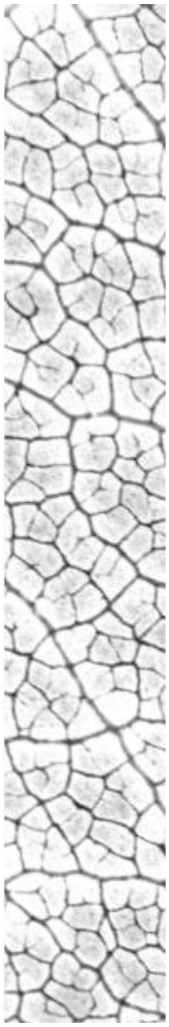

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Effect of Moringa *oleifera* seeds ethanol extract on some coagulant parameters of the blood of rams, bulls, and stallions: In vitro study

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Abstract
This work is conducted on rams(n=15), bulls(n=15), and stallions(n=15) which are divided randomly into three main groups and each group is divided randomly into three sub groups. Moringa oleifera seeds ethanol extract collect and dilute into 25mg extract/ml., 50mg extract/ml. concentrations. After blood collection 5ml. from each animal diluent 25 mg extract/ml. were added directly to rams R1, bulls B1, and stallions S1, diluent 50 mg extract/ml. were added directly to R2, B2, and S2 and R3, B3, and S3 were regarded as control group. Coagulation parameters fibrinogen concentrations, platelet counts, prothrombin time(PT), and activated partial thromboplastin time(aPTT) were measured. The results show that there are coagulant parameters differences between these field animals. There is significant increase of fibrinogen concentrations, platelet counts, and PT whereas there is significant decrease of aPTT of rams, bulls, stallions.

Keywords: Moringa, oleifera, seeds, coagulant, blood, rams, bulls, stallions

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INTRODUCTION

Moringa oleifera plant is widely distributed throughout the earth topics and subtopics. Several African Governments advocate its use as cheap inexpensive component in foods nutritional supplements to patients of HIV/AID (Anwar & Bhanger, 2003). In the west, the powdered seeds of Moringa is one of the best known uses to flocculate contaminants and purify drinking water so it regards better than aluminum sulfate which is slightly toxic when use repeatedly to the nervous system (Etukudo, 2003). The seeds have attractive scientific interest, it contains a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid > 70%) and, after refining, a notable resistance to oxidative degradation (Anwar et al., 2005). Coagulation or blood clot is a complex biological process that ends with stops bleeding. The damaged blood vessel wall will cover with a platelets and clot contains mainly fibrin to cessation the blood loss and stop bleeding (Palta et al., 2014; Jaccob et al., 2019).

To measure the extrinsic pathway of coagulation a PT test must be done. Trinsic clotting factors are activated when tissue thromboplastin and calcium ions are added to plasma, this will be resulting in the formation of thrombin and fibrin clot, so the test will indicate the functions of factors II, V, VII, and X (Palta et al., 2014). The test that use to demonstrate deficiencies within the intrinsic pathway and the final pathway of clotting is aPTT. All factors except factor VII and platelet factor 3 will affect the clotting time (Ochei & Kolhatkar, 2000). Most of the researches were done on the seeds of Moringa oleifera to study its effect on coagulant parameters (Osman et al., 2012). The aim of this work is to study the effect of Moringa oleifera seeds ethanol extract on some coagulant parameters of the blood of rams, bulls, and stallions.

