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#### Araştırma Makalesi– Research Paper

#### THE EFFECTS OF DIFFERENT PRESERVATION METHODS APPLIED DONKEY MILK SAMPLES ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN WISTAR RATS

# EŞEK SÜTÜ ÖRNEKLERİNE UYGULANAN FARKLI MUHAFAZA YÖNTEMLERİNİN WİSTAR SIÇANLARINDA BAZI HEMATOLOJİK VE BİYOKİMYASAL PARAMETRELER ÜZERİNE ETKİLERİ

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#### Özet

Çalışmanın amacını, eşek sütü örneklerine uygulanan farklı muhafaza yöntemlerinin ratlardaki bazı hematolojik [lökositler (WBC), hemoglobin (HGB), hematokrit (HCT), trombositler (PLT), eritrositler (RBC), ortalama korpüsküler hemoglobin üzerindeki etkilerinin araştırılmasıdır. (MCH), ortalama alyuvar hemoglobin konsantrasyonu (MCHC), ortalama alyuvar hacmi (MCV) ve diferansiyel WBC (lenfosit, monosit, nötrofil) sayıları] ve biyokimyasal [(serum kreatin (CREAT), alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), alkalin fosfataz (ALP), trigliseritler (TG), toplam serum proteini (TP), albümin (ALB), globulin (GLOB), üre (UREA), kan üre azotu (BUN), kolesterol (CHOL), glikoz ( Sıçanlarda GLU), kalsiyum (Ca), fosfor (P), sodyum (Na), klor (Cl), potasyum (K) ve ozmolarite (OSM)] parametreler üzerine etkileri oluşturmuştur. Bu amaçla 28 erkek Wistar albino rat; Kontrol (C; n = 7), Çiğ süt (Rm; n = 7), Pastörizasyon (Pst; n = 7) ve Ultrason (Ult; n = 7) grupları olarak 4 eşit gruba ayrıldı. Kontrol grubu hariç sıçanların her birine 4 hafta boyunca Pst ve Ult uygulamalı süt örnekleri (48 ml/gün oranında) verildi. Deney boyunca C grubuna ad libitum olarak sadece standart rat yemi ve içme suyu verildi. Uygulama periyodunun ardından ratlar sakrifiye edilerek hematolojik ve biyokimyasal analizler için kan örnekleri alındı. WBC düzeyleri C'ye göre en yüksek Ult grubunda tespit edildi (p < 0,05). Ayrıca LYM düzeyleri de Ult ve Rm gruplarında C grubuna göre en yüksek bulundu (p < 0,05). Ayrıca çalışmamızda diğer gruplara göre en yüksek olarak tanımlanmıştır (p < 0,05). Öte yandan çalışmamızda Past grubunda serum AST değerleri C, Ult ve Rm gruplarına göre kesin olarak daha yüksekti (p < 0,05). Ayrıca çalışmamızda, Past grubunda serum Cl değerleri C grubuna göre daha yüksekti (p < 0,05). Sonuç olarak, eşek sütü örneklerine uygulanan farklı muhafaza yöntemlerinin sıçanlarda bazı hematolojik ve biyokimyasal özellikler üzerinde potansiyel etkileri olduğu görülmüştür.

Anahtar Kelimeler: Eşek sütü, Ultrasound, Sıçan, Hematoloji, Biyokimya, Pastörizasyon.

