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Investigation of the effects of Afyonkarahisar Region hot springs water on blood calcium and some hormon levels in experimentally-created osteoporosis in rats

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SUMMARY

Today, one of the most common non-communicable diseases, which, according the World Health Organization experts, dominates the structure of human morbidity and mortality is osteoporosis. The aim of this study was to determine the effect of water from the hot springs of the Afyonkarahisar region on the calcium content and the levels of certain hormones in the blood of rats with experimentally induced osteoporosis. 25 female albino rats of the same age were used in the experiment. Ovaries were removed from all animals under anesthesia with ketamine (200 mg/kg) and xylazine (10 mg/kg), after which they were divided into two groups: control (10 animals) and experimental (15 animals). The animals of the control group were given tap water twice a day through an orogastric tube and they were bathed in it for 15 minutes at the same time, the water temperature was (35 ± 2) °C. The animals of the experimental group were given fresh water from the Süreyya I hot spring using the same method. Blood clinical, hematological and biochemical parameters were measured prior to the study, as well as on day 1, 7, 14, and 21 after the ovariectomy operation. The ovariectomy demonstrated inconsistence of the tested blood parameters with the standard ones. In the course of the treatment, by day 21 of the experiment, the parameters normalized, and the most noticeable changes were observed in the rats of the experimental group ($p < 0.05$). The results of the work performed showed that Süreyya I hot spring water contributed to a significant improvement in the clinical, hematological and biochemical blood parameters in rats with osteoporosis, therefore, it can be used for prevention and treatment of this disease in combination with other types of treatment.

Keywords: Afyonkarahisar, balneotherapy, osteoporosis, rat.

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Исследование воздействия термальной воды из источника региона Афьонкарахисар на содержание кальция и уровни некоторых гормонов в крови крыс с экспериментально воспроизведенным остеопорозом

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РЕЗЮМЕ

На сегодняшний день одним из наиболее распространенных неинфекционных заболеваний, которое, по данным экспертов Всемирной организации здравоохранения, занимает ведущее место в структуре заболеваемости и смертности населения, является остеопороз. Целью настоящего исследования было определение влияния воды из термальных источников региона Афьонкарахисар на содержание кальция и уровни некоторых гормонов в крови крыс с воспроизведенным остеопорозом. В эксперименте использовали 25 самок крыс-альбиносов одного возраста. У всех животных под анестезией с использованием кетамина (200 мг/кг) и ксилазина (10 мг/кг) удалили яичники, после чего их разделили на две группы: контрольную (10 особей) и опытную (15 особей). Животных контрольной группы дважды в день через орогастральный зонд выпаивали водопроводной водой и в течение 15 минут в одно и то же время купали в ней, температура воды составляла (35 ± 2) °С. Животные опытной группы в том же режиме получали свежую воду из горячего источника Süreyya I. Клинические, гематологические и биохимические параметры крови измеряли до начала исследования, а также на 1, 7, 14 и 21-е сут после овариэктомии. Показано, что после овариэктомии отмечались отклонения от нормы величин исследуемых показателей крови. В процессе лечения к 21-м сут эксперимента наблюдался процесс нормализации показателей, наиболее заметные изменения произошли у крыс опытной группы ($p < 0,05$). По итогам работы было установлено, что вода из термального источника Süreyya I способствовала значительному улучшению клинических, гематологических и биохимических показателей крови у крыс с остеопорозом, следовательно, ее можно использовать для профилактики и лечения данного заболевания в комплексе с другими видами лечения.

Ключевые слова: Афьонкарахисар, бальнеотерапия, остеопороз, крыса.

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INTRODUCTION

Osteoporosis is a disease characterized by low bone density and increased brittleness in the bones and is one of the common syndromes of the menopause period [1]. It was reported that osteoporosis was responsible for 1,700 bone fractures per day in Europe, and this number will increase with rising in the elderly population and put a great burden on the health system [2]. As a matter of fact, not only the resorption in the bone accelerates in the menopause, but also some changes in many blood parameters, especially hormones, such as estrogen [3].

