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RESEARCH ARTICLE

Determination of Effects on Oxidant Antioxidant Balance and Some Biochemical Parameters Consumption of Bottled, Distilled and Deionized Thermal Water in Rats

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ABSTRACT

The objective of this research is to determine the effects of the consumption of the bottled, distilled and deionized versions of thermal water taken from the Gazlıgöl district in the city of Afyonkarahisar on the oxidant – antioxidant balance and on certain biochemical parameters, along with possible effects on liver and kidney tissue.

The research involved 6 rats in each group. The experiment group was given fresh, bottled, deionized and distilled thermal water for 30 days whereas the control group was given normal water. At the end of the experiment, rats' blood samples as well as liver, kidney and heart tissue samples were taken. The research determined that the hemoglobin level in rats that consumed bottled thermal water is considerably lower than those that consumed fresh and deionized water ($p<0,05$). The zinc level was meaningfully lower in rats that consumed distilled water than those in the other groups ($p<0,05$).

There was no meaningful difference among the groups in terms of the liver and kidney tissues' MDA, GSH and SOD levels. Nevertheless, it was observed that the heart tissues' MDA level was higher in rats that consumed bottled and deionized water than those in other groups ($p<0,01$) whereas the GSH and SOD levels did not change. The histopathological examination of liver and kidney tissue showed certain pathological changes.The conclusion of the research is that thermal water does not have a positive effect on the organism.

Key Words: Biochemical Parameters, Oxidant Antioxidant Balance, Thermal Water.

Sıçanlarda Bekletilmiş, Distile ve Deiyonize Kaplıca Suyu Tüketilmesinin Oksidan Antioksidan Denge ve Bazı Biyokimyasal Parametreler Üzerine Etkisinin Belirlenmesi

ÖZ

Bu çalışmada, Afyonkarahisar ilinin Gazlıgöl bölgesinden alınan kaplıca suyu ve bu suyun bekletilerek, distile ve deiyonize edilerek tüketilmesinin oksidan-antioksidan denge ve bazı biyokimyasal parametreler ile karaciğer ve böbrek dokusuna etkilerinin belirlenmesi amaçlanmıştır. Çalışmada her grupta 6 sıçan yer almıştır. 30 gün boyunca, deney grubundaki sıçanlara taze, bekletilmiş, deiyonize ve distile edilmiş kaplıca suyu, kontrol grubundakilere ise normal su verilmiştir. Deneme sonunda sıçanlardan kan numuneleri ile karaciğer, böbrek ve kalp doku örnekleri alınmıştır.Hemoglobin değeri bekletilmiş kaplıca suyu tüketen sıçanlarda taze ve deiyonize tüketen sıçanlara göre önemli oranda düşük olduğu gözlenmiştir($p<0,05$). Distile edilmiş kaplıca suyu tüketen sıçanlarda Çinko değerinde diğer gruplara göre anlamlı ($p<0,05$) bir düşüş oluşmuştur. Karaciğer ve böbrek dokusu MDA, GSH ve SOD düzeyleri bakımından gruplar arasında anlamlı bir değişim gözlenmemiştir. Buna karşın, kalp dokusunda MDA düzeylerinin bekletilmiş ve deiyonize kaplıca suyu tüketen sıçanlarda diğer gruptakilere göre daha yüksek olduğu ($p<0,01$), GSH ile SOD düzeylerinin ise değişmediği saptanmıştır. Karaciğer ve böbrek dokusu hispatolojik incelemesinde birtakım patolojik değişiklikler tespit edilmiştir. Sonuç olarak çalışmada kullanılan kaplıca suyunun organizmada olumlu bir etki yaratmadığı kanısına varılmıştır.

Anahtar Kelimeler: Biyokimyasal Parametre, Oksidan Antioksidan Denge, Kaplıca Suyu.

