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Toxicological and haemato-biochemical studies of carbofuran and treatment of its toxicity in guinea pigs

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Abstract

Carbofuran (Furadan®, 5G, Padma Oil Company Ltd, Bangladesh) at different five doses i.e. 0.4, 4, 8, 12 and 16 mg/kg body weight were administered orally to five groups of adult guinea pigs (each group consisting of 5 guinea pigs) for studying the toxic symptoms and lethality, haemato-biochemical parameters & treatment of its toxicity in guinea pigs. The lowest dose i.e. 0.4 mg/kg dose of carbofuran was found to be non toxic for adult guinea pigs. However, the dose of 4 mg/kg body weight of carbofuran was found to be toxic without causing any mortality. Whereas the higher three doses i.e. 8, 12 and 16 mg/kg body weight caused 40, 80 and 100% mortality respectively. The toxic symptoms produced by carbofuran treated guineapigs were restlessness, nasal secretion, sneezing, frothy salivation, incoordination, opisthotonus, shivering, muscular fasciculation, respiratory distress, recumbencey, distended abdomen and muscular paralysis, which were characteristic of anti-ChE (Cholinesterase) poisoning. Based on mortality rate, the oral acute LD_{50} was calculated as 7.5mg/kg body weight in adult guinea pigs. The activities of SGPT and SGOT were increased significantly ($p<0.01$) to extent of 22-119% and 48-114% respectively following higher four doses of carbofuran (4, 8, 12 and 16 mg/kg b.wt.). Similarly, total leukocyte count (TLC) was increased significantly ($p<0.01$) upto 4-26% following higher four doses. On the other hand total erythrocyte count (TEC) and haemoglobin (Hb) content were decreased significantly upto 6-30% and 7-31% respectively following higher four doses of carbofuran (4-16 mg/kg). Packed cell volume (PCV) values were not altered following any of the five doses of carbofuran. Blood glucose level was significantly increased ($p<0.01$) to the extent of 9-26% following four doses of Furadan® (4-16 mg/kg bwt.), whereas glucose level was unaffected following nontoxic dose (0.4 mg/kg b.wt.) In survivors, the altered biochemical and hematological values returned to pre-exposure level within 7-14 days of insecticide feeding. On comparing the therapeutic efficacy between atropine sulphate alone and atropine sulphate plus Lasix(R) (frusemide), it could be concluded that the combined therapy was found to be better against Furadan® poisoning in guinea pigs.

Keywords: Carbofuran, Haematobiochemical parameters, Atropine sulphate, Guinea pigs

Introduction

Large-scale manufacture and utilization of pesticide formulations for controlling crop pests and vectors of communicable diseases have caused global concern. The greatly increased use of insecticides and other pesticides has introduced a serious and novel hazard to livestock. It is well documented that a large number of domestic animals die of insecticide poisoning every year mainly due to insecticides. Carbofuran (Furadan®, 5G, Padma Oil Company Ltd, Bangladesh) is a modern organocarbamate insecticide and has been used widely and successfully as effective crop protectant in the field of agriculture. Although

carbofuran is one of the moderate toxic organocarbamate insecticide, but like other organocarbamate insecticides it has also toxic effects to livestock and human beings (Soreq and Zakut 1993, Uludag *et al.* 2001). The detail information on the toxicity of carbofuran is not fully available in guineapigs, a laboratory animal of significant importance. The result of the present studies would certainly facilitate in understanding the possible hazards of carbofuran in guinea pig, which will help in recommending its judicious use in the field of agriculture and livestock in Bangladesh. Keeping these views in mind the present work was undertaken to study the toxicity and effects on some enzymes like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), blood glucose level and on some blood parameters viz. total erythrocyte counts (TLC), total leukocyte counts (TLC), hemoglobin (Hb) content, and pocked cells volume (PCV) in guinea pigs. Attempts were also made to evaluate the efficacy of different therapeutic agents (atropine sulphate alone and atropine sulphate plus frusemide (Lasix®) against carbofuran induced poisoning in guinea pigs.

