STUDIES ON PATHOLOGICAL CHANGES IN ROGOR POISONING IN BUFFALOES *(Bubalus bubalis)*

THESIS

SUBMITTED TO THE FACULTY OF VETERINARY SCIENCE
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR
In partial fulfilment of the requirements
FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY PATHOLOGY

BY

Jageshwar Lal

B. V. Sc. & A. H. (R. A. U.)

JUNIOR RESEARCH FELLOW (R. A. U.)
DEPARTMENT OF PATHOLOGY
BIHAR VETERINARY COLLEGE
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C E R T I F I C A T E - I

Dr. C.D.N. Singh,
B.V.Sc. & A.H., M.Sc. Vet.
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This is to certify that the work embodied in this Thesis entitled "STU-DIES ON PATHOLOGICAL CHANGES IN ROGOR POISONING IN BUFFALOES (Bubalus bubalis)" by Dr. J. Lal submitted to Rajendra Agricultural University, Bihar for award of Master Degree of Veterinary Science (Veterinary Pathology) is the bonafide research work of Dr. J. Lal and was carried out under my guidance and supervision, and that incorporates the results of his independent study.

It is further certified that the assistance received during the course of investigation have been fully acknowledged.

16.5.86

(Dr. D. N. Singh)
Major Advisor

C E R T I F I C A T E - II

We, the undersigned, member of the Advisory Committee of Dr. J. Lal a candidate for the degree of Master of Veterinary Science with major in Pathology have gone through the manuscript of the Thesis and agree that the thesis entitled " STUDIES ON PATHOLOGICAL CHANGES IN ROGOR POISONING IN BUFFALOES (Bubalus bubalis)" may be submitted by Dr. J. Lal in partial fulfilment of the requirement for the degree.

16.5.86

(Dr. C.D.N. Singh)
Chairman, Advisory Committee
and
Major Advisor

Members of advisory committee :

1. Dr. H. N. Thakur 16/5/86

2. Dr. B.N. Prasad 16.5.86.

3. Dr. L.N. Prasad 16.5.86.

C E R T I F I C A T E - III

This is to certify that the thesis entitled "STUDIES ON PATHOLOGICAL CHANGES IN ROGOR POISONING IN BUFFALOES (*Bubalus bubalis*)" submitted in the partial fulfilment of the requirements for the degree of Master of Veterinary Science (Pathology) of the Faculty of Post graduate studies, Rajendra Agricultural University, Bihar was examined and approved on 24.9.

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Members of Advisory Committee :

1. Dr. H.N. Thakur.

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CERTIFICATE - IV

Certified that the research works incorporated in this Thesis have not been published in part or in full in any other journal.

J. Lal

(JAGESHWAR LAL)

A C K N O W L E D G E M E N T S

I express my gratitude to Dr. C.D.N. Singh, B.V.Sc. & A.H., M.Sc. (Vet.) Gold medalist, F.R.C.V.S. (Sweden) Ph.D., Associate Professor, Major Advisor and Guide for his able guidance, constant encouragement and invaluable advice during the course of the manuscript.

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Lastly I express my thanks to all those who helped and co-operate during the work.

J. Lal

(J. LAL)

DEDICATED

TO

MY

LOVING

PARENTS

C O N T E N T S

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INTRODUCTION

INTRODUCTION

Stress on more use of fertilizers as well as pesticides is increasingly being given with the modernisation of agriculture in India. Improvement in agricultural production calls for ever increasing use of fertilizers and pesticides.

India loses its agricultural produce worth seven thousand crores annually due to pests and diseases only. By the turn of the century, the present Indian consumption of 70 thousand tonnes of pesticides per annum is expected to shot up to 200 thousand tonnes. The pesticides, toxic in nature promote poisoning in cattle besides environmental pollution with increasing indiscriminate use.

As there is every chance of toxicity by mishandling of these substances, careful handling of pesticides is needed. The recent disastrous Bhopal gas tragedy due to leakage and inhalation of a toxic substance used in manufacture of pesticide is a point to be remembered.

Organophosphorus insecticides, very potent poisons, may pose serious hazards to animal and human health. Unlike organochloride group, they do not have residues in the environment for a prolonged period. Rogor, a widely used organophosphorus insecticide, is extensively used for protection of crops

from various pests. A detailed experimental work on pathological changes due to Rogor poisoning in buffalo calves has been undertaken in the present study.

Rogor (O, O - dimethyl S-(N-methyl-carbamoylmethyl) phosphorodithioate) a broad spectrum organo phosphorous insecticide has found large scale use as a systemic insecticide in horticulture and also useful against bot fly in sheep.

For dairy calves under 2 weeks of age, the minimum lethal dose is about 50 mg/kg whereas it is 25 mg/kg for cattle of 1 year age. Variable results have been obtained in one year old calves at the dose of 15 mg/kg. Subacute doses of 10 mg/kg has been found to be safe for cattle. It is also reported that continuous 5 day administration of Rogor mixed with feeds @ 5 mg/kg body weight lowered the cholinesterase activity of the whole blood to 21 per cent of the normal values though no poisoning was produced in cattle (Radoleff, 1970).

Buffalo serves as the main milk producers in India and with high fat content in milk. Due to the importance of buffalo in dairy industry, it has been taken up as the experimental animal. In India, there are 61 million buffaloes with average milk yield of 495 kg per lactation with 6.5 to 7.5% fat (Banerjee, 1982).

Vectors of human and animal diseases have been under control due to organophosphorus pesticides, though cases of poisoning in animals and man due to them during manufacturing and use even after great precautionary measures have been of common occurrence.

Occupational hazards due to organophosphorus pesticides have been noticed in men involved in manufacturing, compounding, packaging and aerial spraying (Paul, 1977). Continuous exposure of livestock to low levels of these insecticides ensues from pollution of environment, extensive spraying of pesticides and also from continuous ingestion of treated crops, cereals etc. and initiates a chain of reactions in the ecosystem (Narayan and Verma, 1977). To a variable degree, these are absorbed through the intact skin, mucous membrane and alimentary tract (Jolly, 1957) and thus exceed the minimum lethal dose in many cases.

The object of present work is to find out the toxic effects of Rogor on animal tissues and organs, to study the clinical and pathological and haematological changes, and the Cholinesterase activity in the poisoned buffalo calves.

The pathological findings will also go a long way in initiating and elaborating more research work on Rogor poisoning in buffaloes bringing out news facts to the light.

Considerable potentiality of the organophosphorus pesticides for improving agricultural production has been noted but indiscriminate and careless use of such toxic compounds in agriculture and in livestock may prove fatal for animal life. Investigations on symptoms, clinical or histopathological features, haematological and histopathological changes are scarce in literature.

Hence, the exact position with reference to symptoms and pathology of disease in relation to Rogor, an organophosphorus pesticide and its experimental production for correct diagnosis is called for in respect of effective control. To achieve this, experimental studies have been taken for the compound namely, (ROGOR) to get a true pathological picture.

In short, the main objectives of the present study are :

1. Attempt to study of the incidence of ROGOR poisoning in buffaloes.
2. Study of pathological, haematological changes in experimentally induced "Rogor" poisoning in buffaloes.
3. Study of biochemical change in Cholinesterase activity in experimentally poisoned buffalo calves.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Historical background

Chemical poisoning specially due to organophosphorus poisoning which resulting in quick or sudden death in cattle and buffaloes pose a serious hazard. Few pathological studies of Dimethoate (Rogor) poisoning has been reported in these animals. Farbenindustrie was the first organophosphate used as agricultural insecticide. Since then by 1963, more than 50 thousand organophosphorus compounds have been synthesized. Of these, only three dozens have been produced on commercial basis (Chadwick, 1963).

Lange and Krueger (1932) synthesized DFP (Diisopropyle phosphorofluoridate), the first organophosphorus compound containing P-F linkage. They reported choking, sensation and blurred vision due to inhalation of Dimethyl and Diethyl phosphorofluoridate vapour.

Insecticidal and fungicidal activities of organophosphorus compounds were reported in mid thirties by Lange (1935).

The first agricultural insecticide of this group Bladen (7EPP) was marketed in Germany in 1945 (O'Brien, 1967).

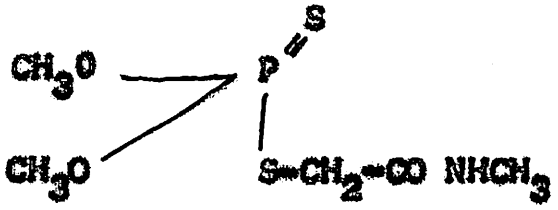
Anticholinesterase activity of organophosphates was first reported by Schrader (1952). Toxic symptoms due to these poisons were attributed to this activity.

