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HAEMATOBIOCHEMICAL CHANGES OF OVINE (*Ovis aries*) BLOOD DURING STORAGE FOR TRANSFUSION

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ABSTRACT

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Haematobiochemical changes of ovine (sheep) blood were investigated during preservation and storage with Citrate Phosphate Dextrose Adenine-1 (CPDA-1) and Acid Citrate Dextrose (ACD) for transfusion. Twelve healthy sheep were selected and divided into two equal groups: group X (n=6) and group Y (n=6). Thirty-five ml of blood was collected from each animal and preserved with CPDA-1 in group X and ACD in group Y under 4°C in refrigerator for 28 days. Haematological changes viz., total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC); and biochemical changes viz., total protein (TP) and pH were evaluated immediately after blood collection and thereafter on day-1, day-3, day-7, day-14, day-21 and day-28 for both groups. In ACD preserved blood; TEC, TLC, Hb and PCV decreased significantly ($P<0.01$) from day-14 onward, whereas in CPDA-1 preserved blood, these parameters decreased significantly ($P<0.01$) from day-21 onward. Blood preserved in ACD showed significant changes ($P<0.01$) in MCV, MCH and MCHC respectively from day-7, day-14 and day-21 onward, whereas blood preserved in CPDA-1 showed no significant changes in the same parameters throughout the experiment. In both groups, no significant changes were noticed in TP but significant changes ($P<0.01$) were observed in pH with the progression of storage period. These findings elicited that both ACD and CPDA-1 exert certain haematobiochemical changes in stored sheep blood, however, CPDA-1 was more efficient than ACD in terms of maintaining proper levels of TEC, TLC, Hb., PCV, MCV, MCH and MCHC during preservation and storage of sheep blood for transfusion.

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INTRODUCTION

Blood transfusion has been used as a life-saving therapeutic procedure in veterinary medicine for a long time since Richard Lower first experimented this technique in animal species in 1665 (Davidow, 2013; Kumar, 2017). It is usually used in critically ill animals with life-threatening anaemia, haemolysis, immune-mediated diseases, severe non-regenerative conditions, neonatal isoerythrolysis, trauma, injury and burn (Tocci, 2010). Transfusion therapy can be used to upgrade the haematobiochemical parameters in the recipients (Suffian and Hossain, 1999; Alam and Hossain, 2005). Various preservatives and additives are used to store blood and blood products (Monica, 2003) intended to be transfused in animals. Citrate-dextrose was found by Rous and Turner in 1916, as an anticoagulant to permit preservation and storage of blood in a refrigerated state for several days post-collection (Klein and Anstee, 2005; Greening *et al.*, 2010). Loutit and others developed the acid citrate dextrose solution in response to the huge demand for stored blood during the Spanish Civil War and World War II, whereas in 1960s, Simon pioneered the use of adenine additives (Dzik, 2008). The ideal duration of storage should be maintained to minimize storage lesions and erythrocyte haemolysis (McDevitt *et al.*, 2011; Solomon *et al.*, 2013). During storage, blood cells undergo biochemical, structural, enzymatic, morphological and functional deterioration due to loss of 2,3 diphosphoglycerate (DPG); depletion of ATP; reduction in Na⁺-K⁺ gradients; increase in osmotic fragility and membrane changes including micro vesiculation and haemolysis (Hess, 2006; Dzik, 2008). Although many of these changes are reversible following transfusion, it is essential to find out the limit of red blood cell (RBC) storage beyond which transfusion is unfavorable. RBC can be stored at 2–6°C in refrigerator up to 42 days (maximum duration may vary depending on the type of additive solution used or local regulatory criteria), whereas for platelets, storage should be at 20–24°C up to 5 days with continuous gentle agitation to maintain maximum biological function, and plasma can be rapidly frozen at less than –25 °C in order to maintain maximum function of labile coagulation factors, such as Factor VIII (Holme, 2005; Hogman *et al.*, 2006; Tinmouth *et al.*, 2006; Greening *et al.*, 2008). White blood cells lose their phagocytic property within 4-6 hours of collection and become non-functional after 24 hours of storage (Thon *et al.*, 2008). However, they do not lose their antigenic property and are capable of sensitizing the recipient to produce non-haemolytic febrile transfusion reactions, and few lymphocytes may remain viable even after 3 weeks of storage (Batham and Nayak, 2018).

