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Investigation of the healing effects of Afyon Province hot spring waters on experimentally-induced fatty liver in mice

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SUMMARY

Due the increasing number of alcoholic fatty liver disease cases in the world, the development of methods for treating this disease is an urgent task. According to different publications mineral water from hot springs has a beneficial effect on liver cells. In this regard, an investigation was performed with the aim to assess the effectiveness of hot spring water from the Süreyya I spring (Afyonkarahisar province) in treatment of fatty liver disease. 50 one-day-old albino mice with an average body weight of 29.6 g were selected for the experiment. The tests of liver tissue, biochemical and hematological blood tests, as well as blood gas tests performed at this stage, demonstrated deterioration in all parameters. To prove the effectiveness of using hot spring water in the treatment of alcoholic fatty liver disease, two groups of 25 mice each were formed. The animals of the control group were given tap water to drink, and were also bathed (daily) in it for one hour. The mice of the experimental group were given the hot spring water to drink and bathed in it for 15 minutes every day. Histological tests and blood tests were performed on day 1, 7, 14, and 21 of the experiment using five animals randomly selected from each group. On day 21 of the experiment, the animals of the experimental group demonstrated a significant ($p < 0.05$) decrease in the total number of leukocytes, neutrophils, monocytes, as well as in the levels of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, low-density lipoproteins, total cholesterol, triglycerides. There was also an increase in erythrocyte, hemoglobin, hematocrit, total protein, albumin and high density lipoprotein levels ($p < 0.05$). The results of histopathological analysis also demonstrated positive dynamics. At the same time, no pronounced positive dynamics was observed in animals of the control group. Moreover, microscopy of liver samples showed an ongoing process of tissue degeneration. The data obtained allow us to conclude that it is advisable to use the hot spring water for the treatment of alcoholic fatty liver disease.

Key words: balneotherapy, biochemistry, hematology, histopathology, fatty liver.

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Исследование лечебного воздействия вод термальных источников провинции Афьон на экспериментально индуцированную жировую дистрофию печени у мышей

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РЕЗЮМЕ

В связи с возрастающим количеством случаев развития алкогольной жировой дистрофии печени в мире актуальной задачей является разработка методов лечения этого заболевания. Из литературных данных известно, что минеральная вода из термальных источников оказывает благотворное влияние на клетки печени. В связи с этим было проведено исследование по оценке эффективности применения термальной воды из источника Süreyya I (провинция Афьонкарахисар) при лечении жировой дистрофии печени. Для эксперимента были отобраны 50 мышей-альбиносов суточного возраста со средней массой тела 29,6 г. Через шесть недель применения этилового спирта у мышей сформировалось ожирение печени. Проведенные на этом этапе исследования тканей печени, биохимический и гематологический анализы крови, а также анализ газового состава демонстрировали ухудшение всех показателей. Для доказательства эффективности применения термальной воды при лечении алкогольной жировой дистрофии печени были сформированы две группы по 25 мышей в каждой. Животных контрольной группы выпаивали водопроводной водой, а также устраивали из нее ежедневные часовые ванны. Мышей опытной группы поили термальной водой, а также купали в ней по 15 мин каждый день. Гистологические исследования и анализы крови проводили на 1, 7, 14 и 21-е сут эксперимента у 5 произвольно выбранных из каждой группы животных. На 21-е сут исследования у животных опытной группы наблюдалось достоверное ($p < 0,05$) снижение общего количества лейкоцитов, нейтрофилов, моноцитов, а также уровней аспартатаминотрансферазы, аланинаминотрансферазы, гамма-глутамилтрансферазы, липопротеинов низкой плотности, концентрации общего холестерина, триглицеридов. Также наблюдалось увеличение уровней эритроцитов, гемоглобина, гематокрита, общего белка, альбумина и липопротеинов высокой плотности ($p < 0,05$). Положительная динамика наблюдалась также по результатам гистопатологического анализа. В то же время у животных контрольной группы ярко выраженной положительной динамики не наблюдалось. Более того, микроскопия проб печени показала продолжающийся процесс дегенерации тканей. Полученные данные позволяют сделать вывод о целесообразности применения термальной воды для лечения алкогольной жировой дистрофии печени.

Ключевые слова: бальнеотерапия, биохимия, гематология, гистопатология, жировая дистрофия печени.

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INTRODUCTION

Fat accumulation exceeding 5% of the total liver mass is called fatty liver disease (FLD) and its incidence is increasing rapidly all over the world [1]. Along with that genetic factors are thought to have impact on the development of the disease, the most important risk factor of alcoholic fatty liver disease (AFLD) is excessive alcohol consumption (> 20 g/day) [2]. Alcohol increases the uptake of fats to liver from the intestine and fatty acid synthesis (lipogenesis) and reduces the digestion of fatty acids (by reducing the beta oxidation of fatty acids), causes fat accumulation in liver cells and ultimately causes AFLD [1, 3]. AFLD can lead to firstly hepatitis and cirrhosis lately [4], and even cancer [5].

During AFLD, hematological, biochemical and histopathological changes are taking place. Alcohol increases the translocation of bacteria from the intestine and leads to increased uptake of bacterial lipopolysaccharides that cause inflammation by activation of Kupffer cells [6]. Therefore, leukocytosis and thrombocytopenia occur frequently in patients with alcoholic steatohepatitis [7]. The most common biochemical finding is elevated transaminases and 2–4 times increase in AST and ALT levels may be seen [8]. The most important histopathological finding of the FDL is the presence of fat vacuoles in hepatocytes in microvesicular or macrovesicular form, or both [9].

It has been reported that hot spring baths contribute significantly to the prevention of hepatitis supporting the incidence of chronic hepatitis by decreasing the portal venous pressure, drinking hot spring water reduces fattening in the liver, stabilizing carbohydrate and lipid metabolism and preventing the progression of pathological process [3].

This study was conducted to determine the efficiency of drinking and bathing applications of Süreyya I hot spring water containing many minerals and compounds that have proven therapeutic efficiency in experimental FLD mice in the treatment of FLD

MATERIALS AND METHODS

Experimental part of this study was made in Experimental Animals Application and Research Center of Afyon Kocatepe University and conducted in accordance with Afyon Kocatepe University Experimental Animals Ethics Committee Instructions (AKUHADYEK) under the report with reference number 42-18 and was supported as Master's Thesis Project by Afyon Kocatepe University Scientific Research Projects Committee (BAPK) under the number 18.SAĞ.BİL.11.