Organophosphates acted as nervous systemic poison causing paralysis in affected livestock (Jolly, 1957).

Dimethoate

Known commercially as 'Rogor', coined by Tata-Fison pesticides Division was found to be a broadspectrum, systemic and contact insecticide against mites, flies and mosquitoes etc. (Tata-Fison, 1966).

Chemically known as O, O-dimethyl S-(N-methyl carbamoyl methyl) phosphorodithioate the empirical formula of Dimethoate is $C_5H_{12}O_3NPS_2$ and shown as follows :



Rogor is available as liquid (30 E.C dimethoate) or as granules (5 and 10% dimethoate).

Incidence of poisoning

Accidental ingestion of pesticide treated crops and also from ingestion of insecticide itself or from dermal use the poisoning ensue.

Experimentally produced toxic hazards of dinitro organophosphates poisoning in man and animals was reported after normal sprayed crops ingestion. Lethal dose of this in animals from few square yards of sprayed crops was observed by McGirr and Papworth (1953).

Parathion poisoning in animals due to feeding of parathion contaminated paddy straw showed bronchial constriction, excessive salivary and bronchial secretion and depression of respiratory centre resulting in asphyxia and finally death of animals (Venkateraman and Jaganathan, 1962).

Tri-orthocresyl phosphate (TOCP) poisoning in cattle due to ingestion of ration contaminated with it showed paralysis and cholinergic signs in cows (Gentile and Gruarian, 1965).

Two of organophosphate poisoning outbreaks in cattle and sheep were described by Poloz et al.(1965) who recorded poisoning in suckling calves fed on parathion poisoned dams.

Intake of food contaminated with parathion resulted in death of 100 persons (Wadhani, 1972).

Rude et al. (1975) noticed parathion poisoning in dairy cattle.

At Doraha in Ludhiana district more than 25 buffaloes of either sex were reported to be poisoned due to ingestion of dimethoate(Singh 1981

Due to dermal application of 44.5% of emulsion accidental dichlorvos poisoning in 34 Holstein and Jersey cows was noticed by Knapp and Garden (1964) although Zurrer (1969) described parathion poisoning from washing with its solution against lice infestation in cattle.

Toxicity signs and symptoms

Three categories of signs and symptoms viz. muscarinic, nicotinic and central nervous effects were observed in organophosphorus pesticide poisoning (Radeleff et al.,1955, Clarke and Clarke, 1967, Blood and Henderson, 1968, Radeleff, 1970 and Wills, 1970).

Salivation, abdominal pain, gastrointestinal hypermotility, bronchial constriction increased secretion of bronchial mucosa, lacrimation, involuntary defecation were observed in muscarinic effects whereas the nicotinic effects were characterised by urination, bradycardia, hypotension, sweating, muscular twichings, fatigue, weakness and lastly paralysis. Restlessness, ataxia, convulsion, loss of reflexes, coma and death were the central nervous system symptom(Radeleff et al.,1955).

Dyspnoea, salivation, stiffness of legs were also noticed in organophosphorus poisoning in cattle and sheep (Radeleff, 1958).

Profuse salivation, dyspnoea, diarrhoea and trembling were noted due to organophosphorus poisoning in cattle (George, 1957) whereas Holmsted et al. (1957) recorded vascular congestion and oedema in experimental poisoning in laboratory animals.

Cattle poisoned with organophosphorus compound exhibited trembling and convulsion (Gaser et al., 1962).

Respiratory failure in organophosphorus poisoning might be due to cardiovascular failure where vasodilation and fall in blood pressure ensued (Kolle, 1965).

Lesions and necrotic changes in heart liver, kidney, salivary glands etc. and in lungs congestion, haemorrhage, and oedema were noticed by Galati (1966).

Acute toxicity

Minimum toxic dose of Rogor in calves was 15-20 mg/kg body weight though 40 mg/kg dose produced severe toxicity symptoms and in doses of 80 mg/kg and above showed lethal effects. Hewtt et al. (1958). Cattle orally administered 20 mg/kg dimethoate revealed toxic symptoms (Drummond, 1959).

Dimethoate poisoning in sheep with a dose of 32 mg/kg body weight was described by Meleny and Peterson (1964).

Minimum toxic oral dose for calves under two weeks was found to be 50 mg/kg body weight although 25 mg/kg turned out to be lethal in one year old cattle (Radeleff, 1970).

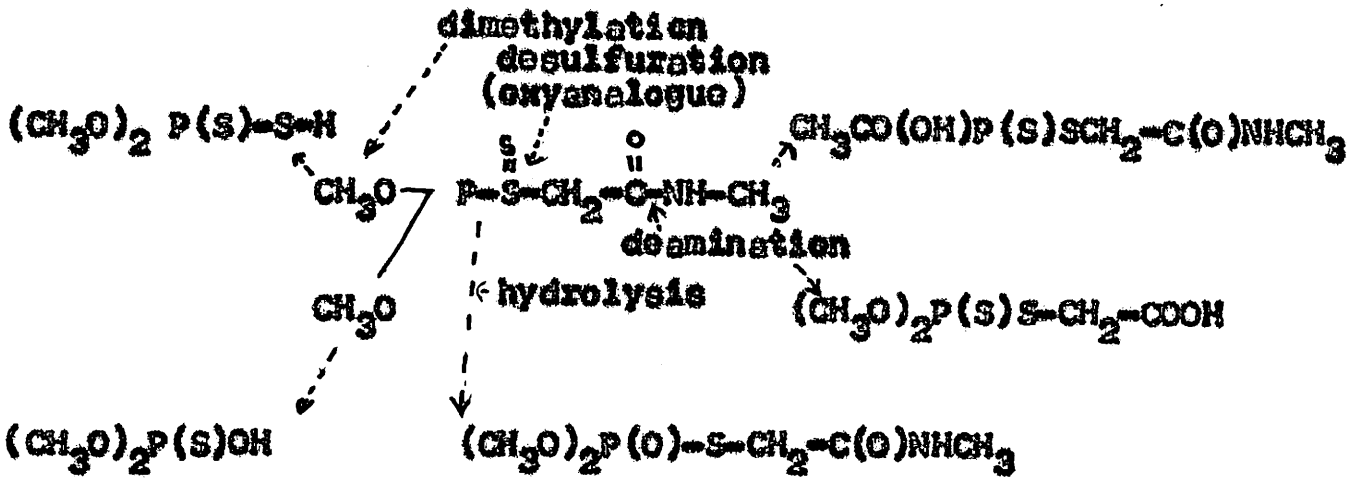
Mitra (1978) used metasystox (100 mg/per os) in buffaloes and reported profuse salivation, increase bronchial secretion, tremors etc. in buffalo calves but no pathologic changes were noticed in animals.

Malik (1978 a) reported loss of appetite, depression, increased salivation, lacrimation and diarrhoea in buffalo though diarrhoea was more pronounced in later stages followed by weakness of hind legs and paralysis.

Metabolism of dimethoate and its relation to toxicity

Organophosphorus insecticides are found to undergo chemical changes in the body of the affected animals. According to O'Brien (1967) the metabolism of Rogor is divided in two phases viz. (i) activative and (ii) degradative phases. In the former, the parent compound is converted to oxy-metabolite which is more toxic (eg. dimethoate to dimethoxon) and degradation of metabolite in the second phase was to demathoxon acid, dimethyl phosphate etc. Less toxicity in mammals compared to those of insects is due to ability of mammals to attack on C-N bond more vigorously than those of the insects.

Dauterman et al. (1950) and Stevens (1971) enunciated the following pathway of metabolism in insects and mammals.



Anticholinesterase activity might be responsible for organophosphate toxicity (Schrader, 1952).

Cholinergic symptoms like muscular tremor, salivation and diarrhoea with DFP intoxication in monkeys and rabbit to be associated with inhibition of brain cholinesterase activity were described by Mazur and Bodansky (1946).

No significance of red blood cell cholinesterase in acute organophosphorus toxicity was found by Frawley et al. (1952).

Haematological studies

Very poor information on haematological studies in buffalo calves in organophosphate toxicity was there.

Leucocytosis and Erythrocytic sedomentation rate increase in rabbits administered Trichlorphon @ dose of 50 mg/kg body weight

were observed by Peterichev and Lazarov (1969).

