Effect of supplementary feeding of vitamin K₁ on difethialone treated Indian gerbil, Tatera indica Hardwicke in laboratory

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Two dosages (1 and 2 mg/kg) of vitamin K₁ supplementation for 5 and 15 days were given to Indian gerbil T. indica fed on difethialone bait (0.0025%) for one day. The results indicated that the lower dosage could not reverse the anticoagulation process, however the period of mortality was considerably increased from 3-9 days (in control) to 5-14 days (5 days supplementation regime). Subsequently when the vitamin K₁ dosage was doubled and given for 15 days, there was 100% reversal of anticoagulation process and all the test gerbils became normal within a month of poisoning with difethialone bait.

Keywords: Anticoagulants, Baits, Difethialone, Gerbils, Plain food, Vitamin K₁ supplemented food

Rodent pest management is largely dependent on usage of toxic rodenticides. Zinc phosphide, an acute rodenticide is being extensively used for the purpose in India and elsewhere.7 12. Besides being highly toxic to non-targets, zinc phosphide induces bait/poison shyness in a variety of pest rodent species.7 13. With the advent of second-generation single dose anticoagulant rodenticides, the whole concept of rodent pest management has been revolutionized in recent years. They act on the blood vascular system of target animals and inhibit blood coagulation mechanism leading to excessive internal haemorrhage and death of the animals within 3-15 days even after one-day exposure. Moreover, due to their requirement in very small dosages (0.0025-0.005%) in baits, these anticoagulants tend to be relatively safer to non-target species. The anticoagulants and vitamin K₁ are known to share the same site of action by blocking the epoxide reductase enzyme system in the blood.6 Thus process of anticoagulation of blood may be reversed by addition of larger doses of vitamin K₁.7 8. Most of the studies on effect of vitamin K₁ on anticoagulant poisoning have been conducted on larger vertebrates,8 9 however, very little information on this aspect is available on rodents. Considering the bioefficacy of difethialone against major Indian pest rodent species and the safety factor involved through antidoting, the present communication makes a maiden attempts to describe and quantify the effect of supplementary feeding of vitamin K₁ on difethialone treated Indian gerbil, Tatera indica Hardwicke in laboratory.

Material and Methods

The Indian gerbils were captured from crop fields and grasslands near Jodhpur (Lat. 26°18'N Long, 73°01'E). It was ascertained that the captured rodents had no previous experience of feeding on anticoagulant baits. In all 30 healthy adult gerbils of weight range 98-120 g were used in different sets of experiment. The test gerbils were weighed and lodged in iron mesh cages (24′x12″x12″) for acclimatization. During this period the gerbils were fed pearl millet grain and water was available to them ad libitum.

Sets I and II—Immediately after exposure of anticoagulant rodenticides, the test animals of these
two sets were fed with arachis oil (2% w/w) smeared pearl millet grains mixed with vitamin K<sub>1</sub> powder @ 1 mg/kg which was almost equal to the active ingredient of difethialone ingested by the gerbils. In Set I the vitamin K<sub>1</sub> supplemented feed was given for 5 days whereas in Set II this period was extended for 15 days.

Sets III and IV—In these sets, the vitamin K<sub>1</sub> doses was doubled i.e. 2 mg/kg mixed in pearl millet grains smeared with 2% arachis oil. This feed was provided to the difethialone (0.0025%) fed gerbils for 5 days (Set III) and for 15 days (Set IV).

Set V—In this set the experimental gerbils were exposed to difethialone (0.0025%) in pearl millet grains for one day and only plain bait (no vitamin K<sub>1</sub> supplement) was offered on subsequent occasions till death of the animals. This set acted as control/check.

Since the symptom of difethialone poisoning appears in the gerbils after 3-4 days of poisoning and lasts upto 14 days, two feeding regimes of 5 and 15 days were experimented for these trials. Consumption pattern and symptoms of poisoning, such as blood discharge from nose, eyes, anus, ear etc., dullness in activity and paralyses of limbs and death of experimental rodents were monitored for 30 days immediately after feeding on difethialone baits.

### Results and Discussion

Difethialone has proved its potency as an effective anticoagulant rodenticide against rodent pest species in India at a relatively lower dosages (0.0025%) in comparison to other rodenticides of the same generation viz., bromadiolone, brodifacoum, flocumafen etc., which are effective at 0.005% in baits. In the present investigation also, difethialone (0.0025%) yielded 100% mortality of Indian gerbils after one day exposure (Table 1). Effect of vitamin K<sub>1</sub> supplementation on difethialone fed Indian gerbils is discussed below.