Various substances in hot spring waters have been shown to provide significant benefits in reducing the symptoms of osteoporosis by reducing the osteoclastic effect [4]. In addition, it is stated that the calcium, which is found in the hot water and fast absorbed in the intestine, and increases the ionized calcium level in the blood and eliminates the calcium deficit. Therefore, the oral intake of the spa waters will provide great benefits in closing the calcium deficiencies [5]. It has been reported that treatment with hot spring waters can also benefit ovaries to work more regularly, actively and for a longer period of time, by helping the circulatory system to work more actively, to help nutrition and regulate tissues [6].

In this study, it was aimed to reveal the effectiveness of Süreyya I hot spring water, which has rich mineral content, especially calcium, in the treatment of osteoporosis.

MATERIALS AND METHODS

The experimental part of this study was carried out in Afyon Kocatepe University Experimental Animals Application and Research Center, in accordance with the Directive of Afyon Kocatepe University Experimental Animals Ethics Committee (AKUHADYEK) with the report number 137-18, and supported by Afyon Kocatepe University Scientific Research Projects Board (AKÜBAPK) as Master's Thesis Project with No. 19.SAĞ.BİL.04.

Animal Material. In this study, 25 female Albino rats of the same age were used. Ten of 25 rats served as control group (CG), while 15 rats assigned as study group (SG). All the rats was kept in plastic cages in Afyon Kocatepe University Experimental Animals Application and Research Center in a stable environment where the same humidity (50–60%) and heat (17–22 °C) conditions were created for 12 hours night and 12 hours day. During the study, the rats were provided to get *ad libitum* rat food.

Creating Experimental Osteoporosis. In this study, ovariectomy operation was performed according to the method reported by M. Berkoz et al. [7]. According to this method ketamine (200 mg/kg) and xylazine (10 mg/kg) anesthesia was administered intraperitoneally, following disinfection and shaving of the operation area in female rats. Then, the abdominal cavities were opened, the tubers were ligated using '0 chrome caTRIGut, and ovaries was removed from uterus. Finally, peritoneum, connective tissue, muscle tissue and skin were sutured. Operation site was disinfected with Batikon.

Groups and Treatment Procedure. A total of 25 rats, 10 from the control group and 15 from the study group, whose ovaries were removed, were taken into the 21-day treatment period according to the following method:

1. Control Group (CG). Totally 10 ovariectomized rats received tap water twice a day with an orogastric tube at the same time every day with the calculation of 1 L/33 kg body

weight. In addition, all rats in this group were bathed at (35 ± 2) °C in tap water for 15 minutes at the same time every day. After bathing, the rats were placed in their cages.

2. Study Group (SG). In order to treat 15 ovariectomized rats in this group, Süreyya I hot spring water was given twice a day with an orogastric tube at the same time every day with the calculation of 1 L/33 kg bw. In addition, all rats in this group were bathed at (35 ± 2) °C in Süreyya I hot spring water for 15 minutes at the same time every day, and following the bath, the rats were placed in their cages.

Süreyya I Spa Spring water, whose terrapeutic effectiveness on osteoporosis was investigated in this study, is a volcanic origin mineral water, containing carbon dioxide and rich in minerals such as calcium, magnesium, and it has a mineral content of more than 4 g (4,046.8 µg/L) per liter.

Blood samples were collected according to the method previously described by H. B. Waynforth and P. A. Flecknell [8] before the study, after the ovariectomy operation, and on days of 1st, 7th, 14th and 21st following ovariectomy operation. Blood samples were collected under ketamine (200 mg/kg) and xylazine (10 mg/kg) anaesthesia acoording to the method described by M. A. Suckow et al. [9].

Clinical Examinations. Body temperatures (T), respiration rates (R) and heart frequencies (P) were measured and recorded in all the rats during the study.

Hematological Examinations. For hematological examinations, blood samples collected into blood tubes containing EDTA, and measured in Chemray Brand blood count commercial test kits (Rayto Life and Analytical Sciences Co., China). Total leukocyte (WBC), erythrocyte (RBC), hematocrit (HCT), hemoglobin (HB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), neutrophils (NEUT), eosinophils (EOS), monocytes (MON) and basophils (BAS) were measured hematologically.