MATERIALS AND METHODS

Under supervisor of the bioethics of the committee of the College of Veterinary Medicine/ University of Al-Qadisiyah, this work was conducted on 15 rams, 15 bulls, and 15 local Arabian stallion lived in the Animal Station of the college in Al-Dewania province 200km south Baghdad, Iraq with routine health care, standard feeding and ad libitum clean water. The fifteen rams were divided randomly into three subgroups denoted R1, R2, and R3 regarded as control counterparts of five rams, in the same manner the other animals were divided randomly and denoted B1, B2, and B3 regarded as control counterparts of five bulls and S1, S2, and S3

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Table 1. The species, sub groups, blood quantity, and the extract diluents that used

No.	Species	Sub group	Blood quantity	Extract diluents mg/ml.
1	Ram	R1(n=5)	5ml.	25
		R2(n=5)	5ml.	50
		R3(n=5)	5ml.	-
2	Bull	B1(n=5)	5ml.	25
		B2(n=5)	5ml.	50
		B3(n=5)	5ml.	-
3	Stallion	S1(n=5)	5ml.	25
		S2(n=5)	5ml.	50
		S3(n=5)	5ml.	-

Fig. 1. Erythrocytes regression of R2X40

denoted and regarded as control counterparts of five stallions.

Preparation of M. oleifera Seeds Extract

Seeds of M. oleifera were dried in the shade, grinded with electrical grinder and left in absolute ethanol for 48 hours in Soxhlet apparatus (Electrothermal, UK). Then the ethanol seeds extract was dried in Rotary Evaporator apparatus, weighed and in distilled water to give final concentrations 25mg extract/ml., 50mg extract/ml. and were refrigerated at 4 ºC until use.

Blood collection

Whole blood 5 ml. was collected aseptically from the jugular vein of each experimental animal and added directly the diluents 25 mg extract/ml. to the blood of R1, B1, and S1 respectively, 50 mg extract/ml. to the blood of R2, B2, and S2 respectively, while the blood of R3, B3, and S3 had no any diluents and regarded as control counterparts.

Erythrocytes agglutination

One drop of blood mixed with one drop of the extract, left for 5 min. and examined by Microscope Olympus, Japan for erythrocyte agglutination. Agglutination degrees were scaled into four degrees, no agglutination, slight agglutination, moderate agglutination, and severe agglutination.

The measurements of fibrinogen concentrations mg/dl, platelets count x$10^{9/}$L., PT (sec.), and aPTT (sec.) according to Thukral et al. (2018) procedure were recorded.

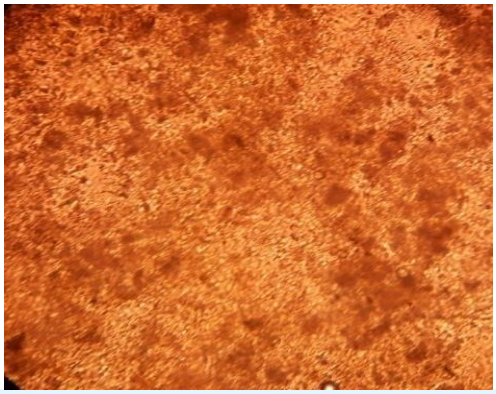

Fig. 2. Erythrocytes regression of B2X40

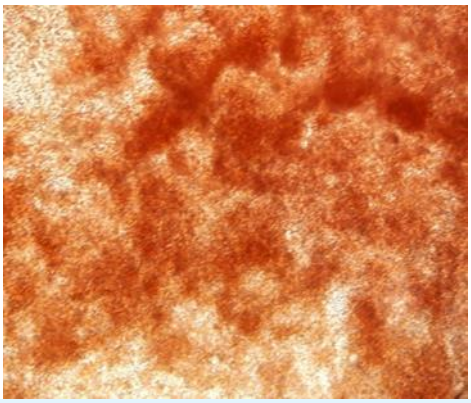

Fig. 3. Erythrocytes regression of S2X40

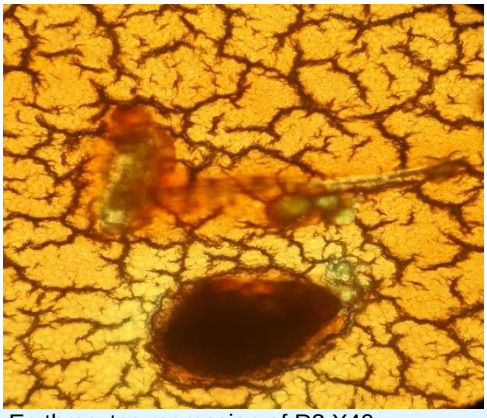

Fig. 4. Erythrocytes regression of R3.X40

STATISTICAL ANALYSIS

The mean values of coagulant parameters were analyzed by student- test using SPSS program version 32 and the variances were regarded significant at P≤0.05.