#### Abstract

The aim of the study was to investigate the effects of different preservation methods applied donkey milk samples on some hematological [leukocytes (WBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), erythrocytes (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and differential WBC (lymphocyte, monocyte, neutrophil) counts] and biochemical [(serum creatine (CREAT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides (TG), total serum protein (TP), albumin (ALB), globulin (GLOB), urea (UREA), blood urea nitrogen (BUN), cholesterol (CHOL), glucose (GLU), calcium (Ca), phospurus (P), sodium (Na), clour (Cl), potasium (K), and osmolarity (OSM)] parameters in rats. For this purpose, 28 male Wistar albino rats were divided four equal groups as Control (C; n = 7), Raw milk (Rm; n = 7), Pasteurization (Pst; n = 7) and Ultrasound (Ult; n = 7). Rm, Pst, and Ult applicated milk samples (at the rate of 48 ml/day) was administrated to each of the rats except C for 4 weeks. Only standard rat feed and drinking water were given ad libitum to C group throughout the experiment. After the application period, rats were sacrified and blood samples were taken for hematological and biochemical analysis. WBC levels were detected the highest in Ult group compared to C (p < 0,05). Besides, LYM levels were also found the highest in Ult and Rm groups when compared to the C group (p < 0,05). In addition, the highest NEU levels were observed in Ult group compared to other groups in our study (p < 0,05). Serum ALP values were defined the highest in Rm group compared to other experimental groups (C, Ult, and Pst) in the present study (p < 0,05). In terestingly. On the other hand, serum AST values were definitively higher in Pst group than C, Ult, and Rm groups in our study (p < 0,05). In addition, serum Cl values were higher in Past group than C group in our study (p < 0,05). In conclusion, different prese

Keywords: Donkey milk, Ultrasound, Rat, Hematology, Biochemistry, Pasteurization.

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### **1. INTRODUCTION**

Donkey milk (DM) historically has been taken into the account as a therapeutic food in many different cultures around the World (Lu et al., 2006, p. 11-18). Therefore, scientific researchers have aimed to discover the ingredients of DM and the presence of potential functional compounds in recent years. Also, potentially bioactive substances such as poly-unsaturated / omega 3 fatty acids, some functional proteins, different vitamins, polar lipids, phytosterols, and compositional variability of the milk were determined by different researchers (Martini et al., 2018, p. 8721-25; Almanto et al., 2019, p. 11-118). Besides, DM composition is found closest to human milk in terms of lactose, protein, and amino acid content (Almanto et al., 2019, p. 11-118). Recently, depends on the rediscovery of its potential protective effects, DM has become popular in Europe and also different Asian countries. As it is well known, China is a big donkey meat - milk producer and consumer (Martini et al., 2021, p. 2-15).

There are many factors including personnel, parlor environment, milking equipment, outer surface of udder tissue, air, water, feed, grass, feces, and soil that affect the microbiota and the quality of raw milk (Rm) (Pilla et al., 2010, p. 392). In addition to its technological importance for the dairy industry, this microbiota generally includes lactic acid bacteria as well as pathogenic bacteria and fungi that cause milk spoilage. For these reasons, pasteurization process is performed to Rm samples in order to destroy the vegetative forms of pathogenic microorganisms (Abdullah et al., 2019, p.157-166). At the same time, pasteurization process is a necessity for RM produce according to the Food and Drug Administration (FDA), Pasteurized Milk Ordinance (PMO). However, it has been reported that thermal pasteurization may lead to quality changes in milk, significant nutrient losses, and the development of protein-based allergic properties. In particular, bioactive petit and lipids associated with immunity are more affected in milk by pasteurization (Roth-Walter et al., 2008, p. 882-890; Abdullah et al., 2019, p.157-166). Depending on these nutrient losses, many alternative methods to pasteurization have been investigated up to now. Examples of alternative methods can be given as X-rays, ultraviolet, oscillating magnetic field (OMF), ohmic heating, and ultrasound applications (Bansal and Chen, 2006, p. 27-33; Soria et al., 2010, p. 323-331). Ultrasound technology, which is one of these new technologies, is realized by the effect of sound waves at frequencies above the threshold of human hearing (> 20 kHz). In addition, ultrasound applications are used in the microbial quality control with changes in the physical and chemical structure of foods (Awad et al., 2012, p. 2; Shershenkov and Suchkova, 2015, p. 1074-1085).

Detection of the hematological [leukocytes (WBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT), erythrocytes (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and differential WBC (lymphocyte, monocyte, neutrophil) and biochemical [(serum creatine (Creat), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides (TG), total serum protein (TP), albumin (ALB), globulin (GLOB), urea (UREA), blood urea nitrogen (BUN), cholesterol (CHOL), glucose (GLU),



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calcium (Ca), phospurus (P), sodium (Na), clour (Cl), potasium (K), and osmolarity (OSM)] parameters of human beings (human and animals) help scientific researchers, doctors and also veterinarians to evaluate normal status of the organism, the clinical diagnosis and prognosis of many animal-human diseases, and also investigation of the therapeutic and prophylactic efficacy of organic-inorganic substances (Donmez et al., 2014, p. 558; Kisadere et al., 2019, p. 817-823).

