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Toxicity of Furdan (Carbofuran) in Nubian Goats

Amani B.A. Abdelmaged [1] and O.S.A. Mohamed [2]

[1] Department of Veterinary Medicine, Pharmacology and Toxicology, College of Veterinary Medicine, Bahri University, Sudan.
[2] Department of Clinical Studies, College of Veterinary Medicine and Surgery, Sudan University of Science and Technology, Khartoum, P.O. Box.407, Sudan.
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Abstract

Oral toxicity of Furdan (Carbofuran) were examined in fiftteen healthy Nubian goat kids 5-6 months old and weighing 10-12 kg.They were divided randomly into 5 groups each of 3. Furdan was dosed at the rate of 10, 5, 2.5 and 1.25 mg/kg body weight/day to groups 1, 2,3 and 4 respectively. Group 5 was control. The most observable clinical signs were excessive salivation, lacrimation, mydriasis, dyspnoea, abduction of forelimbs, convulsive seizures, tremors, paresis of the hindlimbs, imbalance, recumbency and death. Postmortem lesions were varying degrees of haemorrhages and congestion in the brain, heart, lungs, liver, kidneys and small intestines. Histopathological findings were satolytosis in cerebral tissue, pulmonary emphysema and edema, centrilobular hepatocytic degeneration, cortical renal tubular epithelial cells degeneration and glomerular shrinkage and gastrointestinal erosions. The values of Hb, RBC count; MCH and MCHC were decreased while the value of MCV was increased significantly. No significant changes were observed for the values of PCVandWBC and in serum concentrations of Na, K, Mg, Ca, P, Cu, Fe, total proteins, albumin, globulinsl, total bilirubin and urea and for serum activities of ALT and ALP. Significant increases in serum AST activity and decreases in serum concentrations of glucose and iron.

Keywords: Furdan, Toxicity, Nubian Goat.

Introduction

In Sudan, the increase in both human and animal populations necessitates availability of food and fodder. The boost in agriculture production is inseparable from usage of chemicals like fertilizers and pesticides. However, their toxic potential is still alarming particularly with negligence or indiscriminate use. One of the greatest challenges facing the government today is to satisfy the nutritional needs of the growing Sudan population while at the same time preserving resources such as land, water, air, and biodiversity. Livestock are a crucial element in this balancing process. Demand for livestock products is growing and livestock are through their multiple functions, a corner stone of most rural populations in Sudan. Man, to combat pests, uses pesticides to eliminate harmful insects and related creatures. Such chemicals seem to be important at least in near future, but the risk comes from their misuse, mistakes in assessing residue levels and adverse toxicity. Absence of technical information on pesticide storage and disposal, re-use of empty

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containers, lack of linkages between people who have to deal with pesticides, pesticide stockpiles, and the limited resources available for national level training are serious hazard contributing factors. Prehaps the main hazardous risk is the use of agrochemical formulations for eradicating external parasites hosted by the animals especially where agricultural/ animal management is practiced.

Furdan (Carbofuran) is a systemic carbamate insecticide. It is a reversible, direct inhibitor of cholinesterases through carbamoylation of the esteratic site of the enzyme and it has contact activity against pests (Lorgue *et.al*, 1996)

It has one of the highest acute toxicities to humans than any insecticide used on field crops. A quarter teaspoon (1 mL) can be fatal. Birds often eat numerous grains of the pesticide, mistaking them for seeds, and then die shortly thereafter. Before it was banned by US.EPA in 1991, (United States. Enviromental Protection Agency) granular carbofuran was blamed for millions of bird deaths per year. The liquid version of the pesticide is less hazardous to birds since they are not as likely to ingest it directly, but it is still very hazardous (Lorgue *et. al*, 1996).

