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EFFECTS OF ANTICOAGULANT (EDTA AND HEPARIN) ON BLOOD OF GOAT

Sushanto Chandro Roy, Md. Zamal Uddin, Sm. Ahasanul Hamid, Moizur Rahman, Md. Royhan Gofur and S. M. Kamruzzaman*

Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.

***Corresponding author:** S. M. Kamruzzaman; E-mail: drsazadru@gmail.com

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ABSTRACT

Blood is an important tool for accurate diagnosis of disease, forensic investigation, and hematological analysis. However, this phenomenon can be thwarted occasionally if appropriate anticoagulant with storage times is not maintained properly. This research aims to compare and explore the effect of anticoagulants (such as ethylene diamine tetra acetic acid and heparin) and storage time on the hematological parameters in indigenous goats. Twenty goats were enrolled and raised for this purpose. Bloods were collected into two different tubes containing EDTA and heparin and analyzed immediately to evaluate the basal value. All tubes were divided into two aliquots and stored at 4°C and 25°C and were analyzed again at the 24th and 48th hours of collection. The statistical analysis of this result showed that there was no significant difference (P>0.05) in the anticoagulant's effect on the hematological parameters. RBC, Hb, PCV, MCV, MCH, MCHC, total WBC, and Platelets except ESR decreased gradually along with the storage time of up to 48 hours compared to the basal value. The hematological parameters were reduced more significantly (P<0.05) when stored at 25^{0}C rather than 4°C indicating that the parameters remain in better condition in refrigeration. The samples should be stored at 4°C rather than room temperature and be used within 24 hours because the storage time modify the analyzed results. These findings demonstrate that both of the anticoagulants (EDTA and Heparin) show reliable results, therefore it can be used to store blood samples for any diagnostic purpose.

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INTRODUCTION

Hematological analysis is an important and reliable tool used for monitoring and evaluating the health and welfare status of animals specially in small ruminants (Polizopoulou, 2010). Considering the economic values of goats, it is essential to execute in farms, clinical and preclinical cases for sanitary control strategies, prevention or treatment of diseases and ensure good management practices (Gupta et al., 2007). In some cases, blood samples required for hematological analyses collected from animals raised at remotely located area to the laboratory and the analysis of these samples may be delayed (Hulme-Moir, Clark and Spencer, 2006). Hence, the storage conditions of these blood samples may affect test results even false and misleading results can be obtained (Ihedioha and Onwubuche, 2007). This changes are directly related to the storage time and the type of the anticoagulants used (Ochei and Kolhatkar, 2000). The storage conditions also affects the hematological values of blood samples taken from humans and various animal species (Athanasiou et al., 2016; Hadzimusic et al., 2010). Therefore, it is important to ensure packing the samples with ice in insulated containers to minimize these effects (Jones and Allison, 2007; Latimer, Mahaffey and Prasse, 2003). The prolonged storage in particular condition could compromise red blood cell (RBC) properties and volume changes accompanied by alterations in intracellular hemoglobin concentrations (Ho, Sibbald and Chin-Yee, 2003; Van Wijk and Van Solinge, 2005). Room temperature also caused greater RBC swelling to occur after 6-24 hrs, may lead to aberrations, such as increased PCV and decreased MCHC (Piccione et al., 2014).

The choice of anticoagulant and storage time is very important when blood samples are used in laboratorial analysis because blood parameters are likely influenced by these two independent variables (Faggio et al., 2013). There are relatively few reports and evidence of the effects of anticoagulants and storage time on blood parameters of animals specially in the goat. So, it is very important to evaluate the stability of hematological parameters in caprine blood stored at 4°C and 25^0C as well as to compare the hematological parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC and PLT levels) in EDTA and heparin containing tubes at 2 hrs, 24 hrs and 48 hrs of storage.

MATERIALS AND METHODS

Selection and rearing of goats

Twenty goats (Age: 1.5-3 years and body weight: 10 ± 5 kg) were selected and raised during the period of July-December, 2018 in the research and practice farm under the department of veterinary and animal sciences of Rajshahi University. All goats were clinically healthy and free from internal and external parasites as anthelmintic treatment was provided.