In this research project, 50 Albino mice of the same daily age were used. The animals were kept in plastic cages in a stable environment with equal humidity and heat conditions for 12 hours night and 12 hours day at Afyon Kocatepe University Experimental Animals Application and Research Center. During the study, animals were allowed to receive *ad libitum* rat feed.

Six weeks after alcoholic fatty liver procedure was applied in all animals [10], 50 mice which have same body weight average and constitute the study material were divided into two groups as control group (CG) ($n = 25$) and

Table 1
Weight gain, bouyancy and presence of lesions at the stage of before study, after fatty liver disease formation and after treatment in the animals

Таблица 1
Прибавка в весе, самочувствие и наличие поражений до исследования, после ожирения печени и проведенного лечения животных

Time of indicator measurement by groups		Weight gain or loss (g)	Bouyancy / Clinical lesion formation
BS ($n = 50$)		29.6[b] (25.4–32.5)	Bouyancy and appetency (+++), lesion (–)
AFLF ($n = 44$)		31.6[a] (26.3–32.1)	Bouyancy and appetency (+++), 2 mice dead, 4 mice were operatively exed for examinations, lesion (–)
AT 1st day	CG ($n = 21$)	31.5[a] (26.3–32.0)	Bouyancy and appetency (+++), 1 mouse dead, 5 mice were operatively exed for examinations, lesion (–)
	SG ($n = 22$)	31.70[a] (26.4–32.1)	Bouyancy and appetency (+++), 5 mice were operatively exed for examinations, lesion (–)
AT 7th day	CG ($n = 15$)	31.26[a] (26.3–31.64)	Bouyancy and appetency (+++), 5 mice were operatively exed for examinations, lesion (–)
	SG ($n = 15$)	30.02[b] (24.2–30.4)	Bouyancy and appetency (+++), 1 mouse dead, 5 mice were operatively exed for examinations, lesion (–)
AT 14th day	CG ($n = 9$)	30.64[ab] (25.6–31.0)	Bouyancy and appetency (+++), 1 mouse dead, 5 mice were operatively exed for examinations, lesion (–)
	SG ($n = 10$)	30.12[b] (24.0–30.2)	Bouyancy and appetency (+++), 5 mice were operatively exed for examinations, lesion (–)
AT 21st day	CG ($n = 4$)	30.23[b] (25.6–31.0)	Bouyancy and appetency (+++), 4 mice were operatively exed for examinations, lesion (–)
	SG ($n = 5$)	28.02[c] (23.1–28.9)	Bouyancy and appetency (+++), 5 mice were operatively exed for examinations, lesion (–)

[a–c] The values in the column are statistically significant ($p < 0.05$).
[a–c] Значения в столбце являются статистически значимыми ($p < 0,05$).
BS – before study (до исследования), AFLF – after fatty liver formation (после ожирения печени), AT – after treatment (после лечения), CG – control group (контрольная группа), SG –study group (опытная группа).

study groups (SG) ($n = 25$) for a 21-day treatment period equally and randomly. Normal tap water was added to the CG mice' drinkers, while the hot spring water that was brought from the source each day as fresh added to SG mice' drinkers and they were allowed to reach *ad libitum*. Also, CG mice were bathed in the (35 ± 2) °C tap water as same hour every day, SG mice were bathed in the same temperature with fresh hot spring water for 15 minutes, they were dried with a soft towel after bath, blow dryer was installed gently and then they were put in their cages. In the treatment stage, five animals randomly selected after clinical examination were made on 1, 7, 14 and 21 days after treatment in all of the CG and SG group animals (under xylazine (10 mg/kg) and ketamine (100 mg/kg) anesthesia) and blood and liver tissue samples were taken by intracardiac method for hematological, blood biochemical parameters, blood gases analysis and histopathological examinations [11].

Characteristics of Süreyya I Hot Spring: Süreyya I Hot Spring Water which is volcanic spring and has the property of being the only carbon dioxide water of the region, has been reported as sodium bicarbonate, carbon dioxide, fluoride and silicon thermomineral water class by İstanbul University Çapa Medical Faculty Department of Medical Ecology and Hydroclimatology and mineralization in total has been reported as 4046.8 mg/L by İzmir Community Health Laboratory. Although not specified in this report, it is also accepted in calcium water class since its calcium content is > 150 mg/L.

Clinical Examinations. The body weight (T), mobilization, feed and water consumption, whether they developed lesions of mice were examined and body temperatures, heart frequency (P) and respiratory rate (R) of the animals were measured at the determined measurement times.

Hematological Examinations. Hematological parameters such as erythrocyte (RBC), total leukocytes (WBC), hematocrit (HCT), hemoglobin (HB), mean corpusculer volume (MCV), mean corpusculer hemoglobin concentration (MCHC), lymphocyte (LYM), neutrophil (NEUT), eosinophil (EOS), monocyte (MON) and basophil (BAS) were determined using by commercial test kits and Chemray Brand blood counting device.

Blood Biochemical Examinations. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triglyceride (TRIG), total cholesterol (TCHOL), high density lipoprotein (HDL), low density lipoprotein (LDL) and magnesium (Mg) levels were measured on the Cobas Integra

400 Plus Roche Brand analyzer (Roche Diagnostics GmbH, Germany).

Blood Gases Analyses. After blood samples were taken to plastic syringes with heparin supplemented as 500 IU liquid heparin for 1 ml of blood prepared previously, pH, partial carbon dioxide pressure (pCO_2), total carbon dioxide concentration (TCO_2), base excess (BE), bicarbonate (HCO_3^-), chlorine (Cl^-), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) measurements were carried out on portable blood gas analyzer (Edan i15 Veterinary blood gas analyzer) by using commercial cartridges.

Histopathological Examinations. Liver samples were taken under anesthesia of ketamine/xylazine (10 mg/kg/100 mg/kg) [11] one week interval from 5 animals randomly selected from both groups at the determined measurement times. Samples were sent to the relevant laboratories in 10% formol. Histopathological examinations were carried out by experts at Veterinary Control Center Research Institute, Pathology Laboratory in T.R. Ministry of Agriculture and Forestry.

Statistical Analyses. Statistical analyzes of the groups were made according to ANOVA method. Duncan test was used to determine the importance of intragroup differences in the study group. Statistical analyzes were performed using Windows compatible SPSS 18.1 (Inc., Chicago, Il, USA) software. Data were expressed as mean ± standard error and $p < 0.05$ was considered significant.