The oral dose of Furdan in lactating cows is rapidly absorbed, degraded and eliminated where mixed function oxidases are chiefly responsible for its metabolism. The 3-hydroxycarbofuran and 3 ketocarbofuran are the most common carbamate metabolites which are eliminated in urine (92% in 32 hours), none is exhaled and less than 3% is found in faeces and less than 3% is found in milk (Roder, 2001)

The present study was planned and designed to assess and describe the toxicity of Furdan in Nubian goat-kids, as casualties might be expected in farm animals due to inadvertent ingestion and/or its use in eradicating external parasites

Materials and Methods

Animals:

Fifteen, apparently healthy male Nubian goat kids of 5-6 months old and weighing 10-12 kg, were purchased from Hilat Kuku Goats Market, Khartoum North. They were kept in standard pens at The College of Veterinary Medicine and Surgery, Sudan University of Science and Technology.The goats were fed on forage sorghum (*Sorghum vulgarae)* and provided with water *ad libitum*. The animals were kept 30 days for adaptation and acclimatization during which each animal was dosed with Oxytetracycline, Albendazole, and Amprolium for the control of bacterial diseases, worms and coccidiosis respectively. At the end of the adaptation period, the animals were divided randomly into 5 groups each of three. Each group was kept separately.

Furdan (Carbofuran)

A carbamate systemic insecticide. Produced by (FMC) (Agriculture Chemical Group Philadelphia, PA19103 USA), with chemical name [2.3-dihydro-2, 2- dimethyl-7-benzofuranyl –methylcarbamate] and molecular formula ($C_{12}H_{15}NO_3$). Granular formulate 10% was used .Furdan was obtained from Khartoum North State Pesticide Market.

Dosing:

Goats in groups 1, 2, 3 and 4 were drenched daily with Furdan at the rate of 10, 5, 2.5, and 1.25 mg/kg/ b.w. respectively. Goats in group 5 were not drenched and used as controls, for 45 days.

Blood Sampling:

Each experimental animal was subjected to blood sampling on days 1,3, 7, 14, 21, 28, 35 and 45- post dosing. Additional samples were taken from animals in moribund condition. A volume of 10 ml blood was collected from the jugular vein puncture using a disposable 10 ml syringe. Immediately, 1 ml of the collected blood sample was poured into a small clean 5 ml vacutainer containing anticoagulant EDTA (ethylene diamine-

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tetra-acetic acid) for the haematological investigations. Another 1 ml was poured into a small clean 5 ml vacutainer containing fluoride oxalate as an anticoagulant and used immediately for glucose measurement.The remaining blood was kept to clot, centrifuged at 3000 r.p.m. for 5 minutes and sera were collected and kept at -20 °C for serobiochemical analysis.

Clinical Observations

Experimental animals were closely observed for clinical signs and behavioral changes.

Postmortem Examination:

Dead or sacrificed animals were immediately examined for postmortem lesions. Samples from the brain, lungs, heart, liver, spleen, pancreas, kidneys, abomasum, omasum and small intestines were collected and fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned at 5µm and stained with haematoxylin and eosin (H&E) for histopatholoical investigations.

Haematological analysis:

EDTA – anticoagulated blood were investigated according to the methods described by Dacie and Lewis (1991) for haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells count (RBC), white blood cells count, (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) .

Serobiochemical Analysis

Collected sera were analyzed for the activities of aspartate aminotransferase (AST),alanine aminotransferase(ALT,GPT, E.C.2.6.1.2,), alkaline phosphatase (ALP) and for the concentrations of total proteins, albumin , total bilirubin, and urea using commercial kits, (Plasmatec Laboratory Products Ltd., England), and absorbance was measured in a spectrophotometer (Unicam). 8625 uv / vis Spectrometer – PU , England The concentration of serum globulins was obtained by subtracting values of serum albumin from the total proteins. Glucose concentration was measured using enzymatic colorimetric kit (GOD-PAP method. Fouz Diagnostics Laboratory) (FDL) and the concentration was read in a spectrophotometer (Perkin -Elmer 2380, Germany). Serum concentration of sodium (Na), potassium (K), calcium (Ca), inorganic phosphate (PO_4), magnesium (Mg), copper (Cu) and iron (Fe) was determined using the method described by Allen (1989).The sample solution was aspirated along with the standard according to a specific cathode lamp used and they were read out in the atomic absorption spectrophotometer (Perkin – Elmer 2380, Germany) against the deionized water as blank.

Statistical Analysis:

The data were analyzed using Students t- test (Mendenhall, 1971).

Results

Clinical symptoms:

The dosages and animal fate are summarized in Table (1).