Collection and storage of blood samples

Blood samples were collected from each goat and it was put into two different tubes, one contains ethylene diamine tetracetic acid (1 mg EDTA/ml blood) and other contains heparin (50 IU/ml) (Witeska and Wargocka, 2011). Blood samples were stored on “wet ice” (4~6°C) in an insulated container and its temperature was continuously monitored. Samples were tested within 2 hrs after sampling (T2) for determination of the baseline value. Blood samples were carefully divided into two equal aliquot, one aliquot was kept in a refrigerator at 3°C-5°C (average 4°C) while the second aliquot was kept at room temperature (25^0C). Both of the aliquots were stored at 4°C and tested again at 24^{th} and 48^{th}hrs respectively.

Hematological analysis

For hematological examination, the collected samples were transported to the laboratory of the department of veterinary and animal sciences, University of Rajshahi and metro diagnostic centre of Rajshahi, Bangladesh. Before testing, samples were removed from the insulated container and left at room temperature for 20 mins and then gently mixed. The estimation of hematological parameters were carried out by an automated analyzer (Automated Dymind DH-33 Hematology Analyzer) and verified manually by the standard method as described by (Coffin, 1953) and (Lamberg and Rothstein, 1978).

Statistical Analysis

Data were compiled and analyzed using IBM SPSS statistics version 25. One-way repeated measures analysis of variance (ANOVA) was performed in order to determine the effects of anticoagulant by Bonferroni’s confidence interval adjustment at $p<0.05$ level of significance. All data were presented as mean ± SD and p values $p<0.05$ was considered as statistically significant.

RESULT AND DISCUSSION

All of the hematological parameters such as RBC, WBC, Platelet, Hb, PCV, MCV, MCH, MCHC except ESR were decreased gradually over time in both of the EDTA and Heparin containing blood samples when stored at 4°C and room temperature (25°C) respectively but the ESR increased over the time in compare to the base value (Table 1 and Table 2).

Table 1. Hematological parameters of EDTA and heparin treated blood sample stored at 4°C

Parameters	EDTA treated blood sample			Heparin treated blood sample		
	2 hrs	24 hrs	46 hrs	2 hrs	24 hrs	46 hrs
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
RBC($x10^6$/µL)	4.48±0.25	4.16± 0.68	2.85±0.74	4.37±0.35	4.09 ± 0.42	2.96±0.56
Hb (gm/dL)	9.57±0.78	8.75±0.8	7.01±1.5	9.51±0.52	8.5±0.5	6.94±1.0
PCV (%)	35.63±3.29	30.53±4.62	23.85±5.28	34.75±2.06	31.8±2.31	22.71±1.54
ESR (mm/hr)	0.57 ±0.25	0.92±0.34	1.94±0.56	0.62±0.15	0.88±0.28	1.99±0.42
MCV (fL)	34.21±2.56	34.27±2.85	34.32±2.64	33.98±1.85	34.01±2.25	34.09±2.32
MCH (pg)	9.08±1.46	8.91±1.25	7.19±1.15	9.11±1.04	8.79±0.65	7.06±0.53
MCHC (gm/dL)	32.07±1.67	31.08±1.45	28.57±1.84	32.21±1.06	30.99±0.38	28.01±0.25
Total WBC ($x10^3$/µL)	14.85±3.82	12.58±3.45	9.21±4.68	14.62±2.54	12.35±1.83	9.37±3.9
PLT($x10^3$/µL)	266.38±95.61	228.46±87.51	172.44±30.54	272.82±82.53	234.23±75.28	181.53±21.3

Table 2. Hematological parameters of EDTA and heparin treated blood sample stored at room temperature (25°C)