Materials and Methods

Thirty-five apparently healthy adult guinea pigs (*Cavia cobaya*) weighing between 500 and 750gm were purchased and acclimatized to the new environmental condition for a period of one week prior to the commencement of experiment. During the experimental period (14 days) the guinea pigs were kept under good housing conditions and were maintained on a ration consisting of grasses, special type of pillet and water *ad libitum*. Carbofuran (Furadan®, 5G, Padma Oil Company Ltd., Bangladesh) was purchased from local market. All other chemicals were extra pure quality and some were prepared in the laboratory. Based on the results gathered from the preliminary trails conducted in five adult guinea pigs, five doses of carbofuran viz, 0.4, 4, 8, 12 and 16 mg/kg body weight were selected in this study. Thirty adult guinea pigs were randomly divided into six groups (A, B, C, D, E and F), each consisting of five guinea pigs. All guinea pigs were weighed carefully and kept group wise in the special type iron cages. The group of A, B, C, D and E guinea pigs were administered with carbofuran in single oral doses of 0.4, 4, 8, 12 and 16 mg/kg body weight respectively. Guinea pigs of group F was kept as control and not given any insecticide. The requisite quantity of insecticides was diluted in water and was administrated to the guinea pigs by means of a dropper and was kept under close observation for a period of 14 days. During this period, carbofuran exposed guinea pigs were carefully observed for the appearance of any toxic symptoms and lethality. The nature and time of appearance of various toxic manifestations and mortality were carefully recorded in each group of insecticide treated guinea pigs. Oral acute LD_{50} of carbofuran was determined by single oral doses in guinea pigs according to the experimental design adopted by Awal and Alim (1994). Blood samples were collected directly from the heart and ear vein of individual guinea pig at 0 h (pre-dosing period), 6h, 7th day and 14th day of the administration of carbofuran for determination of some blood parameters like TEC, TLC, Hb and PCV as per method cited by Coffin (1955) and some serum enzyme levels like SGPT & SGOT and blood glucose level by using auto analyzer (Model no. Reflotron M-06).

For evaluation of therapeutic efficacy, twelve adult guinea pigs were randomly divided into 3 groups I, II and III, each group consisting of 4 guinea pigs. Carbofuran was administered at a single oral lethal dose (7.5 mg/kg b.wt.) to all the guinea pigs. All the animals of group I, II, and III were treated by administration of atropine sulphate alone at lower dose (0.25 mg/kg b.wt. s/c) & higher dose (0.5 mg/kg bwt. s/c) and atropine sulphate (0.5 mg/kg b.wt. s/c) plus frusemide (Lasix®) respectively when intoxicated animals exhibited severe toxic symptoms characteristics of anti-ChE poisoning and were kept under close observation for a period of 14 days.

The results of the controlled and treated animals were analyzed by using student's 't' test.

Result and discussion

The lowest dose i.e. 0.4 mg/kg of carbofuran did not induce any toxic symptom in adult guinea pigs and this dose may be considered as non-toxic for guinea pigs. The single oral administration of carbofuran in doses of 4, 8, 12, and 16 mg/ kg body weights to adult guinea pigs produced toxic symptoms of varying degrees. The dose of 4mg/kg-body weight induced clinical toxicity of mild degree in all the insecticide exposed guinea pigs of group B without causing any mortality. Toxic symptoms first appeared within 25-30 min after administration of carbofuran. The symptoms observed were restlessness, nasal secretion, sneezing, frothy salivation, and movement of head, incoordination, opisthotonus, shivering, muscular fasciculation and respiratory distress. However, the toxic symptoms started to disappear from 24 h onward and disappeared completely within 30-36 h of insecticide administration. Similarly, the toxic symptoms exhibited by the group C guinea pigs after receiving insecticide at 8 mg/kg body weight were mild to moderate in nature and first symptom appeared within 16-20 min after administration of carbofuran. Symptoms observed in group C were similar to group B guinea pigs but two animal of this group showed recumbency, distended abdomen and muscular paralysis and died at 12 h of carbofuran administration and other three animals recovered within 32-40 h of administration.

The animals of group D (12 mg/kg body weight) produced first toxic symptoms within 8-11 min of carbofuran administration and the toxic symptoms observed were almost similar but slightly severe in nature as observed in group C guinea pigs and ultimately four animals of this group died within 10-15 h of insecticide administration and rest one animal recovered within 36-48 h of insecticide feeding. The dose of 16 mg/ kg body weight of carbofuran in group E produced severe toxic symptoms, which first appeared within 5-8 min of insecticide administration and the toxic symptoms were similar as displayed by the groups C and D animals receiving 8 and 12 mg/kg body weight respectively and ultimately all the five animals of this group died within 4-7 h of insecticide administration. In accordance to the present findings, almost similar toxic symptoms and mortalities were also reported by other workers (Wahbi *et al.*1987, Stec and Marczuk, 1993, Awal *et al.* 1994 and Basheer *et al.* 1999). However, in the present study , onset of toxic symptoms was rapid in comparison to many other organocarbamate insecticides. In addition, opisthotonus and distended abdomen as observed in the present study were not seen by all other organocarbamate insecticides.