TEST RESULTS

In this study, sex was not considered a factor in the statistical analysis of the data.

Clinical Findings

Clinical findings of animals are shown in Tables 1 and 2.

When Table 1 is examined, the body weight (bw) averages of the CG mice was 31.5 g (min 26.3, max 32.0) in the weighings performed at the beginning of the treatment phase and the bw averages of the SG mice was 31.7 g (min 26.4, max 32.1) and it was observed that there was no statistical difference in terms of bw ($p > 0.05$). In bw measurements on the 21st day of the study, mean SG animals were found to be statistically significant lower than the CG ($p < 0.05$). When Table 2 is examined, along with there was no statistically significant difference in terms of T in the measured time periods ($p > 0.05$), when compared with the pre-study, it was found that P and R frequencies showed significant ($p < 0.05$) differences in terms of average after fattening formation, and it was found that these averages were significantly higher in CG animals ($p < 0.05$) compared to SG animals during the treatment process.

Hematological Findings

The hematological examination findings of CG and SG animals are shown in Table 3.

When the Table 3 was examined, it was observed that WBC, NEUT, MON, MCV, MCH levels increased significantly after fattening ($p < 0.05$), whereas RBC, HB, HCT, LYM, MCHC and PLT levels were significantly ($p < 0.05$) decreased. In the post-treatment comparisons, WBC, NEUT, MON, MCV and MCH averages in SG animals were lower ($p < 0.05$) and RBC, HB, HCT, LYM and PLT levels were higher ($p < 0.05$).

Blood Biochemical Findings

The averages of blood biochemical analysis findings are shown in Table 4. It was determined that AST, ALT, GGT, UREA, CREA, TCHOL, TRIG and LDL levels increased significantly ($p < 0.05$) after fattening and TP, ALB, HDL, GLU and Mg levels decreased significantly ($p < 0.05$). Although

Table 2
Statistical comparison of body temperature, pulse frequency and respiratory rate

Таблица 2
Статистическое сравнение температуры тела, частоты пульса и частоты дыхания

Time of indicator measurement by groups		Parameters (X ± SD)		
		T (°C)	P (frequency/min)	R (rate/min)
BS ($n = 50$)		37.20 ± 0.14	341.44 ± 45.10^{f}	126.30 ± 20.00^{e}
AFLF ($n = 44$)		37.40 ± 0.18	432.28 ± 65.30^{a}	240.14 ± 35.00^{a}
AT 1st day	CG ($n = 21$)	37.40 ± 0.10	413.04 ± 53.40^{b}	238.28 ± 33.10^{a}
	SG ($n = 22$)	37.30 ± 0.12	392.05 ± 47.10^{c}	221.24 ± 32.00^{b}
AT 7th day	CG ($n = 15$)	37.30 ± 0.00	402.12 ± 45.30^{bc}	229.00 ± 25.00^{ab}
	SG ($n = 15$)	37.10 ± 0.00	376.18 ± 38.20^{d}	178.10 ± 24.10^{c}
AT 14th day	CG ($n = 9$)	37.20 ± 0.20	398.47 ± 32.00^{bc}	180.34 ± 24.00^{c}
	SG ($n = 10$)	37.10 ± 0.10	360.40 ± 28.40^{e}	145.18 ± 20.00^{d}
AT 21st day	CG ($n = 4$)	37.20 ± 0.12	374.10 ± 35.20^{d}	150.30 ± 22.00^{d}
	SG ($n = 5$)	37.00 ± 0.14	337.18 ± 24.30^{f}	130.34 ± 16.10^{e}

$^{a–f}$The values in the column are statistically significant ($p < 0.05$).

$^{a–f}$Значения в столбце являются статистически значимыми ($p < 0,05$).

BS – before study (до исследования), AFLF – after fatty liver formation (после ожирения печени), AT – after treatment (после лечения), CG – control group (контрольная группа), SG –study group (опытная группа).

