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

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Received: 11/9/2017

Accepted: 7/1/2018

Publishing: 28/6/2018

Summary

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.

Introduction

Historically, serum was preferred as an assay material for determination of extracellular concentration of blood constituents; today plasma is preferred for many but not all laboratory investigations because the constituents in plasma are better reflecting of the pathological situation than serum through some changes of constituents which can be avoided by using anticoagulants (1 and 2). Anticoagulants are a class of drugs that work to prevent blood coagulation (clotting), such substances occur naturally in leeches and blood sucking insects (3). Anticoagulants are additives that inhibit the clotting of blood thereby ensuring that the concentration of the substance to be measured is changed as little as possible before the analytical process (4). Anticoagulants can be used in an injection like heparin or taken orally as coumarin; some anticoagulants are used in medical equipments such as test tubes, blood transfusion bags like ethylene diamine tetra acetic acid (EDTA) and citrate and renal dialysis equipment (5). Anticoagulants are closely related to anti platelets drugs and

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, odium 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, drawling 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, formaldedhyde 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).

Plasma platelets count PPC: The data present in (Table, 1) revealed a significant increase (P<0.05) in PPC of EDTA group as compared to heparin group while the differences were non significant P>0.05 with oxalate and citrate. This result may be attributed to the fact that EDTA can change the shape of platelets and prevent aggregation (19 and 20). While the results of bull blood samples in the heparin anticoagulant group revealed a significant increase (P<0.05) in PPC as compared to the remaining groups. This means that heparin does not produce structural and functional damage to platelets. This finding agrees with (21) and disagrees with (22).

Plasma calcium ion concentration Ca^{++}: The heparin anticoagulant group showed a significant increase (P<0.05) in calcium ion concentration (Table, 1 and 2) as compared with the remaining anticoagulant groups. Ethylene EDTA exert their actions as anticoagulants by inhibiting thrombin in blood and chelating the calcium ions (13).

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.

Groups Parameters	EDTA	Sodium oxalate	Sodium citrate	Heparin	LSD value
Hb gm/100ml	13.3 a ±2.51	11.3 a ±2.48	11.8 a ±2.55	12.5 a ±2.97	7.897
PCV %	28.4 a ±1.50	23.4 a ±2.16	25.0 a ±3.80	27.8 a ±2.60	8.02
Clarity	+++	+++	+++	+++	
Volume Ml	2.8 B ±0.18	3.3 A ±0.15	3.2 A ±0.13	2.7 B ±0.11	0.444
pH	8.8 a ±0.20	8.8 a ±0.31	8.8 a ±0.37	8.6 a 0.24	0.874
PPC Cell	42.8×10^3 ±3.05 A	36.8×10^3 ±2.25 AB	38×10^3 ±2.30 AB	32.4×10^3 ±4.59 B	9.858
Ca mg/100ml	7.9 B ±0.79	8.3 B ±0.78	8.4 B ±0.43	13.2 A ±1.58	2.97

The data expressed as mean± standard error, significant level (P<0.05), capital letters mean significant differences, small letters mean non significant differences (P>0.05).

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.

Groups Parameters	EDTA	Sodium oxalate	Sodium citrate	Heparin	LSD value
Hb gm/100ml	20.7 AB ±1.13	19.1 B ±0.76	18.5 B ±1.11	22.3 A ±1.12	3.139
PCV %	32.6 AB ±1.69	27.4 B ±1.03	26.6 B ±1.86	34.0 A ±2.05	5.10
Clarity	+++	+++	+++	+++	
Volume mL	2.2 a ±0.22	2.7 a ±0.29	2.6 a ±0.34	2.4 a ±0.15	0.796
pH	8.8 a ±0.20	8.6 a ±0.24	8.8 a ±0.20	8.4 a ±0.24	0.670
PPC Cell	40.2×10^3B ±3.97	40.0×10^3B ±2.70	40.1×10^3B ±3.68	42.5×10^3A ±2.62	2.15
Ca mg/100mL	9.2 B ±0.22	11.9 B ±0.95	10.9 B ±1.32	16.8 A ±2.68	4.86

The data expressed as mean ± standard error SE, significant level (P<0.05), capital letters mean significant differences, small letters mean non significant differences (P>0.05).

