

BIOCHEMICAL ALTERATION FOLLOWING SINGLE ORAL DOSES OF PHOSPHAMIDON IN *BUBALUS BUBALIS*

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The single oral administration of phosphamidon in doses of 20, 40 and 80 gm/kg body wt. caused 0, 50 and 100% mortality, respectively and significantly inhibited serum cholinesterase activity to the extent of 47-81 % in male buffalo calves. On the other hand, all the 3 doses of phosphamidon significantly elevated serum levels of aspartate aminotransferase (32-109%), alanine aminotransferase (79-240%), acid phosphatase (80-309%) and alkaline phosphatase (44-148%) in male buffalo calves. Inactivation of serum cholinesterase following 40 mg/kg body wt did not return to control level even on day 28 of insecticide dosing indicating that inhibition of cholinesterase activity produced by phosphamidon is almost irreversible and long-lasting in buffalo calves. Whereas, the altered values of other parameters in surviving animals returned to their normal values within 7-14 days of insecticide exposure in indicating that the damage to the tissues if any, was not of permanent nature.

Key words: Phosphamidon, Aminotransferases, Phosphatases.

Introduction

Phosphamidon (0, 0-dimethyl-0-(2-chloro-2-(diethyl carbamoyl)-1-methylvinyl)-Phosphate), a broad spectrum, systemic and direct acting organophosphorus insecticide has gained widespread popularity as crop protectant in the field of agriculture. Similar to other organophosphorus insecticides, phosphamidon poses potential health hazards to man and livestock. Because of its indiscriminate and inadvertent use in agriculture, animals, besides their accidental ingestion by way of licking of empty containers etc. may be exposed to phosphamidon by the increased utilization of insecticide treated crops, feed, food waste and inhalation from the polluted environment. The information regarding toxicity of phosphamidon is however, absolutely lacking in buffaloes, a species of significant importance among large animals. Accordingly, the present study was aimed to investigate the effects of single oral doses of phosphamidon on serum cholinesterase, aminotransferases and phosphatases in male buffalo calves.

Material and Methods

Twelve clinically healthy male buffalo calves of non-descript breed and weighing between 70-120 kg. were purchased from the local market and acclimatized to the new environmental condition for two weeks prior to the commencement of the experiment. The animals were maintained on green fodder of the season (maize or barseem), wheat straw and water *ad-lib*.

The calves were randomly divided into 3 groups each comprising of 4 animals. All the animals were fasted for 18-20 hrs prior to the administration of insecticide. Based on the results gathered from preliminary trial, phosphamidon

(dimecron, 85% of the active ingredient w/w) was administered in single oral doses of 20, 40 and 80 mg/kg body wt. to 3 groups of calves. The requisite quantity of phosphamidon was diluted with 100 ml of water and was drenched to animals by drenching bottle. After the administration of insecticide all the animals were kept under close observation for a period of 28 days.

Blood samples were collected by jugular venipuncture prior to and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 and 72 hrs and 7, 14, 21 and 28 days after phosphamidon administration. The serum cholinesterase was determined by the method of Ellman spectrophotometric method [1]. The activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and acid phosphatase in serum were determined by the colorimetric procedures as described by Wootton [2]. The statistical significance between two means was calculated by using analysis of variance followed by a critical difference test [3].

Results and Discussion

Phosphamidon in single oral doses of 20, 40 and 80 mg/kg body wt. produced mild to severe toxic symptoms characteristic of anti-cholinesterase poisoning and caused 0, 50 and 100% mortality, respectively in male buffalo calves. The results on the effect of single oral administration of phosphamidon on serum cholinesterase, aminotransferases and phosphatases are presented in Tables 1-5. Higher dose of phosphamidon i.e. 80 mg/kg was lethal within 8 hrs of insecticide administration and hence further biochemical studies in these animals could not be undertaken.

Phosphamidon at all doses (20, 40 and 80 mg/kg) significantly ($P < 0.01$) inhibited the serum cholinesterase activity

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within 15-30 mins of its administration. The maximum inhibition to the extent of 47-81% occurred within 8-12 hrs of administration of all the 3 doses of phosphamidon. Inhibition of serum cholinesterase activity has also been reported in cattle poisoned with organophosphorus insecticides [4]. In the present study, the maximal inhibition of serum cholinesterase enzyme coincided with the severity of toxic symptoms

TABLE 1. EFFECT OF SINGLE ORAL ADMINISTRATION OF PHOSPHAMIDON ON SERUM CHOLINESTERASE IN BUFFALO CALVES.