After administration of dimethoate in sheep at the dose of 2 mg/kg body weight for 240 days Poloz and Kokhtyuk (1970) found fall in haemoglobin and red cell value by 14% and 20% respectively.

Erythrocytopenia, fall in haemoglobin percentage and packed cell volume were noticed in buffalo calves given malathion subacutely (10 mg/kg for 40 days) (Hothi, 1970).

Gallus domesticus given malathion in feeds for a period of 30 days (400 and 800 ppm of malathion) showed leucocytosis (Gupta and Paul, 1972).

Increase in packed cell volume with no change in differential leucocyte count in pony foals, intoxicated with shell 3-D 15803 (16 and 384 mg/kg) was recorded by Bello and Torbet (1972).

Vadlamudi (1974) observed decrease in red cell count and haemoglobin value in buffalo calves spread with 1000 ppm of malathion and 250 ppm of malathion and 250 ppm of sumithion.

No haematological change in horse and ponies orally administered dichlorvos (35.8 mg/kg) was observed by Thomas et al. (1974).

Fall in haemoglobin per cent , red cell counts and leucocytosis in calves orally intoxicated with dimethoate (16 mg/kg for 3 month) were noticed by Abbasov (1974).

Buffalo calves were fed malathion sprayed fodder with 416 ppm concentration for 4 weeks and 0.5 to 1.5 mg/kg for one year exhibited erythrocytopenia and leucocytosis (Gupta and Paul, 1977, 1978 b).

Significant increase in blood clotting time and decrease in plasma total protein and haemoglobin in buffaloes in pesticide toxicity was noticed by Gupta (1978).

Pathological changes

In parathion poisoning in cattle parenchymatous degeneration in liver, kidney were found by Fontenelli (1955).

Necropsy findings, haemorrhagic enteritis with mutiple petechiae and ecchymoses, engorgement of intestinal blood vessles, haemorrhagic kidney and exudate in bronchi were reported by Radeloff (1957) in poisoned experimental animals.

In experimental poisoning of dogs and guinea pigs generalised vascular congestion and pulmonary oedema were reported by Holmstd et al.(1957).

In parathion poisoning in cattle and buffaloes degenerative changes in heart, liver, kidney and congestion & haemorrhage in lungs and pulmonary oedema were evident (Galati, 1966).

Petechiae in meninges, liver and myocardium were also reported by Hothi and Kwatra (1972).

Cholinesterase inhibition

It is a known fact that organophosphorus insecticides exert their effects through the inhibition of cholinesterase enzyme. No direct relationship between cholinesterase inactivation and toxic manifestation has been described(Frawley et al. 1952; O'Brien, 1960; Radeleff, 1970 and Steinberg et al.,1975).

DFP intoxication in monkeys and rabbit were characterised by cholinergic symptoms including muscular tremors , salivation and diarrhoea according to Mazur and Bodnasky (1946).

Frawley et al. (1952) found that in acute insecticide toxicity cholinesterase inhibition has got no significance. Cholinesterase inhibition in brain, red blood cells or plasma does not affect toxic symptoms. No characteristic symptoms were noticed in EPN intoxication in small doses although there was inhibition of cholinesterase activity with lapse of time (Frawley et al. 1952 and Radeleff and Woodard, 1957).

Lovel (1963) noted that neither insecticide properties nor mamallian toxicity exhibited by dimethoate or malathion was related to cholinesterase inhibition in the head of M. domestica or in the brain of rats.

After feeding fodder contaminated with sumithion @ 50 and 200 ppm and malathion 20 and 100 ppm, inhibition of cholinesterase activity both in red cells and plasma of buffalo calves were noted by Vadlamudi and Paul (1974).

Poisoned cattle showed profuse salivation, bradycardia, dyspnoea and reduction of cholinesterase activity in blood (Klee and Raake, 1977).

No correlation between the extent of inhibition of erythrocyte and plasma cholinesterase and clinical manifestation of toxicity were noticed by Gupta and Paul (1977, 1978 b).

Malik et al. (1978 b) noted that 'Hinosani' produced blood cholinesterase inhibition in buffalo calves. This was partially related with severity of intoxication.

Buffalo calves (administered 125 mg/kg body weight malathion orally) exhibited increased trend in cholinesterase inhibition till death by Gupta et al. (1981 b).

Singh (1981) fed buffalo calves orally dimethoate @ 20, 25, 37.5, 40, 50, 100, 200 mg/kg body weight by drenching bottle. Toxic symptoms were produced by the doses of 30 and 50 mg/kg body weight, and 25 and 100 per cent death respectively with these doses. After 36 hours post oral administration of Rogor @ 50 mg/kg body weight showed maximum inhibition of plasma cholinesterase was noticed.

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MATERIALS AND METHODS

MATERIALS AND METHODS

In the present study, a total of 14 normal buffalo calves (aged 1 year) were procured for experimental work. All were of non-descript breed available in the district of Patna. These animals apparently well, were maintained in the available housing condition at Bihar Veterinary College, Patna. The animals were kept under observation for a week prior to administration of dimethoate for the sake of acclimatization. They were given paddy straw and greens and water *ad lib*. These animals were tested clinically before the experiment and they were free from parasitic diseases.

There was one group of 12 animals against 2 animals as control. Control animals were given water in place of dimethoate.

Insecticide used 'Rogor' 30 EC (30% dimethoate as an emulsiable concentration). It is a product of Tata-Fison pesticide division (Rallis India Ltd., Bombay). It is easily available in market. It is in common use in this state for control of insects and pests etc.

Plan of work

The buffalo calves were administered orally at a constant dose rate of 50 mg/kg body weight to produce acute poisoning in them. The lesions developed in the buffalo under acute oral toxicity were main aspects of studies. Prior to

administration of dimethoate for acute poisoning, the animals were off fed for 24 hours.

Parameters studied

Record of symptomatology, toxicity signs and symptoms etc. haematology and plasma cholinesterase of blood, and gross histopathology were the main parameters. Blood samples were obtained from jugular vein at different intervals, before 0 hours of Rogor poisoning and at peak of toxicity symptoms.

Haematological parameters

Haemoglobin (Hb)
Total Erythrocytic count TEC)
Total Leucocytic count (TLC)
Erythrocyte sedementation rate (ESR)
Differential leucocytic count (DLC)

Biochemical parameters

Cholinesterase activity- Plasma cholinesterase was calculated by method of Michel described by Oser (1965).

Histopathological examination

Experimental group of buffalo calves in this plan included twelve animals divided into 3 groups consisting of four animals in each group. There were two animals in a group acting as control which were not given any dimethoate. The buffalo calves of I, II, III group were administered dimethoate

50 mg/kg of body weight orally be means of a drenching bottle. The calculated amount of dimethoate was diluted with 100 ml of water and then administered to the animals.

Control animals were sacrificed after the end of experiment and tissues were collected for histopathology.

Symptomatology, haematology and biochemical analysis were done in all the animals of different groups before and after administration of dimethoate.

The same studies were carried out in control animals which received only water. The blood samples were collected from jugular vein by sterilised glass syringe for haematological studies. In addition to above plan of work, time interval between administration of dimethoate and appearance of clinical symptoms and death were recorded.

Clinical examination

(a) General appearance
(b) Behaviour
(c) Inspection of body region
(d) Pulse rate/min.
(e) Rectal temperature

Haematological examination

The blood was collected in sterile vial containing

an anticoagulent (citrate) in dried form after keeping the vial in hot air oven at 60°C. The following values were calculated.

(a) Total leucocytic count and total Erythrocytic count - Standard method of Boddie (1962).

(b) Differential leucocytic count - standard method of Boddie (1962).

(c) Haemoglobin (Hb%) - determined by Sahlis haemoglobinometer (Oscar W. Schalm, 1967).

(d) Erythrocytic sedimentation rate - Westergren method.

Westergren tube has a total length of 300 mm. and 2 mm diameter and capacity of 1 ml and graduation from 0 to 200 at 1 mm intervals. Blood was drawn into the westergren tube upto 0 mark and the tube was placed in an upright position in a special stand. The fall in erythrocyte sedimentation rate was noted as average per hour and calculated as follows :

$$\frac{\frac{\text{Level at 2 hr}}{2} + \frac{\text{level at 1st hr}}{2}}{2} = \text{Average sedimentation rate mm per hr.}$$

(e) Total erythrocytic count - (TEC) was determined by Neubour's chamber.