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INTRODUCTION

Thermal spring waters have long been used for medicinal purposes and common wisdom has it that they cure all kinds of ailments (Mergen et al., 2006). Thermal tourism started when Romans first built facilities near thermal sources. Today, it is widely practiced in many countries the world over, including Turkey (Erer, 2004). The use of these waters for prophylactic and therapeutic purposes is called spa treatment and the curative effect stems from the heat of the water as well as its mineral content (Karagülle and Karagülle, 2000; Vaccarezza and Vitale, 2010). A recent study showed that in children with chronic rhinosinusitis a15 minute/15 day inhalation of thermal water containing sulfate, sodium and chloride results in the reduction nasal mucosal inflammatory mediators (Passariello et al., 2012). Yet other studies show that many mineral waters, especially those rich in bicarbonate, have an effect on lipoprotein levels and that their consumption causes a reduction in total serum cholesterol and LDL cholesterol levels (Schoppen et al., 2004). Costantino et al. (2012) observed a reduction of the blood glucose levels in hunger in type 2 diabetic patients who consumed sulfur thermal water in addition to their diabetes medications for 2 weeks. Granados et al.(2010) found that the serum glucose levels show a tendency to drop in people who consume a liter of bicarbonate mineral water daily for 8 weeks and maintained that this finding may be due to the relation between glucose and lipid mechanism. Benedetti et al. (2009) argue that the plasma samples taken from people who were given sulfur mineral water for 2 weeks show considerable reductions in the protein and lipid oxidation products caused by free radicals. It is claimed that the consumption of thermal waters alters the oxidant-anti-oxidant level in the blood (Benedetti et al., 2009; Çevik et al., 2011). However, it has also been determined that lipid peroxidation does not change in rats who consume thermal water whereas the glutathione levels rise (Çevik et al. 2011).

One of Turkey's popular spots for thermal tourism is the Gazlıgöl district of the city of Afyonkarahisar. Visitors to the thermal source by undergo bath treatments as well as drink the thermal water on site and bottle it for later consumption. This way they also benefit from a drinking cure. When one takes into consideration the effects of the changes in oxidant-antioxidant balance on the organism and the fact that people consume bottled water that sits, bottled, distilled and deionized thermal water may well affect the oxidant-antioxidant balance of the body as well as the tissues through other biochemical parameters. Therefore, in order to understand the effects of the minerals found in thermal water on the organism, it is important to identify the changes in biochemical parameters along with the oxidant-antioxidant balance caused by the deionization and demineralization of cooled thermal water.

This study was planned in order to find out the effects of the consumption of cooled, distilled and deionized thermal water on the oxidant-antioxidant balance as well as on liver and kidney tissues through certain biochemical parameters and to uncover the mechanisms involved in the cause-effect relationship.

MATERIAL AND METHOD

This project has been carried out with approval no 96 dated 03 October 2011 granted by Afyon Kocatepe University's (AKU) Animal Research Local Ethics Board.

In this research, 30 250-300 gr, male Sprague-Dawley rats were used. The animals were housed in cages in groups of 3, on a 12:12 light - dark cycle and were fed ad libitum. They were separated into five groups with each group containing 6 animals. The thermal water used in the experiment has been taken from a widely used public source in the Gazlıgöl district of the city of Afyonkarahisar. The chemical and physical properties of the water are given in table 1 as reported by the Afyon Municipality Yüntaş Thermal Facility Directorate.

The experiment has been implemented according to the below groups

Group 1: Identified as the control group and was given commercial drinking water (Yıldız^{R}).

Group 2: Given fresh thermal water transported daily from the source.

Group 3: Thermal water was given to the animals after sitting in a bottle for a week.

Group 4: Thermal water was given to the animals after it was deionized.

Group 5: Thermal water was given to the animals after it was distilled.