RESULTS

Erythrocytes regression

The erythrocytes regression of R2 and R3 was moderate (**Fig. 1**) respectively, the erythrocytes regression of B2 andB3 was mild (**Fig. 2**) respectively, the erythrocytes of S2 and S3 was severe (**Fig. 3**) respectively.

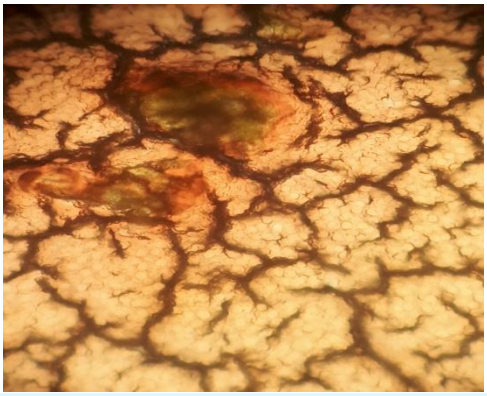

Fig. 5. Erythrocytes regression of B3X40

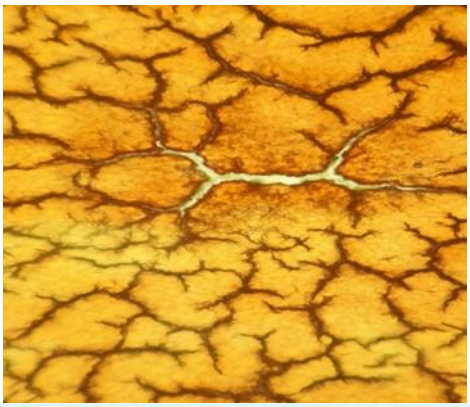

Fig. 6. Erythrocytes regression of S3X40

In rams the results of **Table 2** showed significant increase of fibrinogen concentrations (344.8±8.06), platelet counts (735.8±4.01), PT (30.2±1.03) and decrease of aPTT (100.0±1.11) of R1 respectively, and increase of fibrinogen concentrations (798.4±37.77), platelet counts (867.2±22.39), PT (34.2±0.83) and decrease of aPTT (49.6±1.19) of R2 respectively, whereas the significant mean values of fibrinogen concentrations (204.2±5.0), platelet counts (517.6±35.39), PT (24.8±1.08), and aPTT (198.0±0.96) of R3 respectively were within normal values.

In bulls as showed in **Table 3** there were significant increase of fibrinogen concentration 788.4±2.85, 1093±2.53 of B1 and B2 respectively and significant increase of platelets count 820.2±0.54, 863.8±2.30 of B1 and B2 respectively and significant increase of PT 30.2±0.84, 36.6±1.54 of B1 and B2 respectively and significant decrease of aPTT 101.0±0.70, 71.0±1.09 of B1 and B2 respectively, whereas the significant mean values of fibrinogen concentration280.6±8.84,platelets count 374.2±2.82, PT 25.0±0.94 and aPTT 139.6±0.79 of B3 respectively were within normal values.

In stallions as showed in **Table 4** there were significant increase of fibrinogen concentration482.00±13.55, 487.0±4.24 of S1 and S2 respectively and significant increase of platelets count654.4±12.45, 746.8±11.02 of S1 and S2 respectively and significant increase of PT15.0±0.68, 33.8±0.55 of S1 and S2 respectively and significant decrease of aPTT 41.0±0.71, 21.4±1.92 of S1 and S2 respectively, whereas the significant mean values of fibrinogen concentration579.2±24.01,platelets count400.6±9.73, PT12.4±0.84 and aPTT 54.8±1.14 of S3 respectively were within normal values.