It has been reported that lambs receiving inadequate amount of colostrums after birth have low levels of immunoglobulin, and hence are susceptible to various diseases (Perez *et al.*, 1990). This susceptibility may be reduced through blood transfusion. However, several factors should be considered before blood transfusion, such as transmission reactions, compatibility, cost and availability (Jahan *et al.*, 2019). Large amount of sheep blood is wasted every year at the abattoirs; hence blood sources are adequate, but a suitable technique of blood preservation and storage needs to be developed to promote the transfusion therapy at the time of need. As there are limited observations on storage of sheep blood for transfusion; therefore, this study was conducted to investigate the haematobiochemical changes of sheep blood during storage as well as the efficacy of Citrate Phosphate Dextrose Adenine-1 (CPDA-1) and Acid Citrate Dextrose (ACD) for long term storage of sheep blood for transfusion.

MATERIALS AND METHODS

This experiment was carried out from July to November, 2017 in indigenous sheep at the Department of Surgery and Obstetrics of Bangladesh Agricultural University, Mymensingh-2202.

Experimental design

Study animals

Twelve castrated, healthy and adult indigenous sheep were randomly selected and divided into two groups: group X and group Y. Each group consisted of six animals. Blood samples were collected from the animals in both groups and afterwards, preserved with CPDA-1 solution in case of group X (n=6); whereas for group Y (n=6), blood samples were preserved with ACD solution for further transfusion.

Preservative solutions

The CPDA-1 solution contained citric acid monohydrate 0.3 g, sodium citrate dihydrate 2.6 g, sodium biphosphate monohydrate 0.2 g, dextrose monohydrate 3.2 g, adenine 0.03 g and distilled water 100 ml; whereas the ACD solution consisted of dextrose 1.5 g, trisodium citrate 1.3 g, citric acid 0.5 g and distilled water 100 ml.

Experimental procedure

Blood collection and storage

Blood samples were collected from the sheep in abattoir before being slaughtered. The health status of the selected sheep was physically examined before blood collection. For each animal, thirty-five ml of blood was collected from the jugular vein using a 50 ml sterile plastic syringe with 19 gauge needle. After collection, the blood samples were transferred immediately to the Falcon tubes (15ml) containing CPDA-1 (group X) and ACD (group Y) at a concentration of 1 ml of preservative (ACD/CPDA-1) for every 7 ml of blood. Then the samples were kept under 4°C in refrigerator for 28 days. During storage, the tubes were gently and manually overturned everyday to mix the whole blood with the preservative solutions.

Haematobiochemical examinations

Routine haematology: Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC); and biochemistry: Total Protein (TP) and pH of sheep blood were performed immediately after collection and thereafter on day 1, day 3, day 7, day 14, day 21 and day 28 for both groups. TEC and TLC were estimated through basic haemocytometer method and manual counting. Hb was assessed by Sahli's method with standard color comparator (colorimetric method), whereas Wintrobe haematocrit method was used for PCV. MCV, MCH and MCHC were calculated as $MCV (10^{-15}L \text{ or fl}) = \frac{PCV(\%) \times 10}{\text{no. of erythrocytes per cumm blood} \times 10^6}$, $MCH (10^{-12}g \text{ or pg}) = \frac{\text{hemoglobin (g \%)} \times 10}{\text{no. of erythrocytes per cumm blood} \times 10^6}$ and $MCHC (\%) = \frac{\text{hemoglobin (g \%)} \times 100}{PCV(\%)}$. TP was estimated by biuret method with spectrophotometer, and pH was determined by using a glass electrode pH meter.

Statistical Analysis

The data obtained in this investigation were calculated and represented as 'mean \pm standard error' for all the haematological and biochemical parameters, and one way ANOVA (Analysis of Variance) was performed using SPSS software for analysis. $P < 0.05$ and $P < 0.01$ were considered statistically significant.