For this purpose, it was aimed to investigate the potential protective effects of different preservation methods applied donkey milk samples on some hematological and biochemical parameters in Wistar rats.

# 2. MATERIALS AND METHODS

### 2.1. Ethics

All animal procedures were approved and conducted by following the guidelines of the Experimental Medicine Research and Application Center of Balikesir University Experimental Animal Ethics Committee (Approval no: 2021 / 3-6).

## 2.2. Preparation of different milk samples

Milk samples were taken from 12 healthy donkeys. Then, they were milked in sterile containers (250-500 ml) and immediately brought to the laboratory at +4 C° for analysis. Rm, pasteurized milk (Pst) and ultrasound (Ult)-treated samples were prepared for applications. Slow pasteurization was applied at 65 °C for 30 minutes. Ultrasound was applied to 100 ml of DM with Bandelin Hd 2200.2, probe TT 13 (AMPLICHRON® Germany) ultrasonic homogenizer at 50% wavelength for 10 minutes (During the ultrasound application, the necessary combination of wavelength and time was applied until the temperature value of DM reached + 60 C°). The combination of wavelength and minute in ultrasound application has been tried in the laboratories of Balikesir University Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, and has achieved bacterial inactivation equivalent to pasteurization.

### 2.3. Animals and administration of different milk samples

The animals, that were used in the experiment, were obtained from Balikesir University Experimental Medicine Research and Application Center. In this study, 28 adult male Wistar Albino rats, which were healthy, two months old (~2), and whose body weights  $(350 \pm 5.0 \text{ g})$  were close to each other, were selected and used. Experimental groups were formed as Control group (C; n = 7), Raw milk group (Rm; n = 7), Pasteurization group (Pst; n = 7) and Ultrasound (Ult; n = 7) group. Although they were divided four equal groups, animals were kept in individual cages. Rats were housed in the Balikesir University Experimental Medicine Research and Application Center, in standard plastic rat cages, at  $23 \pm 2$  C0 room temperature,  $55 \pm 10\%$  relative humidity, and 12 hours day/night light period. Ad-libitum standard rat food



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and fresh drinking water (50 ml /day /rat) were given to the animals during the study period. The feeding of rats with different DM samples was carried out by the method applied by Trinchese et al. (2018, p. 9-32). All applications were made with end-of-day control.

After the two-week adaptation period, the following administrations were performed to the rats:

a. C Group: Standard rat feed and drinking water were given ad libitum throughout the experiment.

b. Rm Group: Raw milk (at the rate of 48 ml/day) was given each of the rats in this group for 4 weeks.

c. Pst Group: Pasteurized milk (at the rate of 48 ml/day) was given each of the rats in this group for 4 weeks.

d. Ult Group: Ultrasound applied milk (at the rate of 48 ml/day) was given each of the rats in this group for 4 weeks.

At the end of the 4-weeks trial, the animals were terminated with the cervical dislocation technique under general anesthesia [(ketamine/xylazine (0.1 ml / 100 mg/ body weight)]. Then, a sufficient amount of blood were drawn from the heart through cardiac puncture into tubes with and without anticoagulant. After, the blood samples were centrifuged (3000 rpm, 25 min, Hermle Z380, Rösler, Germany) and their plasma and serum samples were collected. The separated plasma and serum samples were stored in the freezer at - 80 0C until the time of analysis.

# 2.4. Determination of hematological parameters

Plasma WBC counts, HGB values, percentage (%) of HCT, PLT and RBC counts, MCH, MCHC, MCV levels, and differential WBC (lymphocyte, monocyte, neutrophil) counts were measured by using automatic hematology device (Abacus Junior Vet-5, USA)

# 2.5. Determination of the some biochemical parameters

Serum Creat, ALT, AST, ALP, TG, TP, ALB, GLOB, UREA, BUN, CHOL, GLU, Ca, P, Na, Cl, K, and OSM values were analyzed by using an automated biochemistry analyzer (BS-400 PLUS Mindray, Nanshan, Shenzhen, China).