Parameters	EDTA treated blood sample			Heparin treated blood sample		
	2 hrs	24 hrs	48 hrs	2 hrs	24 hrs	48 hrs
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
RBC($x10^6$/µL)	4.48±0.25	3.74± 0.86	2.14±0.69	4.37±0.35	3.67 ± 0.52	2.03±0.83
Hb (gm/dL)	9.57±0.78	7.25±0.58	5.89±1.08	9.51±0.52	7.57±0.73	5.78±1.1
PCV (%)	35.63±3.29	28.43±3.72	21.76±4.17	34.75±2.06	27.98±3.41	21.11±3.37
ESR (mm/hr)	0.57 ±0.25	1.62±0.93	2.71±1.06	0.62±0.15	1.68±0.74	2.83±1.15
MCV (fL)	34.21±2.56	35.36±2.87	38.33±1.28	33.98±1.85	35.29±2.64	38.09±1.22
MCH (pg)	9.08±1.46	7.28±1.21	6.99±1.34	9.11±1.04	7.19±1.01	6.87±0.89
MCHC (gm/dL)	32.07±1.67	30.57±2.38	27.84±2.58	32.21±1.06	30.49±2.11	27.79±2.01
Total WBC ($x10^3$/µL)	14.85±3.82	10.5±3.26	6.91±4.21	14.62±2.54	10.7±1.41	7.07±4.87
PLT($x10^3$/µL)	266.38±95.61	215.41±87.51	142.37±30.54	272.82±82.53	213.25±75.28	146.53±21.36

Effects on Total Erythrocyte Count (TEC)

The findings of this research showed EDTA containing blood samples stored over time can lead to relatively little changes in erythrocytes morphology as well as their fragility in comparison with the heparin containing blood samples. But, in case of both samples, there is a gradual decrease of RBC level along with the storage time (Figure 1). This result has similarities with the studies on human and equine blood samples that reported a decrease volume of RBC stored for several hrs both at room and refrigerator temperature (Faggio et al., 2014). It has been demonstrated that the viability of RBC may be compromised if blood is stored for longer than 96 hrs (Caldwell et al., 2006). Hence, the assessed parameters change significantly from baseline values varies from one species to the other.

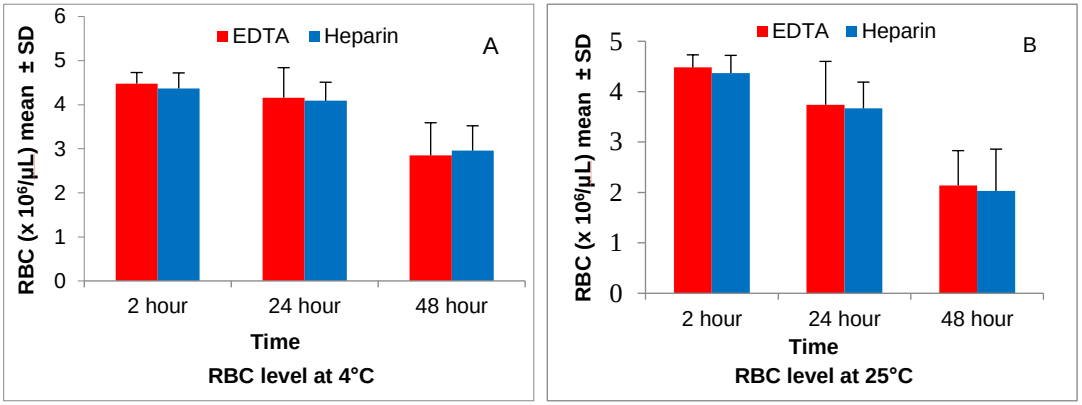

Figure 1. Comparison of RBC between EDTA & heparin treated samples; A & B. RBC (x10^6/cmm) decreased gradually over time in EDTA and heparin containing samples at 4^0C & 25^0C

Effects on Hemoglobin (Hb)

From the exploration of anticoagulant containing samples it was observed that, the Hb values were decreased gradually at 24th and 48thhrs in comparison with the baseline value at 4^0C and 25^0C storage conditions (Figure 2). These results were analogous to some previous studies where significant decrease in Hb was noticed (Phulia et al., 2010; Selvaraj, Mathivanan and Nanjappan, 2004). Generally, high hematocrit and high Hb was related to erythrocyte swelling and hemolysis respectively (Witeska and Wargocka, 2011).