RESULTS

The effects of CPDA-1 and ACD on TEC, TLC, Hb and PCV are presented in Figure 1 and Figure 2, respectively. In case of CPDA-1 group, TEC decreased from 9.41 ± 0.41 million/cumm (control) to 9.19 ± 0.41 , 8.90 ± 0.40 , 8.61 ± 0.40 , 7.88 ± 0.41 , 6.92 ± 0.42 and 5.83 ± 0.41 million/cumm on day-1, day-3, day-7, day-14, day-21 and day-28, respectively where significant changes ($P < 0.05$ and $P < 0.01$) were observed on day-14 and onward; TLC decreased from 9.19 ± 0.53 thousand/cumm (control) to 9.02 ± 0.48 , 8.70 ± 0.48 , 8.31 ± 0.49 , 7.64 ± 0.53 , 6.92 ± 0.57 and 6.03 ± 0.54 thousand/cumm on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.05$ and $P < 0.01$) were observed on day-14 and onward; Hb decreased from 8.80 ± 0.30 g/dl (control) to 8.53 ± 0.31 , 8.19 ± 0.31 , 7.63 ± 0.30 , 6.84 ± 0.25 , 6.15 ± 0.25 and 5.35 ± 0.25 g/dl on day-1, day-3, day-7, day-14, day-21 and day-28, respectively where significant changes ($P < 0.01$) were observed from day-7 onward; PCV decreased from 30.00 ± 0.50 % (control) to 28.90 ± 0.40 , 26.60 ± 0.30 , 25.00 ± 0.50 , 22.90 ± 0.50 , 19.90 ± 0.50 and 16.90 ± 0.50 % on day-1, day-3, day-7, day-14, day-21 and day-28, respectively where significant changes ($P < 0.01$) were observed from day-3 onward.

Whereas for ACD group, TEC decreased from 9.40 ± 0.23 million/cumm (control) to 9.12 ± 0.23 , 8.82 ± 0.23 , 8.53 ± 0.24 , 7.76 ± 0.24 , 6.51 ± 0.29 and 5.34 ± 0.33 million/cumm on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.05$ and $P < 0.01$) were observed on day-7 and onward; TLC decreased from 8.85 ± 0.22 thousand/cumm (control) to 8.60 ± 0.22 , 8.28 ± 0.22 , 7.50 ± 0.13 , 6.08 ± 0.12 , 4.60 ± 0.21 and 3.88 ± 0.22 thousand/cumm on day-1, day-3, day-7, day-14, day-21 and day-28, respectively where significant changes ($P < 0.05$ and $P < 0.01$) were observed on day-3 and onward; Hb decreased from 8.61 ± 0.17 g/dl (control) to 8.28 ± 0.16 , 7.95 ± 0.16 , 7.20 ± 0.23 , 6.11 ± 0.17 , 5.06 ± 0.19 and 4.95 ± 0.21 g/dl on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.01$) were observed from day-3 onward; PCV decreased from 29.88 ± 0.52 % (control) to 28.25 ± 0.37 , 25.38 ± 0.32 , 22.50 ± 0.50 , 19.00 ± 0.65 , 14.13 ± 0.69 and 11.21 ± 0.72 % on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.05$ and $P < 0.01$) were observed on day-1 and onward.