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.

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بعض الصفات الفسلجية والكيموحيوية لنماذج دم الخيول والأبقار تحت تأثير أنواع مختلفة من موانع التخثر

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الخلاصة

الغرض من إجراء هذه الدراسة هو تحديد ومقارنة تأثير موانع التخثر: الأثيلين دايمين تترا اسيتك أسد وصوديوم أوكسالات وصوديوم سترات وهيبارين في عينات الدم من الخيول والأبقار. من خلال قياس (الهيموكلوبين وحجم كريات الدم المرصوص وعدد الصفيحات الدمية في البلازما) وبعض الصفات الفيزيائية للبلازما مثل (درجة النقاوة وحجم البلازما) وبعض الصفات الكيموحيوية للبلازما مثل (درجة الحموضة وتركيزايون الكالسيوم). جمعت خمسة عينات دم من خمسة خيول وخمسة ثيران (20 مل/ عينة) ووزعت بواقع 5مل لكل من أنابيب الاختبار الحاوية على موانع تخثرالاثيلين دايمين تتراستك اسد وصوديوم أوكسالات وصوديوم ستريت والهيبارين في كلية الطب البيطري جامعة ديالى تم فحص الهيموكلوبين وحجم كريات الدم المرصوص مباشرة بعد جمع العينات ومن ثم وضعت انابيب الاختبار في جهاز الطرد المركزي لمدة 15 دقيقة بسرعة 3000 دورة /دقيقة للحصول على بلازما الدم لدراسة الصفات الفيزياوية والكيموحيوية لبلازما الدم المذكورة اعلاه. أظهرت نتائج عينات دم الخيول بأن الارتفاع في الهيموكلوبين وحجم كريات الدم المرصوص كان غير معنوي في مجموعة الاثيلين دايمين تترا استك أسد والهيبارين مقارنة مع مجموعة الاوكسلات والستريت فضلاً عن أن درجة النقاوة كانت عالية في جميع العينات كذلك كانت التغيرات غير معنوية في درجة الحموضة لجميع العينات بينما كان هناك زيادة معنوية في حجم البلازما لمجموعة الاوكسالات والستريت مقارنة مع مجموعة الاثيلين دايامين تترا اسيتك أسد والهيبارين ومن جانب اخر أظهرت مجموعة الاثيلين دايمين تترا اسيتك أسد ارتفاعا معنويا في تركيز الصفيحات الدمية مقارنة مع مجموعة الهيبارين فضلاً عن الانخفاض في تركيز الكالسيوم في كل المجاميع عدا مجموعة الهيبارين، بينما اظهرت نتائج عينات دم الأبقار بان الهيموكلوبين وحجم كريات الدم المرصوص ارتفعت معنويا في مجموعة الهيبارين مقارنة مع مجموعة الاوكسلات والستريت وغير معنوي مع مجموعة الاثلين دايمين تترا اسيتك أسد، كانت درجة النقاوة عالية في جميع نماذج البلازما الى جانب حجم البلازما الذي ارتفاعه غير معنوي في مجموعة الاوكسلات والستريت عن بقية المجاميع، من جانب آخر كان الارتفاع تركيز الصفيحات في البلازما وتركيز ايون الكالسيوم معنوياً في مجموعة الهيبارين عن بقية المجاميع، يستنتج من هذه الدراسة بأن مانع التخثر الاثيلين دايمين تترا اسيتك اسد اكثر ملائمة لدم الخيول. بينما مانع التخثر الهيبارين اكثر ملائمة لدم الأبقار .

الكلمات المفتاحية: مضادات التخثر،اثيلين دايمين تترا اسيتك أسد ،صوديوم اوكسالات، صوديوم سترات، هيبارين، نماذج دم.