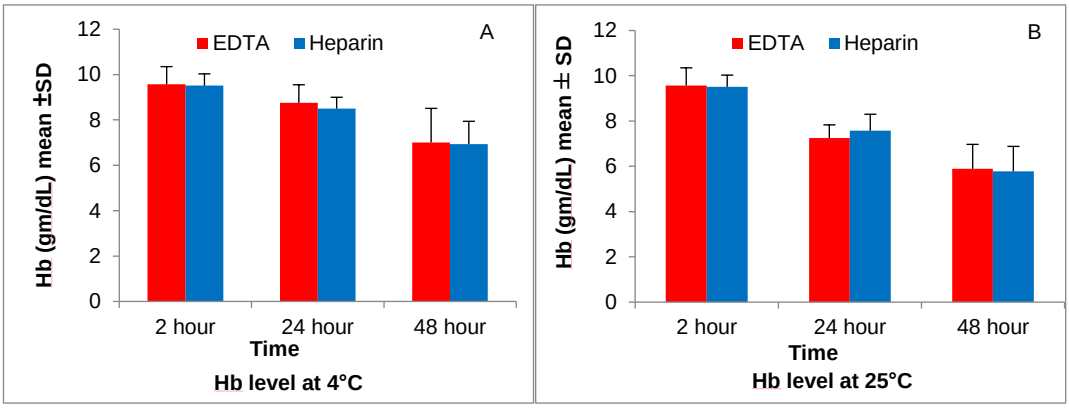

Figure 2. Comparison of Hb between EDTA & heparin treated samples; A & B. Hb (gm/dl) decreased gradually over time in EDTA and heparin containing samples at 4^0C & 25^0C

Effects on Packed Cell Volume (PCV)

The PCV values are decreased gradually over time in comparison with the baseline value at different storage temperature (Figure 3). This finding was similar with previous reports (Gulati et al., 2002) but in dogs, PCV values increased over time (Furlanello et al., 2006). Increased PCV values may be due to increase in environmental temperature. Furthermore, it has been reported that increased in storage temperature may cause decreased in PCV values (Aye, 2012).

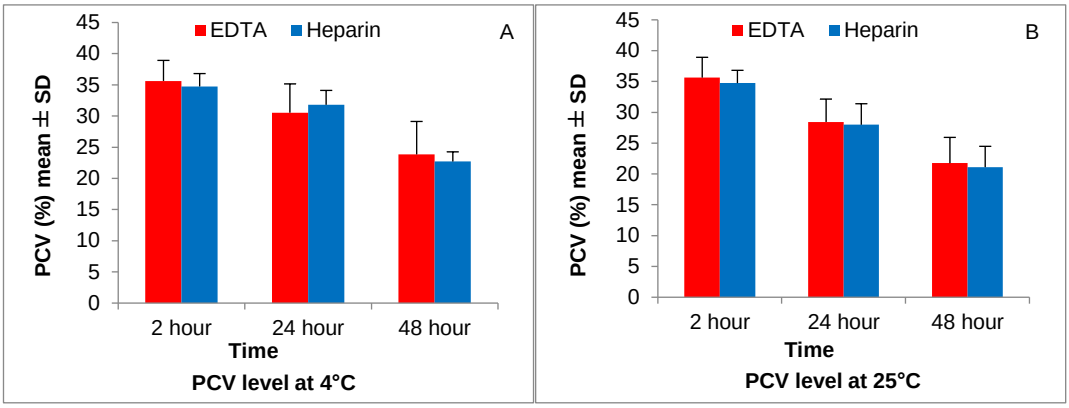

Figure 3. Comparison of PCV between EDTA & heparin containing samples; A & B. PCV (%) decreased gradually over time in EDTA and heparin containing samples at 4^0C & 25^0C

Effects on Erythrocyte Sedimentation Rate (ESR)

It was obviously observed in this study that the ESR values are increased gradually over time (Figure 4). This result was supported by Schalm who revealed that the ESR is inversely related to the number of erythrocytes or PCV (Schalm, Jain and Carroll, 1975). Certain factors of plasma protein particularly the concentration of fibrinogen and globulin might be involved in the ESR.