Goats in group (1) showed after few minutes postdosing signs include uneasiness , restless-ness, excessive salivation, lacrimation ,constri-ction of the pupil (mydriasis), nasal discharge, dyspnoea, grinding of the teeth, abdominal pain, frequent urination, soft faeces, inappetence, abduction of forelimbs , convulsive seizures, tremors, paresis of the hind limbs, imbalance and became recumbent with lateral deviation of the head and neck. All goats in this group died within 15 minutes postdosing.

Group (2) goats showed sings of excessive salivation, inappetence, dyspnoea, abdominal cramps, shivering, bloat, incoordination of movement and the goats became recumbent.These signs started half an hour postdosing and continued for 3 hours then these signs generally disappeared, but the goats were depressed and suffered decrease in appetite and difficult breathing. These

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animals died after 4 hours postdosing between days 7 - 11.

Group (3) goats showed the same clinical signs as in group (2) when started 2 hour postdosing and continued for one hour then diminished gradually. These animals suffered decrease in appetite, depression isolated from each other with lazy movements, licking objects and some of them pushing their heads against the fence and loss of weight in addition there were signs of pneumonia, and abduction of the fore and hindlimbs. The goats in this group died between days 15 - 17.

Group (4) goats showed no clinical signs postdosing throughout the experimental period except that the animals suffered decrease in appetite, loss of weight, became weak, depressed, and dull. These animals were sacrificed on day 45. Group (5) was control and slaughtered on day 45.

Table 1: Dose and fate of Nubian Goats poisoned with Furdan

Groups	Animal No.	Age (months)	Daily oral dose (mg/kg)	Fate of animals(postdosing)
Group (1)	28	8	10.0	Died on day 1 (15 minute after dosing)
	29	9	10.0	Died on day1 (15 minute after dosing)
	30	10	10.0	Died on day 1 (15 minute after dosing)
Group (2)	31	9	5.0	Died on day 7
	32	10	5.0	Died on day 10
	33	10	5.0	Died on day11
Group (3)	34	8	2.5	Died on day 15
	35	9	2.5	Died on day 17
	36	10	2.5	Died on day 17
Group (4)	37	9	1.25	Sacrificed on day45
	38	9	1.25	Sacrificed on day45
	39	10	1.25	Sacrificed on day45
Control group(5)	40	9	0.0	Sacrificed on day45
	41	9	0.0	Sacrificed on day45
	42	10	0.0	Sacrificed on day45

Postmortem Findings:

Postmortem lesions in different organs of goats dosed with Furdan were summarized in Table (2). Generally, there were haemorrhages and congestion in the brain, heart, lungs, liver, kidneys, abomasums, omasum, and small intestines in the different dosed groups. . The heart appeared flabby and the pericardial sac contained turbid fluids (hydropericardium). The emphysema and odema of the lungs were more in group 1 and 2. The liver and the kidneys showed scattered foci of fatty changes. Haemorrhagic foci were seen on the internal surface of the small intestines and abomasums and omasum, with slight sloughing of the mucosa of the abomasum and omasum. No apparent lesions were observed in the rumen, reticulum, pancreas, spleen, cutaneous blood vessels and lymph nodes. No pathological lesions seen in the organs of the control goats.

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Table 2: Postmortem lesions of Goats poisoned with Furdan

Organ	Lesions	Groups				
		1	2	3	4	5
Brain	Congestion	+ + +	++	++	+	-
	Haemorrhage	+ +	+	+	-	-
Lungs	Congestion	+ + +	+ +	+ +	+	-
	Haemorrhage	+ +	+ +	+	+	-
	Odema	++	+ +	++	+	-
	Emphysema	+ + +	+ +	++	+	-
Heart	Congestion	+ + +	+ +	+ +	+	-
	Haemorrhage	+ +	+ +	+ +	+	-
	Hydropericardium	+	+ +	+	+	-
	Flabbiness	+	+ +	+ +	+	-
Kidneys	congestion	+ + +	+ + +	+ +	+	-
	Haemorrhage	+ +	+ +	+	+	-
Liver	Congestion	+ + +	++	+ +	+	-
	Haemorrhage	++	+ +	+	+	-
	Fatty change / necrosis	+	+ +	+ +	+	-
Abomasum and	Erosions	++	+ +	++	+	-
Omasum	Congestion	++	++	++	+	-
	Haemorrhage	++	++	+	+	-
Small	Haemorrhage	++	+ +	+	+	-
Intestines	Congestion	+ +	++	++	+	-

+ = Slight lesions, ++ = Moderate lesions, +++= Severe lesions. (-) = Absence of lesions

Histopathological Findings:

Brain: In cerebellum tissue, there were satolytosis, many neurons stained deeply basiophilic with shrunken appearance, widening of perineurons and perivascular spaces in all treated groups. Areas of haemorrhages were also observed in the cerebrum tissue of groups 1-3 (Figure 1).