30 days after these applications, following a 50mg/kg Ketamine and 10mg/kg Xylazine HCI injection, animals' blood was drawn intracardially and liver and kidney were removed for histopathological examination. For plasma, blood was collected in EDTA (Ethylenediaminetetraacetic acid) hemogram tubes and for serum it was collected in capped tubes. Immediately after collection blood was centrifuged in 4° C at 5000 RPM for 10 minutes to produce serum and plasma. The serum was then tested for the following parameters: aspartate amino transaminase (AST), alanine amino transaminase (ALT), Urea, Creatine, Total Cholesterol, HDL cholesterol, LDL cholesterol, Triglyceride, Calcium, Phosphorus, Glucose. A whole blood erythrocyte package was prepared and Glutathione (GSH), Malondialdehyde (MDA) levels measured. Serums (for total antioxidant activity (TAOA) and SOD measurements) and kidney, liver and heart tissues (for MDA, GSH and SOD measurements) were preserved at -20°C and measured later. Zinc and

copper levels were measured using the ISP via service procurement from Düzen Laboratories in Ankara. AST, ALT, Urea, Creatine, Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglyceride, Calcium, Phosphorus and Glucose levels were measured with a Roche Cobas c501 autoanalyzer (using the following kits in respective order of catalogue number: 0764949, 0764957, 4460715, 4810716, 3039773, 4399803, 0767107, 5061482, 3183793, 4404483) at the AKU, Faculty of Medicine biochemistry laboratory.

Full Blood count has been measured using the AKU Animal Hospital's blood count machine (Mindray BC-2800). MDA assay of the tissue follows the method of Draper and Hardley (1990); GSH assay of tissue and blood follows that of Beutler(1984); Superoxide Dismutase Activity assay follows Sun et al. (1988) and Total Antioxidant Activity Assay follows Koracevic et al. (2001).

For Histopathological assay, kidney and liver tissues were analyzed in tamponed 10% formal saline. Then, they were treated in an automatic tissue processor (Leica TP 1020) with alcohol and xylol series and paraffin blocs were prepared. Out of the latter, 5 μm sections were then cut and stained according to the Hematoxylin and Eosin Method (Luna, 1968). The stained slides were analyzed under a binocular light microscope (Olympus BX 51).

Statistical Analysis

The statistical analyzes were performed using in the SPSS for Windows version 20. Differences in variables among groups were assessed using Kruskal-Wallis H analysis. P values below 0,05 were considered statistically significant. In order to determine from which groups the variance stemmed, a post-hoc analysis was conducted using the conover inman test.

RESULTS

The experiment showed that the parameters under observation had no effect on the AST, ALT, Urea, Creatine, LDL, HDL, total cholesterol, triglyceride, glucose, leukocyte, erythrocyte, thrombocyte, hematocrit, calcium, phosphorus and copper along with the GSH, SOD, TAOA and MDA levels (Table 2).

The hemoglobin level was significantly lower in rats that consumed bottled thermal water as opposed to those that consumed fresh and deionized water (p=0,015). In comparison with other groups, there was a meaningful drop in the zinc level of the rats that consumed distilled thermal water (p=0,022) (Table 2).

There was no meaningful variance among the groups with respect to the MDA, GSH and SOD levels. In spite of this finding, the MDA level in heart tissue was higher in rats that consumed bottled and deionized thermal water than those in other groups (p=0,009) whereas the GSH and SOD levels remained the same (Table 3).

The analysis of groups' liver and kidney tissues showed the following: degenerative changes in the liver tissue (Figure 1) and hyaline casts in their renal tubular lumen were observed in rats that consumed distilled thermal water (Figure 2); The rats that consumed deionized thermal water were observed to undergo degenerative and necrotic changes in hepatocytes (Figure 3) and had hyaline casts in their renal tubular lumen (Figure 4)

DISCUSSION

This article looks at the effects of rats' consumption of fresh, bottled, deionized and distilled versions of thermal water from the Gazlıgöl district of Afyonkarahisar on the oxidant-antioxidant balance and on selected biochemical parameters. Its objective is to determine the possible effects of the minerals in the water and of the bottling process (water sitting) on the organism.