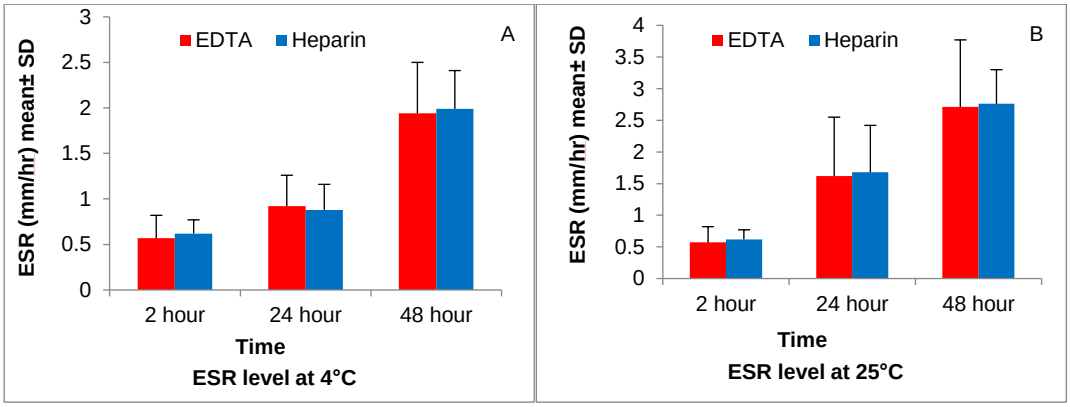

Figure 4. Comparison of ESR between EDTA & heparin containing samples; A & B. ESR (mm/hr) increased gradually over time in EDTA and heparin containing samples at 4^0C & 25^0C

Effects on Mean Corpuscular Volume (MCV)

The MCV values are decreased gradually along with the storage time at 24th and 48th hrs in comparison with baseline value at both of the storage temperature (Figure 5). This finding was similar to previously reported results but there was no change in MCH for up to 4 days after collection of blood (Gulati et al., 2002; Mahmoodi et al., 2006). The increase in MCV is known to reflect the red cell swelling at room temperature (de Baca et al., 2006). RBC swells and increases in size/volume may be due to degenerative changes that permit ingress of water into the cells compromising the membrane stability (Hadzimusic et al., 2010). Refrigerated storage prevents the swelling but membrane fragility decreases with storage time in goats (Okwusidi, 2004).

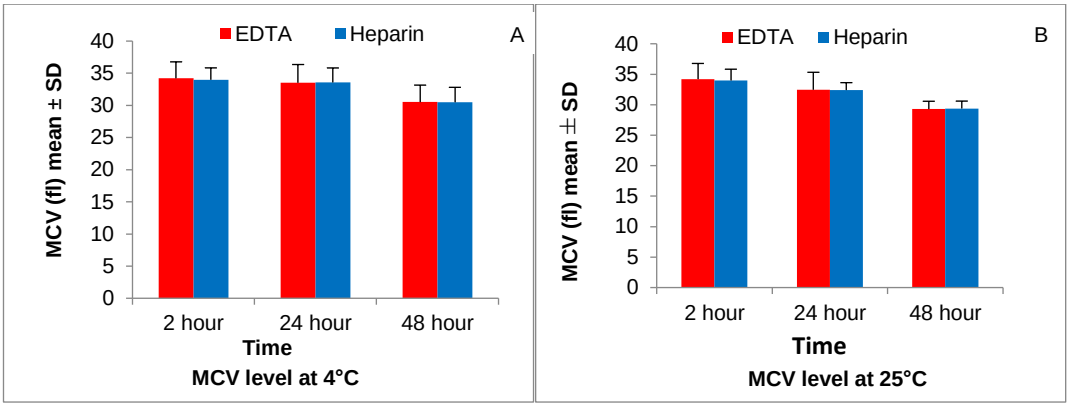

Figure 5. Comparison of MCV between EDTA & heparin containing blood samples; A & B. MCV (fl) decreased gradually over time in EDTA and heparin containing samples at 4^0C & 25^0C

Effects on Mean Corpuscular Hemoglobin (MCH)

The MCH values are decreased gradually over time in comparison with the baseline value at both of the storage temperature (Figure 6). This result was supported by previous study where MCH decreases because the Hb concentration decreases. Hb concentration is decreased may be due to conversion of Hb to degradation intermediates as reported in pigs (Ihedioha and Onwubuche, 2007).