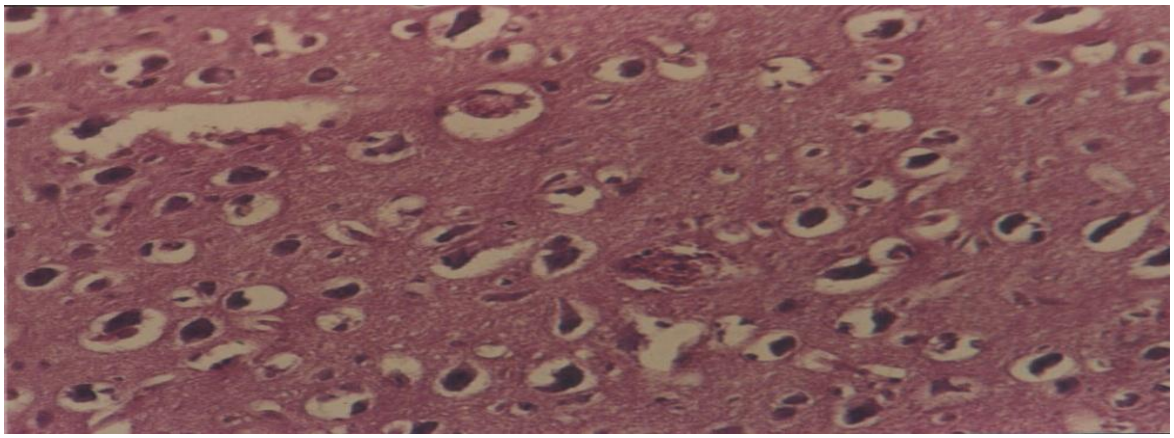

Figure 1: The brain of goat No (36) in group (3) dosed with 2.5 mg/kg/ day of Furdan and died on day (17) postdosing, showing neurons stained deeply basophilic with shrunken appearance, widening of perineurons and perivascular paces. Areas of haemorrhages.(H&E1X25)

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Lungs: - Areas of emphysema, edema thickening of interstitial tissue, with mononuclear cells infiltration with atrophy of many alveoli were seen in goats of groups 1, 2 and 3. These lesions were mild in group (4).

Heart: - hypercellularity, congestion and haemorrhges were observed in group 1, 2 and 3. These lesions were mild in group 4.

Liver: - variable degrees of congestion in the central veins and sinusoids of groups 1, 2 and 3 and there were centrilobular hepatocytic fatty changes which extended to the periportal tract in some instances and accompanied with foci of haemorrhges. These lesions were mild in group 4 (Figure 2).