Table 3
Results of hematology blood tests

Таблица 3
Результаты гематологических исследований крови животных

Time of indicator measurement by groups		Parameters (X ± SD)												
		WBC (10^3/mm^3)	RBC (10^6/mm^3)	HB (g/dl)	HCT (%)	PLT (10^3/mm^3)	MCV (fl)	MCH (pg)	MCHC (g/dl)	LYM (%)	NEUT (%)	EOS (%)	MON (%)	BAS (%)
BS ($n = 50$)		8.30 ± 1.40^{f}	7.93 ± 1.16^{a}	13.42 ± 1.24^{a}	43.23 ± 1.24^{a}	284.90 ± 35.24^{a}	53.48 ± 0.26^{g}	16.48 ± 3.06^{c}	31.18 ± 2.03^{a}	61.42 ± 5.20^{a}	33.30 ± 3.20^{f}	2.10 ± 1.00	3.40 ± 0.20^{d}	NS
AFLF ($n = 44$)		15.40 ± 3.20^{a}	4.48 ± 0.43^{d}	8.27 ± 3.43^{d}	32.48 ± 3.22^{ef}	186.24 ± 32.08^{e}	72.21 ± 5.14^{a}	18.21 ± 6.11^{a}	25.41 ± 3.12^{d}	47.68 ± 6.40^{f}	45.20 ± 4.10^{a}	2.14 ± 0.10	6.30 ± 1.60^{a}	NS
AT 1st day	CG ($n = 21$)	15.28 ± 3.10^{a}	4.62 ± 0.64^{d}	8.26 ± 2.05^{d}	31.98 ± 3.06^{f}	184.38 ± 47.86^{e}	68.36 ± 4.04^{b}	17.87 ± 4.23^{b}	25.56 ± 3.11^{d}	48.26 ± 5.30^{ef}	45.10 ± 4.30^{a}	2.23 ± 0.12	5.20 ± 1.23^{b}	NS
	SG ($n = 22$)	14.06 ± 2.30^{bc}	5.83 ± 0.57^{c}	9.54 ± 2.28^{c}	33.46 ± 2.68^{e}	198.12 ± 55.46^{de}	57.14 ± 4.08	16.15 ± 3.22^{c}	28.51 ± 3.24^{c}	52.18 ± 4.40^{d}	42.20 ± 3.60^{b}	2.16 ± 0.04	4.10 ± 1.22^{c}	NS
AT 7th day	CG ($n = 15$)	14.40 ± 2.34^{b}	5.38 ± 1.10^{c}	8.90 ± 2.11^{d}	32.88 ± 2.27^{e}	186.27 ± 38.22^{e}	61.03 ± 4.28^{c}	16.37 ± 2.11^{c}	27.12 ± 2.23^{c}	49.27 ± 5.10^{e}	44.40 ± 3.00^{a}	2.16 ± 0.20	5.00 ± 1.32^{b}	NS
	SG ($n = 15$)	13.34 ± 2.16^{c}	6.80 ± 1.24^{b}	10.38 ± 2.00^{bc}	37.40 ± 2.06^{c}	214.45 ± 33.26^{c}	55.23 ± 3.32^{e}	15.32 ± 2.08^{d}	27.70 ± 2.03^{c}	55.18 ± 4.34^{c}	40.20 ± 2.30^{c}	2.40 ± 1.40	3.04 ± 1.20^{d}	NS
AT 14th day	CG ($n = 9$)	13.40 ± 2.04^{c}	5.79 ± 1.23^{c}	9.41 ± 2.08^{c}	33.29 ± 2.08^{e}	196.02 ± 27.62^{de}	57.22 ± 3.20^{d}	16.21 ± 1.24^{c}	28.04 ± 1.16^{c}	51.24 ± 4.10^{d}	42.50 ± 2.18^{b}	2.18 ± 0.06	5.69 ± 1.42^{ab}	NS
	SG ($n = 10$)	10.28 ± 1.54^{e}	7.86 ± 1.02^{a}	11.27 ± 1.68^{b}	40.16 ± 1.43^{b}	248.27 ± 28.48^{b}	51.23 ± 2.35^{f}	14.25 ± 1.00^{e}	28.14 ± 0.68^{c}	57.04 ± 3.28^{b}	36.28 ± 2.00^{e}	2.15 ± 0.00	4.03 ± 0.50^{c}	NS
AT 21st day	CG ($n = 4$)	12.04 ± 2.18^{d}	6.23 ± 1.16^{b}	9.87 ± 1.24	35.87 ± 1.66^{d}	201.26 ± 24.32^{d}	57.52 ± 2.22^{d}	15.60 ± 0.55^{d}	27.43 ± 0.55^{c}	54.16 ± 4.24^{c}	38.14 ± 2.10^{d}	2.16 ± 0.00	5.10 ± 0.40^{b}	NS
	SG ($n = 5$)	9.02 ± 1.23^{f}	7.98 ± 1.18^{a}	12.98 ± 1.18^{a}	43.07 ± 1.18^{a}	279.84 ± 21.16^{a}	53.86 ± 1.16^{g}	16.21 ± 0.43^{c}	30.04 ± 0.38^{b}	61.80 ± 2.40^{a}	33.12 ± 1.60^{f}	2.10 ± 0.20	3.28 ± 0.30^{d}	NS

[a-g] The values in the column are statistically significant ($p < 0.05$).

[a-g] Значения в столбце являются статистически значимыми ($p < 0,05$).

BS – before study (до исследования), AFLF – after fatty liver formation (после ожирения печени), AT – after treatment (после лечения),
CG – control group (контрольная группа), SG –study group (опытная группа), NS – non-significant (не значимо).
WBC –white blood cells (лейкоциты), RBC – red blood cells (эритроциты), HB – hemoglobin (гемоглобин),
HCT – hematocrit (гематокрит), PLT – platelets (тромбоциты), MCV – mean corpusculare volume (средний объем эритроцитов),
MCH – mean corpuscular hemoglobin (среднее содержание гемоглобина в эритроците),
MCHC – mean corpuscular hemoglobin concentration (средняя концентрация гемоглобина в эритроците),
LYM – lymphocyte (лимфоциты), NEUT – neutrophils (нейтрофилы), EOS – eosinophils (эозинофилы),
MON – monocyte (моноциты), BAS – basophils (базофилы).