# 2.6. Statistical analysis

Statistical analysis of the data was done using the analysis of variance (ANOVA) followed by Duncan's test using the SPSS 25.0 program (SPSS, Inc., Chicago, IL). Data were considered significant at  $p \le 0.05$ .



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# **3. RESULTS**

### 3.1. Hematological Parameters

In our study, WBC levels were detected the highest in Ult group compared to C (p < 0,05). Besides, WBC levels were statistically higher in Ult, Rm, and Pst groups than C group (p < 0,05). LYM levels were also found the highest in Ult and Rm groups when compared to the C group (p < 0,05). In addition, C group had the lowest LYM count in the present study. NEU levels were determined the lowest in C group compared to the other experimetal groups (p < 0,05). The highest NEU levels were observed in Ult group compared to other groups in our study (p < 0,05). It was not detected any significant change among the experimental groups according to other hematological parameters in the present study (p > 0,05), shown in Table-1.

Table-1. Some hematological parameters of the experimental groups.

Parameters	Groups	n	Mean±SE
WBC (10 <sup>9</sup> /L)	Raw (Rm)	7	8,62±0,21 <sup>ab</sup>
	Pasteurized (Pst)	7	6,85±0,77 <sup>b</sup>
	Control (C)	7	4,76±0,51°
	Ultrasound (Ult)	7	9,17±0,93ª
LYM (109/L)	Raw (Rm)	7	6,14±0,11ª
	Pasteurized (Pst)	7	4,58±0,64 <sup>b</sup>
	Control (C)	7	3,26±0,34°
	Ultrasound (Ult)	7	5,84±0,42ª
MON (109/L)	Raw (Rm)	7	0,28±0,13
	Pasteurized (Pst)	7	$0,18{\pm}0,11$
	Control (C)	7	0,19±0,11
	Ultrasound (Ult)	7	0,40±0,18
NEU (109/L)	Raw (Rm)	7	2,19±0,10 <sup>ab</sup>
	Pasteurized (Pst)	7	2,08±0,17 <sup>bc</sup>
	Control (C)	7	1,31±0,10°
	Ultrasound (Ult)	7	2,92±0,48 <sup>a</sup>
<b>RBC (1012/L)</b>	Raw (Rm)	7	9,91±0,84
	Pasteurized (Pst)	7	9,05±0,18
	Control (C)	7	9,58±0,30
	Ultrasound (Ult)	7	9,26±0,23
HGB (g/dL)	Raw (Rm)	7	13,60±0,21
	Pasteurized (Pst)	7	13,62±0,69
	Control (C)	7	13,50±0,23
	Ultrasound (Ult)	7	13,94±0,16
HCT (%)	Raw (Rm)	7	47,52±2,96
	Pasteurized (Pst)	7	43,25±0,98
	Control (C)	7	46,72±1,13
	Ultrasound (Ult)	7	45,38±1,05
MCV (fL)	Raw (Rm)	7	48,29±0,89
	Pasteurized (Pst)	7	47,86±0,34
	Control (C)	7	48,86±0,55
	Ultrasound (Ult)	7	49,14±0,63
MCH (pg)	Raw (Rm)	7	14,18±0,91
	Pasteurized (Pst)	7	$15,04{\pm}0,17$
	Control (C)	7	$14,17\pm0,49$
	Ultrasound (Ult)	7	$15,08\pm0,27$

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MCHC (g/dL)	Raw (Rm)	7	29,17±1,43
	Pasteurized (Pst)	7	31,50±0,30
	Control (C)	7	$28,95{\pm}0,69$
	Ultrasound (Ult)	7	$30,78{\pm}0,53$
PLT (109/L)	Raw (Rm)	7	985,43±243,52
	Pasteurized (Pst)	7	589,71±37,67
	Control (C)	7	741,86±45,65
	Ultrasound (Ult)	7	675,43±44,20

a,b,cP < 0,05 considered statistically significant in the same column.

