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

Israa Abdul Wadood Muhammad Ali

Department of Internal and Preventive Medicine College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

(Received 1 November 2015, Accepted 29 November 2015)

Keywords: Warfarin, vitamin K, coagulation factor.

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, severe pale mucous membrane in gum, hemoptysis, blood vomit, weakness, excitement, nose bleeding, eye bleeding, protruded of eye, congestion of gum, melena and incoordinated. there is significant decrease (P<0.01) in mean value of red blood cell count in dog treated with 3mg warfarin (4.09 ×10^6 ± 0.254) as well as hemoglobin level (9.613
g/dl ± 0.6085), packed cell volume (23.133 ± 0.592), and there is an increase in mean of MCV (72.40 fl ± 1.29), MCH (23.86 pg ± 0.52) and MCHC (32.78 g/dl ± 0.50) which indicate macrocytic anemia.

Also dogs treated with 5 mg warfarin showed that the mean of red blood cell count was 3.81 × 10^6 ± 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 a significant increase in mean of MCV, MCH, and MCHC 77.07 fl ± 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 3 mg of warfarin, 1.336 ± 0.146, 1.036 ± 0.1074 min. Respectively when compared with control animals 0.601 ± 0.0863, 0.153 ± 0.003 min. and There is significant decrease (P > 0.05) in mean value of platelets count 253.80 × 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 5 mg 1.855 ± 0.2039, 1.401 ± 0.1051 min. respectively, whereas the mean of platelet 240.40 × 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, as Warfarin 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 K1, 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 for normal 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 to 19 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 obtained 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, dogs began to exhibit 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 ± 0.17 minute and Activated Partial Thromboplastin Time (APTT) time 1.2 ± 0.11 minute with significant difference (P<0.01) compared with control 0.74 ± 0.24 and 0.15 ± 0.05 minute respectively. And protruded of eyes (retrobulbar hemorrhage), congestion of mucus membrane of gum when the mean of PT time 2.4 ± 0.08 minute and APTT time 2.2 ± 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**

<table>
<thead>
<tr>
<th>Signs</th>
<th>PT/min.</th>
<th>Control/min.</th>
<th>PPT/min.</th>
<th>Control/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>weakness, excitement, epistaxis, and eye bleeding</td>
<td>1.82 ± 0.17</td>
<td>0.74 ± 0.24</td>
<td>1.2 ± 0.11</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>And retrobulber of eyes (retrobulbar hemorrhage), congestion of mucus membrane , Scant feces , Incoordination</td>
<td>2.4 ± 0.08</td>
<td>2.2 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 $1.07 \pm 0.21$ min. and APTT 1.4 $\pm 0.12$ min. with significant variation ($P<0.05$) when compared with control 0.63 $\pm 0.108$ min. and 0.105 $\pm 0.102$ respectively the animal showed signs of sever eye congestion, depression, weakness, and lameness when mean of PT and APTT reach 1.50 $\pm 0.176$ and 1.26 $\pm 0.014$ min. respectively.

when mean of PT and APTT 2.33 $\pm 0.65$ and 1.31 $\pm 0.31$ 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 $\pm 0.32$ min. and APTT time 1.27 $\pm 0.048$ min. when mean of PT time 3.23 min. and APTT time 1.20 min., the animal had died (table 2).

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

<table>
<thead>
<tr>
<th>Sign</th>
<th>PT/min.</th>
<th>Control/min.</th>
<th>APTT/min.</th>
<th>Control/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematuria and vaginal bleeding, severe eye congestion, depression,</td>
<td>$1.07 \pm 0.2$</td>
<td>$1.4 \pm 0.12$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>weakness, and lameness</td>
<td>1.07 $\pm 0.2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bleeding in toe, excessive salivation, severe pale mucous membrane,</td>
<td>$2.33 \pm 0.65$</td>
<td>$0.63 \pm 0.108$</td>
<td>$1.31 \pm 0.13$</td>
<td>$0.105 \pm 0.102$</td>
</tr>
<tr>
<td>coughing with blood, blood vomit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weakness, excitement, epistaxis, eye bleeding, protruded of eyes</td>
<td>$3.4 \pm 0.32$</td>
<td></td>
<td>$1.27 \pm 0.048$</td>
<td></td>
</tr>
<tr>
<td>(retohedral hemorrhage), congestion of mucous membrane, Melena,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>incoordination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 of the 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 ± 0.25 when camper with control 6.52 ± 0.49 also there is significant decrease(P<0.05) in mean of hemoglobin concentration 9.61±0.6085gm/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 MCHC 32.78 ±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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean, TSE of RBC×10⁶</th>
<th>Mean, TSE of Hb g/dl</th>
<th>Mean, TSE of PCV%</th>
<th>Mean, TSE of MCV/fl</th>
<th>Mean, TSE of MCH/pg</th>
<th>Mean, TSE of MCCHC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.52±0.49 a</td>
<td>11.77±1.19a</td>
<td>34.01±2.88 a</td>
<td>70.87±7.5a</td>
<td>24.18±0.41 a</td>
<td>33.53±7.2a</td>
</tr>
<tr>
<td>3mg</td>
<td>4.09±0.25 b</td>
<td>9.61±6.0 b</td>
<td>23.13±0.59 b</td>
<td>69.10±1.08 b</td>
<td>23.86±2.5 b</td>
<td>32.78±0.50 b</td>
</tr>
<tr>
<td>5mg</td>
<td>3.81±0.23 b</td>
<td>8.30±1.006b</td>
<td>28.50±2.51 b</td>
<td>77.07±1.31b</td>
<td>22.74±4.9 b</td>
<td>31.38±2.9 b</td>
</tr>
</tbody>
</table>

