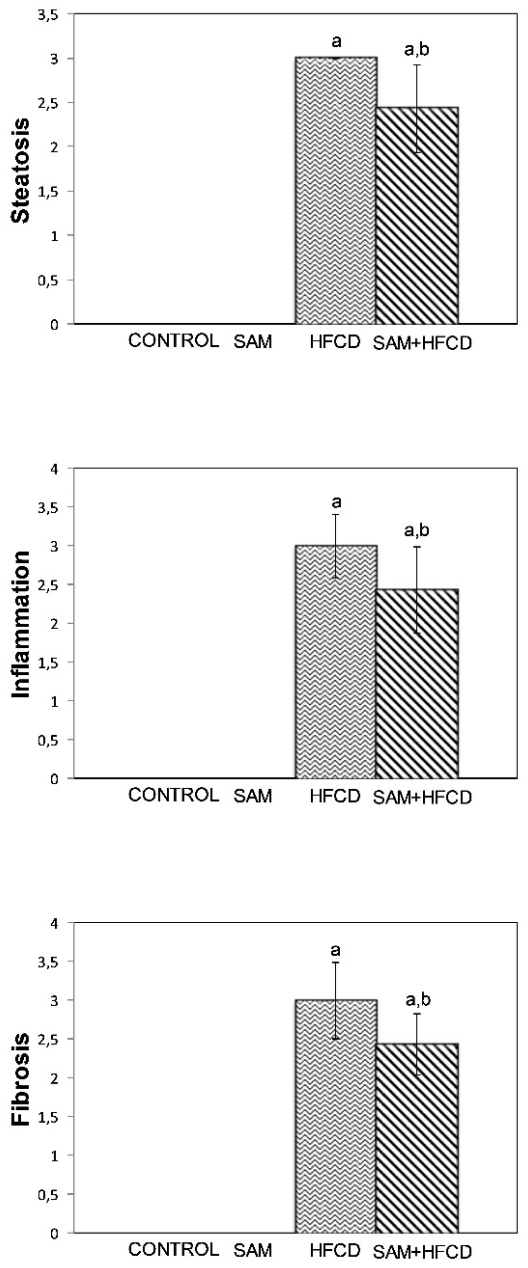

Figure 2: The effect of S-adenosylmethionine treatment on hepatic steatosis, inflammation, and fibrosis scores in guinea pigs fed a high-fat/high-cholesterol diet. No steatosis, inflammation and fibrosis were seen in the control and S-adenosylmethionine groups. The high-fat/cholesterol diet group exhibited severe macrovesicular steatosis, along with fibrous bands and mild chronic infiltration between the central and portal veins. The high-fat/high-cholesterol diet + S-adenosylmethionine groups showed decreases in steatosis inflammation and fibrosis scores (Mean±SD; n=6; each). Groups: Control; SAM; HFCD; SAM + HFCD. HFCD: high-fat/cholesterol diet. SAM: S-adenosylmethionine. [a]: $p<0.05$ as compared to control, [b]: $p<0.05$ as compared to HFCD group

cholesterol and dietary fat (29,30). Therefore, HFCD is recognised as a suitable dietary experimental model for inducing hepatic steatosis and inflammation and NASH in mice and rats (29, 30). Unlike rats and mice, guinea pigs and humans share an LDL-dominant lipoprotein profile and show a high degree of similarity with humans in terms of hepatic lipid metabolism, inflammation, and fibrogenesis (32). Guinea pigs are susceptible to HFCD-induced NASH and are used as a suitable model to study the pathogenesis of the disease (31, 33).

In guinea pigs fed on HFCD for 10 weeks, the increases were detected in ALT and AST activities, indicating liver damage, TC and inflammatory cytokine (TNF-α and IL-6) levels as well as HOMA-IR values in their serum of guinea pigs. Hepatic TC, TG and Hyp levels, profibrotic α-SMA and TGF-β1 protein expressions were increased. Hepatic histopathological observations also showed significant increases in steatosis, inflammation, and fibrosis scores in this group. All these results demonstrated the occurrence of NASH due to HFCD feeding, which is characterised by increased steatosis, inflammation, fibrosis and hepatocyte necrosis, consistent with previous reports (29-31). HFCD feeding also caused an increase in ROS levels in the liver of guinea pigs. As is known, ROS produced by CYP2E1 plays an important role in triggering oxidative stress in NASH (1, 34). In this study, increased ROS levels were associated with higher formation of lipid peroxides (such as TBARS, DC) and protein oxidation products (such as PC). The decrease in the antioxidant parameters (GSH and FRAP) reflects the deficiency in the antioxidant power. The results of the study indicate that a pro-oxidant state is present in the livers of guinea pigs with NASH, as previously reported (35-37).