#### **3.2.** Biochemical Parameters

Serum ALP values were defined the highest in Rm group compared to other experimental groups (C, Ult, and Pst) in the present study (p < 0,05), interestingly. On the other hand, serum AST values were definitively higher in Past group than C, Ult, and Rm groups in our study (p < 0,05). Although, ALB and GLOB values were not affected from the different milk applications, ALB/GLOB ratio was observed the lowest in Pst group compared to the other experimental groups (p < 0,05). In addition, serum Cl values were higher in Pst group than C group in our study (p < 0,05). There was no significant change among the experimental groups according to other biochemical values in the present study (p > 0,05), shown in Table-2.

Parameters	Groups	n	Mean±SE
ALP (U/L)	Raw (Rm)	7	203,14±17,81ª
( )	Pasteurized (Pst)	7	162,86±9,19 <sup>b</sup>
	Control (C)	7	162,00±9,92 <sup>b</sup>
	Ultrasound (Ult)	7	158,57±13,79 <sup>b</sup>
AST (U/L)	Raw (Rm)	7	114,00±5,98 <sup>b</sup>
	Pasteurized (Pst)	7	208,43±50,31ª
	Control (C)	7	125,57±10,21 <sup>b</sup>
	Ultrasound (Ult)	7	127,14±14,69 <sup>b</sup>
ALT (U/L)	Raw (Rm)	7	77,61±3,77
	Pasteurized (Pst)	7	90,51±8,53
	Control (C)	7	86,45±8,51
	Ultrasound (Ult)	7	76,14±2,96
ALB (g/dL)	Raw (Rm)	7	2,81±0,02
	Pasteurized (Pst)	7	$2,64{\pm}0,24$
	Control (C)	7	$2,94{\pm}0,06$
	Ultrasound (Ult)	7	$2,88{\pm}0,01$
GLOB (mg/dL)	Raw (Rm)	7	$1,82{\pm}0,05$
	Pasteurized (Pst)	7	1,91±0,15
	Control (C)	7	$1,90\pm0,06$
	Ultrasound (Ult)	7	2,04±0,06
ALB/GLOB	Raw (Rm)	7	1,54±0,04ª
	Pasteurized (Pst)	7	1,36±0,05 <sup>b</sup>
	Control (C)	7	1,55±0,05ª
	Ultrasound (Ult)	7	1,42±0,04 <sup>ab</sup>
TP (g/dL)	Raw (Rm)	7	4,24±0,41
	Pasteurized (Pst)	7	4,55±0,38
	Control (C)	7	$4,84{\pm}0,09$
	Ultrasound (Ult)	7	4,92±0,06
UREA (mg/dL)	Raw (Rm)	7	44,28±2,03