Blood Biochemical Examinations. Gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB) and glucose (GLU), triglyceride (TRIG), total cholesterol (TCHOL), high-density lipoprotein (HDL), low density lipoprotein (LDL) was measured in Cobas Integra 400 Plus (Roche Diagnostics GmbH, Germany) analyzer using commerical kits. In addition, estradiol (E2), calcitonin (CT) and calcium (Ca) measurements were made with ChemWell, Chromate 4300 Elisa Reader device (Awareness Technology, Inc., USA) using commercial Elisa kits (Sunred Biological Technology Co., Ltd, China).

Statistical Analyses. Analysis of variance (ANOVA) were used for statistical analyses. Intra-group differences were revealed by Duncan test. For statistical analyses, Windows-compatible SPSS Statistics 18.1 (IBM, USA) package program was used, and $p < 0.05$ was determined as an important value.

Table 1
Statistical comparison of body temperature, pulse frequency and respiratory rate

Таблица 1
Статистическое сравнение температуры тела, частоты пульса и дыхания у животных

Time of indicator measurement by groups		Parameters (X ± SD)		
		T (°C)	P (frequence/min)	R (rate/min)
BS ($n = 25$)		37.30 ± 0.30	313.16 ± 45.22^{d}	104.23 ± 32.23^{d}
AOF ($n = 25$)		37.30 ± 0.20	309.18 ± 43.24^{d}	103.12 ± 30.20^{d}
AT 1st day	CG ($n = 10$)	37.20 ± 0.30	312.26 ± 41.14^{d}	104.18 ± 26.34^{d}
	SG ($n = 15$)	37.30 ± 0.20	333.27 ± 32.21^{cd}	109.16 ± 24.12^{c}
AT 7th day	CG ($n = 10$)	37.30 ± 0.20	313.12 ± 22.18^{d}	105.13 ± 18.24^{d}
	SG ($n = 15$)	37.30 ± 0.20	346.12 ± 21.12^{c}	116.20 ± 12.22^{b}
AT 14th day	CG ($n = 10$)	37.30 ± 0.20	314.11 ± 10.25^{d}	106.04 ± 10.10^{d}
	SG ($n = 15$)	37.20 ± 0.20	357.22 ± 11.28^{b}	118.45 ± 8.10^{a}
AT 21st day	CG ($n = 10$)	37.20 ± 0.20	313.13 ± 6.13^{d}	105.16 ± 4.24^{d}
	SG ($n = 15$)	37.30 ± 0.20	464.31 ± 7.16^{a}	119.21 ± 3.16^{a}
Normal physiological parameters		37.00–38.00	360.00 ± 3.30	100.90 ± 4.40

$^{a–d}$ The values in the column are statistically significant ($p < 0.05$).
$^{a–d}$ Значения в столбце являются статистически значимыми ($p < 0,05$).
BS – before study (до исследования), AOF – after ovariectomy (после овариэктомии), AT – after treatment (после лечения), CG – control group (контрольная группа), SG – study group (экспериментальная группа).

TEST RESULTS

Although no age difference was detected between the groups ($p > 0.05$), the mean bw before starting the study (310.2 g) was found to be statistically significantly higher ($p > 0.05$) than the mean bw after ovariectomy operation (286.4 g). The most important loss of body weight was mesured in SG rats (283.2 g) on 21st day of treatment.

Clinical Findings

The clinical findings determined for both groups are presented in Table 1. According to this Table, while no significant difference was observed in periods measured in terms of T ($p > 0.05$), it was determined that P and R statistically significant differences ($p < 0.05$) occurred, and the highest levels were formed on day 21 in SG animals.

Hematological Findings

The results of the hematological measurements shown in Table 2. According to this Table; it was determined that mean WBC, NEUT and MCV increased significantly ($p < 0.05$) following ovariectomy operation. Conversely, mean RBC, HB, HCT, LYM and MCHC decreased significantly ($p < 0.05$). With the start of the treatment period, mean RBC, HB, HCT, LYM, and MCHC levels increased in both groups, but these increases were statistically more significant ($p < 0.05$) in SG animals. However, it was observed that the biggest heamtological changes in the last day of study (21th day) in SG animals.