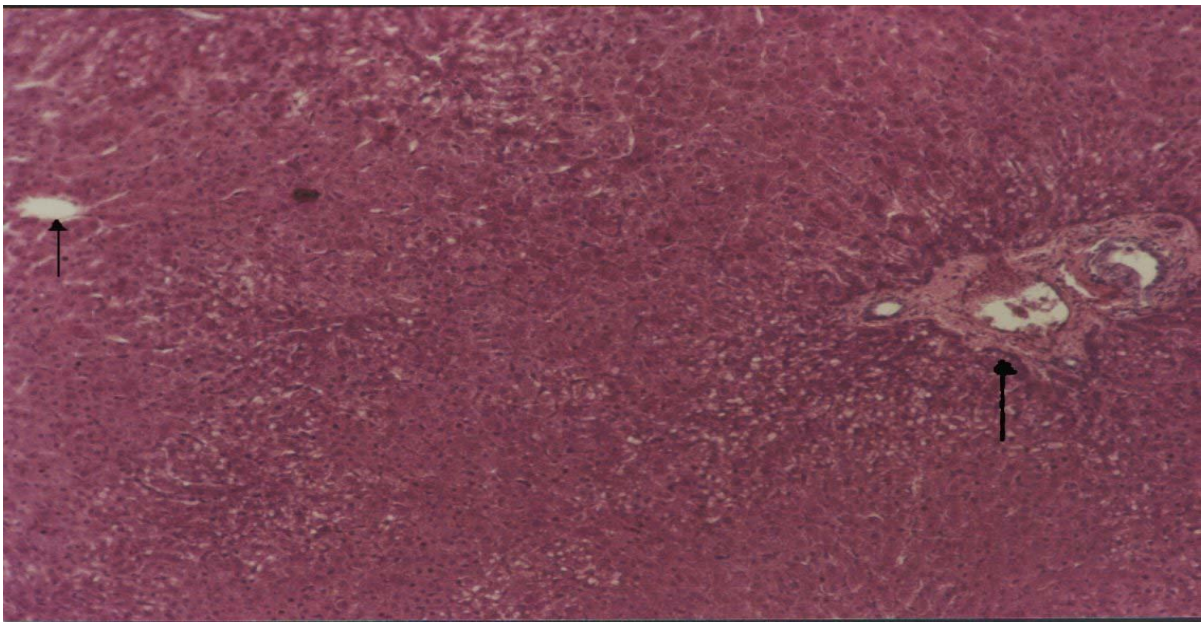

Figure 2: The liver of goat No (33) in group (2) dosed with 5 mg/kg /day of Furdan and died on day 11 postdosing, showing variable degrees of congestion of central veins and sinusoid and there were centrilobular hepatocytic fatty changes the fatty changes extended to periportal tract. Foci of haemorrhges were also seen (H&E, X10).

Kidneys: excessive dilatation and degeneration of some renal cortical tubules and many of the medullary tubules contained homogenous eosinophlic hyaline cast especially in groups1 and 2 and mild in groups 3 and 4. Moreover, many of the glomeruli were shrunken and lobulated.

Small intestines: acute catarrhal enteritis, congestion of the blood vessels and capillaries particularly in the submucosa and heavy infiltration of mononuclear cells (mainly lymphocytes) on the mucosa of groups 1- 4. In addition to excessive destruction of mucosal glands and desquamation of the lining epithelium. Erosions in the omasum and abomasums submucosa. No lesions were observed in the spleen and pancreas. No postmortem lesions were observed in control group.

Haematological and Serobiochemical Findings:

Haematological and serobiochemical findings of goats dosed with Furdan were summarized in Tables (3, 4 and 5). No haematological and serobiochemical data were obtained for goat kids in group 1,

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because none of them survived for more than 15 min. postdosing.

Significant decreases were observed in RBCs count,($p < 0.01$) and in Hb,PCV, MCH and MCHC ($p < 0.05$) and significant increases ($p < 0.05$) were detected in the MCV of groups 2, 3 and 4 .No significance change was observed in WBC in goats. No haematological changes of significance were observed in goats of the control group (5). No significant ($p > 0.05$) changes were observed in serum concentrations of Na, K, Ca, P, Mg, Fe and Cu in any of the tested groups. No changes were also observed in serum concentration of total proteins, albumin, globulins, urea and bilirubim and in the serum activity of ALT and ALP in any of the tested groups. Significant increase ($p < 0.05$) in serum activity of AST and significant decrease ($p < 0.05$) in the serum glucose and Fe concentration was observed in goat kids of groups 2, 3 and 4. No significant changes were observed in goat kids of control group (5).

Table 3: Haematological values of Nubian Goats poisoned with Furdan.(M±SD)

Parameters Subgroups	Hb (g/dL)	PCV (%)	RBC ($\times 10^6$/µL)	MCV (fl)	MCH (pg)	MCHC (g/dL)	WBC (per/µL)
group2	8.1	28.1	4.7	59.8	17.2	28.8	13800
(5.0mg/kg)	±1.23	±1.31	±0.55	±0.15	±1.17	±2.11	±1.72
	*	NS	**	*	*	*	*
Group3	7.9	31.8	5.3	60.0	14.9	24.8	11000
(2.5mg/kg)	±1.42	±1.29	±0.49	±0.18	±1.15	±2.08	±1.61
	*	NS	**	*	*	*	*
Group4	7.0	28.6	4.8	59.6	14.6	24.5	11500
(1.25mg/kg)	±1.29	±1.19	±0.62	±0.22	±1.14	±2.08	±1.59
	*	NS	**	*	*	*	*
Group5	10.0	32.0	10.0	32.0	10.0	31.3	8100
(Control)	±1.27	±1.16	±0.52	±0.41	±1.37	±2.03	±0.9