Table-2. Some biochemical parameters of the experimental groups

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	Pasteurized (Pst)	7	45,92±2,48
	Control (C)	7	44,24±0,89
	Ultrasound (Ult)	7	46,67±2,33
CREAT (mg/dL)	Raw (Rm)	7	0,57±0,03
	Pasteurized (Pst)	7	$0,50{\pm}0,03$
	Control (C)	7	$0,46{\pm}0,05$
	Ultrasound (Ult)	7	$0,53{\pm}0,03$
BUN (mg/dL)	Raw (Rm)	7	20,68±0,95
	Pasteurized (Pst)	7	21,44±1,15
	Control (C)	7	20,66±0,41
	Ultrasound (Ult)	7	21,79±1,08
BUN/CREAT	Raw (Rm)	7	36,97±2,69
Derweiten	Pasteurized (Pst)	7	43,91±5,13
	Control (C)	7	52,04±10,12
	Ultrasound (Ult)	7	41,83±3,29
CHOL (mg/dL)	Raw (Rm)	7	35,86±2,65
CHOL (ing/uL)	Pasteurized (Pst)	7	$43,43\pm1,30$
	Control (C)	7	$38,43\pm3,25$
	Ultrasound (Ult)	7	$38,43\pm 3,23$ $38,14\pm 3,49$
TC (mg/dL)		7	72,38±4,94
TG (mg/dL)	Raw (Rm) Pasteurized (Pst)	7	72,38±4,94 74,84±19,78
	Control (C)	7	$90,12\pm6,83$
	Ultrasound (Ult)		
		7	58,54±4,88
GLU (mg/dL)	Raw (Rm)	7	269,80±31,73
	Pasteurized (Pst)	7 7	231,00±21,12
	Control (C)		249,22±24,73
<b>O</b> ( ) <b>H</b> )	Ultrasound (Ult)	7	255,22±26,05
Ca (mg/dL)	Raw (Rm)	7	10,15±0,33
	Pasteurized (Pst)	7	11,75±1,97
	Control (C)	7	12,17±0,83
	Ultrasound (Ult)	7	10,40±0,30
P (mg/dL)	Raw (Rm)	7	8,13±0,62
	Pasteurized (Pst)	7	7,09±0,27
	Control (C)	7	6,97±0,20
	Ultrasound (Ult)	7	7,60±0,42
Na (mmol/L)	Raw (Rm)	7	123,54±0,97
	Pasteurized (Pst)	7	123,72±0,46
	Control (C)	7	123,35±1,04
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	Ultrasound (Ult)	7	124,80±0,51
Cl (mmol/L)	Raw (Rm)	7	87,00±2,45 <sup>ab</sup>
	Pasteurized (Pst)	7	92,00±2,74 <sup>a</sup>
	Control (C)	7	84,43±1,08 <sup>b</sup>
	Ultrasound (Ult)	7	88,57±1,28 <sup>ab</sup>
K (mmol/L)	Raw (Rm)	7	5,01±0,31
	Pasteurized (Pst)	7	$4,82\pm0,18$
	Control (C)	7	$4,93{\pm}0,07$
	Ultrasound (Ult)	7	$4,78\pm0,28$
Na/K	Raw (Rm)	7	25,16±1,41
	Pasteurized (Pst)	7	25,87±0,93
	Control (C)	7	25,05±0,52
	Ultrasound (Ult)	7	26,60±1,47
Osmolarity	Raw (Rm)	7	269,46±2,51
(Osmol/L)	Pasteurized (Pst)	7	267,95±1,37
	Control (C)	7	267,93±2,51
	Ultrasound (Ult)	7	264,98±4,36
$b\mathbf{D} < 0.05$ as a side and as	tatistically significant in		<b>2</b> 0 139 0- 138 0

 $^{a,b}P \le 0.05$  considered statistically significant in the same column.



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#### **4. DISCUSSION**

In our study, different DM treatments (48 ml/day for 4 weeks, p.o) led to an increase in WBC counts of Ult, Rm, and Pst groups, especially in the Ult, compared C group animals, interestingly. Besides, LYM counts were observed the highest in Ult and Rm groups compared to the C group. The highest NEU counts were determined in Ult group compared to other groups in our study. In contrary, C group had the lowest LYM and NEU counts in the present study. In addition, it was not detected any significant change among the experimental groups according to the MON counts in our study. Chihara et al. (1992, p. 57-77) informed that DM could induce a remarkable increase in cytokine production, which is enhaged to an immune response, and result in maturation, differentiation, and proliferation of immun cells for defence mechanisms of the organism, of interleukin-2 (IL-2), interferon-gamma (IFN-g), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-a), and interleukin-1-beta (IL-1b) from LYM and macrophages. Mao et al. (2009, p. 703-708) also reported that DM could promote the cytokine potentiation response of precursor T cells and macrophages in vitro. These changes may be occurred due to the varying lysozyme content depending on the different preservation methods applied to donkey milk or hygiene of applications. In the present study, it was not defined any significant alteration among the experimental groups according to the other hematological parameters RBC, HCT, HGB, MCV, MCH, MCHC, and PLT. Deeba et al. (2020, p. 915-923) reported that diabetic altered levels of RBC, HGB, MCV, and MCH were significantly improved by different treatments of camel milk (CM) and buffalo milk (BM) in rabbits. Fisberg et al. (2010, p. 526-537) also informed that goat's milk (GM) (300 mL, for 4 months, p.o) treatment increased RBC count, however decreased MCV and MCH values in children. Khalid et al. (2014, p.1465-1468) aslo suggested that non-significant differences were observed in some hematological parameters including RBC, Hb, PCV, MCH, MCHC, MCV, and WBC of the CM group (5mL/kg for 3 weeks) compared with C group rats that was corresponding with our results. In the literature, we could not find any significant information about the effects of DM treatment on above mentioned parameters in rats. It may be observed due to different gender, milk content and treatment period.