The research shows that the ALT and AST levels, which are among the most widely used enzymes in the analysis of tissue metabolism changes and whose serum levels rise in all diseases that cause liver damage were not affected by the experiment. The values obtained in the research are compatible with normal values in regards to the ALT levels reported in rats (www.jcam.com.tr/files/KATD-604.pdf. 17.12.2015) whereas observed AST levels were higher than normal. The findings of this research support a previous statement that ALT levels do not change in rats that consume thermal water (Çevik et al., 2011). Nonetheless, despite the fact that AST and ALT levels did not change, the histopathological examination of the rats' livers shows that the consumption of deionized thermal water causes degenerative changes in hepatocytes (Figure 1), whereas distilled thermal water consumption causes degenerative and necrotic changes in hepatocytes (Figure 3). While it is assessed that the changes in the hepatocytes may not have caused an enzyme leak that would have elevated the serum enzyme levels, It is obvious that more research is needed to uncover the mechanisms of these changes in hepatocytes that do not affect the serum enzyme levels.

The research also shows that the experiment had no effect with respect to the level of urea that is synthesized (Sözbilir and Bayşu, 2008) in the liver for the detoxification and removal of ammonia from the body, the latter produced from protein metabolism in the organism and highly toxic for cells. Additionally, the experiment did not have an effect on the creatine levels, which were analyzed in order to ascertain whether kidney functions were regular. The findings obtained in this research are compatible with those of the earlier study (Çevik et al., 2011) and suggest that the consumption of the fresh, bottled, deionized and distilled forms of thermal

water did not have an effect on the serum urea and creatine levels that were measured to determine the effects of the water on kidney functions. At the same time, despite the fact that the urea and creatine levels remained unchanged, the histopathological examination of kidney tissue found hyaline casts in the renal tubular lumen of the distilled and deionized thermal water groups (Figures 2 and 4).

Research conducted on mice suggests that the consumption of distilled water may cause an increase in urinary excretion, extracellular fluid volume and water consumption. It is also suggested that when the mineral requirement cannot be met through food consumption minerals are increasingly excreted from the body and a general disorder may follow.

Further, it is emphasized that there may be morphological changes in the kidneys that can amount to glomerular atrophy (World Health Organization 2004). In light of the data at hand, kidney tissue changes that took place in the distilled and deionized groups can be related to the insufficient mineral intake that results from the absence of minerals in the water. Further, the fact that the kidney function indicators have not changed despite the change in kidney tissue may well mean that the tissue changes have not yet had an effect on the indicators. The possible effects of demineralized water on kidney tissue need to be explored in further research.

In contrast to research that states that the HDL and LDL levels are affected by the consumption of mineral-rich water (Nasuti et al., 2005). and that, depending on the duration of consumption, the total cholesterol and LDL cholesterol levels decrease while HDL levels increase (Granados et al., 2010), this research found that the consumption of either fresh, bottled, deionized and deionized water does not affect the blood total cholesterol, HDL and LDL cholesterols. This finding supports an earlier research that argued that a 4-week consumption of thermal water does not affect the blood total cholesterol, HDL and LDL levels in rats (Çevik et al., 2011). Similarly, it was determined in the present research that the experiment did not affect the blood glucose levels. This finding is not compatible with that which argues that in humans who consume 1 liter of sodium-bicarbonate rich water everyday for 8 weeks, the level of insulin hormone remains unchanged whereas the glucose level tends to decrease (Granados et al.,2010). Granados et al. (2010) argue that the decrease in the glucose level with a steady level of blood insulin hormone in the sodium-bicarbonate rich water intake is due to the relationship between glucose and lipid metabolism. The present research observed that the serum glucose levels obtained from all of the groups remain within the normal levels reported for rats (www.jcam.com.tr/files/KATD-604.pdf 17.12.2015). These findings indicate that the level of minerals in the thermal water was not enough to affect the bodily cholesterol and glucose metabolism. Contrary to other research that argues that thermal water, especially of the sulfured variety shows an antioxidant effect (Benedetti et al., 2009; Çevik et al., 2011; Costantino et al., 2012) the present research found that the water used had no effect on the levels of the following indicators based on an analysis of the blood to determine the changes in the oxidant – antioxidant balance in blood and tissue: Lipid Peroxidation indicators: MDA and Antioxidant indicators: GSA, SOD and TAOA.