In chronic liver diseases, SAM levels decreased due to increased use as an antioxidant and/or a decrease in its synthesis (8, 17, 38). Since methionine adenosyltransferase 1 (MAT1), the key enzyme in SAM synthesis, is an enzyme sensitive to oxidation, it may be inhibited under conditions where oxidative stress is induced (17, 38). In MAT1-deficient mice, steatosis was detected to progress towards NASH, and SAM treatment reduced hepatic damage, ALT and AST activities, and TG levels in these mice (9). Therefore, it has been suggested that SAM homeostasis may have an active role in the pathogenesis of NASH and that SAM supplementation can be used as a therapeutic agent in NAFLD/NASH (8, 12).

Some investigators have tested whether SAM treatment is effective in experimental dietary models of NAFLD/NASH. SAM treatment was able to ameliorate fatty acid-induced lipid accumulation and oxidative stress through promoting β-oxidation in hepatocyte cultures (39). Similarly, the administration of SAM decreased fatty liver and oxidative

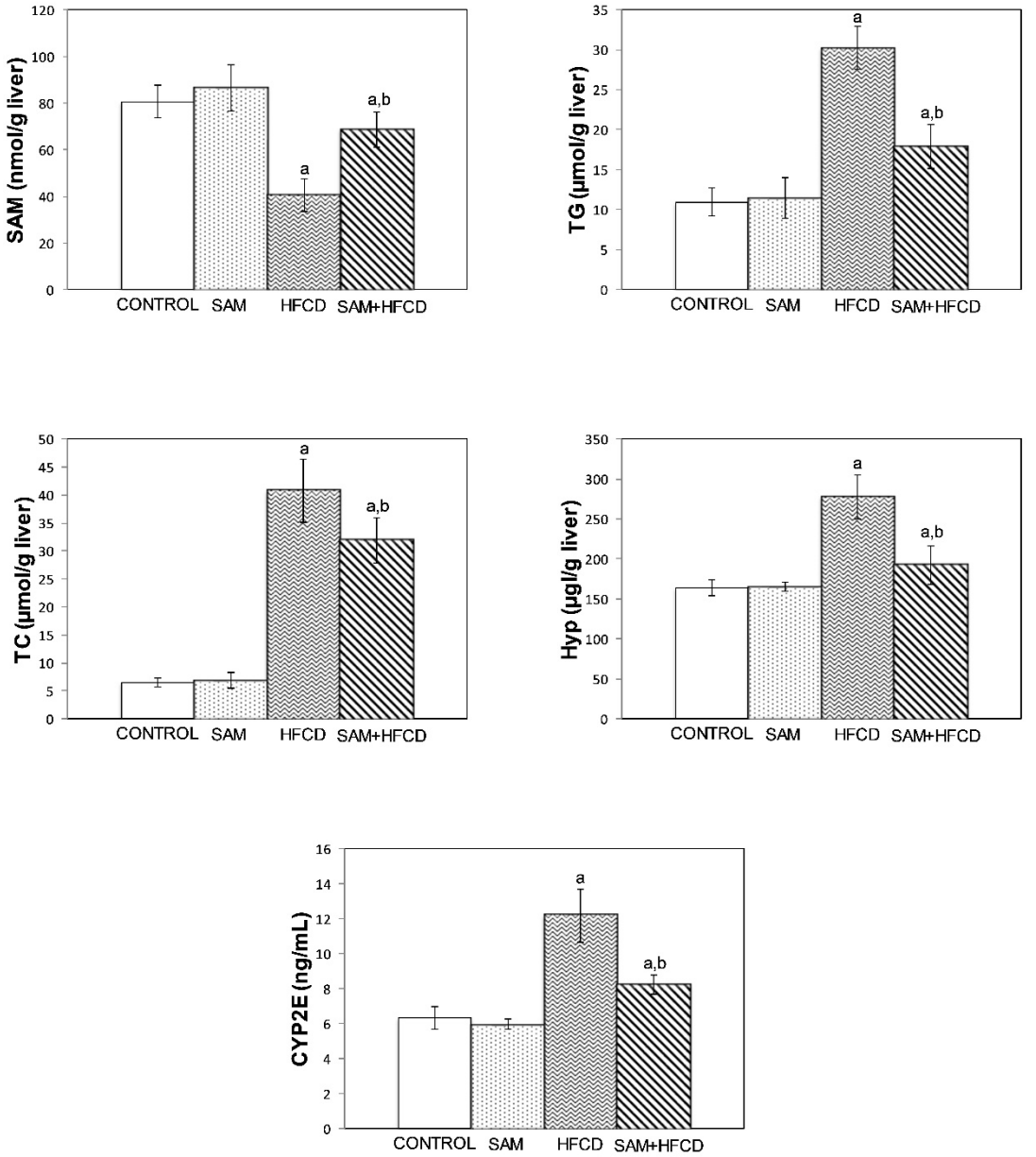

Figure 3: The effect of S-adenosylmethionine treatment S-adenosylmethionine, hepatic total cholesterol, triglyceride, hydroxyproline (Hyp), and cytochrome P450-2E1 (CYP2E1) levels in guinea pigs fed on a