EVALUATION OF SOME HEMATOLOGICAL PARAMETERS AND CLINICAL SIGNS AFTER REPEATED EXPOSURE TO WARFARIN IN DOGS

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ABSTRACT

Warfarin poisoning in dogs is not unusual which is used as a rodenticide. Competitive inhibition of vitamin K with an incomplete synthesis of the coagulation factors II, VII, IX and X can lead to a significant bleeding tendency.

The study was conducted at college of veterinary medicine / Basrah university to Evaluate clinical ,hematological and clotting indices in dogs in case of warfarin poisoning, which include twelve dogs of both sex at age about three years old.

The animals divided to three groups equally .first group treated with 3mg warfarin tablet given orally daily,the second group treated with 5mg until the signs of poisoning appears While the third group untreated as a control.

The results showed that the first group exhibited signs of warfarin poisoning like hematuria, vaginal bleeding, severe eye congestion, limping, bleeding in toe, excessive salivation, severe pale of mucus membrane in gum, hemoptysis, also the second group exhibited signs of warfarin poisoning after ten days the signs was hematuria and vaginal bleeding ,the animal showed signs of severe eye congestion, depression, weakness, and lameness ,bleeding in toe and then excessive salivation ,sever pale mucous membrane in gum, hemoptysis, blood vomit. weakness, excitement,nose bleeding, eye bleeding,protruded of eye,congestion of gum, melena and incoordination. there is significant decrease ($P<0.01$) in mean value of red blood cell count in dog treated with 3mg warfarin ($4.09 \times 10^6 \pm$ o.254) as well as hemoglobin level(9.613

g/dl±o.6085), packed cell volume (23.133 ±0.592) . and there is increase in mean of MCV (72. 40 fl±1.29) , MCH (23.86pg±0.52) and MCHC (32.78 g/dl ±0.50) which indicate macrocytic anemia .

Also dogs treated with 5mg warfarin showed that the mean of red blood cell count was 3.81 $\times 10^6 \pm$ 0.2347, mean of Hb was 8.30 ±1.006 g/dl and PCV 28± 2.510 with significant decrease ($P<0.01$) in these parameters and there is significant increase in mean of MCV,MCH. and MCHC 77.07fl±1.31 , 22.74 pg±0.49, 31.38 g/dl ±0.294 respectively .

There is significant increase($P<0.01$) in mean of prothrombin time and activated partial Thromboplastin Time in dogs treated with 3mg of warfarin, 1.336 ± 0.146 , 1.036 ± 0.1074 min.Respectively whencompared with control animals 0.601± 0.0863 , 0 .153±0.003min. and There is significant decrease ($P>0.05$) in mean value of platelets count 253.80 $\times 10^3$g/l ± 18.31 compared with control group 448.12 ±52.24 . ,also there is significant increase($P<0.01$) in PT. and APTT. when treated with 5mg 1.855 ±0.2039 , 1.401 ±0.1051 min.respectively,whereas the mean of platelet 240.40 $\times 10^3$ g/l ± 5.39 with significant differences($P<0.01$) .

INTRODUCTION

Rodenticides are one of the most common poisonings in veterinary medicine. (1). ,as anticoagulant rodenticides are the largest group of pesticides used for control of harmful rodents (2,3). All anticoagulants have the basic coumarin or indanedione nucleus. The “first-generation” anticoagulants (warfarin, pindone, coumafuryl, coumachlor, isovaleryl indanedione) which require multiple feedings to result in toxicity**,** asWarfarin was the first compound marketed as an anticoagulant rodenticide (4 ,5).

.The active ingredient warfarin is found in a variety of commercial rodenticides., is used for controlling rats and house mice in and around homes, animal and agricultural premises, and commercial and industrial sites. It is odorless and tasteless and effective in very low dosages ,repeated ingestion is needed to produce toxic symptoms(6 ,7,8).

anticoagulant prevent the clotting of blood , These agents are commonly used and are one of the most common household poisons, accounting for a large number of accidental poisoning among dogs. As
ingestion of dead or alive poisoned rodents generally, the mechanism of anticoagulant rodenticides
action is to bind and inhibit enzyme complexes responsible for the recycling of vitamin K_1, thus creating a series of deleterious clotting and coagulation impairments(9,10).
When ingested by an animal, anticoagulants block the synthesis of vitamin K, an essential component fornormal blood clotting, which results in spontaneous and uncontrolled bleeding (11,12,13).Common signs of *anticoagulant*Rodenticides toxicosis include anorexia, weakness, coughing, epistaxis and dyspnea, although other signs such as hematuria, lameness or seizures have been reported. Since many of the clinical signs are non-specific in nature(14).
the present study conducted to observe clinical signs , assessment hematological changes and some clotting factors in dogs after warfarin administration .