Table 4
Blood biochemical findings of the animals

Таблица 4
Результаты биохимических исследований крови животных

Time of indicator measurement by groups		Parameters (X ± SD)													
		AST (IU/L)	ALT (IU/L)	GGT (IU/L)	TP (g/dl)	ALB (g/dl)	GLU (g/dl)	Mg (mmol/L)	TLPD (mg/dl)	TCHOL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TRIG (mg/dl)	CREA (mg/dl)	UREA (mg/dl)
BS ($n = 50$)		94.62 ± 4.24	58.68 ± 6.18^{f}	3.55 ± 0.14^{e}	55.74 ± 6.18^{a}	34.14 ± 0.48^{a}	1.68 ± 0.12^{a}	1.63 ± 0.18^{a}	218.44 ± 23.28^{e}	88.71 ± 8.04^{f}	42.82 ± 6.40^{a}	68.24 ± 17.32^{e}	95.12 ± 15.1^{e}	0.58 ± 0.05^{f}	8.65 ± 3.18^{e}
AFLF ($n = 44$)		248.74 ± 16.14^{a}	165.84 ± 14.23^{a}	8.68 ± 2.13^{a}	36.04 ± 5.32^{de}	22.02 ± 0.54^{g}	0.89 ± 0.24^{e}	0.64 ± 0.04^{d}	459.48 ± 45.26^{a}	176.18 ± 46.12^{a}	23.28 ± 9.23^{e}	136.28 ± 23.44^{a}	207.25 ± 34.12^{a}	3.64 ± 1.05^{a}	15.42 ± 6.27^{a}
AT 1st day	CG ($n = 21$)	243.12 ± 14.27^{a}	167.49 ± 15.16^{a}	8.45 ± 0.54^{a}	35.42 ± 6.04^{e}	23.32 ± 0.48^{f}	0.90 ± 0.20^{e}	0.65 ± 0.16^{d}	458.21 ± 47.14^{a}	174.24 ± 48.13^{a}	23.31 ± 8.65^{e}	135.45 ± 25.16^{a}	206.43 ± 35.21^{a}	3.63 ± 1.12^{a}	15.32 ± 7.12^{a}
	SG ($n = 22$)	242.26 ± 16.23^{a}	162.47 ± 16.32^{a}	8.32 ± 0.47^{a}	37.10 ± 7.23^{d}	24.04 ± 0.46^{ef}	0.91 ± 0.19^{e}	0.68 ± 0.22^{d}	456.03 ± 48.18^{a}	173.45 ± 45.71^{a}	24.12 ± 9.27^{e}	134.12 ± 23.04^{a}	203.18 ± 37.14^{a}	3.62 ± 1.16^{a}	15.04 ± 6.45^{a}
AT 7th day	CG ($n = 15$)	222.18 ± 14.43^{b}	151.48 ± 14.30^{b}	8.18 ± 0.36^{a}	38.18 ± 5.44^{cd}	25.18 ± 0.43^{e}	0.94 ± 0.18^{e}	0.73 ± 0.12^{d}	432.27 ± 35.86^{b}	167.16 ± 38.27^{ab}	25.16 ± 12.21^{e}	130.28 ± 21.25^{ab}	202.28 ± 25.34^{a}	3.54 ± 1.04^{a}	14.03 ± 4.16^{b}
	SG ($n = 15$)	187.74 ± 15.65^{d}	132.61 ± 13.26^{c}	7.44 ± 0.28^{b}	41.32 ± 4.66^{c}	28.15 ± 0.34^{cd}	1.28 ± 0.14^{c}	0.92 ± 0.04^{bc}	345.27 ± 27.21^{d}	122.05 ± 27.34^{d}	35.48 ± 11.51^{c}	112.31 ± 15.14^{c}	178.27 ± 18.23^{b}	2.14 ± 0.64^{c}	11.21 ± 3.09^{d}
AT 14th day	CG ($n = 9$)	204.18 ± 12.32^{c}	141.74 ± 14.25^{bc}	7.69 ± 0.24^{b}	39.23 ± 5.45^{cd}	27.64 ± 0.33^{d}	1.02 ± 0.16^{d}	0.86 ± 0.03^{c}	416.25 ± 31.14^{c}	160.21 ± 28.12^{b}	27.28 ± 10.21^{de}	126.05 ± 14.48^{b}	184.24 ± 19.40^{ab}	2.72 ± 1.02^{b}	12.36 ± 3.07^{c}
	SG ($n = 10$)	161.28 ± 11.43^{f}	106.43 ± 9.46^{e}	5.03 ± 0.12^{d}	47.08 ± 3.46^{b}	31.82 ± 0.35^{b}	1.48 ± 0.13^{b}	1.20 ± 0.04^{b}	276.28 ± 22.21^{e}	106.12 ± 11.22^{e}	40.14 ± 7.22^{b}	83.26 ± 7.41^{d}	130.34 ± 12.22^{d}	0.96 ± 0.27^{e}	9.01 ± 1.16^{e}
AT 21st day	CG ($n = 4$)	183.78 ± 10.44^{e}	129.75 ± 8.55^{d}	6.65 ± 0.11^{c}	42.21 ± 4.33^{c}	29.18 ± 0.26^{c}	1.06 ± 0.09^{d}	0.92 ± 0.03^{bc}	398.00 ± 13.15^{c}	144.21 ± 12.23^{c}	30.16 ± 8.13^{d}	108.07 ± 8.10^{c}	158.21 ± 13.11^{c}	1.48 ± 0.15^{d}	11.27 ± 1.20^{d}
	SG ($n = 5$)	114.76 ± 9.20^{g}	62.48 ± 5.42^{f}	3.88 ± 0.07^{e}	55.19 ± 2.24^{a}	33.18 ± 0.17^{ab}	1.69 ± 0.05^{a}	1.64 ± 0.01^{a}	203.28 ± 11.23^{e}	87.18 ± 5.03^{f}	43.02 ± 5.14^{a}	67.32 ± 6.13^{e}	101.20 ± 9.20^{e}	0.60 ± 0.04^{f}	8.42 ± 0.22^{e}

$^{a-g}$ The values in the column are statistically significant ($p < 0.05$).

$^{a-g}$ Значения в столбце являются статистически значимыми ($p < 0,05$).

BS – before study (до исследования), AFLF – after fatty liver formation (после ожирения печени), AT – after treatment (после лечения),
CG – control group (контрольная группа), SG –study group (опытная группа).
AST – aspartate transaminase (аспартатаминотрансфераза), ALT – alanine aminotransferase (аланинаминотрансфераза),
GGT – gamma-glutamyl transferase (гамма-глутамилтрансфераза), TP – total protein (общий белок), ALB – albumin (альбумин),
GLU – glucose (глюкоза), Mg – magnesium (магний), TLPD – total lipids (общие липиды), TCHOL – total cholesterol (общий холестерин),
HDL – high-density lipoproteins (липопротеины высокой плотности), LDL – low density lipoprotein (липопротеины низкой плотности),
TRIG – triglycerides (триглицериды), CREA – creatinine (креатинин), UREA – мочевина.