Blood Biochemical Findings

The blood biochemical measurements shown in Table 3. According to this Table; it was seen that mean GGT, AST, LDL, TRIG, TCHOL and GLU increased significantly ($p < 0.05$), whereas mean ALB, TP, CT, E2, HDL and Ca decreased significantly ($p < 0.05$) following ovariectomy operation. On the other hand, it was determined that a reverse course was shaped in terms of the mentioned parameters, and the most important changes occurred in SG rats and on the 21st day of the study ($p < 0.05$) during treatment period.

DISCUSSION AND CONCLUSION

In our study, it was observed that the mean bw decreased after the ovariectomy operation, and the lowest

Table 2
Results of hematology blood tests

Таблица 2
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 (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	LYM (%)	NEUT (%)	EOS (%)	MON (%)	BAS (%)
BS ($n = 25$)		13.56 ± 3.12^{c}	8.57 ± 4.11^{a}	13.26 ± 3.22^{a}	42.23 ± 5.12^{a}	49.26 ± 5.32^{g}	15.48 ± 3.21	31.40 ± 3.23^{ab}	70.14 ± 12.22^{a}	27.14 ± 5.18^{f}	3.30 ±1.10^{a}	0.45 ± 0.10^{b}	NS
AOF ($n = 25$)		18.29 ± 4.16^{a}	6.48 ± 5.22^{c}	10.37 ± 4.12^{b}	37.46 ± 7.22^{c}	57.20 ± 7.18^{ab}	16.01 ± 3.43	27.71 ± 5.18^{c}	56.43 ± 7.44^{d}	43.10 ±6.34^{a}	1.20 ± 1.30^{d}	0.30 ± 0.20^{c}	NS
AT 1st day	CG ($n = 10$)	18.47 ± 4.08^{a}	6.23 ± 3.43^{c}	10.28 ± 4.32^{b}	37.12 ± 6.44^{c}	58.97 ± 6.20^{a}	16.52 ± 3.28	27.70 ± 4.22^{c}	55.28 ± 6.47^{d}	43.20 ± 3.20^{a}	1.24 ± 0.50^{d}	0.27 ± 0.10^{c}	NS
	SG ($n = 15$)	17.34 ± 4.22^{ab}	6.78 ± 3.27^{c}	10.79 ± 3.37^{b}	38.12 ± 5.32^{bc}	56.28 ± 5.44^{b}	15.90 ± 2.09	28.29 ± 4.19^{bc}	55.14 ± 6.13^{d}	43.30 ± 3.10^{a}	1.45 ± 0.40^{c}	0.20 ± 0.20^{d}	NS
AT 7th day	CG ($n = 10$)	17.16 ± 3.44^{ab}	6.96 ± 2.23^{bc}	11.04 ± 2.23^{bc}	38.14 ± 3.32^{a}	54.81 ± 4.18^{c}	15.89 ± 2.53	26.43 ± 3.23^{c}	56.18 ± 4.36^{e}	43.10 ± 1.40^{a}	1.14 ± 0.30^{ab}	0.30 ± 0.20^{c}	NS
	SG ($n = 15$)	15.45 ± 3.14^{b}	7.48 ± 2.31^{b}	12.34 ± 2.43^{ab}	40.12 ± 2.23^{ab}	53.60 ± 4.24^{bc}	16.59 ± 2.16	30.78 ± 3.41^{b}	60.11 ± 4.21^{c}	38.10 ± 1.10^{c}	1.50 ± 0.40^{c}	0.40 ± 0.28^{b}	NS
AT 14th day	CG ($n = 10$)	16.01 ± 2.26^{b}	7.04 ± 1.12^{b}	11.19 ± 1.36^{bc}	39.16 ± 2.09^{a}	55.61 ± 4.12^{d}	15.91 ± 2.06	28.58 ± 2.48^{bc}	58.48 ± 3.26^{d}	40.40 ± 1.40^{b}	1.70 ± 0.30^{bc}	0.30± 0.20^{c}	NS
	SG ($n = 15$)	14.05 ± 2.16^{bc}	8.03 ± 1.06^{ab}	13.24 ± 1.34^{a}	41.48 ± 1.16^{ab}	51.66 ± 3.14^{e}	16.50 ± 1.18	31.90±2.78^{ab}	61.24 ± 1.13^{b}	36.27 ± 1.30^{d}	2.10 ± 0.30^{b}	0.40 ± 0.40^{b}	NS
AT 21st day	CG ($n = 10$)	15.87 ± 1.08^{b}	7.34 ± 0.36^{bc}	11.45 ± 0.34^{bc}	39.15 ± 0.37^{b}	53.34 ± 3.17^{bc}	15.63 ± 1.46	29.27 ± 1.46^{b}	57.28 ± 0.57^{d}	39.60 ± 0.60^{b}	2.00 ± 0.40^{b}	0.50 ± 0.20^{ab}	NS
	SG ($n = 15$)	13.08 ± 1.22^{c}	8.69 ± 0.27^{a}	13.75 ± 0.28^{a}	42.65 ± 0.32^{a}	49.09 ± 2.16^{gf}	15.83 ± 1.43	32.24 ± 1.39^{a}	63.24± 0.66^{b}	34.20 ± 0.50^{e}	3.10 ± 0.30^{a}	0.60 ± 0.30^{a}	NS