Preparation of blood film - Even blood films were prepared with polished and absolutely, clean slides. The smears

were stained by Leishman's stain. 200 leucocyte were connected in each slide following battlement system (1 mm down, 1 mm across and 1 mm above).

Urine examination

Urine samples were collected in clean and dry test tubes and was examined for routine values.

Biochemical examination

Plasma cholinesterase (PChE) activity determined by method of michel - cited by Oser (1965) 0.2 ml of plasma was diluted to 10 ml with water and mixed 1ml of this diluted plasma was transferred to a small beaker containing 1 ml of buffer solution II and was placed in a thermostatically regulated water bath at 25°C for 10 minutes, then the pH of the mixture was measured using a pH meter and reading the 0.01 unit. The beaker was returned to thermostat. The time was noted and then 0.2 ml of 0.165 M acetyl choline solution was added with rapid mixing. Subsequent steps were carried out for calculation.

Calculation :

The cholinesterase activity of the sample in units of Δ pH per hour was calculated as follows :

$$\Delta \text{ pH/hr} = \frac{(pH_1 - pH_2 - b)}{t} f$$

Where pH_1 and pH_2 are initial and final pH, t= time in hours between mixing acetyl choline and b, f are correction factors.

Postmortem examination

After death of experimental animals, a thorough postmortem examination was done. At first, animals were examined were externally and then deskinned. The cartasses were opened by standard procedures. The various parts of body were examined systematically and lesions in them were recorded.

Histopathological examination

The small pieces of various organs such as liver, lung, kidney, brain, muscle, heart were collected and fixed in (10% formal saline solution). Paraffin was used as empedding material and sections were taken at 5 to 6 micron thickness by hand driven microtome. These sections where stained by routine Haemotoxillin and Eosine method (Lillie, 1954) and studied under miscroscope.

Statistical analysis

Calculation of mean, SE and t tests were conducted as per Snedecor and Cochran (1967).

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RESULTS

RESULTS

The material consisted of experimental fatal cases of dimethoate poisoning in buffalo. Buffalo calves were procured from a local commercial supplier for recording of symptomatology, haematology and tissue changes. Only buffalo calves clinically free from diseases (parasitic diseases etc.) for three successive days, were selected for dimethoate administration to produce experimental intoxication. Dimethoate (30 mg/kg) was administered to a group of 12 male buffalo calves against 2 which acted as control.

Due to faulty use of dimethoate by farmers two buffaloes were reported to be dead in the district of Madhubani. Diagnosis of experimental dimethoate poisoning was based on its known administration per os. All the poisoned cases died within two days of oral intake. Results have been judged by all or none standards i.e. reproduction of fatal disease was considered as positive.

The gross lesions in buffaloes were restricted to symptoms developed owing to rapid course of dimethoate poisoning. Symptoms appeared within 21 hrs of drug administration. No rise of body temperature in experimental buffalo calves was noticed rather the temperature became subnormal before death.

Pathologic changes in buffalo calves in accidental cases of dimethoate poisoning in buffalo calves

The affected buffaloes were showing dullness and depression, twitching of muscles, muzzle and incoordination of movement. There was profuse salivation, muscular fasciculation and dyspnoea. Finally they became comatosed and died.

Gross changes

Frothy exudate was present at the nostrils and mouth. The eye balls were sunken with dull coat. The lungs were oedematous and blood flowed from cut surfaces. Liver was swollen and gall bladder distended with bile. Dilated right ventricle contained partially clotted blood and kidneys were swollen. There were hyperaemia or haemorrhagic changes in abomasum and intestine.

Microscopic appearance

The central veins in hepatic lobule were distended and hepatocyte in central lobular area were swollen, granular and showed degenerative changes. There were degenerative changes in lining of epithilial cell of kidneys alongwith focal areas of haemorrhage. The heart lost striation and focal haemorrhage and fragmentation in heart muscles was present. In sections of

lungs stained with haematoxylin and eosine showed hyperaemia and the alveolar walls were prominant. Pink stained fluid, erythrocytes etc. with marked oedema were found in alveolar spaces.

Acute experimental dimethoate poisoning in buffaloes

Dimethoate 50 mg/kg body weight was given per os to produce poisoning in a group of twelve buffalo calves. All the buffalo calves were dead on 2nd day of poisoning (Table 1).

Toxicity signs and symptoms

Acute toxicity signs developed in the buffalo calves administered dimethoate. Table 2 shows the symptoms and signs which were noticed. The time of onset and peak toxicity and mortality with 50 mg/kg body weight dimethoate has been given in table 2. The onset of symptoms were noticed after 18-21 hrs and the peak toxicity after 25-30 hrs and after 36-41 hrs all the animals were dead. The buffaloes were dull depressed and off feed, saliva dribbled from their mouth. Profuse lacrimation and severe twitching of the muzzle were seen. There was laboured breathing, bradycardia and the limbs got paralysed. The animals suffered from asphyxia and all of them ultimately died. The time required for obtaining different stages (i.e. onset, peak, dyspnoea, coma and death in acute oral toxicity of dimethoate) poisoning in buffalo calves are inforporated in table 3.

The average hours for onset of toxicity was(19.83±0.297) as against the culmination into death of the experimental animals after (37.83±0.4582) hours. Temperature of intoxicated animals became subnormal before death.(Table 4). Respiration and pulse rates of these animal came down. All the animals exhibited incordinated movements. There was loss of appetite which developed after poisoning. Circling movements were noticed in three of the poisoned animals after 27 hrs of poisoning which later culminated in paralysis of hind legs after 34 hrs of intoxication. Open mouth breathing with onset of symptoms were noted in all the calves. From the begining upto 21st hrs, there was moderate respiratory distress which later turned to difficult and laboured breathing as time passed. All of them were comatosed before death. Slowing of breathing till their end was also marked. Loud pulmonary rates were detectable by stethoscope. There were severe twitchings and faciculations of muscles. Four poisoned buffaloes were markedly depressed with their heads thrown back on their flanks. Of the poisoned group, eight buffaloes showed diarrhoea, swaying gait from the first day onwards. The muscular paralysis, laying down condition and asphyxia characterised all the animals second day before death. On the second day animals failed to stand. Animals of the poisoned group exhibited sweating, lacrimation, excessive salivation and involuntary defaecation. There was significant decrease in respiration and rectal temperature though no significant difference in urine was revealed on examination.

Ha-ematology

There was significant decrease in total erythrocytic count (4.20±0.348 million/cmm), haemoglobin per cent (8.52±0.150) though significant increase in total leucocytic count (10.83±0.348 thousand/cmm) and erythrocytic sedimentation rate (113.33±0.74 mm/hr) were observed (Table 5) in poisoned animals. Differential leucocytic count (per cent) revealed significant increase in lymphocytes (73.58±0.9728) whereas the neutrophils (23.08±0.8569) decreased significantly The value of monocytes (1.75±0.2176), Eosinophils (1.00 ± 0.3482) and basophils (0.50±0.1508) were found to be non-significant.

Biochemical

Significant increase in plasma cholinesterase (PChE) inhibition per cent values was noted in experimental group of acute poisoned buffalo calves per os (Table 6). The plasma cholinesterase inhibition per cent (81.58±0.908)in the post administration period of the experimental group showed highly significant difference over that of the control one.

Pathological changes

Gross pathology : External examination of four poisoned buffaloes revealed sunken eye balls. Blood tinged fluid was

found to escape from their nostrils. Dribbling of frothy exudate from mouth was also noticed. The coat was dull. Muscles were found to be dry and sticky to touch on deskining.

Lungs

There were few sub pleural irregular depressed areas in both the swollen and enlarged lungs. From the cut surface of lungs, blood tinged watery fluid escaped. The lungs were of firm consistency. Trachea consisted of blood tinged frothy materials. The lungs showed pink or greyish white sub pleural raised areas above the level of surrounding tissues (Fig. 1 and 2).

Liver

The livers of the experimentally poisoned buffaloes were invariably swollen and reddened with rounded edges. A few reddish sub capsular foci were noticed in the livers of five poisoned buffaloes. Full gall bladders distended with greenish and ropy bile were observed in case of 8 buffaloes of experimental group. There was flow of blood on the cut surfaces. Pink stained fluid consisting of few erythrocytes in the interlobular spaces were also noticed. Greyish white patches in liver of 3 dead buffaloes were encountered.