high-fat/cholesterol diet (HFCD). (Mean±SD; n=6; each). CYP2E1: cytochrome P450-2E1, HFCD: high-fat/cholesterol diet, S-adenosylmethionine, Hyp: hydroxyproline, total cholesterol: TC, triglyceride: TG. [a]: $p<0.05$ as compared to the control, [b]: $p<0.05$ as compared to the HFCD group

stress in the liver of rats fed a high fructose diet induced NAFLD (13). Moreover, in mice fed an MCD diet, SAM administration reduced liver damage by increasing hepatic SAM and GSH levels and by downregulating the expression of inflammatory and fibrogenic cytokines (16). On the other hand, angiotensin II (Ang II), as the main component of the renin-angiotensin system (RAS), is known to influence lipid metabolism and insulin sensitivity via its receptor, Ang II type 1 receptor (AT1R), thereby contributing to NAFLD progression (40). SAM was reported to prevent intrahepatic RAS activation by upregulating the expression of the AT1R-associated protein (ATRAP), an inhibitor of AT1R. Therefore, it has been suggested that SAM may be useful as a therapeutic agent in the prevention of NAFLD/NASH through this mechanism (15). Indeed, it has been reported that both hepatic SAM levels and ATRAP protein expression are decreased in patients with NAFLD. Similar results were also found in HFCD-fed rats, andwhen SAM