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±0.08 minute and 0.15±0.003 mintand 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 5mg 1.85±0.20 and 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±0.88 in 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± Std error of PT/minute</th>
<th>Mean ± Std error of APTT/minute</th>
<th>Mean ± SE of Plate Latex×10³</th>
<th>Mean ± SE of PV/fl</th>
<th>Mean ± SE of PDW %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.62 ± 0.08 a</td>
<td>0.153 ± 0.003 a</td>
<td>448.12 ± 52.40 a</td>
<td>7.13 ± 0.63 a</td>
<td>5.62 ± 0.55 a</td>
</tr>
<tr>
<td>3 mg</td>
<td>1.33 ± 0.14 b</td>
<td>1.03 ± 0.107 b</td>
<td>253 ± 18.31 b</td>
<td>9.26 ± 0.74 b</td>
<td>8.21 ± 0.88 b</td>
</tr>
<tr>
<td>5 mg</td>
<td>1.85 ± 0.20 b</td>
<td>1.40 ± 0.10 b</td>
<td>240.40 ± 5.39 b</td>
<td>7.36 ± 0.29 b</td>
<td>8.05 ± 0.59 b</td>
</tr>
</tbody>
</table>

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 الاعلامات السريري وبعض القيم الدمويه بعد التعرض المتكرر للوارفارين في الكلاب

إسراء عبد الودود محمد علي
فرع الطب الباطني والوقائي ،كلية الطب البيطرى ، جامعه البصرة ،العراق.

الخلاصة

يعتبر تسمم الكلاب بمادة الوارفارين شائع حيث يستخدم كقاتل للجرذان يعمل على التنافس مع فيتامين K و بذلك يكون هناك نقص بعوامل التخثر II, VII, IX, X هذا يؤدي إلى الزيادة في ظرف الدم. اجريت هذه الدراسة في كلية الطب البيطرى، جامعة البصرة لتقسيم الاعلامات السريري وبعض القيم الدموية و عوامل التخثر في حالة تسمم الكلاب بمادة الوارفارين. تضمنت الدراسة اثنا عشر جنود ذكور اجتازوا تراث في اعمارهم ثلاث سنين بحيث تم تقسيم الحيوانات إلى ثلاثة مجموعات متساوية. تم تجريب المجموعة الأولى 3 مليم من مادة الوارفارين عند طريق الفم يوميا في المجموعة الثانية 5 مليم من مادة الوارفارين يوميا أما المجموعة الثالثة اعتبرت كسيطرة.
اظهرت نتائج الدراسة علامات التسمم في الكلاب في المجموعة الأولى حيث تم تسجيل علامات ضعف التهيج، النزف من العين، بول دموي، وعلامات عصبية، ظهور الدم بالبراز، نزف من الأنف، بروز العين، وانقباض الجدار المخاطي للثة.

اما المجموعة الثانية اظهرت علامات البول الدموي، النزف المهبل، اختبائه حاد في العين، نزف من الأصابع، زيادة الالعاب، شحوب للجدران، رفع العين، وانقباض الجدار المخاطي للثة.

وكذلك بنيت الدراسة انخفاض معنوي (p<0.05) في عدد كرات الدم الحمراء 4.09±0.25 وكذلك في معدل تركز خضاب الدم والحجم الخلايا المضغوط 23.13±0.59 على التوالي. ونحو زيادة في معدل الحجم الكريات ومعدل خضاب الكريات ومعدل تركز خضاب الكريات 1.29±0.52 و 32.78±0.60. مما يدل على حدوث فقر الدم. وكذلك بالنسبة للحيوانات المعاشة 5 ملغ/كم³ فإن معدل كرات الدم الحمراء 2.31±0.83 في معدل خضاب الدم بالخلايا المضغوط 8.30±0.40. ونحو زيادة في معدل الحجم الكريات ومعدل خضاب الكريات 31.87±0.40 و 22.74±0.49 على التوالي. أما معدل سباق الخثران و معدل زمن حركة الخثران الجزئي في الكلاب المعاشة بـ 3 ملغ/كم³ وارفارين 1.33±0.14.

وقد قا مقا مقارنة عداد القرص الدموي مع وجود فارق معنوي (p<0.05). عند المقارنة مع مجموعه السيطرة 60.10±0.10. ونحو زيادة مع وجود فارق معنوي (p<0.05) في معدل عدد الأعراض الدموي 253.80±0.05. ونحو زيادة مع وجود فارق معنوي (p<0.05) في معدل عدد الأعراض الدموي 240.40±0.10. ونحو زيادة مع وجود فارق معنوي مقارنة بـ 253.80±0.05. مع وجود فارق معنوي مقارنة بـ 240.40±0.10.

References

6. United States Occupational Safety and Health Administration (OSHA) (16 August 1996). "Documentation for Immediately Dangerous To Life or Health


8- Justine A. Lee (2012 ) in Dog Toxins & Poisons ,pet health Net work .


17- 285-291.