In the liver, kidney and heart tissues, MDA, GSH and SOD levels were also not affected. These findings indicate that the mineral level of thermal water used in the experiment was not enough to affect the bodily oxidant – antioxidant balance. The fact that in the present research the MDA levels in heart tissue were higher in rats that consumed bottled water suggests that the changes that may have happened while the water was sitting may have produced an oxidative effect and affected the heart tissue.

The present research found that the experiment did not affect rats' levels of serum calcium, phosphorus and copper. It was further observed that the measured serum calcium and phosphorus levels were within the normal values reported for rats (Sharp and Regina, 1998). Nonetheless, the copper and zinc levels were below the reported normal values. Taking into account the argument that calcium-rich mineral waters increase the serum-calcium level but that this increase is dependent on the water's mineralization level (Albertini et al., 2007), it can be argued that the mineral level in the thermal water was not enough to affect the blood mineral levels of the rats. Indeed, previous research (Çevik et al., 2011) indicated that the serum copper and zinc levels do not change in rats that consumed thermal water for 4 weeks. This research is in parallel with our present study in its argument that the copper level does not change. Despite the fact that these minerals remain unchanged, the level of serum copper decreased meaningfully in rats that consumed distilled thermal water. Because the minerals in the water were removed following its distillation, this decrease causes only the minerals in the diet to be absorbed through the digestive tract. Even though the level of minerals in rats' feed was not measured for this research, it is possible that the decrease in the serum zinc levels of the rats that consumed distilled thermal water may be due to the low level of zinc in the feed.

Our research shows that with regards to blood cells, leucocyte, erythrocyte and thrombocyte as well as hematocrit levels were not different; the hemoglobin level was lower in rats that consumed bottled thermal water than that of the rats that consumed fresh and deionized water. The hemoglobin level for the latter group was within the normal values

reported for rats (Sharp and Regina, 1998). It is concluded that the difference is insignificant.

CONCLUSION

The present research determined that thermal water taken from a publicly used source in the Gazlıgöl district of Afyonkarahisar did not affect the parameters used in the experiment. It has been further concluded that these findings will contribute to further scientific evaluations of the benefits of thermal water. However, it is also assessed that more research is needed to determine the histopathological effects of the long-term consumption of thermal water particularly on tissues.

Table 1: Physical and chemical properties of water received from Gazlıgöl-Afyonkarahisar Region
Tablo 1: Gazlıgöl Bölgesindeki su kaynağından alınan suyun kimyasal ve fiziksel özellikleri

Physical and chemical properties	**Value (mg/L)**
Color	0
Tubidity in ntu	3.8
pH	7.3
Total permanent hardness	270
TDS	2670
Anionic	<0.02
Magnesium ion	22
Calsium ion	72
Manganese ion	<0.1
Iron ion	0.3
Nitrogen from nitrite	0.001
Nitrogen from nitrate	<0.05
Nitrogen from ammonia	<0.02
Sulfate ion	<10
Floride ion	1.3
Cadmium ion	<0.0005
Chromium ion	<0.05
Cyanide ion	<0.001
Silver ion	<0.05
Lead ion	<0.05
Copper ion	<0.5
Zinc ion	<0.1
Chloride ion	145
Chlorine	<0.018
Carbondioxide	232
Phenol	<0.001

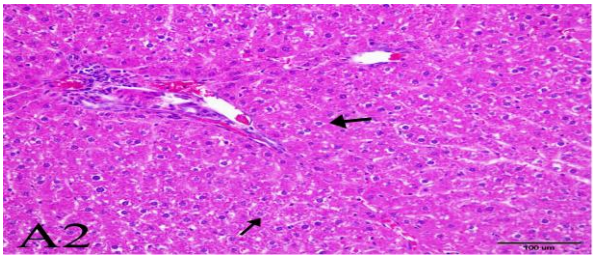

Figure 1: Distilled thermal water group liver tissue histopathologic appearance: Degenerative changes in hepatocytes.
Şekil 1: Distile kaplıca suyu grubu karaciğer dokusu histopatolojik görünüm: Hepatositlerde dejeneratif değişiklikler.