$^{a–f}$ 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), AOF – after ovariectomy (после овариэктомии), AT – after treatment (после лечения),
CG – control group (контрольная группа), SG – study group (опытная группа).
WBC – white blood cells (лейкоциты), RBC – red blood cells (эритроциты), HB – hemoglobin (гемоглобин),
HCT – hematocrit (гематокрит), 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 3
Blood biochemical findings of the animals

Таблица 3
Результаты биохимических исследований крови животных

Time of indicator measurement by groups		Parameters (X ± SD)											
		AST (IU/L)	GGT (IU/L)	ALB (g/dl)	TP (g/dl)	GLU (g/dl)	E2 (pg/L)	CT (pg/ml)	Ca (mg/dl)	TCHOL (mg/dL)	HDL (mg/dl)	LDL (mg/dl)	TRIG (mg/dl)
BS ($n = 25$)		153.25 ± 43.20^{c}	13.24 ± 3.12^{c}	40.20 ± 12.23^{ab}	66.62 ± 21.44^{a}	120.14 ± 32.18^{f}	14.48 ± 4.24^{a}	5.27 ± 2.22^{a}	6.28 ± 2.18^{a}	91.82 ± 9.27^{f}	45.12 ± 10.25^{a}	67.48 ± 14.17^{f}	97.23 ± 23.14^{e}
AOF ($n = 25$)		175.68 ± 52.30^{a}	17.48 ± 6.24^{a}	34.48 ± 18.10^{d}	54.48 ± 32.26^{d}	156.16 ± 27.28^{a}	1.03 ± 0.36^{d}	2.16 ± 1.43^{e}	3.46 ± 1.24^{d}	139.34 ± 25.23^{a}	26.17 ± 12.32^{c}	138.44 ± 19.56^{a}	213.48 ± 37.23^{a}
AT 1st day	CG ($n = 10$)	176.48 ± 51.13^{a}	17.56 ± 6.44^{a}	34.10 ± 17.30^{d}	55.14 ± 31.23^{d}	147.13 ± 25.12^{b}	0.56 ± 0.13^{de}	2.28 ± 1.34^{e}	3.53 ± 1.14^{d}	138.31 ± 22.16^{a}	26.40 ± 10.03^{c}	138.41 ± 17.24^{a}	214.44 ± 38.56^{a}
	SG ($n = 15$)	172.14 ± 50.21^{a}	17.04 ± 5.16^{a}	35.14 ± 16.40^{d}	56.34 ± 27.33^{d}	138.13 ± 13.48^{c}	1.54 ± 0.10^{d}	2.33 ± 1.25^{e}	3.67 ± 1.10^{d}	133.17 ± 21.34^{b}	27.14 ± 10.43^{c}	132.45 ± 15.12^{b}	207.21 ± 35.23^{a}
AT 7th day	CG ($n = 10$)	172.14 ± 50.21^{a}	17.04 ± 5.16^{a}	35.14 ± 16.40^{d}	56.34 ± 27.33^{d}	138.13 ± 13.48^{c}	1.54 ± 0.10^{d}	2.33 ± 1.25^{e}	3.67 ± 1.10^{d}	137.22 ± 18.48^{a}	26.13 ± 9.20^{c}	137.36 ± 12.44^{a}	213.44 ± 31.09^{a}
	SG ($n = 15$)	174.18 ± 37.40^{a}	16.87 ± 3.32^{a}	35.34 ± 11.18^{d}	56.45 ± 28.22^{a}	139.34 ± 11.13^{d}	0.32 ± 0.10^{e}	2.88 ± 1.32^{d}	3.41 ± 1.12^{d}	124.24 ± 15.67^{c}	36.21 ± 7.24^{b}	114.11 ± 12.32^{c}	176.14 ± 24.45^{b}