In the present study, serum ALP values were detected the highest in Rm applied rats compared to C. In addition, serum ALP levels did not change in Ult and Pst groups, interestingly. Abdel-Mobdy et al. (2021, p. 7958-7960) suggested that CM administration caused to decrease of ALP values in rats exposed to gamma-radiation. We could not find any significant information about the effects of different milk samples on ALP values in rats. On the other hand, serum AST values were definitively higher in Pst group than C, Ult, and Rm groups in our study. Conversely, we did not obtain any changes in ALT values of the experimental groups in our study. Deeba et al. (2020, p. 915-923) informed that CM and BM administrations ameliorated the serum AST and ALT values in alloxan-induced diabetic rats. Miao et al. (2016, p. 89-96) also claimed that oral administration of fermented milk suplemention with synbiotics did not alter serum ALT and AST values in rats. Ali et al. (2017, p. 17-22) reported that CM administration led to decrease of serum ALT and AST values in



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diabetic female albino rats. Also, Xu et al. (2015, p.1-6) offered that GM administration generally induced to reduce of ALT and AST values in weaned rats. Differences may be observed due to different gender, application time-dose-methods or quality changes of milk due to different preservation methods.

Although, ALB and GLOB values were not affected from the different milk applications, ALB/GLOB ratio was observed the lowest in Pst group compared to the other experimental groups. In addition, TP values were not affected from different donkey milk samples in our study. Debaa et al. (2020, p. 915-923) suggested that CM and BM administrations decreased to the diabet-induced increased TP values in rabbits. On the other hand, CM and BM administrations caused to increase of ALB values in rabbits in the same study. Miao et al. (2016, p. 89-96) informed that oral administration of fermented milk suplemention with synbiotics increased the serum TP and ALB values in female Wistar rats, however, male rats were not affected from these applications. Xu et al. (2015, p.1-6) reported that GM administration increased to the TP and GLOB values, however, decreased the ALB values compared to C groups in weaned rats. It may be occured due to changing of milk content and quality depend on the application methods or different immune responses of animals.

In our study, serum UREA, CREAT, and BUN values did not influenced from different type of DM administrations. Debaa et al. (2020, p. 915-923) reported that CM and BM administrations increased to the serum UREA levels, but did not effect the CREAT values compared to placebo group in a previous study. Besides, Xu et al. (2015, p.1-6) informed that GM administration did not alter serum BUN and CREAT values in weaned rats. On the other hand, Ali et al. (2017, p. 17-22) suggested that cow milk (CM) administration increased to the BUN and CREAT levels when compared to C group in rats. The obtained different results may have occurred depending on the type or characteristics of the milk samples.

Serum CHOL, TG, and also GLU values were not influenced from different type of DM administrations in our study. Singh et al. (2019, p. 332-335) reported that supplemention of Lactobacillus plantarum and L. casei based probiotic milk powder led to decrease of serum CHOL and TG values in Charles Foster rats. Xu et al. (2015, p.1-6) also claimed that GM administration did not affect the serum TG values in weaned rats. Miao et al. (2016, p. 89-96) also informed that milk supplemention with synbiotics reduced to the serum TG and GLU values in Wistar rats. In addition, Deeba et al. (2020, p. 915-923) suggested that CM and BM administrations caused to reduce of serum GLU values in diabetic rabbits. These results may be obtained due to unaffected benefical gluco-metabolic properties of DM by different preservation methods.

In the present study, administration of different type of DM samples did not affect the serum Ca, P, Na, K, and OSM values except Cl, interestingly. In addition, serum Cl values were significantly higher in Pst group than C group in our study. Shokrollahi et al. (2013, p. 184-190) suggested that administration of the enriched CM with selenium (Se) and vitamine E (Vit-E) to newborn goat kids did not lead to change in serum Ca values. We could not get any



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information about the effects of different milk administrations on serum mineral values of the rats in the literature.

### **5. CONCLUSION**

In conclusion, different preservation methods applied donkey milk administration exhibits potential affects on some hematologic and biochemical characteristics in rats.

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