Some physiological and chemical properties of horse and cow blood samples under effect of some different types of anticoagulants

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Abstract

The purpose of the present study is to determine and compare the anticoagulants, Ethylene diamine tetra acetic acid, Sodium oxalate, Sodium citrate and heparin in blood samples of horse and bull, through estimation of (Hemoglobin, Packed Cell Volume and Plasma Platelets Count) and plasma physical properties (clarity and volume) and some chemical properties (pH and calcium ion concentration). Five Blood samples were obtained from five stallions and five bulls (20 ml/sample) and were divided as 5 ml in four test tubes containing Ethylene diamine tetra acetic acid, sodium oxalate, sodium citrate and heparin at the College of Veterinary Medicine, University of Diyala. Hemoglobin and packed cell volume were estimated immediately and then the blood samples were centrifuged for 15 min. at 3000 rpm to obtain plasma for evaluating the physical and chemical properties specified above. The horse blood samples pertaining data had no significant elevation in hemoglobin concentration and packed cell volume in Ethylene diamine tetra acetic acid group as compared to other groups, beside having no significant changes in pH between the four treated groups while there was a significant increase in plasma volume of oxalate and citrate as compared to Ethylene diamine tetra acetic acid and heparin, and there was a significant increase in plasma platelet count of Ethylene diamine tetra acetic acid group as compared to heparin. As well there was a significant decrease of calcium ion concentration in Ethylene diamine tetra acetic acid, oxalate and citrate as compared to heparin, while the results of bull blood samples revealed that the hemoglobin and Packed Cell Volume were significantly higher in heparin group than in the oxalate and citrate groups with non significant differences with Ethylene diamine tetra acetic acid group. The plasma obtained from different types of anticoagulants appeared with a high degree of clarity, and the volume of plasma had no significant increase in oxalate and citrate group as compared to other groups. The Plasma Platelet Count and calcium concentration significantly increased in heparin group as compared to other groups. In conclusion, the Ethylene diamine tetra acetic acid was more reliable for horse blood samples while the anticoagulant heparin was more reliable anticoagulant for bull blood samples.

Keywords: Anticoagulants, Ethylene diamine tetra acetic acid, Sodium oxalate, Sodium citrate, Heparin, Blood samples.
thrombolytic drugs by manipulating the various pathways of blood coagulation cascade that build upon the initial platelets thrombus (6). The anticoagulants which are used in this study are: EDTA strongly and irreversibly chalets calcium ion to prevent blood from clotting (7); Oxalate has a mechanism similar to that of citrate used in florid oxalate tubes used to determine glucose and lactate; Citrate is in liquid form; it binds to calcium ion but not as strongly as EDTA (8); and heparin is a biological substance usually made from pig intestine, it work by activating anti thrombin III which blocks thrombin from clotting (9). The effect of various type of anticoagulants on plasma biochemistry have been studied in man and various animals but limited information exist for horse and bull blood components and plasma; Therefore, this project was designed to determine the effect of these anticoagulants on some horse and bull blood samples components and plasma physical and chemical properties through studying the following parameters: Packed cell volume (PCV), Hemoglobin (Hb), plasma clarity, plasma volume, plasma pH, plasma platelets count, and plasma calcium ion concentrations.

Materials and Methods
Preparation of anticoagulants: EDTA, 0.1 ml 10% dissolved EDTA solution for 5 ml of blood. Sodium oxalate, 0.5 ml of 3.8% solution of sodium oxalate for 5ml of blood. Sodium citrate, 0.5 ml of 3.8% solution of sodium citrate for 5 ml of blood. Heparin, 100 unites for 5 ml (8).

Five blood samples (20 ml/ sample) were obtained from five horses (stallions) and five blood samples (20ml/ sample) from five cows (bulls), 2-3 years old at College of Veterinary Medicine/ University of Diyala. The blood samples were collected from jugular vein by disposable syringe gage 16, divided as 5 ml/ test tubes containing the following anticoagulants: EDTA, Sodium oxalate, sodium citrate and heparin. Parameters assist in this study: These blood samples were analyzed within 3hrs of collection in physiology laboratory for assisting the following parameters: Physiological, including hemoglobin and packed cell volume measured by kit of Hb testing system. Then the blood samples test tubes were centrifuged at 3000rpm for 15 minutes to obtain plasma for assisting the following plasma chemical parameters: Degree of plasma clarity, volume of plasma, drawing the plasma by mechanical graduated pipette from each sample. pH of plasma; by putting few drops of plasma samples on the pH paper and then being compared with pH paper colors standard after 3 minutes.