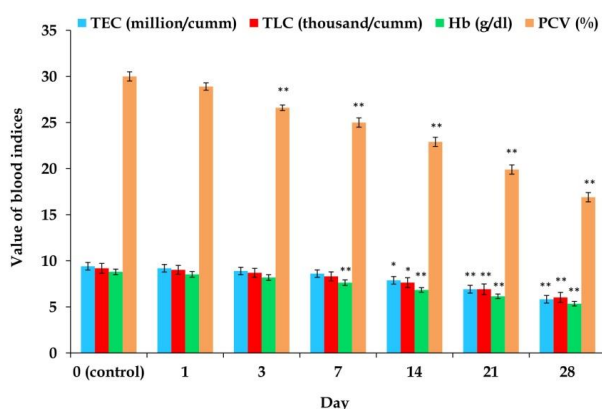


Figure-1: Effects of CPDA-1 on TEC, TLC, Hb and PCV

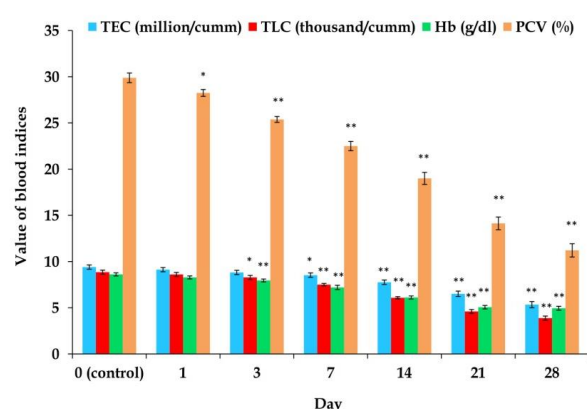


Figure-2: Effects of ACD on TEC, TLC, Hb and PCV

± = Standard Error, ** = Significant at 1% level of significance, * = Significant at 5% of significance

The effects of CPDA-1 and ACD on MCV, MCH and MCHC are presented in Figure 3 and Figure 4, respectively. In case of CPDA-1 group, MCV decreased insignificantly from 32.22 ± 1.25 fl (control) to 31.82 ± 1.33 , 30.27 ± 1.12 , 29.52 ± 1.52 , 29.43 ± 1.64 , 29.31 ± 1.89 and 29.19 ± 2.27 fl on day-1, day-3, day-7, day-14, day-21 and day-28 respectively; MCH decreased insignificantly from 9.44 ± 0.42 pg (control) to 9.37 ± 0.42 , 9.29 ± 0.42 , 8.95 ± 0.41 , 8.83 ± 0.54 , 8.68 ± 0.65 and 8.49 ± 0.82 pg on day-1, day-3, day-7, day-14, day-21 and day-28 respectively; MCHC increased insignificantly from 29.39 ± 1.12 % (control) to 29.56 ± 1.10 , 30.77 ± 1.18 , 30.84 ± 1.14 , 30.97 ± 1.43 , 31.16 ± 1.70 and 32.00 ± 2.01 % on day-1, day-3, day-7, day-14, day-21 and day-28 respectively. On the other hand, for ACD group, MCV decreased from 31.90 ± 0.95 fl (control) to 31.15 ± 0.99 , 28.94 ± 1.06 , 26.60 ± 1.21 , 24.78 ± 1.49 , 22.06 ± 1.61 and 20.45 ± 1.83 fl on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.01$) were observed from day-7 onward; MCH decreased from 9.19 ± 0.24 pg (control) to 9.11 ± 0.25 , 9.04 ± 0.26 , 8.48 ± 0.25 , 7.92 ± 0.29 , 7.34 ± 0.51 and 6.98 ± 0.75 pg on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.01$) were observed from day-14 onward; MCHC increased from 28.87 ± 0.65 % (control) to 29.31 ± 0.59 , 31.36 ± 0.74 , 32.09 ± 0.83 , 32.46 ± 1.56 , 34.12 ± 2.47 and 36.47 ± 2.69 % on day-1, day-3, day-7, day-14, day-21 and day-28, respectively where significant changes ($p < 0.01$) were observed from day-21 onward.