NS =not significant, * = Significant at (P< 0.05) ,* * = Significant at (P< 0.01) SD= Stander Deviation

Table 4: Changes in serum concentrations of some minerals and trace elements of Nubian goats dosed with Furdan (M±SD)

Main effects	Na (mEq/L)	K (mEq/L)	total Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Fe (µg /dL)	Cu (µg /dL)
Subgroup(2)	120.0	5.0		4.9	1.4	57.9	43
(5.0mg/kg)	±1.02	±0.71		±0.09	±0.07	±1.0	±0.25
	NS	NS		NS	NS	NS	NS
Subgroup(3)	121.1	5.0		4.4	1.3	57.9	45
(2.5mg/kg)	±2.0	±0.22		±0.36	±0.06	±0.26	±0.07
	NS	NS		NS	NS	NS	NS
Subgroup(4)	118.0	4.9		4.3	1.3	55.0	44
(1.25mg/kg)	±0.75	±0.34		±0.23	±0.06	±1.24	±0.06
	NS	NS		NS	NS	NS	NS
Subgroup(5)	120.4	4.9		4.3	1.3	56.0	42
Control	±0.5	±0.08		±0.29	±0.14	±1.21	±0.1

NS =not significant

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Table 5: Changes in serum concentrations of Nubian goats dosed with Furdan (M±SD)

Main effects	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Urea (mg/dL)	Total Bilirubin (mg/dL)	Glucose (mg/dL)	AST (IU)	ALT (IU)	ALP (IU)
Subgroup(2)	7.4	3.4	4.0	24.1	0.4	29.5	42.0	9.6	86.4
(5.0mg/kg)	±0.2	±07	±0.17	±0.23	±0.09	±2.07	±0.77	±0.57	±0.26
	NS	NS	NS	NS	NS	**	*	NS	NS
Subgroup(3)	7.3	3.4	3.9	23.4	0.3	26.0	40.0	10.0	84.9
(2.5mg/kg)	±0.2	±0.91	±0.18	±0.36	±0.31	±1.87	±0.81	±0.53	±0.31
	NS	NS	NS	NS	NS	**	*	NS	NS
Subgroup(4) (1.25mg/kg)	7.5	3.5	4.0	24.1	0.3	29.0	43.0	9.9	86.1
	±07	±0.13	±0.14	±0.4	±0.22	±2.7	±0.56	±0.36	±0.27
	NS	NS	NS	NS	NS	**	*	NS	NS
Subgroup(5) (Control)	7.4	3.4	4.0	22.9	0.2	55.2	27.8	8.8	86.0
	±0.06	±0.24	±0.05	±0.31	±0.1	±2.91	±0.68	±0.31	±0.34

NS =not significant, *= Significant at (P< 0.05),* * = Significant at (P< 0.01)

Discussion

The objective of this study was to describe the toxicity of furdan in Nubian goat- kids. The present study showed that single oral dose of 10 mg/kg killed the animals within 15 minutes postdosing, while daily dosages of 5and 2.5 mg/kg killed the animals within 7-17 days this result is in accord with Lorgue *et al.*, (1996) who mentioned that the LD50 of carbofuran in sheep equal to 9 mg/kg b.w. However, daily dosages of 1.25 mg/kg caused no deaths. The cause of death is commonly attributed to respiratory failure, resulting most often from inhibition of central (medullary) respiratory drive, as well as from excessive bronchial secretions and bronchospasm (all regarded as muscarinic sites of action), potentially coupled with depolarizing blockade at the neuromuscular junctions (a nicotinic site), which further impairs diaphragm and intercostals muscles contraction (James, 1990). Goats which were drenched 1.25mg/kg/day survived until slaughtered on day 45 .This might be due to the fact that the carbamlyloted enzyme undergoes spontaneous and rapid reactivation (Lorgue *et. al*, 1996)

In the present study the activity of acetylcholinestrase was not measured in serum or tissues because carbofuran is a member of carbamates and it is a

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cholinesterase inhibitor (Clarke and Clarke 1981). Clinical signs in this study were typical to that described by Radostits (2005). As a result of acetylcholinestrase inhibition in the cholinergic receptors.