The single oral administration of carbofuran @ 0.4, 4, 8, 12, and 16 mg/kg body weight in groups A to E caused 0, 0, 40, 80 and 100% mortality respectively in adult guineapigs. On the basis of data obtained from acute toxicity study, oral acute LD_{50} was calculated and found to be 7.5-mg/kg body weight in adult guinea pigs.

The activities of SGOT were significantly elevated with a peak rise to the extent of 48-114% following 4, 8, 12 and 16 mg/kg body weight in guinea pigs (Table - 1). Similarly, the values of SGPT were also increased upto 22-119% after administration of 4-16 mg/kg body weight (Table - 1). In accordance to the present finding, the increased levels of SGOT and SGPT have been reported in animals exposed to organocarbamate insecticide sevin (Wahbi *et al.* 1987). The exact mechanism involved in the mediation of such effects has not been conclusively elucidated. However, the increase in serum aminotransferases (SGOT and SGPT) has been suggested as due to cellular damage, increase plasma membrane permeability or their altered metabolism. The oral administration of organocarbamate esters is known to severely affect the liver, an organ primarily involved in their activation and/or detoxification. Blood glucose level was significantly increased ($p<0.01$) to the extent of 9-26% following four doses of Furadan® (4-16 mg/kg bwt.) whereas glucose level was unaffected following nontoxic dose (0.4 mg/kg bwt.) Hyperglycemia observed in the present study may be related to the increased secretion of adrenaline and glucocorticoids implicating both adrenal medullary and cortical activities as reported for organophosphorus insecticides (Awal and Malik, 1992).

Table 1. Effect of single oral administration of carbofuran on SGOT, SGPT (U/L) and blood glucose (BG) level (gm/dl) in adult guinea pigs

Group	Dose (mg/kg b.wt.)	Parameters	Time of blood collection			
			0h	6h	7th day	14th day
A n=5	0.4 mg	SGOT	33.65±0.65	33.00±0.89	33.82±0.81	33.82±0.75
		SGPT	17.62±0.54	17.89±0.55	17.68±0.72	17.64±0.56
		BG	114.25±2.60	120.40±2.92	116.90±2.80	114.68±2.79
B n=5	4 mg	SGOT	32.88±0.95	47.35±0.92**	33.05±0.75	32.95±0.88
		SGPT	16.78±0.62	19.89±0.68**	17.19±0.72	16.89±0.81
		BG	115.98±3.20	126.10±3.05**	114.70±3.05	113.85±2.98
C n=5	8 mg	SGOT	34.35±0.86	52.18±98**[b]	34.95±0.48[b]	34.45±0.98[b]
		SGPT	17.52±0.83	28.97±0.72**[b]	17.90±0.97[b]	17.70±0.81[b]
		BG	112.90±3.05	132.48±3.65**[b]	118.95±3.40[b]	116.80±3.25[b]
D n=5	12 mg	SGOT	33.65±0.88	58.48**[C]	33.75[C]	33.75[C]
		SGPT	16.58±0.92	32.89**[C]	17.48[C]	16.98[C]
		BG	116.75±3.10	141.25**[C]	118.19[C]	118.10[C]
E n=5	12 mg	SGOT	32.65±0.51	-	-	-
		SGPT	17.72±0.68	-	-	-
		BG	115.75±3.12	-	-	-
F n=5	control	SGOT	32.98±0.87	33.82±0.64	32.15±0.87	32.48±0.72
		SGPT	17.60±0.87	16.25±0.52	16.96±0.72	16.45±0.55
		BG	114.75±3.05	110.35±2.99	114.90±3.18	117.25±2.98

Values represent the mean ±SE of five guinea pigs unless otherwise stated.
b = Values of three guinea pigs; c = value of one guinea pig, ** Significant at ($p<0.01$)

The single oral administration of carbofuran at higher four doses i.e. 4, 8, 12, and 16 mg/kg body weight significantly decreased the TEC (6-30%) and Hb content (7-31%) in guinea pigs (Table-2). On the other hand, TLC was increased significantly upto 4-26% after administration of higher four doses of carbofuran (Table-2). PCV value was not influenced significantly following any of the doses of carbofuran (Table-2). The findings of the present results on TEC, TLC, Hb, PCV values are in accordance with the findings of Kossakowski and Lysek (1982), Wahbi *et al.* (1987), Awal *et al.* (1994) and Bakre *et al.* (1995). The exact mechanism by which hematological parameters were altered due to oral administration of carbofuran in adult guinea pigs could not be explained fully in the present study. It may be assumed that toxic effect induced by carbofuran on bone marrow may be responsible for erythropenia and leucocytosis observed in this study (Awal and Mailk, 1986). However, the altered biochemical and hematological parameters returned to their pre-exposure levels within seven days of insecticide dosing indicating that the damage to some organs, if any, was not permanent in nature.