Heart

In 7 intoxicated buffaloes right ventricles of the

heart were dilated. Sub epicardial haemorrhagic foci were also noted. The ventricular walls were congested and partially clotted blood was present in their cavities. In case of 4 dead buffaloes of experimental group chicken fat clot was observed. There was clear groove between left and right ventricle indicating dilatation of right ventricle. The auricles also contained clotted blood. The cardiac muscles were soft in consistency.

Pancreas

Moderately swollen with few reddish areas in pancreas was observed in 8 dead buffaloes of experimental group.

Alimentary tract

There were petechiae and oedematous areas and ecchymoses in mucosae of abomasum (fig. 3) and the small intestinal tract was covered with blood-tinged watery material. Swollen intestinal mucosae with oedematous reddish patches were met with in case of 8 dead buffaloes. Hyperaemic patches of abomasal mucosa were found in six out of twelve dead buffalo calves of the experimental group.

Spleen

Swollen and hyperaemic spleens were noticed in six out of 12 dead experimental calves .

Kidneys

Swollen reddened kidneys with blood flowing out of cut surfaces were also noted. Four dead buffalo calves exhibited reddish foci or stripesin the cortex of kidneys. The capsules were easily detached from them. Moderately pale and swollen kidneys were marked in two dead experimental buffalo calves.

Brain

The meninges of brain were reddened, the vessels in the meninges of brain were engorged (Fig. 4). When they were cut there were minute spots of haemorrhages on cut surfaces. Meningial hyperaemia was noticed in nine of experimentally dead buffaloes. There were reddish stripes on cut surfaces of cerebellum.

Muscles

Muscles fibres did not exhibit any gross lesion.

Histopathology

Lungs : There were areas of hyperaemia oedema and presence of pink stained fluid in inter lobular spaces. Blood vessels in peribronchial spaces are filled and capillaries were engorged with blood at places (Fig. 5). The bronchi were dilated and contained exudates rich in red cell alongwith a few round cells (Fig. 6 and 7). The alveoli were filled with pink stained

homogenous proteinous material alongwith red cells and a few lymphocytes (Fig. 8). Thickened alveolar walls due to excessive number of erythrocytes in alveolar capillaries as well as due to infiltration of monenuclear cells and round cells were also noted. The haematoxylin and eosin stained sections of lungs revealed distended alveoli with broken walls intercommunicating between alveoli (Fig. 9).

Liver

Livers of all the animals of toxic group showed distended sinusoids and central veins (Fig. 10). The liver cells in hepatic cord of lobule were dissociated and disorganised in several places (Fig. 11). The liver cells were moderately swollen, rounded and granular. Red cells were noticed in distended sinusoids. The vessels in portal triad were engorged with erythrocytes. In livers of three buffaloes there were foci of haemorrhages (Fig. 12). The liver cell showed degenerative changes. The central e vein was very much dilated due to hyperaemia.

Heart

In the heart muscles the striations in muscle fibre were not visible and showed fragmentation at places. The vessels in interstitium were engorged. There were also few focal haemorrhages.

Pancreas

In pancreas in seven buffaloes the islet cells were found to be swollen and depleted in number (Fig. 13). The vessels were swollen and engorged with blood. The islet cells were swollen, granular and very discrete.

Spleen

In spleen, the Malphigian's corpuscles at places were depleted of lymphocytes. There were areas of haemorrhages. The blood vessels were also congested.

Kidneys

In kidneys the epithelial cells lining the tubules were swollen and desquamated to form clumps in lamina (Fig.14) . Epithelial cells was swollen, granular and exhibit degenerative changes (cloudy swelling). In kidneys of six fatal cases there were pink stained material in lamina. Vessels in interstitium of kidneys were congested. The Bowman's capsule were very much dilated. There were areas of focal haemorrhage in kidneys of five animals (Fig. 15).

B r a i n

Marked perineural oedema showed evidence of empty spaces around the neurons in eight dead buffaloes of toxic group (Fig. 16). There was also irregular areas of liquefaction necrosis and also perivascular oedema. The neurons

showed neuronophagia. Anoxic changes were noticed in brain sections. The neurons were swollen and nuclei in neurons eccentric in position in some cases in position in some cases. Glial cells (microglia or oligo-dendroglia)appeared around the dying neuron and showed satellotosis and neurono-phagia. Increased accumulation of microglial cell in brains of three dead buffaloes were met with. In brain, there were irregular empty spaces in cerebellum of two of the dead buffaloes. The perivascular spaces were enlarged dilated and contained empty spaces (Fig. 17).

Alimentary tract

The intestine showed epithelial cells lining crypts of Liberkhun desquamated at places to form clumps in their lumina.

Muscles

Muscle fibres of thigh showed loss of striations in two buffaloes.

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Table - 1 : Experimental Dimethoate Poisoning

Insecticide	Toxic dose	Survival time (in hrs)
Rogor (Dimethoate)	50 mg/kg	36 - 41 (12)*

* Figure in parenthesis indicates number of animals.

Table - 2 : Studies on acute oral toxicity of dimethoate poisoning in buffalo calves.

Dimethoate dose(mg/kg)	Approximate time of appearance of toxicity signs & symptoms					
	Onset	Peak	Dyspnoea	Coma	Death	Remarks
	Off fed,mild depression, salivation, twitching of muzzle	Muscular fasciculations,incordination, rigidity of limbs, deep depression,paddling of limbs,hypersalivation, open mouth breathing with groaning rales				
50	18 - 21 hr (12)	25 - 30 hr (12)	30-33 hr (12)	34-40 hr (12)	36-41 hr (12)	100% mortality (12)

Figures in parentheses indicate number of animals exhibiting toxicity signs.

Table - 3 : Showing mean time in hours required for attaining different stages of toxicity in experimental Rogor poisoning in buffalo calves.

	Appearance/onset	Peak	Dyspnoea	Coma	Death
Mean±SE (n) in hrs.	19.83±0.297 (12)	26.5±0.379 (12)	30.75±0.279 (12)	36.75±0.543 (12)	37.83±0.4582 (12)

The figures in parentheses indicate the number of animals.

Table - 4 : Table showing the mean values with different physiological parameters in pre-toxic and post toxic stage in experimental Rogor poisoning in buffalo calves.

Stages of	Mean Rectal temp calves±SE (n) °F	Mean respiration per minute±SE (n)	Mean pulse rate/ mm ± SE (n)
Pretoxic	101.25±0.90a (12)	10.25±0.131C (12)	54.92±0.229 f (12)
Post toxic	100.21±0.115*ab (12)	8.50±0.1508**d (12)	53.58±0.933 f (12)
Control	101.25±0.250a (2)	10.50±0.499c (2)	53.50±1.90 f (2)

Those with same superscripts are nonsignificant for each parameter.

* significance at 5% (P/0.05)

** significance at 1% (P/0.01)

Table - 5 : Showing the haematological parameters in buffalo calves under experimental Roger poisoning in both pre and post toxic stage.

Parameters	Control group	Experimental group	
		Pre-toxic stage	Post toxic stage
TEC (10^6)	5.05±0.0204 (2)	5.13±0.0810 (12)	4.20±0.348** (12)
TLC (10^3)	8.05±0.0204 (2)	8.79±0.179 (12)	10.83±0.348* (12)
Hb% (g%)	10.25±0.0204 (2)	10.330±0.1102 (12)	8.52±0.150** (12)
ESR (mm/hr)	97.5±0.50 (2)	97.25±0.67 (12)	113.33±0.74** (12)
DLC Monocytes (%)	2.00±0 (2)	1.92±0.3352 (12)	1.75±0.2176 (12)
Lymphocytes (%)	68.50±0.2041 (2)	65.92±0.8915 (12)	73.58±0.9728* (12)
Neutrophils (%)	28.00±0 (2)	30.83±0.8602 (12)	23.08±0.8569** (12)
Eosnophils (%)	1.50±0.2041 (2)	1.08±0.3931 (12)	1.00±0.3482 (12)
Basophils (%)	0 (2)	0.17±0.0849 (12)	0.50±0.1509 (12)

** Significant at 1% (P/0.01) * Significant at 5% (P/0.05)
Figures in parentheses indicate the number of animals.

Table - 6 : Showing plasma cholinesterase (PChE) inhibition per cent of buffalo calves under experimental Rogor poisoning 8 hours post toxicity.

Stage	Mean ± S E (n)
post toxicity	81.58±0.908** (12)
Control	2.50±0.204 (2)

** Significant at 1% (P<0.01).

Figures in parentheses indicate the number of animals.