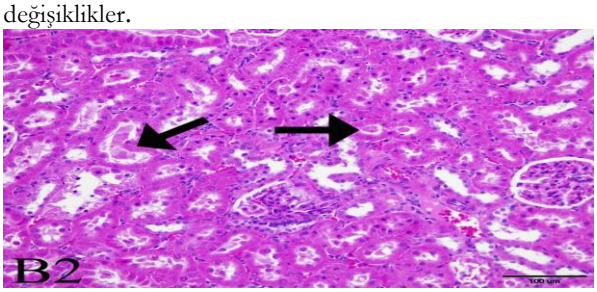

Figure 2: Distilled thermal water group kidney tissue histopathologic appearance: Tubulus lumen hyaline cylinders
Şekil 2: Distile kaplıca suyu grubu böbrek dokusu histopatolojik görünüm: Tubulus lumenlerinde hyalin silindirleri

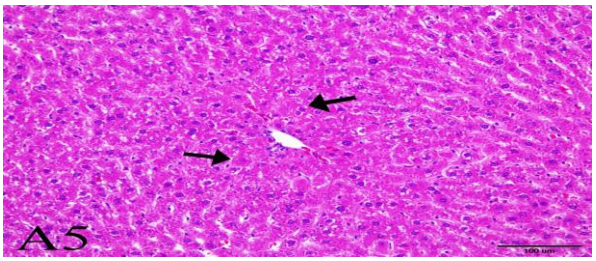

Figure 3: Deionized thermal water group liver tissue histopathologic appearance: Degenerative and necrotic changes in hepatocytes.
Şekil 3: Deiyonize kaplıca suyu grubu karaciğer dokusu histopatolojik görünüm: Hepatositlerde dejeneratif ve nekrotik değişiklikler.

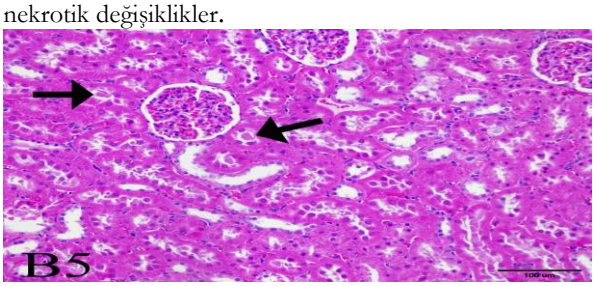

Figure 4: Deionized thermal water group kidney tissue histopathologic appearance: Tubulus lumen hyaline cylinders

Şekil 4: Deiyonize kaplıca suyu grubu böbrek dokusu histopatolojik görünüm: Tubulus lumenlerinde hyalin silindirleri.