Table 5
Blood gases findings of the animals

Таблица 5
Результаты исследования газового состава крови

Time of indicator measurement by groups		Parameters (X ± SD)									
		pH	pCO_2 (mmHg)	HCO_3^- (mmol/L)	BE (mEq/L)	TCO_2 (mmol/L)	LACT (mmol/L)	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	Ca^{2+} (mmol/L)
BS ($n = 50$)		7.35 ± 0.02^{a}	43.18 ± 0.32^{a}	23.54 ± 0.36^{a}	-2.0 ± 0.02^{a}	15.32 ± 0.44^{a}	4.45 ± 0.58^{cd}	8.48 ± 1.05^{a}	157.40 ± 1.42^{e}	113.29 ± 1.35^{h}	10.31 ± 1.33^{a}
AFLF ($n = 44$)		7.24 ± 0.03^{f}	35.64 ± 1.33^{e}	14.86 ± 0.26^{g}	-11.4 ± 0.04^{f}	12.27 ± 2.30^{f}	6.68 ± 1.27^{a}	5.34 ± 1.26^{d}	243.70 ± 12.18^{a}	187.30 ± 9.20^{a}	6.28 ± 1.32^{c}
AT 1st day	CG ($n = 21$)	7.24 ± 0.03^{f}	35.19 ± 1.50^{e}	14.64 ± 0.50^{g}	-11.6 ± 0.04^{f}	12.05 ± 2.22^{f}	6.60 ± 1.23^{a}	5.19 ± 1.27^{d}	234.28 ± 11.20^{a}	183.32 ± 9.22^{a}	6.43 ± 1.04^{c}
	SG ($n = 22$)	7.27 ± 0.02^{e}	33.62 ± 1.34^{e}	14.94 ± 0.38^{f}	-10.6 ± 0.03^{e}	11.61 ± 1.40^{e}	5.88 ± 1.41^{b}	5.12 ± 1.16^{d}	202.27 ± 7.14^{b}	171.40 ± 5.30^{b}	7.28 ± 0.64^{d}
AT 7th day	CG ($n = 15$)	7.26 ± 0.01^{e}	34.19 ± 1.45^{d}	14.13 ± 0.30^{b}	-10.8 ± 0.03^{e}	11.70 ± 1.35^{a}	6.18 ± 1.40^{b}	5.23 ± 1.02^{d}	217.26 ± 5.40^{ab}	166.18 ± 6.23^{c}	7.01 ± 0.55^{b}
	SG ($n = 15$)	7.35 ± 0.02^{cd}	38.65 ± 1.31^{c}	14.85 ± 0.27^{c}	-10.8 ± 0.03^{c}	12.08 ± 0.34^{c}	4.94 ± 0.52^{c}	6.46 ± 0.78^{c}	174.32 ± 4.13^{c}	139.18 ± 3.42^{d}	9.27 ± 0.36^{b}
AT 14th day	CG ($n = 9$)	7.30 ± 0.02^{d}	35.24 ± 1.38^{cd}	20.63 ± 0.20^{e}	-4.2 ± 0.01^{d}	12.62 ± 0.28^{d}	5.86 ± 0.78^{b}	5.88 ± 0.64^{cd}	204.24 ± 4.10^{ab}	145.20 ± 3.36^{e}	8.45 ± 0.43^{c}
	SG ($n = 10$)	7.38 ± 0.02^{b}	41.07 ± 0.33^{b}	23.52 ± 0.22^{b}	-1.2 ± 0.01^{b}	14.70 ± 0.26^{b}	4.67 ± 0.28^{c}	7.38 ± 0.45^{b}	158.22 ± 2.46^{d}	123.28 ± 1.60^{g}	10.12 ± 0.26^{a}
AT 21st day	CG ($n = 4$)	7.32 ± 0.01^{d}	36.19 ± 0.37^{c}	18.02 ± 0.10^{d}	-7.1 ± 0.02^{d}	12.66 ± 0.21^{c}	5.15 ± 0.36^{bc}	6.78 ± 0.36^{c}	179.21 ± 2.76^{c}	134.26 ± 0.43^{f}	9.02 ± 0.17^{b}
	SG ($n = 5$)	7.42 ± 0.01^{a}	43.82 ± 0.03^{a}	27.51 ± 0.12^{a}	-3.1 ± 0.01^{a}	16.01 ± 0.12^{a}	4.12 ± 0.18^{d}	8.68 ± 0.19^{a}	152.27 ± 2.33^{d}	111.28 ± 0.31^{h}	10.89 ± 0.14^{a}

[a-h] The values in the column are statistically significant ($p < 0.05$).

[a-h] Значения в столбце являются статистически значимыми ($p < 0,05$).

BS – before study (до исследования), AFLF – after fatty liver formation (после ожирения печени), AT – after treatment (после лечения), CG – control group (контрольная группа), SG – study group (опытная группа).

pH – hydrogen ion concentration (концентрация ионов водорода), pCO_2 – CO_2 partial pressure (парциальное давление углекислого газа), HCO_3^- – bicarbonate (бикарбонат), BE – base excess (сдвиг буферных оснований), TCO_2 – total CO_2 (общая концентрация углекислого газа), LACT – lactate (лактат), K^+ – potassium (калий), Na^+ – sodium (натрий), Cl^- – chlorine (хлор), Ca^{2+} – calcium (кальций).

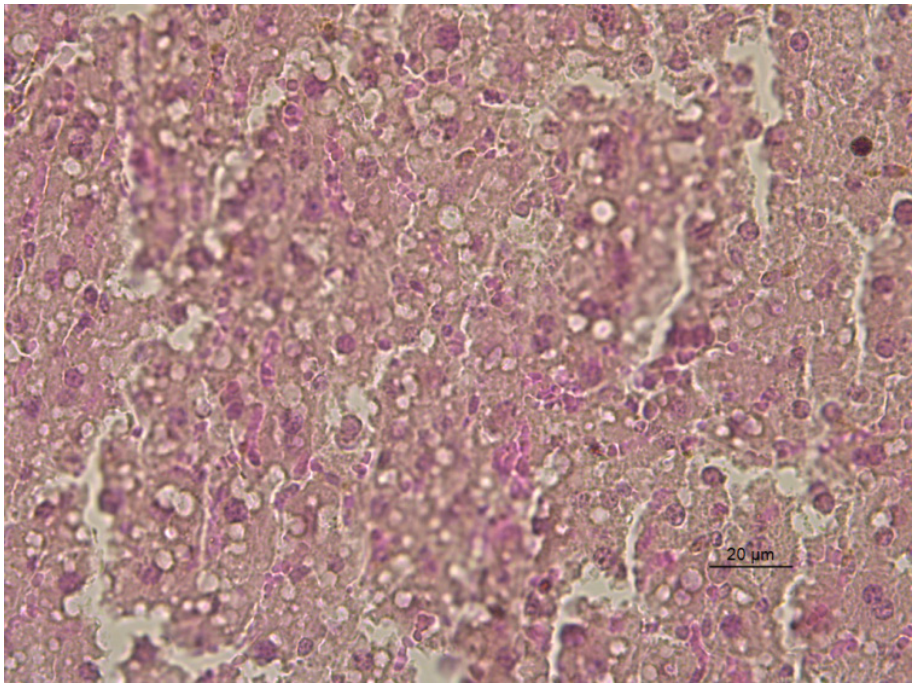

Fig. 1. Formation of fatty liver in mice treated with ethyl alcohol (10×–40×)

Рис. 1. Поражение печени у мышей после применения этилового спирта (увеличение 10×–40×)

TP, ALB, HDL, GLU and Mg levels were increased and AST, ALT, GGT, UREA, CREA, TCHOL, TRIG and LDL levels were decreased in both groups after treatment, in terms of increasing and decreasing parameters, it was observed that the changes in SG animals were statistically significant ($p < 0.05$) than CG animals.

Blood Gases Findings

Statistical comparisons of blood gas analysis results are presented in Table 5 below.

When this table is examined, it was observed that pH, partial CO_2, pCO_2, BE, HCO_3^-, Ca^{2+} and K^+ levels decreased as a result of fatty liver, whereas LACT, sodium Na^+ and Cl^- levels increased, in terms of this increase and decrease in the group comparisons, the highest levels ($p < 0.05$) occurred in SG animals in terms of rate and amount of healing.