Fig. 1 and 2 The lungs were swollen and enlarged, pink or greyish white sub pleural raised areas above the level of surrounding tissues.

Fig.3 Petechiae and ecchymoses in mucosa of abomasm.

Fig. 4 The vessels in meninges of brain were engorged.

Fig.5 There were hyperaemia, oedema present in lungs H & E x 100.

Fig.6 and 7 The bronchi of lungs were dilated and engorged with blood at places H & E x 100.
H & E x 400,

Fig. 8 The alveoli were filled with pink stained proteinous material.
H & E x 100

Fig.9 The alveoli were distended and walls broken intercommunicating between alveoli
H & E x 100

Fig.10 The liver showed distended sinusiod and central veins
H & E x 100

Fig.11 The liver cells in hepatic cord of lobule were dissociated and disorganised
H & E x 100

Fig.12 The vessel in portal triad were engorged with erythrocytes.
H & E x 100

Fig.13 The islet cells were swollen, granular and depleted
H & E x 100

Fig. 14. The epithelial cell lining the tubie in kidneys were swollen and desquameted
H & E x 100

Fig.15. Focal haemorrhage in kidneys
H & E x 100

Fig.16. Marked perineural edema evidence of empty spaces in brain H & E x 400

Fig. 17. Perivascular spaces were enlarged H & E x 100

DISCUSSION

D I S C U S S I O N

Very scanty information on pathologic changes of acute dimethoate poisoning in buffaloes were met with in literature. Results of the present study might be useful and available in elucidation of the pathogenesis of dimethoate poisoning.

Acute dimethoate poisoning was produced in a small group of 4 buffaloes (twelve buffalo in all included in present study) by administering dimethoate (50 mg/kg body weight) through drench. Results obtained after poisoning were compared with those of control as well as the value of same group of buffalo before administration of poison.

Organophosphorus insecticide include very strong toxic chemicals such as dipterex, malathion, dimethoate, chlorophon etc. Excessive accumulation of acetyl choline at the nerve ending is believed to be responsible for exhibiting of toxic symptoms. When organophosphorus inhibits cholinesterase for hydrolysis, cholinergic symptoms appear in animals. Ladell (1961) consider death in animal due to asphyxia due to anticholinesterase activity in brain. In the present study there was marked perineural oedema and neuronophagia. There is also perivascular oedema. Such changes also give rise to other nervous symptoms.

As pointed by Clarke and Clarke (1967), the nervous symptoms also result from prolonged cerebral anoxia and degenerative changes. The symptoms observed in buffalo calves given 50 mg/kg dimethoate were depression, dullness, marked salivation, lacrimation, twitching of muzzle and muscles. The respiration was much laboured and there was also bradycardia. Subnormal temperature before death was noticed in each case. Clarke and Clarke (1975) observed depression in dimethoate poisoning in sheep and Radeleff (1970) also found dyspnoea, salivation, abdominal discomfort and stiff limb in dimethoate poisoning. Their observations were almost similar to the present findings. The toxicity symptoms were in general agreement with those of Radeleff (1958), George (1957), Gaser et al., (1962), Galati (1966) in cattle and buffaloes.

The symptoms and lesions in buffaloes were due to inhibition of cholinesterase in dose of 50 mg/kg body weight of dimethoate. The extent of inhibition has been described to be dose dependant by several workers. Frawley et al. (1952), Vadlamudi and Paul (1974) observed marked plasma cholinesterase inhibition in dimethoate poisoning in buffalo calves. Toxicity signs of dimethoate poisoning noted in present studies agreed mostly with those of Gupta et al. (1981 b) and Singh (1981).

Buffaloes under the present experiment showed toxicity signs in dimethoate poisoning and signs included depression and cholinergic signs like, salivation, incoordination, muscular fasciculation, rigidity of limb, swaying movement, groaning, rales and open mouth breathing etc. Watery discharges from mouth and nostril were noted. Later they became comatosed and died.

The islet cells of Langerhans were swollen, more granular, very few in number. Hyperglycaemia described by several workers in dimethoate poisoning in animals such as in buffalo (Gupta, 1977); Singh (1981) might be attributed to pathological change in islet cells of Langerhans. Weiss et al. (1964) and Rosen et al. (1958) also suggested that liberated catcholamines from adrenal medulla due to accumulated acetyl choline gluco-corticoid might be responsible for hyperglycaemia in animals.

Significant change in total erythrocytic count, total leucocytic count and haemoglobin per cent in buffalo calves administered dimethoate 50 mg/kg were noticed. Total leucocytic count showed significant rise. Abbasov (1974) also described leucocytosis in calves administered 60 mg/kg dimethoate. Erythrocytopenia and leucocytosis in buffalo calves as observed in the present study were similar to the observations of several workers Hothi and Kwatra (1972), Vadlamudi and Paul (1974), Gupta and Paul (1977), Singh(1981).

There was hyperaemia in gastro-intestinal tract of the poisoned buffalo. Distened gall bladders with bile were noticed. Such grossly observed findings were similar to that of malathion poisoning in buffalo calves (Vadlamudi and Paul, 1974, Singh, 1981).

In sections of lung, liver and brain congestion and haemorrhages were seen. There were also satellitosis and neuronophagia in brain. Kokhtyuk (1970), Gupta (1977) and Singh (1981) also reported similar findings. Haemorrhage in gastro-intestinal tract of cattle and sheep intoxicated with dimethoate was also reported by Radeleff and Woodard (1957).

Salivation, muscular paralysis, shooting diarrhoea, prostrate condition, laboured breathing were very much evident on the 2nd day and 100% mortality observed the same day. Decrease in pulse and respiration rate of experimentally poisoned buffalo calves was also noticed. In only two cases the bronchi showed haemorrhagic exudate whereas in others the bronchioles were empty and dilated and there was excess of pink stained fluid, a few erythrocyte and few round cells in the alveoli. The lesions in lungs were essentially acutely odematous and led to development of anoxic stage and asphyxia in the affected animals. The lesions in the spleen were found to be similar to those reported by Singh et al. (1984).

According to Clarke and Clarke (1975) there was no significant pathological change resulting from organophosphorus compound leading to acute toxicity. Lesions such as pulmonary oedema, gastroenteritis led to appearance of toxic signs like asphyxia, diarrhoea etc. The suspected cases of organophosphorus poisoning can be determined by plasma cholinesterase activity as the value was very much inhibited in experimentally poisoned buffalo calves. Symptoms of dyspnoea, excessive salivation and stiff limbs were typical symptoms which were in close proximity to those described by Radeloff (1970). Asphyxia was the main cause of death in dimethoate poisoning. The lesion in liver were similar to those reported by Fontenelli (1959). Denz (1951) reported depletion of lymphocyte in the spleen. In the present case there was depletion of lymphocytes in the spleen in some cases. Gall bladders were distended with bile in all the buffaloes showed retention of bile due to poor contraction ability of the walls of gall bladder. Fragmentation of cardiac muscle fibres and degenerative changes in cardiac muscle led to fall in cardiac activity and evidences of bradycardia in experimental buffalo calves were noticed.

Respiratory failures accompanied by cardiavascular failure were reported to be the cause of death in such cases (Goodman and Gillman, 1975).

SUMMARY

SUMMARY

Pathologic changes in acute dimethoate poisoning in buffalo calves have been described in the present study. The buffalo calves given 50 mg/kg dimethoate showed marked salivation, muscular fasciculation, lying down, condition etc.

All the symptoms noted were the symptoms recorded in buffalo calves which were under dimethoate poisoning at the dose rate of 50 mg/kg body weight.

Plasma cholinesterase value inhibition per cent markedly increased on dimethoate administration.

Pulse and respiration rates were reduced in toxicated animals.

Significant fall in total erythrocytic count and haemoglobin per cent, and marked leucocytosis in poisoned animals was noted.

Histopathological changes in organs i.e. liver, lungs, kidneys, brain were of hyperaemic, haemorrhagic and degenerative in nature.

It may be concluded from the study of histopathological changes of different buffalo calves that Rogor might be a drug possesing nephrotoxic, hepatotoxic and neurotoxic porpensities. Oedema was found to be more or less consistent change in the lungs of the affected buffalo calves.

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BIBLIOGRAPHY

B I B L I O G R A P H Y

Abbasov, T.G. (1974) Chronic toxicity for calves of trichlorphon, dimethoate and fenitrotheon problemy Veterinarnoi Sanitarii 48, 102-109.