AT 14th day	CG ($n = 10$)	171.16 ± 22.10^{a}	16.48 ± 2.26^{a}	36.09 ± 10.11^{a}	57.14 ± 22.12^{c}	132.13 ± 11.12^{d}	1.30 ± 0.00^{a}	2.85 ± 1.48^{d}	3.64 ± 0.46^{d}	135.56 ± 14.12^{b}	27.02 ± 7.33^{c}	136.47 ± 9.13^{b}	211.07 ± 25.32^{a}
	SG ($n = 15$)	156.10 ± 18.10^{c}	13.08 ± 2.34^{c}	40.30 ± 7.32^{ab}	61.44 ± 17.08^{b}	124.22 ± 10.22^{e}	3.30 ± 0.00^{b}	4.13 ± 0.32^{b}	4.76 ± 0.58^{bc}	116.26 ± 10.33^{d}	37.28 ± 5.18^{b}	103.46 ± 8.26^{d}	154.21 ± 22.21^{c}
AT 21st day	CG ($n = 10$)	167.20 ± 11.10^{ab}	16.07 ± 1.28^{a}	37.03 ± 5.12^{c}	58.14 ± 12.18^{c}	131.16 ± 8.24^{de}	1.20 ± 0.00^{a}	3.04 ± 0.56^{c}	3.71 ± 0.43^{d}	134.20 ± 9.27^{b}	27.24 ± 5.43^{c}	134.12 ± 6.14^{b}	209.03 ± 14.27^{a}
	SG ($n = 15$)	151.48 ± 9.10^{c}	12.98 ± 1.14^{c}	41.10 ± 4.14^{a}	67.03 ± 9.16^{a}	118.33 ± 7.12^{f}	3.20 ± 0.00^{b}	4.27 ± 0.48^{b}	5.45 ± 0.37^{b}	105.48 ± 6.25^{e}	37.24 ± 4.11^{b}	83.29 ± 5.47^{e}	121.18 ± 13.14^{d}

$^{a–f}$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), AOF – after ovariectomy (после овариэктомии), AT – after treatment (после лечения),
CG – control group (контрольная группа), SG – study group (экспериментальная группа).
AST – aspartate aminotransferase (аспартатаминотрансфераза),
GGT – gamma-glutamyl transferase (гамма-глутамилтрансфераза), ALB – albumin (альбумин),
TP – total protein (общий белок), GLU – glucose (глюкоза), E2 – estradiol (эстрадиол), CT – calcitonin (кальцитонин),
Ca – calcium (кальций), TCHOL – total cholesterol (общий холестерин),
HDL – high-density lipoprotein (липопротеины высокой плотности),
LDL – low density lipoprotein (липопротеины низкой плотности), TRIG – triglyceride (триглицериды).

mean bw was obtained in SG rats treated with Süreyya I hot spring water. This finding was found to be consistent with findings reported by researchers [10] who found that treatment with mineral waters reduced lipid absorption, but increased burning.