Table 2. Effect of single oral administration of carbofuran on certain hematological parameters in adult guinea pigs

Group	Dose (mg/kg b.wt.)	Hematological parameters	Time of blood collection (h)			
			0	6	7^{th} day	14^{th} day
A n=5	0.4	TEC (million/cu.mm)	5.53±0.08	5.44±0.07	5.49±0.05	5.50±0.06
		TLC (thousand/cu.mm)	7.78±0.06	7.76±0.06	7.78±0.07	7.81±0.07
		Hb (gm%)	10.80±0.16	10.52±0.13	10.70±0.14	10.75±0.15
		PCV (%)	42.75±0.20	42.40±.21	42.60±0.21	42.57±1.20
B n=5	4	TEC (million/cu.mm)	5.67±0.07	5.20±0.06**	5.63±0.06	5.65±0.06
		TLC (thousand/cu.mm)	7.99±0.04	8.15±0.09**	7.99±0.05	7.94±0.03
		Hb (gm%)	10.90±0.14	10.30±0.12*	10.65±0.13	10.80±0.12
		PCV (%)	41.53±0.38	40.80±0.42	41.25±0.38	41.39±0.37
C n=5	8	TEC(million/cu.mm)	5.70±0.07	5.19±0.03 [b] **	5.55±0.06[b]	5.64±0.06[b]
		TLC (thousand/cu.mm)	7.77±0.05	9.21±0.03 [b] ** (29.5%)	7.89±0.04[b]	7.81±0.06[b]
		Hb (gm%)	11.20±0.15	8.95±0.10** [b]	11.95±0.14[b]	11.10±0.15[b]
		PCV (%)	42.16±0.36	41.25±0.41 [b]	41.80±0.40 [b]	42.33±0.47[b]
D n=5	12	TEC(million/cu.mm)	5.53±0.07	4.04 ** [c]	5.52[c]	5.54[c]
		TLC (thousand/cu.mm)	7.63±0.07	9.56 ** [c]	8.12[c]	7.81[c]
		Hb (gm%)	11.65±0.18	8.20** [c]	10.95[c]	11.35[c]
		PCV (%)	42.75±0.26	42.50 [c]	42.75[c]	42.00[c]
E n=5	16	TEC(million/cu.mm)	5.75±0.05	-	-	-
		TLC (thousand/cu.mm)	7.64±0.05	-	-	-
		Hb (gm%)	10.60±0.16	-	-	-
		PCV (%)	42.70±0.23	-	-	-
F n=5	Control	TEC(million/cu.mm)	5.56±0.08	5.53±0.07	5.52±0.06	5.55±0.06
		TLC (thousand/cu.mm)	7.73±2.02	7.73±0.04	7.75±0.02	7.75±0.04
		Hb (gm%)	10.35±0.13	10.10±0.14	10.50±0.15	10.70±0.14
		PCV (%)	42.44±0.21	42.61±0.20	42.50±0.15	41.77±0.33

Values represent the mean ±SE of five guinea pigs unless otherwise stated
b = Values of three guinea pigs, c = value of one guinea pig
*Significant at ($p<0.05$); ** Significant at ($p<0.01$)

After administration of atropine sulphate alone at lower dose (0.25mg/kg bwt. s/c) toxic symptoms disappeared gradually but reappeared soon. Although atropine sulphate was repeated at 30, 75, 110, 200 and 280 min after first injection in group I animals. This dose (0.25 mg/kg) of atropine was only 60% effective whereas atropine sulphate at higher dose (0.5 mg /kg bwt, s/c) followed by its repeated administration at 30, 75, 110, 200 and 280 min after first injection in group II animals afforded 80% protection against Furadan(R) induced toxicity and lethality. The surviving animals of these two groups (I & II) recovered to normal within 10-20 h of atropine sulphate therapy and remained healthy during the 14 days of observation period. On the other hand, the combined administration of atropine (0.5 mg /kg bwt, s/c) along with a diuretic Lasix (R) (Frusemide 5 mg/ kg bwt. im) afforded complete protection (100%) against Furadan(R) induced toxicity and lethality in guinea pigs. The toxic symptoms disappeared gradually and all animals in this group (III) became apparently normal within 8-12 h of treatment. Clarke *et al.* (1981) reported that along with atropine sulphate administration of a diuretic agent is quite helpful for complete protection of furadan induced poisoning. On comparing the efficacy between atropine sulphate alone and atropine sulphate plus Lasix(R), it may be concluded that the combined therapy was found to be better treatment against furadan poisoning in guinea pigs.

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