Histopathological Findings

Histopathologically, it was found that the fattening was in the form of intrastoplasmic, microvesicular and partly

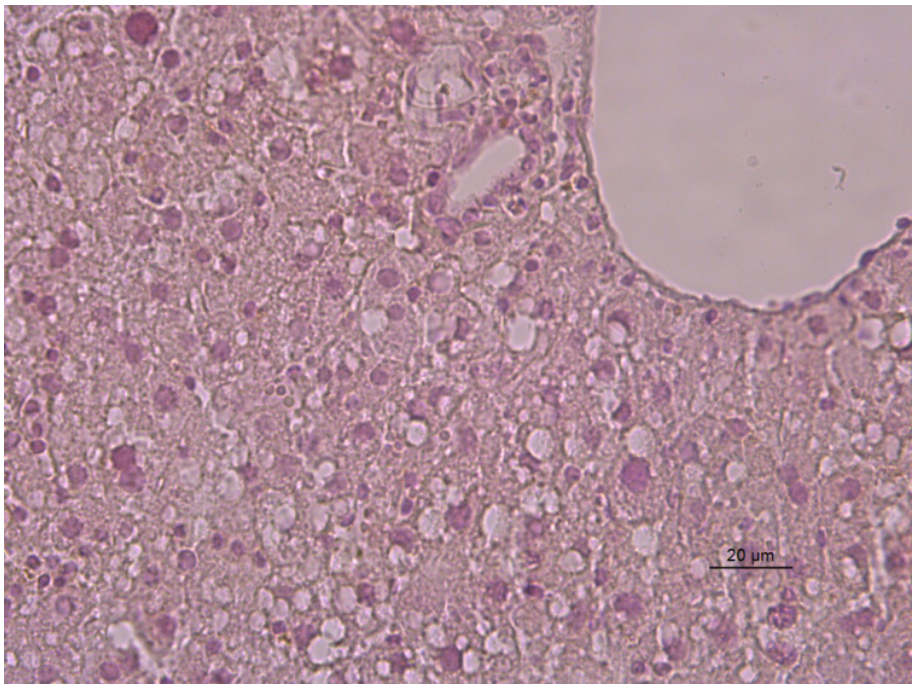

Fig. 2. Ongoing parenchymal degeneration and fattening at the end of day 21 in control animals (10×–40×)

Рис. 2. Паренхиматозная дегенерация печени на 21-е сут у животных контрольной группы (увеличение 10×–40×)

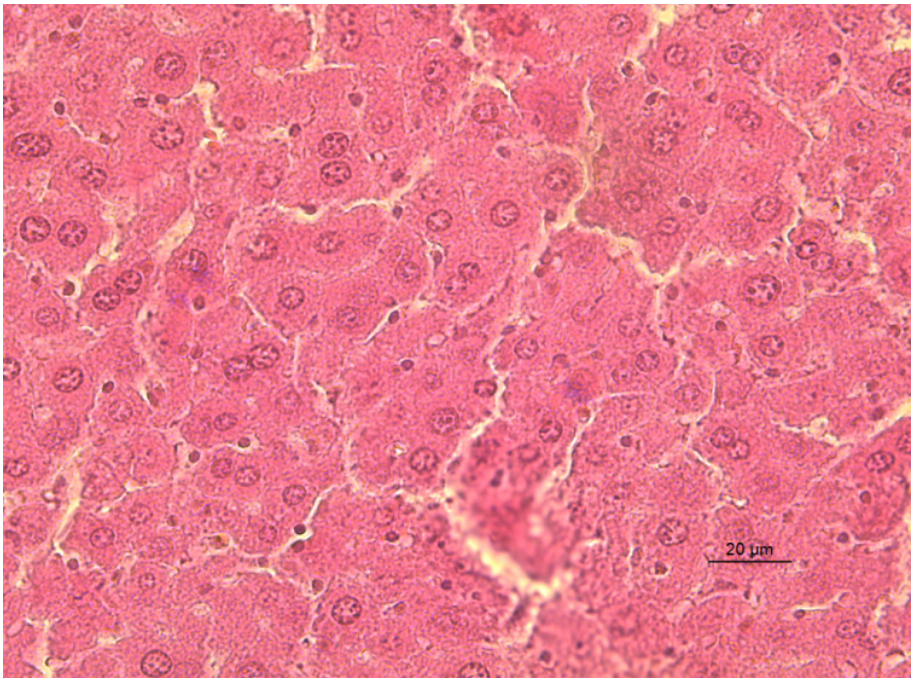

Fig. 3. Normal liver appearance in mice treated with Süreyya I hot spring water at the end of day 21 (10×–40×)

Рис. 3. Внешний вид печени у мышей, получавших воду из термального источника Süreyya I, на 21-е сут исследования (увеличение 10×–40×)

macro-microvescular fattening, and intense parenchymal degeneration and necrosis with severe fattening were partly observed (Fig. 1) after fatty liver formation.

In the comparisons at the end of the 21st day, which is the last day of treatment, it was found that fat formation continued in CG animals treated with tap water (Fig. 2), whereas fat and necrosis cases improved significantly in SG animals treated with hot spring water (Fig. 3). Sections and pictures of each measurement time are archived.

DISCUSSION AND CONCLUSION

It was found that the average bw (31.6 g) after fattening formation was higher compared to the average bw (29.6 g) prior to fattening formation, but the bw average of CG animals (30.23 g) was higher than the average of SG (28.02 g) on the last day of treatment. This finding is consistent with the studies [3] reporting that EtOH causes to weight gain by fastening the liver and that the treatment with hot spring waters leads to weight loss by increasing fat burning and reducing fat intake from the intestines. It was shown that respiratory rate and heart frequencies were statistically significant ($p < 0.05$) in animals whom fatty liver is formed, and these findings were found to be in compliance with the studies indicating that the increased heart rate would be accompanied by increased respiratory frequency [10].

It was observed that WBC, LYM numbers which were initially high, were decreased in the SG animals which drink Süreyya I water and are daily bathed with this water. These findings were found to be compatible with the studies [12] indicating that hot spring waters had an immunosuppressive effect and that T-lymphocytes in blood decreased significantly in hyperthermal baths, and that hyperthermal waters provoked ACTH hormone level and cortisol production and caused T-lymphocytopenia and eosinopenia.