Plasma platelets count: Hemocytometer slide, pipette of RBC red blood cell and Ress-Echar solution (sodium citrate 3.8 gm, formaldehyde 40% 0.2 ml, Brilliant Cresyl Blue 0.05 gm, distilled water 100 ml. To perform a hand count, a known volume of the sample is loaded into a hemocytometer grid and platelets are counted under a light microscope (10). Plasma calcium ion concentration: O- cresolphthalein complex method.

All data were performed on the basis of one way analysis of variance (ANOVA), at P<0.05 significantly level and specific group differences were determined using least significant differences (LSD) test (11).

Results and Discussion
Hemoglobin concentration and PCV: the result of horse blood samples study demonstrate that there was no significant P>0.05 elevation of Hb concentration and PCV in EDTA and heparin groups as compared to oxalate and citrate groups. Determination of blood parameters are helpful in assessing the healthy status of animals (12). The higher PCV and Hb values in EDTA in horse blood samples in respect to sodium oxalate and sodium citrate are directly related with higher RBC found in EDTA treated sample and indicate a better preservation of cells in EDTA treated samples. This result agrees with (13), while the results of cow blood samples showed that the anticoagulant heparin group revealed a significant elevation P<0.05 as compared to oxalate and citrate with non significant differences P>0.05 with EDTA anticoagulant group. This result may be attributed to the base that heparin protects the red blood cell membrane shape and osmolarity. This finding comes in line with
(14); the decrease of Hb and PCV in oxalate and citrate may be due to dilution effect.

Plasma clarity: All types of anticoagulants in (Table, 1 and 2) produce a high degree of plasma clarity because no hemolysis were detected in any sample. This result agrees with (15).

Plasma gulant on horse plasma volume are present in (Table, 1) and indicate that there were a significant P<0.05 increase in plasma volume of oxalate and citrate groups as compared to EDTA and heparin. This may be attributed to rouleaux formation and erythrocyte sedimentation rate. Rouleaux formation is the result of the aggregation of RBCs in linear stacks and depends on the number of RBCs and their tendency to aggregate. Rouleaux formation is a characteristic finding in healthy horses, as a result of weak surface changes on RBC membranes (16). The bull plasma volume in table (2) appeared that there was no significant increase in plasma volume in oxalate and citrate anticoagulant groups as compared to EDTA and heparin anticoagulant groups. This may be attributed to diluting effect (17).

Plasma concentration of H+ ion (pH): the results illustrated in (Table, 1 and 2) showed that the various types of anti coagulant had no significant effect on H+ ion concentration. In veterinary diagnostics the blood pH is an important indicator of homeostasis, which means that H+ ion concentration is kept within certain limits (18).

Table, 1: The effect of different types of anticoagulants on Haemoglobin, packed cell volume and plasma clarity, volume, pH, platelets count and calcium concentration of horse blood samples.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>EDTA</th>
<th>Sodium oxalate</th>
<th>Sodium citrate</th>
<th>Heparin</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb gm/100ml</td>
<td>13.3 ±2.51</td>
<td>11.3 ±2.48</td>
<td>11.8 ±2.55</td>
<td>12.5 ±2.97</td>
<td>7.897</td>
</tr>
<tr>
<td>PCV %</td>
<td>28.4 ±1.50</td>
<td>23.4 ±2.16</td>
<td>25.0 ±3.80</td>
<td>27.8 ±2.60</td>
<td>8.02</td>
</tr>
<tr>
<td>Clarity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Volume Ml</td>
<td>2.8 B</td>
<td>3.3 A</td>
<td>3.2 A</td>
<td>2.7 B</td>
<td>0.444</td>
</tr>
<tr>
<td>pH</td>
<td>8.8 a</td>
<td>8.8 a</td>
<td>8.8 a</td>
<td>8.6 a</td>
<td>0.874</td>
</tr>
<tr>
<td>PPC Cell</td>
<td>42.8×10³ ±0.20</td>
<td>36.8×10³ ±0.31</td>
<td>38×10³ ±0.13</td>
<td>32.4×10³ ±0.37</td>
<td>9.858</td>
</tr>
<tr>
<td>Ca mg/100ml</td>
<td>7.9 B</td>
<td>8.3 B</td>
<td>8.4 B</td>
<td>13.2 A</td>
<td>2.97</td>
</tr>
</tbody>
</table>