Set I—Despite an intake of 4.25±0.28g of difethialone treated baits, the test gerbils in this set consumed vitamin K<sub>1</sub> supplemented feed at the rate of 4.12±0.67g/day with a total intake of 20.38±3.53 during 5 days (Table 1). Additional supply of vitamin K<sub>1</sub> @ 1mg/kg for five days could not help the poisoned gerbils to recover which was evident from the drastic fall in plain food consumption during post treatment period (2.93±0.58g/day) resulting in mortality of all the test gerbils within two weeks of poisoning. Analysis of consumption and mortality data for individual test animal revealed that one animal (gerbil no. 1) consumed significantly lower amounts of vitamin K<sub>1</sub> supplemented food for 4 days (1-1.60g) and died on 5<sup>th</sup> day only, whereas other five test animals consumed 2.80-8g vitamin K<sub>1</sub> treated food during this period (Fig. 1). These observation indicated that vitamin K<sub>1</sub> supplementation @ 1mg/kg for five days doubled the mean duration of death (10.17 days) as compared to control (5.5 days), but was not enough to reverse the anticoagulation effect of difethialone.

Set II—In this set the vitamin K<sub>1</sub> supplementation was continued for two weeks resulting in increased total intake of the supplemented feed (45.38±4.59g), however mean consumption of this feed per day was at par (3.27±0.22g) with previous set. Similar trend was noticed in plain food consumption also during post treatment periods (Table 1). Data presented in Fig. 2 showed that two test rodents (No.4 and 6) died on 11<sup>th</sup> and 14<sup>th</sup> day of poisoning due to blood loss during the vitamin K<sub>1</sub> administration period. Remaining

### Table 1—Effect of supplementary feeding of vitamin K<sub>1</sub> on difethialone fed Tatera indica Hardwicke

<table>
<thead>
<tr>
<th>Experiment Set</th>
<th>Difethialone Bait (0.0025%) consumption (g/100g B.Wt.)</th>
<th>Vitamin K&lt;sub&gt;1&lt;/sub&gt; supplemented food</th>
<th>Post treatment food consumption*</th>
<th>Mortality % (n/N)</th>
<th>Mean days to death</th>
<th>Survival % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean consumption/day</td>
<td>Mean total consumption</td>
<td>Mean consumption/day</td>
<td>Mean total consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set-I</td>
<td>4.25±0.28</td>
<td>4.12±0.67</td>
<td>20.38±3.53</td>
<td>2.93±0.58</td>
<td>14.72±1.91</td>
<td>100</td>
</tr>
<tr>
<td>Set-II</td>
<td>4.03±0.24</td>
<td>3.27±0.22</td>
<td>45.38±4.59</td>
<td>2.83±0.41</td>
<td>16.23±6.9</td>
<td>83 (6/6)</td>
</tr>
<tr>
<td>Set-III</td>
<td>4.07±0.33</td>
<td>3.82±0.26</td>
<td>19.09±1.29</td>
<td>3.56±0.35</td>
<td>43.94±17.35</td>
<td>83 (5/6)</td>
</tr>
<tr>
<td>Set-IV</td>
<td>4.45±0.27</td>
<td>4.04±0.32</td>
<td>60.58±4.80</td>
<td>4.72±0.21</td>
<td>70.87±3.21</td>
<td>Nil</td>
</tr>
<tr>
<td>Control Set-V</td>
<td>4.43±0.16</td>
<td>-</td>
<td>-</td>
<td>2.65±0.53</td>
<td>13.12±3.95</td>
<td>100 (6/6)</td>
</tr>
</tbody>
</table>

*Values expressed in g/100g body weight are mean ± SE
Figs 1-3—Consumption of difethialone bait, Vitamin K₁ supplemented food and plain food by T. indica in different experimental sets. [1: Experimental set-I, 2: Experimental set-II, 3: Experimental set-III]
Figs 4-5—Consumption of difethialone bait, Vitamin K₁ supplemented food and plain food by T. indica in (4) Experimental set-IV; and (5) Control set

four rodents (1,2,3,5) showed steady pattern of consumption up to 15 days. After cessation of vitamin K₁ supplement three of the four rodents (No. 1,2 and 3) died on 19th, 16th and 20th day after poisoning, respectively, however, gerbil No.5 recovered from anticoagulant effect and became normal during fourth week. This indicated that increase in period of vitamin K₁ supplement does have some positive effect on recovery of rodents from anticoagulant poisoning.