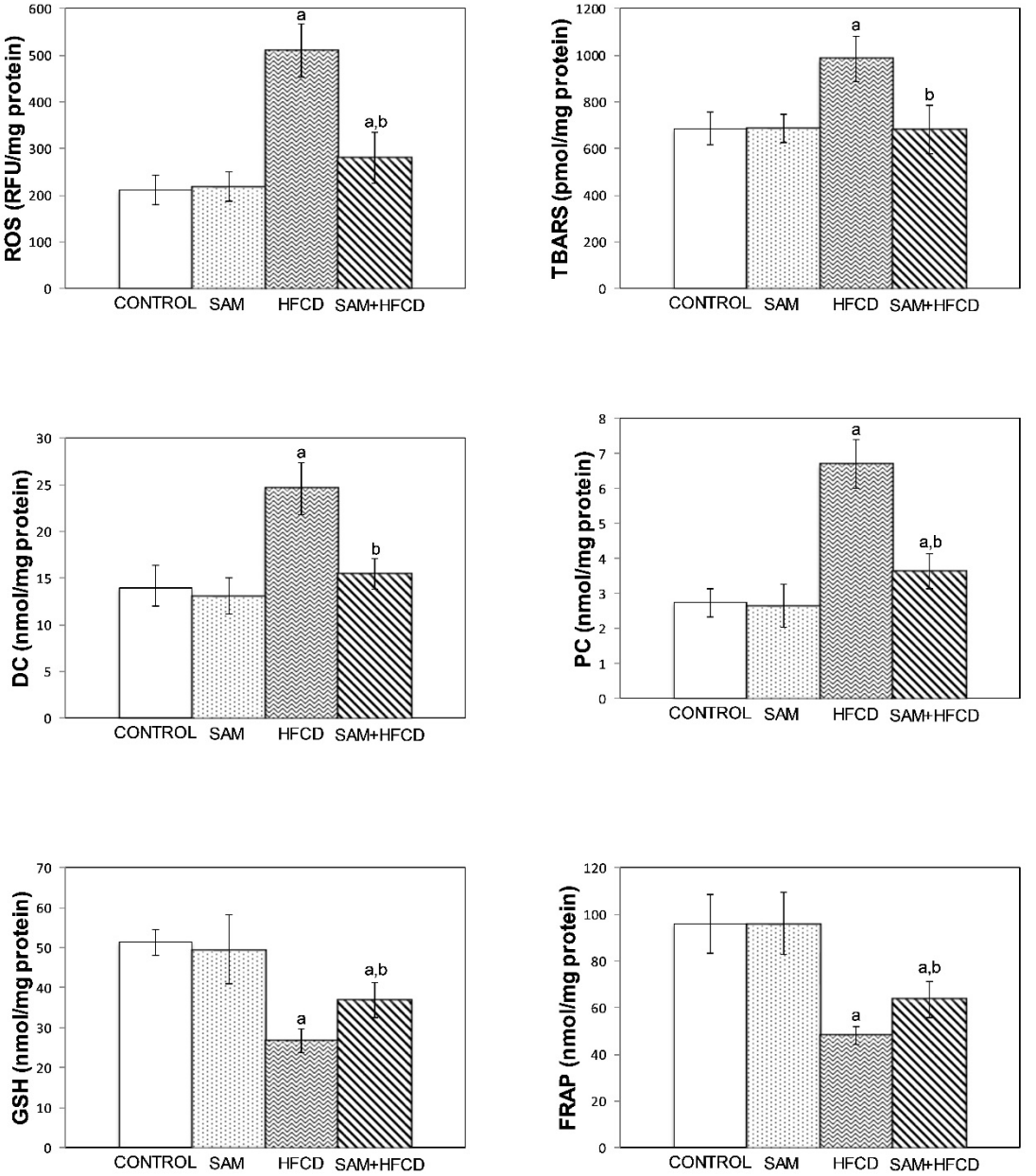

Figure 4: Hepatic reactive oxygen species, thiobarbituric acid reactive substances, diene conjugate and protein carbonyl, glutathione and ferric reducing antioxidant power levels in guinea pigs fed on a high-fat/cholesterol diet. (Mean±SD; n=6 each). DC: diene conjugate, FRAP: ferric reducing antioxidant power, GSH: glutathione, HFCD: high-fat/cholesterol diet, PC: protein carbonyl, ROS: reactive oxygen species, TBARS: thiobarbituric acid reactive substances. [a]: $p<0.05$ compared to control, [b]: $p<0.05$ compared to HFCD group

was administered to these rats, the decreased ATRAP expression was restored and steatosis alleviated (14). In our previous study, SAM was administered to guinea pigs together with HFCD for six weeks and SAM was found to have a preventive effect on NASH (18). In contrast, SAM treatment alone was not effective, but its combination with dilinoleoylphosphatidylcholine (DLPC) prevented CYP2E1 activation, TG accumulation, oxidative stress and fibrotic changes in the liver of rats fed on a high-fat diet (41). However, in these experimental studies, SAM was administered along with different dietary models of NAFLD/NASH in the same period.