Table 2:Blood parameters after water application

Tablo 2: Su uygulamaları sonrasında kan parametreleri

	Control Mean Rank Median (25%-75% quartile)	Fresh thermal water Mean Rank Median (25%-75% quartile)	Bottled thermal water Mean Rank Median (25%-75% quartile)	Deionized thermal water Mean Rank Median (25%-75% quartile)	Distilled thermal water Mean Rank Median (25%-75% quartile)	p
ALT (U/L)	13,00 43,40 (41,57-51,82)	16,67 46,20 (45,32-49,12)	14,92 45,30 (40,37-55,30)	14,75 46,60 (41,27-50,32)	18,17 48,80 (42,47-57,50)	0,876
AST(U/L)	15,83 124,30 (112,90-213,75)	22,33 161,45 (142,27-186,35)	15,50 133,35 (112,15-204,95)	12,67 128,25 (104,85-141,97)	11,17 118,70 (101,27-148,65)	0,223
CREATININE (mg/dL)	16,17 0,31 (0,27-0,35)	15,42 0,32 (0,27-0,35)	14,08 0,30 (0,26-0,35)	20,00 0,34 (0,29-0,39)	11,83 0,30 (0,25-0,32)	0,588
UREA(mg/dL)	10,33 56,55 (54,05-64,90)	14,33 56,65 (54,45-60,25)	16,00 57,25 (52,75-63,32)	21,00 66,20 (54,72-69,32)	15,83 67,40 (61,50-72,90)	0,337
TOTAL CHOLESTEROL (mg/dL)	16,83 56,65 (50,60-59,12)	12,00 52,60 (47,82-58,15)	14,92 51,60 (50,50-59,02)	21,33 61,30 (53,32-66,85)	12,42 51,15 (48,00-59,27)	0,344
TRIGLYCERIDE (mg/dL)	10,33 49,00 (43,67-54,00)	14,33 53,15 (42,52-71,85)	16,00 59,40 (43,57-67,77)	21,00 68,00 (58,67-85,27)	15,83 51,75 (48,50-79,12)	0,337
HDL(mg/dL)	18,92 44,45 (40,47-45,80)	12,67 40,15 (37,02-43,95)	12,67 38,45 (37,52-45,10)	21,08 45,45 (40,85- 51,95)	12,17 38,55 (35,07-45,45)	0,246
LDL(mg/dL)	17,17 9,25 (8,17-11,10)	14,58 9,10 (7,52-10,20)	15,17 9,50 (6,65-10,75)	17,50 10,65 (6,12-11,92)	13,08 7,85 (6,72-11,20)	0,902
GLUCOSE (mg/dL)	11,83 171,60 (147,20-186,77)	11,17 169,25 (134,10-201,72)	13,67 164,95 (145,20-224,55)	19,50 200,80 (163,52-241,65)	21,33 221,35 (189,15-243,00)	0,157
MDA(nmol/mL)	23,17 2,59 (2,15-5,17)	14,92 1,95 (1,59-3,52)	15,00 2,09 (1,76-2,45)	13,25 1,90 (1,85-2,15)	11,17 1,77 (1,63-2,38)	0,168
GSH(g/L)	14,08 30,80 (26,57-34,40)	18,75 33,80 (29,35-5402)	7,92 25,65 (19,52-29,22)	17,92 38,70 (25,27-47,82)	18,83 36,45 (27,50-75,35)	0,150
TAOA(mmol/L)	11,42 0,72 (0,48-0,95)	12,83 0,84 (0,50-0,92)	16,42 0,93 (0,59-0,99)	16,50 0,84 (0,62-1,17)	20,33 0,96 (0,73-1,28)	0,434
SOD(U/mgHb)	13,50 1,84 (0,78-3,38)	21,17 2,61 (2,09-5,53)	16,83 1,97 (1,15-5,53)	8,50 1,26 (0,96-1,62)	17,50 2,21 (1,49-3,20)	0,134
CALCIUM(mg/dL)	19,17 10,20 (9,97-10,39)	9,92 9,74 (9,46-10,10)	14,00 10,05 (9,63-10,30)	46,50 10,22 (9,64-10,33)	15,67 9,92 (9,79-10,55)	0,426
PHOSPHORUS (mg/dL)	18,75 8,32 (7,75-8,75)	18,33 8,23 (7,71-8,85)	19,33 8,41 (7,74-8,75)	13,50 7,88 (7,26-8,54)	7,58 7,32 (7,18-7,91)	0,101
COPPER(µg/dL)	20,33 110,00 (102,50-117,50)	15,92 105,00 (100,00-107,50)	15,92 105,00 (95,00-111,25)	15,75 105,00 (85,00-116,25)	9,58 85,00 (80,00-110,00)	0,326
ZINC(µg/dL)	23,00 140,00 (135,00-140,00)[a]	16,08 130,00 (122,50-136,25)[a]	14,25 120,00 (116,25-137,50)[ab]	17,67 137,50 (107,50-141,25)[a]	6,50 112,50 (100,00-120,00)[b]	**0,022**
LEUKOCYTE m/mm³	10,00 8,59 (7,83-15,28)	18,25 8,35 (6,59-11,49)	14,42 7,26 (6,50-8,71)	18,17 9,43 (8,24-11,53)	16,67 9,32 (7,50-10,94)	0,451
ERYTHROCYTES m/mm³	8,17 8,05 (7,87-8,52)	21,25 8,98 (8,38-9,19)	13,75 8,41 (8,29-8,67)	17,58 8,69 (8,41-8,79)	16,75 8,47 (8,35-8,95)	0,115
PLATELET m/mm³	14,67 1044,50 (759,25-1276,75)	17,67 1070,00 (987,50 - 1237,25)	16,67 1105,50 (984,50 -1241,50)	14,33 1001,00 (938,75 - 1201,50)	14,17 1015,50 (890,75 - 1222,75)	0,943
HEMOGLOBIN g/dl	12,90 17,30 (16,65-17,65)[ab]	22,08 17,70 (17,47-18,45)[a]	6,25 16,85 (16,50-17,20)[b]	19,00 17,75 (17,15-18,25)[a]	14,42 17,20 (17,15-17,80)[ab]	0,015
HEMATOCRIT %	9,92 44,95 (42,25-46,07)	20,25 46,90 (45,30-48,75)	14,83 46,00 (44,72-46,82)	17,50 46,25 (45,30-47,25)	15,00 45,60 (44,57-47,00)	0,339