Banerjee, G.C. (1982) A text book of Animal Husbandry 5th ED, Oxford and IBH Publishing Co., New Delhi-1.

Bello, T.R. and B.J. Torbet (1972) Toxicity of an organophosphate anthelmintic (shell S.D. 15803) at excessive dosage in two months old pony foals Am.J. Vet. Res. 33, 329-334.

Blood, D.C. and J.A. Henderson (1968) Veterinary Medicine 3rd Ed. pp 804. Bailliere Tindal and Cassel London 4th Ed.

Boddie, G.F. (1962) Diagnostic methods in Veterinary medicine Fifth ed. Oliver and Boyd Edinburg and Longon pp 35-60.

Chadwick, L.E. (1963) Action on insects and other invertibrates. In "agents" Ed. Koelle, G.B., Handb.exp. Pharmak. Supp. Verlag, Berlin.

Clarke, E.G.C. and M.L. Clarke (1967). Garner's Veterinary and toxicology 3rd Edn. pp 242-257. Bailliere Tindallcassel. London.

Clarke, E.G.C. and M.L. Clarke (1975) Veterinary Toxicolgoy 1st Edn. Baillere, Tindall, London.

Dauterman, W.C., J.E. Casid, J.B. Knaak and T.Kowalczyk. (1959) Bovine metabolism and residues associated with oral administration of dimethoate to rats and three lactating cows. J. Agr. Food. Chem. 7., 188-193.

Danz, E.A. (1951). Jour. of Path. and Bact. 63 pp 81. cited by Jones and Hunt (83).

Drummond, R.O. (1959). Tests with dimethoate for systemic control of cattle grubs. J. Econ. Entomol 52; 1004-1006.

Fontonelli, E. (1955). Su due casidi avvelenamento nel bovine da estere dell' acido tiofosforice (Parathion) 200 profilassi 10: 486-492.

Frawley, J.P., E.C. Nagan and O.G. Fitzhugh (1952). A comparative pharmacological and toxicological study of organic phosphate anticholinesterase compounds. J. Pharmac. Exp. Ther. 105, 156-165.

Galati, P. (1966) Lesions in cattle and buffaloes dying of acute paration poisoning Vet. Bull.37: 3354.

Gentile, G. and R. Gruarien (1965). Triorthocresyl phosphate poisoning in cattle, Nuava Vet 41: 115-125 (Abs) in Vet. Bull. 36 : 741.

George, T. Woodard (1957). The treatment of organic phosphate insecticide poisoning with atropine sulfate and 2 PAM (2- pyridinium Aldomime Methiodide Vet. Med. 52, 571-577.

Goodman, L.S. and A. Gilman (1975). "The pharmacological Basis of Therapeutics" 5th Edn. pp. 457-458. The Mac Millan Co., London.

Gupta, O.P. (1969). Elementary Biostatistics."Souvenir". Indian Pharmacological society, Lucknow".

Gupta, P.K. and B.S., Paul (1972). Effect of malathion on blood cholinesterase and its toxicity in Gallus domesticus. Indian J. Exp. Bio. 9; 455-457.

Gupta, R.C. (1978). Influence of Malathion (O.O. dimethyl dithiophosphate of diethyl mercapto succinate) on some biochemical and haematological parameter during dermal toxicity study in Bubalus bubalis. I.J. Anim. Sc. 48 (6) 428.

Gupta, R.C. and B.S. Paul (1977). Sub acute toxicity study of malathion is Bubalus bubalis (buffalo)pesticides 9 (8) : 26-29.

Gupta R.C. and B.S. Paul (1978 a). No effect level of malathion (O.O. dimethyl dithiophosphate of diethyl mercapto, succinate) in Bubalis bubalis. Bull Envir. Cont. & Toxicol 20; 819-825.

Gupta, R.C. and B.S. Paul (1978 b). Influence of malathion (O, O. dimethyl dithiophosphate of diethyl mercaptosuccinate) on body enzymes in dermal subacute toxicity in studies in Bubalus-bubalis. Species Bull. Environ. Cont - toxicol. 20, 911-918.

Gupta, R.C., J.K. Malik and B.S. Paul (1981 a) Acute toxicity study, Fenitrothion induced biochemical changes in male rats. Indian J. Pharmac. 13.

Gupta, R.C., Nem Singh, B.S. Paul and M.S. Kwatra (1981 b). Role of residual estimation, cloinicobiochemical and pathological changes in diagnosis of toxicity in Bubalis bubalis caused by S (1, 2 di(ethoxy carbamyl) ethyl O'Q' dimethyl phosphodithioate). Indian J. Anim. Sci. 5.

Gupta, R.C. and B.S. Paul , (1981 c) Antidotal treatment of organophosphorus insecticide malathion toxicity in buffalo calves (unpublished data dept. of Phar. PAU., Ludhiana.

Guser, V.F., A.A. Stupinikov, A.F., Bashmurin, T.A. Motrich and E.A. Vilner (1962) Toxicity of dithiophos (Sulfotep for cattle,. Veterinariya, 7, 84.

Hewitt, R.J., Emro, J. Entwistle, J. Pankavich, R.Thorson, W. Wallace and E. Waletzky, (1958). Carbamoyol alkyl phosphorodithiates as chemotherapeutic agents: Effect of diethoate against Grubs in cattle. J. Econ. Entomol. 51, 445.

Holmstd, B. et al. (1957). The pathology of Experimental Cholinesterase inhibitor. Poisoning Acta Phaxmacol. et toxicol. 13; 337. cited by Jone and Hunt (1983).

Hothi, D.S. (1970). Toxicity of Aldrin and Malathion in buffalo calves M.Sc. Thesis submitted to P.A.U., Ludhiana.

Hothi, D.S. and M.S. Kwatra (1972). Aldrin and Malathion poisoning in experimental buffalo calves. J. Res. 9, 346.

Johnson, W.P. (1960). The effect of dimethoate on Dermatobia hominis in cattle. Am. J. Vet. Res. 21, 1046-1048.

Jolly, W.D. (1957). The toxicity of organophosphorus insecticides. Vet. Res. 79 : 796 - 800.

Jones, T.C. and R.D. Hunt (1983). Veterinary Pathology 5th Ed., Indian Ed. Varghese Company, Bombay.

Karczmar, A.G. (1970). Introduction in "Anticholinesterases" International Encyclopaedia of Pharmacology and Therapeutics. Editor Karczmor, A.G. Vol. I,pp. 1-44. Pergamon Press, New York.

Klee, W. and W. Rakee (1977). Primary symptoms continuous salivation Report on an outbreak in a cattle herd Praktis chetrerast 57: 467-470. Vet. Bul. 47-65.

Knapp, G.W. and A.P. Garden, (1964). Accidental exposure of dairy cows to excessive amount of dichlorvos. J. Dairy Sci. 57, 790-791.

Kolle, G.B. (1965). Neurohumoral transmission and autonomic nervous system. In "The Pharmacological Basis of Therapeutics" Editor. Goodman, L.S. and A. Gilman 3rd Edn pp 399-440. The macmillan Co., New York.

Kokhtyuk, F.F. (1970) Histopathology of chronic fosfamid (dimethoate) poisoning in sheep Vet. Bull 41 :581.

Ladell, W.S.S. (1961). Pros. R. Soci Med. 54405 cited by clarke and Clarke, 1967.

Lange, W. (1935). Fort Schritte auf dem Gebiete der Darstellung und Verwendung von Fluorverbindungen. Chem. Ztg. 59: 393 (cited by Karczmar, 1970).

Lange, W. and G.V. Krueger (1932). Ber Dt. Chem. Ges.65 : 1598-1601 (cited by Goodman and Gilman, 1975).

Lange, W. and G.V. Krueger (1932). Ber Dt. Chem. Ges.65: 575-880 (cited by Karczmar, 1970).

Lien, D.H., G.A. Maylin, R.B. Hilman, W.C.H. Rebhun, J.D. Henion and J.G. Eyel (1982). Chlorpyritos toxicity in dairy cattle cornell veterinarian 72, pp.80.

Lillie, R.D. (1954). Histopathologic technic and practical histochemistry 3 : 170-177. The Black storm Division, M.C. Growth,. Hill, Book Co., London.

Lovel, J.B. (1963). The relationship of anticholinesterase activity penetration and insects and mammalian toxicity of certain organophosphorus insecticides J. Econ. Entomol. 56 : 310 -317.