Boyd (1988) mentioned that ALT is present in high activity in liver of small mammals (rats, cats, dogs) but not in large mammals (pigs, sheep, cattle, horses). Kenneth *et. al* (2003), mentioned that the magnitude of the increase in enzymatic activity does not necessarily correlate with clinical manifestation of hepatic insufficiency.

In the present study Furdan caused hepatotoxicity although there were no changes in the activities of serum ALT and ALP and in the concentrations of bilirubin and total proteins. This is evidenced by the pathological lesions such as congestion, haemorrhges and centrilobular hepatocytic necrosis and mild increases in the serum AST activity .Robert and Charles(2004) mentioned that AST is not liver-specific enzyme and it was found in most tissues such as liver, intestines and muscles. Coles (1986) suggests that ALP as an indicator of liver insufficiency or obstructive jaundice in cattle sheep and goat is not sensitive.

No changes were observed in serum urea or any of electrolytes measured. Renal pathology showed excessive dilatation of renal tubules and many of them degenerated or contained hyaline casts. Some glomeruli shrinked, lobulated or disappeared. This indicates that renal toxicosis in the earlier stages and a considerable mass of renal parenchyma is functioning.

The decrease in serum blood glucose might be attributed to either starvation or release of adrenaline due to nicotinic cholenergic receptors at the adrenal medulla Brander *et al.,* (1991). Steven and Michael (2002) reported that catecholamine activity alters blood glucose concentration by several mechanisms, of which stimulation of β-adrenergic leads to stimulation of pancreatic β-cells to increase insulin release.

In the present study treated groups suffered anaemia because the RBC count, Hb and MCHC were decreased while the MCV and WBC were increased although the WBC count showed no significance. The type of anaemia was regenerative macrocytic hypochromic and attributed to the various degrees of haemorrhages which appeared in the different body vital organs. Also the slight decrease in iron concentration might be due to loss of appetite, gastroenteritis and haemorrhages.

The present study concludes that Furdan at 2.5 mg/kg/day and above is fatal while 1.25 mg/kg/day is toxic to Nubian goat kids and then the use of the agrochemical formulation by farmers causes serious hazards and must be avoided.

Further studies are needed to study the carcinogenic and teratogenic effect of Carbofuran on different species of farm animals. In addition, the residues of the Furdan in different body tissues and organs should be determined especially for those animals exposed to carbofuran and consequently human consumption of their milk and meat.

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سمية الفيوردان في الماعز النوبي

اماني بشرى عبدالماجد[1] و عثمان سعد علي محمد[2]

1. قسم الدراسات الإكلينيكية كلية الطب البيطري – جامعة بحري
2. قسم الدراسات الإلكلينيكية – كلية الطب البيطري جامعة السودان للعلوم والتكنولوجيا

المستخلص:

تم في هذه الدراسة اختبار اثر السمية الفمية للمبيد الحشري الزراعي الفيوردان. تم استخدام 15 من الاغنام النوبية عمر (5 – 6 شهور) واوزانها (10–12 كجم) كحيوانات تجارب حيث قسمت الي خمسة مجموعات في كل مجموعة 3 اغنام واعطيت المجموعات مبيد الفيوردان جرعات عن طريق الفم بمعدل 10و5 و2.5 و1.25 ملجرام / كيلو جرام / اليوم . الاغنام التى سممت بالجرعات العالية من مبيد الفيوردان اظهرت سيلان اللعاب وضيق فى حدقة العين و الترنح و الاضجاع و الموت السريع. اما الاعراض التشرحية فهى الاحتقان والنزف فى كل من المخ، القلب ، الرئتين ، الكبد ، الكليتين و الامعاء . يوجد تغير معنوى فى قيمة كلا من عدد كريات الدم البيضاء و مكداس الدم وتركيز الصوديوم ، البوتاسيوم الماغنسيوم ، الكالسيوم ، الفسفور ، الحديد , النحاس ، البوروتين ، الالبيومين ، القلوبيولين اليوريا ونشاط انزيم ALT و ALP وتوجد زيادة فى تركيز البيليروبين و انخفاض فى تركيز الجلكوز فى مصل الدم .

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