MATERIAL AND METHODS

Experimental study have been performed in college of veterinary medicine / Basrah university, The study include twelve' dogs of both sexes , 3 years old, animal weights (12 to19 kg) , animals examined clinically for any disease detection before ,also animals treated with Ivermectin 0.3ml/10kg.BW. to exclude internal and external parasite and the.Animals of the study divided in to three equal groups each group contained four animals treated with warfarin daily until signs of poisoning observed , The first group treated with 3mg warfarin tablet ,The second group treated with 5mg ,and the third group without treatment as a control group. two blood samples were daily obtaineed from cephalic vein, 2ml with anticoagulant(EDTA) for (RBC, Hb ,PCV and RBC indices ,platelets count(PLT),platelets volume (PV) and platelets distribution width (PDW)) use (hematology analyzer,Genex,USA) , and 5ml with anticoagulant (sodium citrate) centrifuged for obtaining plasma to evaluate coagulation marker {Prothrombin Time(PT) Activated Partial Thromboplastin Time (APTT)}.

1- Prothrombin Time (PT) procedure:

- PT (second) measurement of reference plasma control and patients plasma according to manufacturer instruction (BILABO.SAS,Maizy,France).

2-**Activated Partial Thromboplastin Time** (APTT)

- APTT (second) measurement of reference plasma control and patients plasma according to manufacturer instruction (BILABO.SAS,Maizy,France)

All data were subjected to Statistical analyser ,the significance of variation were statistically analyzed using (spss) one way anova .

Results and discussion

the Present study revealed that dogs which treated with warfarin 3 mg orally examined daily, doges began to exhibited signs of warfarin poisoning after month from the beginning of treatment , the signs weakness, excitement, epistaxis, and eye bleeding when mean of prothrombin time (PT) 1.82 $\mp$ 0.17 mintut . and Activated Partial Thromboplastin Time (APTT) time 1.2 $\mp$ 0.11 minute with significant difference($P<0.01$) compared with control 0.74 $\mp$0.24 *and* 0.15 $\mp$ 0.05 *minut* respectively .and protruded of eyes (retrobular hemorrhage), congestion of mucus membrane of gum when the mean of PT time 2.4 $\mp$0.08 minute and APTT time 2.2 $\mp$0.08 minute and then animals show melena and incoordination movement table(1) .

Table(1):signs of warfarin poisoning with 3mg warfarin orally for one month

Signs	PT/min.	Control/min.	PPT/min.	Control/min.
weakness, excitement, epistaxis, and eye bleeding	1.82$\mp$0.17	0.74 $\mp$0.24	1.2$\mp$0.11	0.15 $\mp$ 0.05
And retrobuber of eyes (retobular hemorrhage), congestion of mucus membrane ,Scant feces ,Incoordination	2.4$\mp$0.08		2.2$\mp$ 0.15	

While in second group which treated with 5 mg warfarin , after ten days the animal began to show signs of warfarin poisoning , the signs was hematuria and vaginal bleeding when mean value of PT reach 1.o7 ∓0.21 min. and APTT 1.4 ∓ 0.12 min. with significant variation (P<0.05) when compared with control 0.63 ∓0.108 *mint.* and 0.105 ∓ 0.102 respectively the animal showed signs of sever eye congestion, depression, weakness, and lameness when mean of PT and APTT reach 1.50 ∓ 0.176 and 1.26 ∓ 0.014 min. respectively.

when mean of PT and APTT 2.33 ∓ 0.65 and 1.31 ∓0.31 min. respectively the animals show bleeding in toe and then excessive salivation ,severe pale mucous membrane, hemoptysis, blood vomit.weakness, excitement,nose bleeding, and eye bleeding,protruded of eye,congestion of mucosa of gum, melena and incoordination, when mean value of PT time 3.4 ∓ 0.32 min. and APTT time1.27 ∓0.048 min. when mean of PT time 3.23 mint. and APTT time 1.20 min., the animal had die (table 2) .