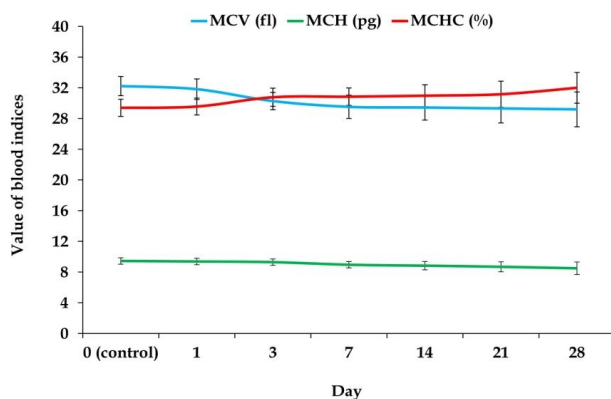


Figure-3: Effects of CPDA-1 on MCV, MCH and MCHC

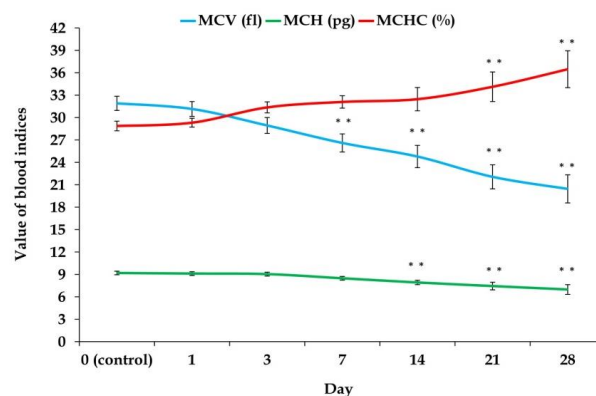


Figure 4: Effects of ACD on MCV, MCH and MCHC

± = Standard Error, ** = Significant at 1% level of significance, * = Significant at 5% of significance

The effects of CPDA-1 and ACD on TP and pH are presented in Figure 5 and Figure 6, respectively. In case of CPDA-1 group, TP decreased from 5.62 ± 0.09 g% (control) to 5.59 ± 0.08 , 5.48 ± 0.09 , 5.48 ± 0.08 , 5.44 ± 0.08 , 5.40 ± 0.08 and 5.34 ± 0.08 g% on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant change ($P < 0.01$) was only observed on day-28; pH decreased from 7.13 ± 0.01 (control) to 7.05 ± 0.01 , 6.95 ± 0.01 , 6.90 ± 0.02 , 6.86 ± 0.01 , 6.82 ± 0.01 and 6.77 ± 0.01 on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.01$) were observed from day-1 onward. On the contrary, for ACD group, TP changed insignificantly from 5.66 ± 0.09 g% (control) to 5.72 ± 0.09 , 5.80 ± 0.09 , 5.71 ± 0.09 , 5.66 ± 0.09 , 5.56 ± 0.09 and 5.49 ± 0.09 g% on day-1, day-3, day-7, day-14, day-21 and day-28 respectively; pH decreased from 7.04 ± 0.02 (control) to 6.99 ± 0.01 , 6.85 ± 0.02 , 6.75 ± 0.03 , 6.59 ± 0.02 , 6.51 ± 0.02 and 6.42 ± 0.02 on day-1, day-3, day-7, day-14, day-21 and day-28 respectively where significant changes ($P < 0.01$) were observed from day-3 onward.

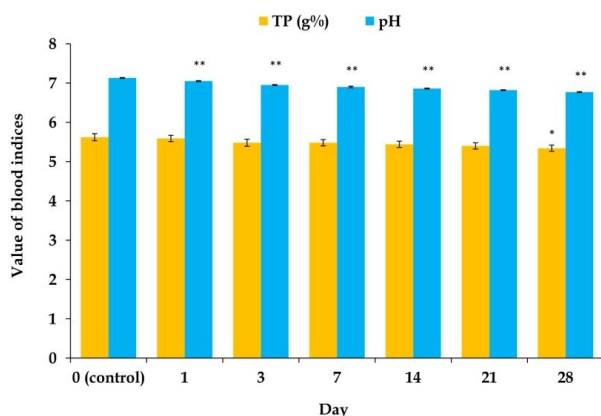


Figure-5: Effects of CPDA-1 on TP and pH

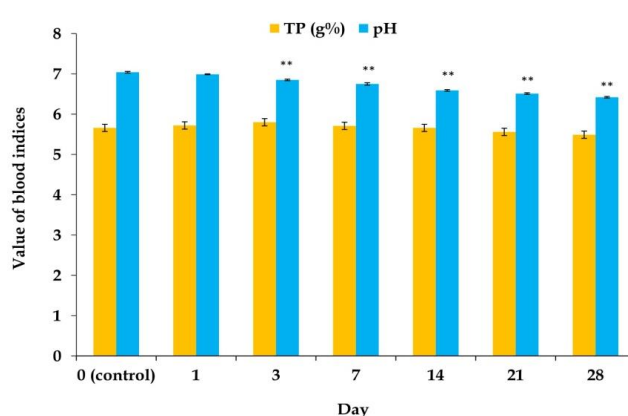


Figure-6: Effects of ACD on TP and pH

± = Standard Error, ** = Significant at 1% level of significance, * = Significant at 5% of significance