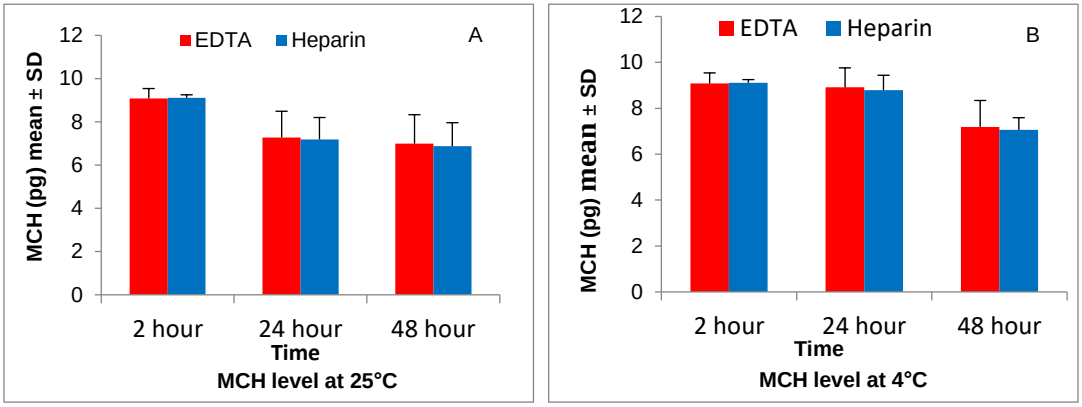

Figure 6. Comparison of MCH between EDTA & heparin containing blood samples; A & B. MCH (pg) decreased gradually over time in EDTA and heparin containing samples

Effects on Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC values are decreased gradually along with the storage time in comparison with the baseline value at both of the storage temperature (Figure 7). In this explore it was found that, decrease in MCHC values was related to the change of Hb and the increase in HCT. The values of mean corpuscular volume (MCV) significantly increased while MCHC indicate macrocytic and hypochronicanaemia probably due to the increased activity of bone marrow and deficiency of some hemopoietic factors (Caillard, 2002).

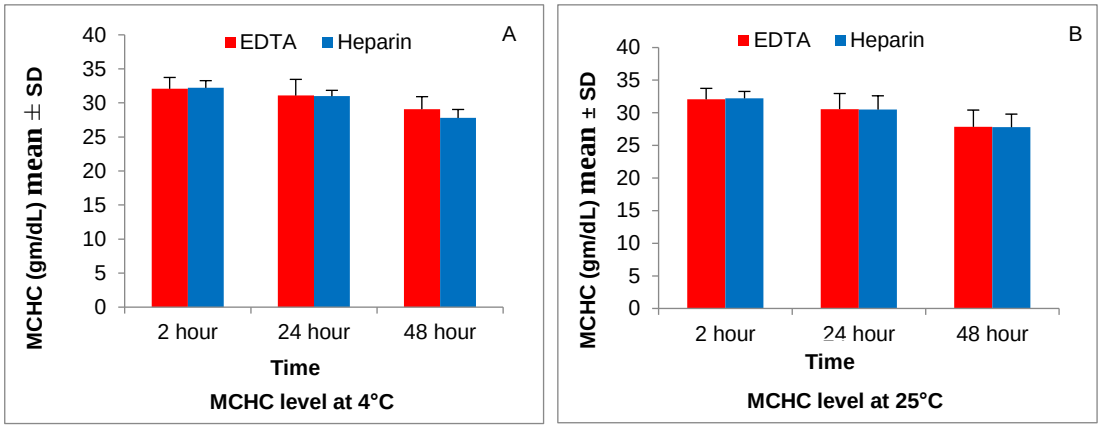

Figure 7. Comparison of MCHC between EDTA & heparin containing blood samples; A & B. MCHC (gm/dl) decreased gradually over time in EDTA and heparin containing samples

Effects on total WBC

The total WBC values were decreased gradually over time at 24th and 48th hrs in comparison with the baseline value at both of the storage temperature (Figure 8). This result consentient with the report of de Baca who found that WBC count reduce after blood collection (de Baca et al., 2006). In another study it was noticed that, bovine and caprine blood samples when stored at 5°C and 30°C for 120 hrs, neither of the storage temperatures caused any alteration in the WBC levels in bovine blood but significant decrease in WBC levels of caprine blood (Ihedioha and Onwubuche, 2007). A possible reason for that the heparin treated blood is recognition of presence of anticoagulants as foreign bodies within the cells thereby stimulating the production of more cells (WBC) to fight against them as self-defense (Faggio et al., 2013).