Table(2):signs of warfarin poisoning with 5mg.orally for ten days administered

Sign	PT/min.	Control/min.	APTT/min.	Control/min.
hematuria and vaginal bleedingsever eye congestion, depression, weakness, and lameness	1.07 ∓0.21	0.63 ∓0.108	1.4 ∓ 0.12	0.105∓0.102
bleeding in toe, excessive salivation, sever pale mucous membrane , coughing with blood, blood vomit	2.33 ∓ 0.65		1.31 ∓0.13	
weakness, excitement, epistaxis, eye bleeding, protruded of eyes (retobular hemorrhage), congestion of mucus membrane ,Melena ,incoordination	3.4∓0.32		1.27 ∓0.048	

Toxic doses of anticoagulants cause damage to capillaries, increasing their permeability, causing diffuse hemorrhag. These effects are gradual developing over several days. In the final stage of intoxication, there is collapses in hypovolemic circulatory shock or severe anemia and the animal dies (15).Repeated ingestion of warfarin may causes the same haemorrhagic risks as acute exposure because ofthe depleting effects of warfarin in serum for clotting factors. With anticoagulant rodenticidetoxicity,hemorrhage can occur in a variety of locations and most typically as body cavity effusions or pulmonary parenchymal bleeding. Patients can show a several of different clinical signs, including both nonspecific (anorexia, lethargy, weakness) and specific manifestations (cough, dyspnea, hemoptysis,lameness, hematuria, bruising, exophthalmos, pharyngeal swelling, CNS signs, epistaxis, melena (16) .

Clotting factors are depleted, with three to five days being the most common time occur, depending on the agent consumed as The clinical signs can vary, but they are always due to the coagulopathy (17),also other researcher showed same signs of warfarin poisoning (18,19,20,21) .

Hematological parameter in dog treated with 3mg warfarin showed significant decrease ($P<0.05$) in mean of red blood cell count 4.09 ± o.25 when camper with control 6.52 ± 0.49 also there is significant decrease($P<0.05$) in mean of hemoglobin concentration 9.613±o.6085gm/dl while in control 11.77±1.19gm/dl,also the mean of packet cell volume 23.13 ±0.59 with significant different($P<0.05$) from control 34.01 ± 2.88 and there is increase in mean of MCV 72. 40 fl. ±1.29 , MCH 23.86±0.52 and MCHC32.78 ±0.50 which indicate macrocytic anemia .and in dogs treated with 5mg warfarin there is significant decrease ($P<0.05$) in mean of red blood cell count 3.81± 0.23, mean of Hb. 8.30±1.006 gm/dl.and PCV 28± 2.51 and there is significant increase in mean of MCV,MCH. And MCHC 77.07±1.31 and 22.74 ±0.49 and 31.38 ±0.29 respectively , Table (3)

Table (3) Means and Std. Error of blood parameters of dogs administered warfarin orally 3mg and 5mg and control.

Groups	Mean,∓SE of RBC×10^6	Mean,∓SE of Hb g/dl	Mean,∓SE of PCV%	Mean,∓SE of MCV/fl	Mean,∓SE of MCH/pg	Mean,∓SE of MCCHC g/dl
Control	6.52∓.49 a	11.77∓1.19a	34.01∓2.88 a	70.87∓.75 a	24.18∓0.41 a	33.53∓.72 a
3mg	4.09∓.25b	9.61∓.60 b	23.13 ∓0.59 b	69.10 ∓ 1.08b	23.86∓.25 b	32.78∓0.50 b
5mg	3.81∓.23b	8.30 ∓1.006b	28.50 ∓ 2.51b	77.07∓1.31b	22.74∓.49 b	31.38 ∓.29 b

The small letter a,b indicate the values is significant ♦♦(P<0.05)

. There is significant increase (P<0.05)in mean of prothrombin time and activated partial Thromboplastin Time after one month of beginning of treatment with 3mg of warfarin, 1.33± 0.14 min. , 1.03 ± 0.10 min. , respectively when camper with control animals 0.60± 0.08minute and 0.15±0.003mintand after that the dogs began to exhibited signs of warfarin poisoning which explain above also There is significant decrease (P>0.05) in mean of platelet count 253.80 ± 18.31 from control group 448.12 ±52.24 .

And there is significant increase(P<0.05) in PT.and APTT. when treated with 5mg1.85±0.20and 1.40 ±0.10 min.respectively,while the mean of platelet 240.40 ± 5.39 with significant variation (P<0.05) ,also there is a significant increasing in mean of platelets volume and platelets distribution width(P<0.05)9.26±0.74,8.21 ± 0.88in dogs treated with 3mg warfarin and 7.27±0.29 ,8.05 ±0.59 in 5mg warfarin respectively table(4).Other researcher(14,19,20,21, 22)showed same changes in clotting indices which indicated in present study.