In the current study, guinea pigs were given HFCD for 10 weeks and SAM treatment was administered in the last 4 weeks of this treatment. Thus, we investigated whether SAM may show a therapeutic potential in pre-existing

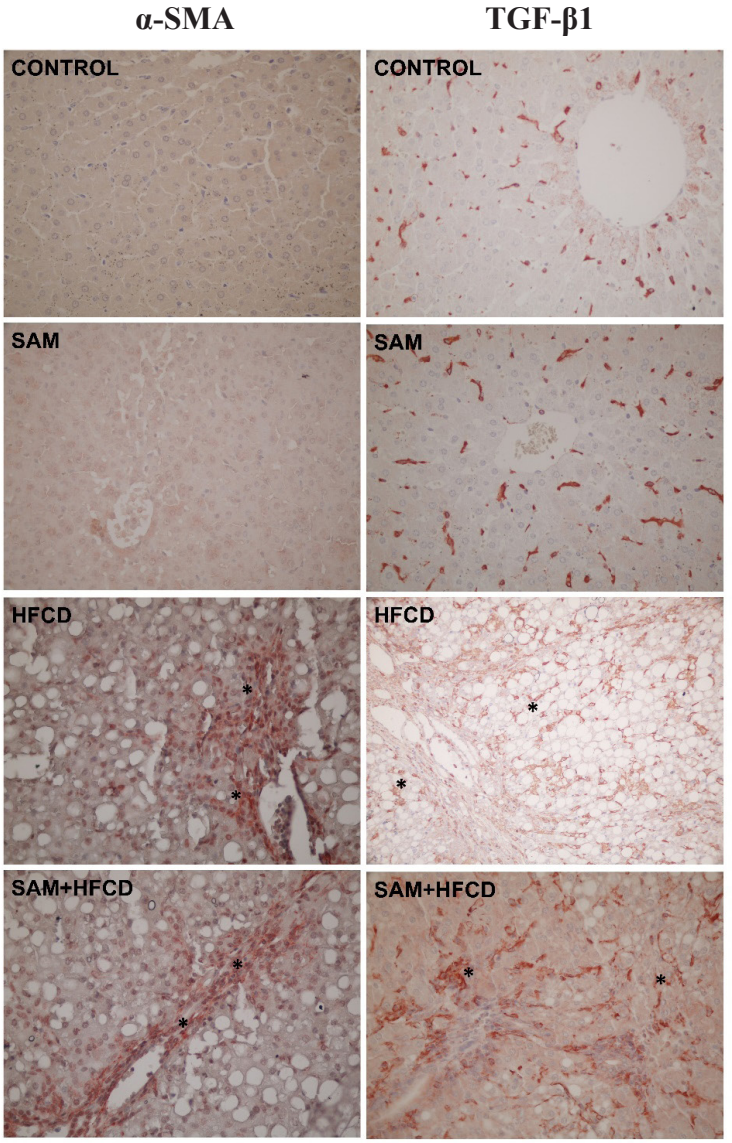

Figure 5: The effect of S-adenosylmethionine treatment on hepatic α-smooth muscle actin and transforming growth factor-β1 protein expression of guinea pigs fed a high-fat/cholesteroldiet (x400). Groups: Control; SAM; HFCD; SAM + HFCD. While no abnormal staining was observed in the control and SAM groups, positive staining was observed in the HFCD and SAM+HFCD groups (asterix). Positive staining was detected in the cells within the fibrous bridging areas between the central vein and the portal region with α-SMA, as well as in the immature mesenchymal cells with the TGF-β1. α-SMA: α-smooth muscle actin HFCD: high fat/cholesterol diet, S-adenosylmethionine, TGF-β1: transforming growth factor- β1

NASH. It was observed that SAM treatment decreased ALT and AST activities, TC, TNF-α and IL-6 levels in serum along with insulin resistance in guinea pigs with NASH. In addition, SAM treatment decreased hepatic TC, TG and Hyp levels and α-SMA and TGF-β1 protein expressions; histopathological improvements were observed with a decrease in steatosis, inflammation and fibrosis scores. Furthermore, a decrease in CYP2E1 levels and improvement in the pro-oxidant-anti-oxidant balance were detected, indicating that SAM effectively reduces oxidative stress in HFCD-induced NASH.

In conclusion, SAM seems to alleviate hepatic injury and metabolic disorders in HFCD-induced NASH by reducing lipogenesis, oxidative stress, inflammation, and fibrosis. In addition to the preventive effect of SAM on NASH observed in our previous study (18), this study demonstrated that SAM may also have therapeutic efficacy.

Ethics Committee Approval: Ethics committee approval was received for this study from the İstanbul University Animal Care and Use Ethics Committee (Date: 02.03.2108, No: 2018/18).

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