Malik, J.K. (1978 a). Experimental. Subacute toxicity and anticholinesterase action of O, ethyl - S-diphenyl phosphore dithiate in buffalo calves. IJ. Anim.Sc. 48, 398.

Malik, J.K., Verma, S.P., Garg, B.D. and Ahmad, A. (1978 b). Studies on blood enzyme during acute toxicity of O-ethyl S-S-diphenyl. phosphorodithioate (Hinson) in buffalo calves. Indian J. Pharmac. 10; 7-14.

Mazur, A. and Sedmesky, D. (1946). The mechanism of in vitro and in vivo inhibition of cholinesterase activity by diso propyl tiurophosphate J. Biol. Chem. 163, 261-276.

Mc Girr, J.L. and Papworth, D.S. (1953). Toxic hazard of the newer insecticide. Vet. Rec. 65; 857-862.

Meleney, W.P. and Peterson, H.O. (1964) The relationship of Shelfage to toxicity of dimethoate to sheep. J. Am. Vet. Med. Ass. 144, 756-758.

Mehrotra, K.N. (1986). "National conference on long term and short term hazards of pesticide and strategies for their safe use". Feb. 24-26, 1986. Organised by the National Academy of Science, India, Allahabad.

Mitra, P.K. (1978). Acute oral toxicity of metasystox in buffalo calves. I.J. Exp. Bio. 48 : 358.

Narayan, K.G. and Verma, R.K. (1977). Use of animals as indicators of environmental pollution with pesticides. Symposium on living in pesticide polluted environment held at H.A.U., Hissar, Nov. 2-4.

O'Brien, R.D. (1960). Toxicophosphorus esters pp 67-187, 374-376. Academic press, New York.

OšBrien, R.D. (1967). Insecticides Action and Metabolism. Academic Press, New York.

Oser, B.L. (1965). Hawk's Physiological Chemistry. Fourteenth Ed. Mac. Graw Hill Book Company, London. New York.

Paul, B.S. (1977). Hazards and toxicity of long term and low level exposure to organophosphate insecticides in domestic animals. Symposium on living in pesticide polluted environment held at H.A.U., Hissar, Nov. 2-4.

Peterichev, M.H. and Lazarov, V.P. (1969). Change in the organ of Laboratory animals during intoxication with trichlorphon. Rev. Med. Vet. 120, 545-552.

Poloz, D.D., V.A. Pletskii and T.B. Bainuradov (1965). Prophylaxis and diagnosis of chronic poisoning with organophosphorus compounds. Veterinariya 42: 73-76 (Abst. in Vet. Bul. 36 : 1125).

Poloz, D.D. and F.P. Kokhtyuk (1970). Chronic poisoning of sheep with fosfamid (dimethoate) Vet. Bul. 41: 581.

Radeleff, R.D., Woodard, G.T., Bickerson, W.J. and Bushland, R.C. (1955). Acute toxicity of chlorinated hydrocarbon and organic phosphorus insecticides for livestock, U.S.D.A. Tech. Bul. 34; 1122.

Radeleff, R.D. and Woodard, T.G. (1957). The toxicity of organophosphorus insecticide to livestock. J. Am. Vet. Med. As. 130, 215-216.

Radeleff, R.D. (1958). The toxicity of insecticides and herbicides to livestock. Adv. in Vet. Sc. 4 :265-576.

Radeleff, R.D. (1970). Veterinary Toxicology 2nd Edn. pp. 221-222 Lea and Fibiger Philedelphia.

Rosen, F;N.R. Roberts, Budnick, L.E. and Michol, N.A. (1958). An enzymatic basis for the gluconeogenic hydrocortisone. Science 127 :2873 288.

Rude, H; J. Christensen and O. Karlog (1975).Parathion poisoning in cattle. Nordisk Vetar. 27: 373-377(Abst. in Vet. Bul : 46 : 306).

Schalm, Oscar, W. (1965). Veterinary Hematology 2nd ED. Lea & Pibiger, Philadelphia.

Schrader, G. (1952). Anful Weinheim, verlag ehemic monographic No.62 (cited by Karczmar, 1970).

Singh, K.K., Jha, G.J., Singh, P.N., Chauhan, H.V.S. (1984). Pathophysiology of acute aldrin intoxication in goats. Indian. J. Anim. Sc. 54 (3) 215-220.

Singh, S.D. (1981). Toxicological and Biochemical studies on O.I. Dimethyl, S.N. Methyl carbamoyl,phosphorodithiate (dimethoate) in Bubalus bubalis species, Ph.D. thesis submitted to P.A.U., Ludhiana.

Snedecor, G.W. and W.G. Cochran (1967). Statistical methods 6th Ed. Oxford and IBH Publishing Co., New Delhi.

Soni, B.K., Kumaran, J.D.S., Singh, S.P. (1967). Laboratory Manual of Experimental Physiology. 2nd Ed. U.P.A.U. Pantnagar, Nainital, U.P.

Steinberg, G.M., Mednick, M.L., Maddox, J. Rice, R. and J. Cramer (1975). A hydrophobic binding site acetyl cholinesterase. J. Med. Chem. 18 : 1056-1061.

Steinberg, G.M. (1974). Phosphonofluridate inhibition of acetyl cholinesterase. An exceptionally rapid reactent Biorg. Chem. 3, 81-86.

Stevens, W.R. (1971). Pesticides in the environment. Vol. I. pp 167-169. Mercal Decker inc. New York.

Tata-Fison (1966). Technical Manual 'Rogor'. Tata Fison pesticide divison Rallis India Ltd., Bombay.

Thomas, R.B., F.A. Grace and J.T. Bethy (1974). Effects of organophosphorus anthelmintice on blood cholinesterase values in horse and ponies. Am. J. Vet. Res. 35, 72-73.

Vadlamudi, V.P. (1974). Sub acute oral toxicity studies of sumithion and malathion in buffalo calves. Satellite symposium on effect of pesticides on physiological system held at P.A.U.

Vadlamudi, V.P. and B.S. Paul (1974). Oral Toxicity studies of sumithion and Malathion in buffalo calves. International congress of physiological sciences. Satellite symposium on effects of pesticides on physiological system. Ludhiana.

Vadlamudi, V.P. and Paul, B.S. (1978). A note on oral toxicity of O, O. dimethyl O-(S-methyl. 4 nitrophenyl) Phosphorothioate and its effect on blood cholinesterase in Indian buffalo. I. J. Anim. Sc. 48 (1) 596.

Venkataraman, V. and Jaganathan, P.D. (1962). A note on parathion poisoning in animals. Indian Vet. J. 39 : 657 - 677.

Wadhani, A.M. (1972). Harmful effects of pesticides.Publ. Indian Council of Agril. Research, New Delhi.

Weiss, L.R., J. Bryant and O.G. Fitzhugh (1964). Blood sugar levels following acute poisoning with parathion and seven in three species. Toxicol. App. Pharmacol. 6 : 363.

Wills, J.H. (1970). Toxicity of anticholinesterases and treatment of poisoning - In " Anticholinesterases International Encyclopadia of Pharmacology and therapeutics". Editor karozmar, A.G. Vol. I pp 365-469 Pergmon Press, New York.

Zurrer, O. (1969). Parathion poisoning in cattle Schweizer Arch. Tierhieilk 3; 355-357 (Abst. in Vet. Bull. 40 : 290 (1970).

**

Vadlamudi, V.P. and Paul, B.S. (1978). A note on oral toxicity of O, O. dimethyl O-(S-methyl. 4 nitrophenyl) Phosphorothioate and its effect on blood cholinesterase in Indian buffalo. I. J. Anim. Sc. 48 (1) 596.

Venkataraman, V. and Jaganathan, P.D. (1962). A note on parathion poisoning in animals. Indian Vet. J. 39 : 657 - 677.

Wadhani, A.M. (1972). Harmful effects of pesticides.Publ. Indian Council of Agril. Research, New Delhi.

Weiss, L.R., J. Bryant and O.G. Fitzhugh (1964). Blood sugar levels following acute poisoning with parathion and seven in three species. Toxicol. App. Pharmacol. 6 : 363.

Wills, J.H. (1970). Toxicity of anticholinesterases and treatment of poisoning - In " Anticholinesterases International Encyclopadia of Pharmacology and therapeutics". Editor karozmar, A.G. Vol. I pp 365-469 Pergmon Press, New York.

Zurrer, O. (1969). Parathion poisoning in cattle Schweizer Arch. Tierheilk 3; 353-357 (Abst. in Vet. Bull. 40 : 290 (1970).

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