DISCUSSION

The present study evaluated the effects of CPDA-1 and ACD on the haematobiochemical parameters of sheep blood during storage for transfusion. TEC, TLC, Hb. and PCV decreased throughout the experiment as compared to the control values in both groups, as also observed by other investigators (Hess, 2006; Ahmed *et al.*, 2009). The decrement in the values of these parameters might be due to the haemolysis that occurs during storage (Ahmed *et al.*, 2009; Jahan *et al.*, 2019). The erythrocyte haemolysis can be attributed to several causes including: old erythrocyte age (Emmanuel, 2001), progressive structural and functional changes during storage (Koch *et al.*, 2008), improper storage of blood (higher than 8°C in blood bank refrigerator) or blood bags leading to decrease in 2,3-diphosphoglycerate (DPG) which is very important to preserve erythrocyte and maintain physiological functions (Jahan *et al.*, 2019). Moreover, storage initiates the breakdown of leukocytes to release their constituents such as hydrogen peroxide and proteases which damage the erythrocytes (Nelson, 1991; Jahan *et al.*, 2019). The changes in leukocyte counts are most likely due to the changes in the sum effects of the loss of individual cell characteristics specifically degeneration as the cell ages (Elemchukwu *et al.*, 2014). Hb decreased throughout the experiment in both groups due to denaturation and alteration of pH during storage which is in agreement with the findings of Bhomia *et al.* (2016) and Jahan *et al.* (2019). Likewise, PCV decreased throughout the experiment in both groups due to haemolysis of RBC. The presence of adenine improved the synthesis of adenosine triphosphate (ATP) in CPDA-1 preserved blood that prolonged the viability of blood cells at 4°C during storage, which is in harmony with the findings of Rejane *et al.* (2014) and Jahan *et al.* (2019). Hence, CPDA-1 preserved blood showed less alteration in these parameters than ACD preserved blood as compared to the control values.

In both groups, decrement in MCV and MCH was observed as compared to the control values during the entire experiment with the progression of storage, which is collateral with other investigations (Suffian and Hossain, 1999; Fazio *et al.*, 2012; Fazio *et al.*, 2017). The decrement in MCV and MCH might be due to the damage of erythrocytes as storage-related degenerative changes lead the widening of the pores on the surface of erythrocytes, permitting the ingress of water into the cells (Fazio *et al.*, 2017). However, increment in MCHC was observed in both groups, which is consistent with the findings of Chen *et al.* (1999) and Nemec *et al.* (2005). This might be ascribed to the shrinkage of erythrocytes in a hypertonic medium due to the osmotic withdrawal of water from the cells, leading to a reduction in PCV, and this discrepancy leads to a reduction in MCV and increase in MCHC (Bush, 1998).

Changes in TP were found in both groups during the storage as compared to the control values. CPDA-1 preserved blood showed gradual decrease in TP with the progression of storage which might be due to the temperature above 0°C that denatures the protein by acting on non-covalent (for example, hydrogen, hydrophobic and electrostatic) bonds which are considered important in stabilizing the secondary and tertiary structure (Hashmi *et al.*, 2001). On the contrary, ACD preserved blood showed an initial increase and later decrease in TP with the progression of storage. This might be due to the fact that haemoglobin strongly absorbs light at 540 nm, and haemolysis therefore increases the absorption in this wavelength affecting the concentration of different analytes which are measured in the same wavelength range. The initial rise in protein concentration is because of optical interference as haemoglobin interferes with protein as it falls in the same absorbance range and intercellular leakage of TP when estimated by biuret method. Similar false elevated level of TP was also observed by Roman *et al.* (2009) and Jahan *et al.* (2019).

This study showed decreased pH throughout the experiment in both groups as during storage there was the formation of lactic acid due to anaerobic glycolysis. These findings are correlated with the reports of Verma and Dahiya (2015) and Jahan *et al.* (2019). Low pH reduces the formation of 2,3-DPG (Castilho *et al.*, 2003), which was found more common in case of ACD than CPDA-1 during the experiment.

CONCLUSIONS

CPDA-1 and ACD solutions asserted certain haematobiochemical changes in sheep blood during storage, however, CPDA-1 was comparatively better than ACD for maintaining the feasible levels of haematobiochemical parameters of sheep blood stored at 4°C up to 28 days for transfusion.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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