Time after admin.	Dose of phosphamidon (mg/kg)		
	20	40	80
0 min.	1920.50 ± 20.25	1880.00 ± 10.00	1870.50 ± 12.50
15 mins	1870.00 ± 16.50	1812.50 ± 12.20**	1780.00 ± 10.00**
30 "	1810.00 ± 13.00**	1705.00 ± 15.30**	1642.00 ± 12.50**
1 hr.	1752.00 ± 8.65**	1555.00 ± 9.50**	1437.00 ± 9.50**
2 hrs	1613.50 ± 9.60**	1358.00 ± 7.90**	1209.50 ± 6.50**
4 "	1425.50 ± 10.80**	1159.00 ± 11.00**	928.00 ± 8.50***
6 "	1303.50 ± 14.25**	948.00 ± 13.00**	554.50 ^c -
8 "	1138.00 ± 9.20**	760.00 ± 9.00**	330.00 ^c -
12 "	1017.00 ± 6.60**	530.00 ± 11.00**	- -
24 "	1091.00 ± 10.50**	681.00 ± 12.20***	- -
48 "	1340.50 ± 16.00**	932.50 ± 16.20***	- -
72 "	1612.50 ± 15.20**	1180.50 ± 12.50***	- -
7 days	1890.00 ± 14.50	1330.00 ^b -	- -
14 "	1908.00 ± 16.50	1515.00 ^b -	- -
21 "	1922.00 ± 18.30	1675.00 ^b -	- -
28 "	1916.00 ± 15.50	1773.00 ^b -	- -

Values given above are expressed as U/L and represent the mean ± SE of 4 animals unless otherwise stated; (a). Values are mean ± SE of 3 animals; (b). Values are mean of 2 animals; (c). Values of one animal. * = P<.05; **=P<.01.

TABLE 2. EFFECT OF SINGLE ORAL ADMINISTRATION OF PHOSPHAMIDON ON SERUM ASPARTATE AMINOTRANSFERASE IN BUFFALO CALVES.

Time after admin.	Dose of phosphamidon (mg/kg)		
	20	40	80
0 min.	59.72 ± 0.57	59.74 ± 1.07	64.77 ± 0.46
15 mins.	61.42 ± 0.46	62.25 ± 1.32	70.92 ± 1.48**
30 "	63.09 ± 0.56**	67.00 ± 0.65**	80.40 ± 1.01**
1 hr.	65.05 ± 0.53**	72.58 ± 0.78**	97.99 ± 2.43**
2 hrs.	67.28 ± 0.84**	77.61 ± 0.72**	114.46 ± 3.04**
4 "	69.79 ± 0.72**	85.71 ± 1.47**	125.81 ± 2.32***
6 "	72.03 ± 0.72**	95.20 ± 1.47**	130.65 ^c -
8 "	74.26 ± 0.72**	105.25 ± 2.55**	135.12 ^c -
12 "	76.88 ± 1.15**	110.55 ± 1.29***	- -
24 "	78.55 ± 2.27**	110.92 ± 2.69***	- -
48 "	72.41 ± 1.58**	104.22 ± 10.01***	- -
72 "	67.28 ± 2.11*	99.75 ± 15.50***	- -
7 days	63.37 ± 1.15*	75.37 ^b -	- -
14 "	61.70 ± 0.28	67.0 ^b -	- -
21 "	60.01 ± 0.29	54.21 ^b -	- -
28 "	59.72 ± 0.34	63.09 ^b -	- -

Values given are expressed as nmol pyruvate formed/min/ml and represent the mean ± SE of 4 animals unless otherwise stated; a. Values are mean ± SE of 3 animals; b. Values are mean of 2 animals; c. Values of one animal. * = P<.05; **=P<.01

following all the doses of phosphamidon. The maximal inactivation in serum cholinesterase was recorded at 8-12 hrs when phosphamidon exposed animals displayed severe toxic manifestation. These findings are in contrast to previous studies suggesting lack of correlation between cholinesterase inhibition and severity of toxic manifestations [5]. In surviving animals, recovery of serum cholinesterase was monitored for 28 days. No relationship between disappearance of apparent toxicity and recovery of serum cholinesterase could be

TABLE 3. EFFECT OF SINGLE ORAL ADMINISTRATION OF PHOSPHAMIDON ON SERUM ALANINE AMINOTRANSFERASE IN BUFFALO CALVES.