Table (4) means and standard error of clotting marker of control and dogs with warfarin poisoning

Groups	Mean ∓Std error ofPT/min	Mean∓Std error of APTT/min	Mean ∓SEof Plate Late×1o^3	Mean∓SEof PV/fl	Mean∓SEof PDW %
Control	0.62∓0.08 a	0.153 ∓ 0.003 a	448.12∓52.40 a	7.13∓0.63 a	5.62∓0.55 a
3mg	1.33∓0.14 b	1.03∓0.107 b	253 ∓18.31 b	9.26∓0.74 b	8.21∓0.88 b
5 mg	1.85∓0.20 b	1.40∓0.10 b	240.40∓5.39 b	7.36∓0.29 b	8.05∓0.59 b

The small later a,b indicate the values is significant ♦♦(P<0.05)

Anticoagulant rodenticides inhibit vitamin K epoxide reductase, resulting in a lack of active vitamin K. This mechanism contributes to blood clotting factors (II, VII, IX, and X) that are not carboxylated and remain nonfunctional (23,3). So when any dog exhibited signs which explained above and which have bleeding tendency and the causes not well clear we must done laboratory examination include clotting markers to confirm if there is accidental exposure to poisoning such as warfarin and must treated immediately .

تقيم العلامات السريريه وبعض القيم الدمويه بعد التعرض المتكرر للوارفرين في الكلاب

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الخلاصة

يعتبر تسمم الكلاب بماده الورفرين شائع حيث يستخدم كقاتل للجرذان يعمل على التنافس مع فيتامين ك وبذلك يكون هناك نقص بعوامل التخثر II, VII, IX X وهذا يؤدي الى الميل للنزف .

اجريت هذه الدراسة في كلية الطب البيطري\جامعة البصرة لتقييم العلامات السريرية والصورة الدموية وعوامل التخثر في حالة تسمم الكلاب بمادة الوارفارين، تضمنت الدراسةاثنا عشر حيوان ذات اجناس مختلفة تراوحت اعمارها ثلاث سنين ،حيث تم تقسيم الحيوانات الى ثلاثة مجاميع متساوية ، تم تجريع المجموعة الاولى 3 ملغم من مادة الوارفارين عن طريق الفم يوميا ،المجموعة الثانية 5 ملغم من مادة الوارفارين يوميا ،اما المجموعة الثالثة اعتبرت كسيطرة.

اظهرت نتائج الدراسة علامات التسمم في الكلاب في المجموعة الاولى حيث تم تسجيل علامات الضعف ،التهيج، النزف من العين ، بول دموي، كدمات، علامات عصبية ، ظهورالدم مع البراز، ترنح، النزف من الانف ،بروز العين ، واحتقان الجدار المخاطي لّلثة .

اما المجموعة الثانية اظهرت علامات البول الدموي ،النزف المهبلي ،احتقان حاد في العين ،عرج، نزف من الاصابع ،زيادة اللعاب ، شحوب حاد للجدار المخاطي ،نفث الدم اثناء السعال عن طريق الفم .

وكذلك بينت الدراسة انخفاض معنوي ($p< 0.05$) في عدد كريات الدم الحمراء 0.25±4.09 وكذلك في معدل تركيز خضاب الدم والحجم الخلايا المضغوط 0.59± 23.13 , 9.61±o.60 على التوالي .وهناك زياده في معدل الحجم الكريات ومعدل خضاب الكريات ومعدل تركيز خضاب الكريات 1.29±40 ، 0.52±23.86 و 32.78 0.50± مما يدل على حدوث فقر الدم . وكذلك بالنسبه للحيوانات المعامله ب5 ملغرام فان معدل عدد كريات الدم الحمراء 0.23 ±3.81 ، معدل تركيز خضاب الدم 1.006± 8.30 و معدل الحجم الخلايا المضغوط 2.51 ±28 مع وجود فارق معنوي ($p< 0.05$) في هذه الفحوصات ، وكذلك هناك زياده في معدل الحجم الكريات ومعدل خضاب الكريات ومعدل تركيز خضاب الكريات 1.31± 77.07 و0.49± 22.74 و0.29± 31.38 على التوالي . اما معدل سابق الخثرين و معدل زمن حرك الخثرين الجزيئي في الكلاب المعاملة ب3ملغرام وارفرين0.14 ± 1.33.

و 0.10 ±1.03 / دقيقه على التوالي مع وجود فارق معنوي ($p< 0.05$) عند المقارنة مع مجموعه السيطره ±60. 0.003±0.15 ,00.08 0/ دقيقه. وكذلك هناك انخفاض معنوي ($p< 0.05$) في معدل عد الاقراص الدمويه 253.80 18.31 ± عند المقارنه مع مجموعه السيطره52.24± 448.12 ، وكذلك هناك زياده معنويه في معدل سابق الخثرين ومعدل زمن حرك الخثرين الجزيئي في الكلاب المعاملة ب 5 ملغرامو ارفرين0.20± 1.85 و 1.40 0.10± على التوالي بينما معدل عد الصفيحات الدمويه5.39 ± 240.40 مع وجود فارق معنوي مقارنه بمجموعه السيطره ($p< 0.05$) .

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