DISCUSSION

To avoid the efficacy of the different sexual states of females due to gonad hormones, this approach is conducted on field male animal's rams, bulls, and stallions. The estrogen has effects on thrombin formation and classically attribute the hepatic derived coagulation factors (Dupuis et al., 2019). As the pharmaceutical value of Moringa oleifera plants and its wild natural distribution in the middle and southern of Iraq field animals are choose whereas recently Singnap et al. (2019) and Osman et al. (2012) have similar studies about the effect of M. oleifera seed extract on blood coagulation parameters in Albino rats.

Lectins are proteins have a capability of erythrocytes agglutination (Hope & Ifeanyi, 2019). This agglutination

Table 2. Mean± SE of the platelets count x10^9/L., PT (sec.), and the aPTT (sec.) of the rams

Species	Sub groups	Fibrinogen concentration Mg/dl	Platelets count x10^9/L	PT (sec.)	aPTT (sec.)
Ram	R1	344.8±1.06 B	735.8±4.01 B	30.2±1.03 B	**100.0±1.11 C**
	R2	798.4±1.77 A	867.2±2.39 A	34.2±0.83 A	**49.6±1.19 B**
	R3	204.2±5.0 C	517.6±5.39 C	24.8±1.08 C	**198.0±0.96 A**

* Different letters denote significant variances at P≤0.05

Table 3. Mean± SE of the platelets count x10^9/L., PT (sec.), and the aPTT (sec.) of the bulls

Species	Sub groups	Fibrinogen concentration Mg/dl	Platelets count x10^9/L	PT (sec.)	aPTT(sec.)
Bull	B1	788.4±2.85 C	820.2±0.54 B	30.2±0.84 A	**101.0±0.70 C**
	B2	1093.2±2.53 B	863.8±2.30 A	36.6±1.54 C	**71.0±1.09 B**
	B3	280.6±8.84 A	374.2±2.82 C	25.0±0.94 B	**139.6±0.79 A**

* Different letters denote significant variances at P≤0.05

Table 4. Mean± SE of the platelets count x10^9/L., PT (sec.), and the aPTT (sec.) of the stallions

Species	Sub groups	Fibrinogen concentration mg/dl	Platelets count x10^9/L	PT (sec.)	aPTT (sec.)
Stallion	S1	482.00±13.55 B	654.4±12.45 B	15.0±0.68 B	**41.0±0.71 C**
	S2	487.0±4.24 B	746.8±11.02 A	33.8±0.55 A	**21.4±1.92 A**
	S3	579.2±24.01 A	400.6±9.73 C	12.4±0.84 C	**54.8±1.14 B**

* Different letters denote significant variances at P≤0.05

is explained by Santos et al. (2009) that may occur due to the differences between electrical charges on the surfaces of particles. The results suggest that there are significant reduce in fibrinogen concentrations, may be due to the effect of lectins which may hydrolyze fibrinogen. The results of the approach suggest that in-vitro use of M. oleifera seed extract affects significantly on some blood coagulation parameters which are included. In all animals there are reduce of fibrinogen concentrations, increase of platelet counts, prolong of PT whereas a decrease of aPPT. These results may be due to the facts that a lot of studies are documented. M. oleifera seeds have contains of lectins and thrombin like enzymes (Zubcevic et al., 2016). In the field of traditional medicine, the seed extract of M. oleifera can be used as a coagulant probably because of its contain of calcium which is one of the coagulant factors (Hope & Ifeanyi, 2019).

CONCLUSIONS

The results showed that the seed extract of M. oleifera had different in-vitro coagulant effects on coagulant parameters of different animals field. So far it could be useful as anticoagulant in traditional medicine.

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