Time after admin.	Dose of phosphamidon (mg/kg)		
	20	40	80
0 min.	47.11 ± 1.06	46.55 ± 2.02	51.54 ± 2.1
15 mins	49.88 ± 0.64	52.09 ± 1.43	62.07 ± 2.02*
30 "	52.65 ± 0.56**	60.40 ± 1.39**	79.08 ± 1.57**
1 hr.	56.53 ± 1.43**	70.93 ± 0.90**	109.73 ± 1.12**
2 hrs	59.85 ± 1.57**	81.46 ± 1.06**	137.43 ± 4.70**
4 "	64.29 ± 1.57**	95.32 ± 0.90**	163.29 ± 2.79***
6 "	69.83 ± 2.64**	110.83 ± 0.90**	168.46 ^c -
8 "	75.37 ± 3.13**	125.24 ± 2.93**	175.12 ^c -
12 "	84.23 ± 5.50**	136.69 ± 3.23***	- -
24 "	79.80 ± 6.00**	143.34 ± 3.91***	- -
48 "	74.26 ± 6.37**	133.00 ± 12.22***	- -
72 "	67.61 ± 4.20**	119.70 ± 24.45***	- -
7 days	57.64 ± 1.57**	72.04 ^b -	- -
14 "	52.09 ± 1.43*	63.18 ^b -	- -
21 "	47.11 ± 1.06	50.99 ^b -	- -
28 "	47.10 ± 1.66	47.66 ^b -	- -

Values given are expressed as n mol and pyruvate formed/min/ml and represent the mean ± SE of 4 animals unless otherwise stated. a = value are mean ± SE of 3 animals; b= Values are mean of 2 animals; c= Values of one animal; * = P<.05; **= P<.01.

TABLE 4. EFFECT OF SINGLE ORAL ADMINISTRATION OF PHOSPHAMIDON ON SERUM ACID PHOSPHATASE IN BUFFALO CALVES.

Time after admin.	Dose of phosphamidon (mg/kg)		
	20	40	80
0 min.	4.03 ± 0.05	4.20 ± 0.15	3.94 ± 0.23
15 mins	4.21 ± 0.05*	4.74 ± 0.17	4.87 ± 0.21*
30 "	4.56 ± 0.15*	5.40 ± 0.17**	6.64 ± 0.29**
1 hr.	4.87 ± 0.17**	6.07 ± 0.14**	9.30 ± 0.54**
2 hrs	5.22 ± 0.21**	6.90 ± 0.12**	12.39 ± 0.82**
4 "	5.75 ± 0.28**	7.66 ± 0.44**	14.18 ± 0.45***
6 "	6.33 ± 0.26**	8.50 ± 0.30**	14.69 ^c -
8 "	6.82 ± 0.27**	9.20 ± 0.26**	16.11 ^c -
12 "	7.26 ± 0.24**	10.09 ± 0.41***	- -
24 "	6.82 ± 0.39**	10.15 ± 0.56***	- -
48 "	5.93 ± 0.51**	8.85 ± 1.25***	- -
72 "	5.27 ± 0.38*	7.96 ± 1.96***	- -
7 days	4.65 ± 0.34*	5.14 ^b -	- -
14 "	4.20 ± 0.26	4.16 ^b -	- -
21 "	3.89 ± 0.07	3.98 ^b -	- -
28 "	3.98 ± 0.09	3.81 ^b -	- -

Values given are expressed as nmol phenol liberated/min/ml and represent the mean ± SE of 4 animals unless otherwise stated; a= Values are mean ± SE of 3 animals; b= Values are mean of 2 animals; c= Values of one animal. * = P<.05; **=P<.01

TABLE 5. EFFECT OF SINGLE ORAL ADMINISTRATION OF PHOSPHAMIDON ON SERUM ALKALINE PHOSPHATASE IN BUFFALO CALVES.