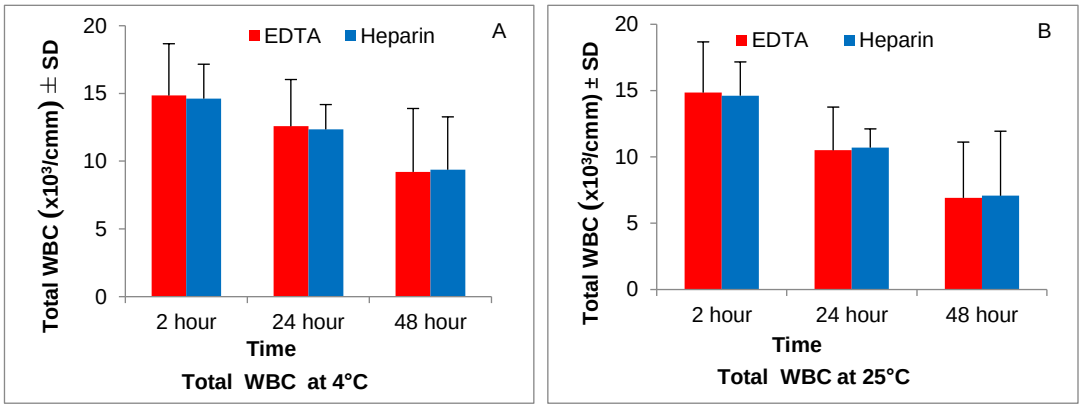

Figure 8. Comparison of WBC between EDTA & heparin containing blood samples; A & B. WBC (x 10^3/cmm) decreased gradually over time in EDTA and heparin containing samples

Effects on Platelets (Plt)

The platelet values were decreased gradually like WBC (Figure 9). Similar findings were reported by Caillard who observed that, platelet counts are the most unstable during storage of canine whole blood tested as early as 6 hrs after sampling. The change of shape in platelets may be the result of microtubule disassembly which may also contribute to reduce survival when stored at 4°C (Caillard, 2002). Moreover, in platelet aggregation and activation, the intracellular Ca^{2+} concentration plays a vital role (Kamruzzaman et al., 2011; Kamruzzaman and Rasool, 2016). On the other hand, another report showed increases in platelets counts after 48 hrs incubation and elevated temperature (Mahmoodi et al., 2006). This happens may be due to the mechanism of laboratory effect that raise the temperature cusses to changes in platelets morphology and movement (Qi, Yatomi and Ozaki, 2001).

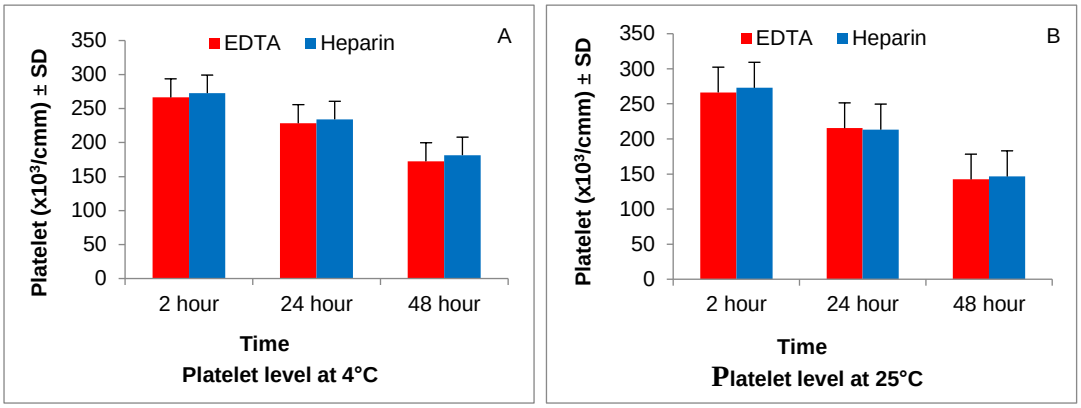

Figure 9. Comparison of platelet between EDTA & heparin containing blood samples; A & B. Platelet (x 10^3/cmm) decreased gradually over time in EDTA and heparin containing samples

CONCLUSION

In conclusion, from this research it is clear that there is no significant difference in the effect of EDTA and heparin on the hematological parameters in caprine blood. EDTA is cost effective and easily available in respective of our country. Therefore, it is advisable to the veterinarian and other personnel for using the EDTA as anticoagulant for any diagnostic purpose.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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