Chronic alcohol consumption has been reported to lead to elevated MCV levels with leukocytosis and thrombocytopenia [13], and leucocytosis and thrombocytopenia have been tended to normal levels upon discontinuation of alcohol consumption [14]. The fact that the lower MCV, WBC and higher PLT levels were obtained in SG animals which drank Süreyya I hot spring water compared to CG and initial measurements fattening, and the that this case reached the most significant level in the last week of treatment support these researches' declarations. Increased MCV has been reported to be important biomarkers of chronic alcohol dependence [15], and high MCV levels accompanied by clinical inflammation syndrome resulting in Mg deficiency in leukocyte and macrophage activation and excessive production of free radicals [16]. Since the Süreyya I hot spring water used in our study is rich in Mg, it is thought that Mg may contribute to the normalization of leukocytosis and high MCV levels by showing anti-inflammatory effect.

In our study, in animals with fatty liver, it was found that TRIG, LDL, TCHOL levels were significantly increased, HDL cholesterol levels were decreased, but in the treatment of animals treated with hot spring water, however, in the SG animals given hot spring water with the beginning of the treatment period, a reverse course was formed in these parameters and the improvement in the lipid profile gradually increased and the best results were obtained in the animals in the SG and in the last week. These findings are consistent with the findings of the researchers [17] who reported that acute or chronic natural use of mineral waters had significant regulatory effects on serum lipid profile. As a matter of fact that, C. L. Hsu et al. [18] reported that rich mineral waters decreased TCHOL levels by increasing fecal cholesterol and bile acid excretion in feces.

The spectacular effects of rich mineral waters on obesity have been revealed by the stimulation mechanism of

mitochondrial genesis and the identification of components controlling energy release from fats. Mg and Ca are the main components that play a role in reducing fat [19]. Süreyya I hot spring is rich in Mg and Ca ions. Mg reduces lipid accumulation due to high cholesterol intake [20]. Y. Kishimoto et al. indicated that Mg uptake also inhibits intestinal fat absorption and may improve postprandial hyperlipidemia in healthy individuals [21]. M. Kimura et al. showed that mineral water containing 600 and 1,000 ppm Mg could reduce cholesterol levels by 18% and 15%, respectively [22]. It was reported that Mg-rich water decreases lipid peroxidation by increasing hepatic low-density lipoprotein receptor and cholesterol-7a-hydroxylase (CYP7A1) gene activation that play a role in cholesterol catabolism, so leads to decrease in TCHOL and LDL levels [23]. However, there are studies that report that Mg-rich mineral waters treat fatty liver by inhibiting cholesterol and fatty acid synthesis by increasing the AMP-activated protein kinase enzyme level [24].

Calcium in the diet prevents adipocyte lipid accumulation and weight gain, increases lipolysis and thus significantly accelerates weight loss. Moreover, it has recently been shown that calcitriols released in response to low calcium diets stimulate Ca flow in human adipocytes and thus support adiposity [25]. In spite of all these positive effects, it has been reported that Mg and Ca do not have an effect alone in reducing fat and other elements contribute to it [26]. HCO_3^- is the leading element of these elements, and water with rich HCO_3^- has been reported to have a reducing effect on total and LDL cholesterol [17]. In our study, Süreyya I hot spring water used for treatment purposes is included in the bicarbonate hot spring water class and has a high concentration of HCO_3^- besides high Mg and Ca levels. As a matter of fact, in the measurements we made, the group with the highest increase in blood HCO_3^- levels was determined to be the SG group and also the lowest levels of TCHOL, LDL and TRIG were obtained in this group. It has also been reported that Cl^- containing bicarbonate water stimulates bile acid excretion and reduces TRIG concentration in the intestine [27].

In our study, it was found that the measured levels of AST, ALT, GGT, UREA and CREA were found to be high in the measurements following the fattening and TP, ALB, GLU, HDL levels were low. With the commencement of the treatment period, in SG animals given Süreyya I hot spring water, a continuous positive improvement was observed in these parameters until the last week of the study when compared with CG animals. Similar findings were found in a study C. Pereira et al. reporting that high levels of AST, ALT, GGT, UREA, CREA decreased in mice with metabolic syndrome, whereas TP, ALB, HDL levels increased [16]. Plasma AST and ALT are considered important markers in the detection of liver damage [28]. I. Chen et al. argued that treatment with high mineral waters reduced lipid accumulation in the liver by increasing daily fecal lipid and bile acid output [29]. Also, it was reported that rich mineral waters reduced lipid peroxidation and related hepatic malondialdehyde (MDA) content in the livers and prevent hepatic damage, boron and Mg contained in it increased antioxidant capacity against oxidative stress and decreased high ALT and AST levels [18].

It was reported that ALB, TP, Mg and K levels were significantly decreased in patients with AFLD similar to our findings, whereas GGT and bilirubin levels were elevated and these findings were important markers of alcohol-related severe fat [30]. In our study, high levels of GGT and low levels of TP, ALB, Mg and K were found to support the findings of these researchers. At the end of the treatment period, the fact that the most important healings related to these parameters are detected in SG animals proves the effectiveness of treatment with hot spring water. As a matter of fact, it has been reported that hot spring water and baths normalize the intake of minerals and proteins from the intestines by reducing portal venous pressure [9].

Overdose and excessive consumption of alcohol cause ketoacidosis associated with metabolic acidosis [27]. In this case, a decrease in blood pH, pCO_2 and HCO_3^- levels and an increase in LACT levels are detected [31]. In our study, similar findings were found in animals with fatty liver formed. However, it was observed that after starting treatment with Süreyya I hot spring water in SG animals, a reversal of these parameters started and withdrawn to physiological limits towards to end of the treatment, metabolic acidosis due to lactic acidosis improved when compared to CG. L. Xu et al. reported that LACT levels decreased significantly after 21-day balneotherapy, along with there was an increase in TCO_2 levels, this increase was not statistically significant.

In our present study, in histopathological sections of liver tissue samples taken after the formation of fatty liver intrastoplasmic, microvesicular and partly macro-microvesicular fat was observed, and intense parenchymal degeneration and necrosis foci with severe fattening were partly observed. These data are consistent with the studies that report that up to 90% of chronic alcohol users will experience steatosis in the centrilobular or perivenular area [33]. In the histopathological comparisons at the end of the 21st day, it was found that although fattening in liver and necrosis healed in SG animals treated with hot spring water, fattening were still continued in CG animals treated with tap water. This finding has been detected for the first time and constitutes a reference.

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