In our current study, P frequencies and R rates in SG rats which were treated with hot spring water were higher. Y. Agishi claimed that hot spring water caused an increase in circulating flow, resulting in vasodilation in peripheral vessels and step-up in P frequency and R rate [11].

D. L. Millis et al. reported that leukocytosis occurred in the hemogram after the experimental ovariectomy operation in the dogs, while neutrophilia, lymphopenia and eosinopenia were noticeable in the differential blood picture [12]. In our study, similarly measurements were determined following the ovariectomy operation. Moreover, it was found that the percentages of NEUT increased, while the percentages of LYM and EOS decreased. With the transition to the treatment period, it was determined that WBC and NEUT levels decreased in SG rats treated with Süreyya I hot spring water. This finding is consistent with some researchers [13] who reported that hot mineral waters suppress the immune system and that adrenocorticotropic hormone and cortisol release in baths made with these waters increase and lead to a decrease in T-lymphocytes. In addition, it has been reported that Mg deficiency has an encouraging effect on the occurrence of inflammatory symptoms, therefore it may have an effect on leukocytosis and macrophage activation [14]. In our current study, the detection of lower mean WBC acount in SG rats treated with Mg rich Süreyya I hot spring water was found to be compatible with findings found out by these above researchers.

As in humans, the increase in turnover due to rapid bone loss in the early period of the absence of estrogen is observed in rats [15]. Although there are 3 types of estrogen, E1 (estrone), E2 (estradiol), E3 (estriol), E2 measurements are used in the determination of estrogen levels, since estradiol (E2) is the strongest and most produced type of estrogen [16]. Indeed, estrogen has a direct effect on bone structure, acting on calcium metabolism, bilateral effect on calcium absorption and excretion [17]. In this study, determining that E2 and Ca levels decreased after ovariectomy operation supports the findings of these researcher. However, an increase of E2 and Ca levels in SG rats can be accepted as an evidence that the spa treatment we applied increased E2 and Ca levels.

In our study, it was also determined that AST, GGT and GLU levels measured after ovariectomy operation were high, whereas E2, CT, Ca, TP and ALB levels were low. After treatment period, E2, CT, Ca, TP and ALB levels were higher in SG rats which treated with Süreyya I hot spring water when compare to CG rats which treated with tap water. Additionally, AST and GGT levels were still higher in CG animals, unlike SG rats.

It has been reported that LDL, TRIG and TCHOL levels increase significantly in the case of menopause, whereas HDL levels decrease [18]. Similar findings were determined following ovariectomy operation. With the start of treatment, complete improvement in lipid profile was observed; LDL, TRIG and TCHOL levels decreased, while HDL levels increased in SG rats which treated with Süreyya I hot spring water. These data confirm the findings of some researchers [19] who reported that mineral waters were highly effective in normalizing the degraded blood lipid levels. Abnormal lipid profile was still observed in CG rats even on day of 21 of the treatment.

Calcitonin (CT) is a hormone produced by thyroid gland C cells that increases calcium and phosphate accumulation in the bone and slows the osteolysis process by decreasing osteoclast activity [20]. In our study, although CT levels decreased after ovariectomy operation, it was found that by the onset of the treatment period, CT levels significantly increased in SG animals, while there was an opposite situation in CG animals. Our findings was in line with the findings obtained by M. Cecchettin et al. who reported that spa treatments had positive effects on bone density and increased CT levels in women with osteoporosis in the postmenopausal period undergoing spa treatment [21].

Some researchers reported that bone-derived osteocalcin hormone regulates the insulin secretion of the pancreas, while estrogen has a direct effect on it [22]. It has also been reported that blood GLU levels have a direct relationship with CT levels so that glucagon infusions decrease CT levels, while Ca infusions decrease glucagon levels and lead to an increase in CT levels [23]. Higher GLU levels following ovariectomy in this study was in full agreement with results of another study [24]. On the other hand, increased GLU levels were observed in CG animals even at the and of the study, despite normalized in SG rats.

Consequently, it has been determined that Süreyya I hot spring water was very successful in treatment of osteoporosis in the rats with osteoporosis. Hence, it can be used safely in removing unwanted symptoms of osteoporosis itself or when combined with other medical treatments.

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