Table, 2: The effect of different types of anticoagulants on Haemoglobin, packed cell volume and plasma clarity, volume, pH, platelets count and calcium concentration of cow blood samples.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>EDTA</th>
<th>Sodium oxalate</th>
<th>Sodium citrate</th>
<th>Heparin</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb gm/100ml</td>
<td>20.7 AB ±1.13</td>
<td>19.1 B ±0.76</td>
<td>18.5 B ±1.11</td>
<td>22.3 A ±1.12</td>
<td>3.139</td>
</tr>
<tr>
<td>PCV %</td>
<td>32.6 AB ±1.69</td>
<td>27.4 B ±1.03</td>
<td>26.6 B ±1.86</td>
<td>34.0 A ±2.05</td>
<td>5.10</td>
</tr>
<tr>
<td>Clarity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Volume mL</td>
<td>2.2 a</td>
<td>2.7 a</td>
<td>2.6 a</td>
<td>2.4 a</td>
<td>0.796</td>
</tr>
<tr>
<td>pH</td>
<td>8.8 a</td>
<td>8.6 a</td>
<td>8.8 a</td>
<td>8.4 a</td>
<td>0.670</td>
</tr>
<tr>
<td>PPC Cell</td>
<td>40.2×10³ ±0.20</td>
<td>40.0×10³ ±0.24</td>
<td>40.1×10³ ±0.20</td>
<td>42.5×10³ ±0.24</td>
<td>2.15</td>
</tr>
<tr>
<td>Ca mg/100ml</td>
<td>9.2 B</td>
<td>11.9 B</td>
<td>10.9 B</td>
<td>16.8 A</td>
<td>4.86</td>
</tr>
</tbody>
</table>

In conclusions of this study, the EDTA is regarded as the reliable anticoagulant for horse blood sample for hematology estimation while heparin is regarded as the reliable anticoagulant for bull blood sample for hematology estimation. The horse plasma pH
was stable in EDTA rather than sodium oxalate and heparin, while bull plasma pH was slightly acidic in heparin group other than the remaining groups. The elevation in horse PPC in EDTA indicate the efficiency of its anticoagulant activity. The elevation in bull PPC and Calcium ion concentration in heparin indicates the efficiency of its anticoagulant activity.

References


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بعض الصفات الفسليجية والكيموحيوية لنماذج دم الخيول والأبقار تحت تأثير أنواع مختلفة من موائع التخثر
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الخلاصة
الغرض من إجراء هذه الدراسة هو تحديد ومقارنة تأثير موائع التخثر: الإثيلين دايمين تترا استك أسد و صوديوم أوكсалات و صوديوم سترات والهيبارين في عينات دم الخيول والأبقار. من خلال قياس (الهيموكلوبين و حجم كريات الدم المرصوص وعدد الصفائح الدموية في البلازما) وبعض الصفات الفيزيائية للبلازما مثل (درجة النقاوة وحجم البلازما) وبعض الصفات الكيموحيوية للبلازما مثل (درجة الحموضة وتركيزات الكالسيوم). جمعت خمسة عينات دم من خمسة خيول وخمسة ثيران (20 مل/عينة) ووزعت بواقع 5 مل لكل من أنابيب الاختبار الحاوية على موائع تختصر الإثيلين دايمين تترا استك أسد و صوديوم أوك살ات و صوديوم سترات والهيبارين في كلية الطب البيطري جامعة ديالى تم فحص الهيموكلوبين وحجم كريات الدم المرسوم مباشرة بعد جمع العينات ومن ثم وضعت أنابيب الاختبار في جهاز الطرد المركزي لمدة 15 دقيقة بسرعة 3000 دورة/دقيقة للحصول على بلازما الدم لدراسة الصفات الفسيولوجية والكيميائية لبلازما الدم المذكورة أعلاه. أظهرت نتائج عينات دم الخيول بأن الارتفاع في الهيموكلوبين وحجم كريات الدم المرسوم كان غير معنوي في مجموعة الإثيلين دايمين تترا استك أسد و الهيبارين، في حين كانت درجة النقاوة عالية في جميع العينات، وتغيرات غير معنوية في درجة الحموضة لجميع العينات بينما كانت هناك زيادة معنوية في حجم البلازما لمجموعة الأوكساكات والستريات مقارنة مع مجموعة الإثيلين دايمين تترا استك أسد والهيبارين ومع تزايد معنوي في حجم البلازما. كما أنه، تركيز الصفيحات الدمية ارتفع معنويًا في مجموعة الهيبارين عن بقية المجموعات. بالنسبة لعناصر الكالسيوم، كان الارتفاع في تركيز الصفيحات الدمية وتركيز أيون الكالسيوم معنويًا في مجموعة الهيبارين عن بقية المجموعات. استنتج من هذه الدراسة أن مانع التخثر الإثيلين دايمين تترا استك أسد و صوديوم أوكساكات، صوديوم سترا، هيبارين، نماذج دم.