Time after admin.	Dose of phosphamidon (mg/kg)		
	20	40	80
0 min.	68.01 ± 1.16	68.36 ± 0.89	64.71 ± 0.40
15 mins.	69.78 ± 0.89	70.84 ± 1.00	73.22 ± 0.85**
30 "	71.55 ± 1.23	76.15 ± 0.89**	86.50 ± 2.75**
1 hr.	74.03 ± 1.46**	81.82 ± 0.68**	103.53 ± 3.15**
2 hrs.	78.28 ± 1.46**	86.78 ± 0.68**	133.51 ± 0.79**
4 "	82.17 ± 1.53**	93.51 ± 0.58**	148.5 ± 1.36***
6 "	86.78 ± 1.34**	102.01 ± 2.38**	155.30 ^c
8 "	91.74 ± 2.03 **	107.32 ± 2.55**	160.75 ^c
12 "	95.99 ± 4.15**	113.81 ± 1.25***	
24 "	97.76 ± 2.38**	106.73 ± 6.20***	
48 "	93.16 ± 2.03**	95.40 ± 13.95***	
72 "	84.30 ± 1.87**	92.56 ± 16.10***	
7 days	75.45 ± 1.86*	72.26 ^b	
14 "	70.84 ± 0.58	69.43 ^b	
21 "	67.30 ± 0.71	69.43 ^b	
28 "	67.65 ± 0.68	67.3 ^b	

Values given are expressed as nmol phenol liberated/min/ml and represent the mean ± SE of 4 animals unless otherwise stated. a = value are mean ± SE of 3 animals; b = Values are mean of 2 animals; c = Values of one animal; * = P < 0.05; ** = P < 0.01.

established in animals survived after phosphamidon administration. The toxic symptoms in surviving animals disappeared within 12-24 hr whereas cholinesterase activity during this period remained significantly inhibited. Similar results have been reported with other organophosphorus insecticides in different species [6]. It is obvious from data shown in Table 1 that in animals given 20 mg/kg dose of phosphamidon the level of serum cholinesterase recovered to their control values within 7-14 days after its administration, whereas this enzyme activity did not return to normal level completely even on day 28 in animals survived after 40 mg/kg dose. The results indicate that inhibition produced by phosphamidon is almost irreversible and long-lasting in buffalo calves.

Phosphamidon in single oral doses of 20, 40 and 80 mg/kg body wt. significantly elevated the values of serum aminotransferases and phosphatases within 15-60 mins of its administration. The increase in the serum levels of aspartate and alanine aminotransferases to the extent of 32-109 and 79-240% respectively were observed within 8-24 hrs of administration of all the 3 doses of phosphamidon. Although the serum levels of aminotransferases have been reported to be markedly elevated in animals exposed to several other organophosphorus insecticides, the exact mechanism involved in elevation of these enzymes have not been conclusively postulated. Several workers have suggested that such effect is the result of cellular damage [7] and/or increased plasma membrane permeability [8]. In addition, factors such as increased synthesis or decreased enzyme degradation may also be involved [9]. The oral administration of organophosphorus

esters is known to severely affect the liver, an organ primarily involved in their activation and/or detoxication [10]. In addition, organophosphorus compounds have been reported to dramatically increase the permeability of artificially prepared lipid membranes [11]. It has been reported that increased levels of aminotransferases in plasma of rats intoxicated with fenitrothion were due to more synthesis of these enzymes in brain, liver, kidney and testes which subsequently released them into blood stream [12]. Comparatively higher serum levels of alanine aminotransferases observed in the present study following phosphamidon administration may be due to faster leakage of this enzyme from the cells than that of aspartate aminotransferase [13].

Similar to aminotransferases, serum phosphatases were also significantly elevated. The elevation in the serum acid phosphatase and alkaline phosphatase to the extent of 80-309 and 44-148% occurred within 8-24 hrs of phosphamidon administration, respectively. Marked elevation in the serum levels of phosphatases have been reported in buffalo calves intoxicated acutely with fenitrothion, dichlorvos and monocrotophos [14-16]. The increase in blood levels of alkaline phosphatase may be due to damage to the liver, small intestine, bone, kidney which may liberate this enzyme into the blood stream [17-18]. Acid phosphatase, on the other hand, is a lysosomal enzyme and is also stimulated in cases of imminent or present tissue damage [19]. The relatively greater effect of phosphamidon on acid phosphatase than alkaline phosphatase indicates that the insecticide has profound effect on lysosomes. The complete recovery of serum aminotransferases and phosphatases in phosphamidon exposed surviving animals indicates that damage to the tissues if any, was not of permanent nature.

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