Table 3: Kidneys, liver, and heart tissue GSH, SOD and MDA levels after water applications

Tablo3: Su uygulamaları sonunda , böbrek, karaciğer ve kalp dokusunda GSH, SOD ve MDA düzeyleri

	Control **Mean Rank** **Median (25%-75% quartile)**	**Fresh thermal water** **Mean Rank** **Median (25%-75% quartile)**	**Bottled thermal water** **Mean Rank** **Median (25%-75% quartile)**	**Deionized thermal water** **Mean Rank** **Median (25%-75% quartile)**	**Distilled thermal water** **Mean Rank** **Median (25%-75% quartile)**	**p**
KİDNEY TISSUE						
GSH (U/g tissue)	12,17 3,58 (2,35-4,74)	15,25 4,03 (3,58-4,33)	13,33 3,50 (2,39-5,37)	20,83 5,00 (3,58-5,74)	15,92 4,03 (2,84-5,19)	0,484
SOD (U/ µg protein)	16,58 11,15 (8,97-15,79)	17,83 11,27 (9,53-15,8)	18,17 12,9 (9,3-14,88)	8,92 8,65 (6,69-11,08)	16,00 11,75 (7,03-19,49)	0,350
MDA (nmol/g tissue)	21,33 4,49 (3,59-4,77)	9,75 3,03 (2,53-3,46)	16,83 3,22 (3,18-4,43)	12,25 3,18 (2,96-3,57)	17,33 3,72 (2,79-4,75)	0,170
LİVER TISSUE						
GSH (U/g tissue)	10,42 2,54 (1,08-2,91)	16,33 3,06 (2,39-3,84)	18,58 3,73 (1,86-4,48)	15,17 2,98 (1,60-4,21)	17,00 3,21 (1,15-4,66)	0,561
SOD (U/ µg protein)	18,33 16,21 (8,43-19,91)	8,33 10,21 (6,41-11,84)	16,83 13,19 (10,74-1654)	19,00 14,63 (11,68-17,99)	15,00 13,32 (10,37-14,91)	0,222
MDA (nmol/g tissue)	13,33 1,08 (0,56-1,92)	21,17 2,01 (1,00-2,30)	11,17 0,94 (0,81-1,26)	15,42 1,01 (0,92-1,82)	16,42 1,31 (1,13-1,42)	0,358
HEART TISSUE						
GSH (U/g tissue)	15,75 3,95 (2,90-5,11)	16,17 4,33 (2,12-5,25)	15,33 4,25 (2,27-5,22)	16,67 3,80 (3,05-5,25)	13,58 3,88 (2,54-4,62)	0,980
SOD (U/ µg protein)	15,33 28,25 (13,54-37,03)	17,50 26,53 (16,93-45,19)	17,17 21,29 (17,85-77,88)	9,83 17,18 (13,02-26,18)	17,67 28,13 (18,09-38,58)	0,497
MDA (nmol/g tissue)	7,33 0,65 (0,46-0,86)[c]	10,00 0,77 (0,70-0,81)[bc]	23,25 1,23 (1,08-1,74)[a]	18,33 1,13 (0,76-1,29)[ab]	16,30 0,93 (0,74-1,20) [abc]	0,009

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