Set III—Mean daily consumption of vitamin K₁ supplemented food and mortality pattern were almost similar to those recorded in set II. Increased dosage of vitamin K₁ (2 mg/kg) fed to poisoned gerbils for 5 days registered total intake of vitamin K₁ treated food upto 19.09±1.29 g and that of plain food was 43.94±17.35 g (Table 1). Animal wise analysis of data (Fig. 3) showed that all the six rodents showed steady pattern of consumption except one gerbil (No. 1), which registered a significant decline in feed consumption and died on seventh day of poisoning. Mortality pattern of gerbils even after consuming higher dosages of vitamin K₁ supplement for 5 days was almost similar to that of set II as all the rodents except No.3 died within 7-18 days. Mean days to death was increased two and a half times (14.0 days) as compared to control (5.5 days) (Table 1). The surviving rodents steady recovery, which was evident from the pattern of plain bait consumption after 5 days of vitamin K₁ supplementation.

Set IV—When the dosages (2 mg/kg) as well as the period of vitamin K₁ supplement was increased (15 days) the test rodents registered complete reversal from anticoagulant poisoning. Here, all the six rodents showed steady pattern of consumption of vitamin K₁.
bait all through. Average daily intake of vitamin K\textsubscript{1} supplemented food and plain food was at par i.e., 4.04±0.32 and 4.72±0.21 g, respectively. Similarly there was no significant difference in mean total consumption of both types of food offered to the poisoned gerbils for 15 days each (Table 1). In case of two experimental gerbils (Nos. 4 and 5) the consumption of vitamin K\textsubscript{1} supplemented feed was no doubt dropped on 5\textsuperscript{th} and 3\textsuperscript{rd} day, but on subsequent days it increased and maintained a steady pattern. The consumption of plain food too remained steady (2.95-6.0 g/100g body wt. per day) even after the cessation of vitamin K\textsubscript{1} supplemented bait after 15 days and all the test gerbils recovered from toxic effects of difethialone within a month of poisoning (Fig. 4).

Control — In the control set, where the gerbils were not provided with vitamin K\textsubscript{1} supplement, all the test gerbils succumbed to the poison bait after single day exposure of difethialone (0.0025%). Mean consumption of plain food after poisoning was expectedly decreased to 2.65±0.53 g/day (Table 1). Symptoms of anticoagulant poisoning were visible quite early and all the test rodents died between 3 and 9 days (Fig. 5).

The results showed that supplementary feeding of vitamin K\textsubscript{1} at a dose equivalent to the active ingredient (a.i.) of difethialone ingested by the test gerbils was not sufficient for the recovery of poisoned animals even if it was administered for 15 days. The period of mortality was no doubt prolonged considerably (11-20 days against 3-9 days in control). On doubling the vitamin K\textsubscript{1} dosage (2 mg/kg) with an enhanced feeding period of 15 days, the gerbils registered complete reversal from the toxicsosis caused due to feeding on difethialone baits. Hadler and Buckle\textsuperscript{15} have also reported that for reversal of anticoagulation frequent administration of vitamin K\textsubscript{1} is necessary until the anticoagulant was cleared from the system.

The results clearly indicated that with the additional supply of vitamin K\textsubscript{1} the process of prothrombinopenia i.e. deficiency of prothrombin factors in blood set in by difethialone may have antagonized after the vitamin K\textsubscript{1} competitively joins the active sites, resulting into reversal of anticoagulation. Brodifacoum poisoning in dogs was antidoted with vitamin K\textsubscript{1} therapy either through feeding or injection daily for three weeks @ 2 mg/kg\textsuperscript{8}. Similarly Reitmeyer et al.\textsuperscript{16} reported improvement in coagulation time in anticoagulant intoxicated dogs after a maintenance dose of vitamin K\textsubscript{1} @ 1-2.6 mg/kg twice daily for 3-10 weeks. Nee et al.\textsuperscript{17} analysed the induction of prothrombin synthesis by K vitamins with that of brodifacoum treated rats for reversing the excessive oral anticoagulation.

Thus the present findings revealed that lethal action of anticoagulant could be reversed by feeding vitamin K\textsubscript{1} supplement for 15 days at 2 mg/kg dose which was almost double the a.i. of anticoagulant ingested by the target rodents.

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References