# STUDIES ON INDUCED ZINC PHOSPHIDE POISONING IN CANINES AND ITS THERAPY

BY

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B. V. Sc.



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## CERTIFICATE

Mr. MOHD. GHOUSE, has satisfactorily prosecuted the course of research and that the thesis entitled **STUDIES ON INDUCED ZINC PHOSPHIDE POISONING IN CANINES AND ITS THERAPY** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

Date: 30th June 1993

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## CERTIFICATE

This is to certify that the thesis entitled **STUDIES ON INDUCED ZINC PHOSPHIDE POISONING IN CANINES AND ITS THERAPY** submitted in partial fulfilment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE (VETERINARY MEDICINE)** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Mr. Mohd. Ghouse under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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## DECLARATION

I, **Mr.MOHD. GHOUSE** hereby declare that the thesis entitled **STUDIES ON INDUCED ZINC PHOSPHIDE POISONING IN CANINES AND ITS THERAPY** submitted to the Andhra Pradesh Agricultural University for the degree of Master of Veterinary Science is a result of the original research work done by me. I further declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Date: 30th June 1993

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## ABSTRACT

An experimental study of Zinc phosphide posioning was carried-out at Department of Medicine, College of Veterinary Science Rajendranagar, Hyderabad, to study the following parameters such as clinical signs, haematological changes, biochemical profiles, gross post-mortem lesions and also to work-out a therapeutic regime.

Twenty, non-descript dogs of either sex in the age group of 1 to 5 years were divided randomly into three groups. Group one animals served as healthy control, group two animals served as experimental control and group three animals were subjected to a therapeutic trial. The toxicity was induced by oral feeding of zinc phosphide at the rate of 40 mg per Kg body weight given with feed as a single dose. The dogs started showing the signs of poisoning within two hours of feeding.

The clinical signs such as repeated vomitings, the vomitus having garlic - like odour, abdominal pain, howling and yelping, salivation, restlessness, muscular tremors, shaking of head, increased respirations, accelerated pulse and rise in body temperature were observed. At the terminal stages there was dyspnoea, convulsions and coma followed by death.

The heamatological changes were decrease in total leucocyte count ($8.9 \times 10^3 \pm 0.60 \times 10^3$) as against $11.5 \times 10^3 \pm 0.51 \times 10^3$), decrease in neutrophils (43.0 $\pm$ 0.25 as

against 69.0 ± 0.34%) and increase in lymphocytes (47.0 ± 0.28 as against 21.0 ± 0.10 %). There was also decrease ($P<0.01$) in haemoglobin level (11.0 ± 0.75 gram per cent as against 15.5 ± 0.15), packed cell volume (31.0 ± 0.36%, 44.4 ± 0.36%) clotting time (0.45 ± 0.06 minutes as against 3.5 ± 0.28 minutes).

The biochemical profiles showed increase ($P<0.01$) in blood glucose level and Alanine amino transferase (ALT) enzyme in the poisoned group of animals. The values were 70.0 ± 1.75, 72.5 ± 1.12 as against 50.0 ± 2.24, and 21.0 ± 1.32 in the healthy control group of dogs respectively.

The post-mortem findings revealed congestion and haemorrhages of all the visceral organs, the liver and kidney dark in colour, swollen and congested. When stomach was opened, the garlic-like odour was evident. Gastric mucosa was congested. The microscopic examination of liver revealed extensive haemorrhages in liver parenchyma, degenerative changes of hepatic cells and mild necrosis. The kidney had wide areas of necrosis and extensive intertubular haemorrhages in kidney parenchyma.

The treatment regime consisted of gastric lavage using saline solution for the removal of toxic material. Infusion of Ringer's lactate, a multi-electrolyte solution, was useful to replace the electrolytes and fluid loss and to reduce the acidity. Dexona was used as a powerfull anti-inflammatory and for re-establishing tissue perfusions. Livobex was used as supportive therapy for the regeneration of hepatic cells and to protect the liver from fatty degenerative changes.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| B | Basophils |
| Cmm | Cubic millimeters |
| dl | Decilitre |
| DLC | Differential leucocyte count |
| E | Eosinophils |
| EDTA | Ethylene diamine tetra acetic acid |
| fig | Figure |
| g | Gram |
| Hg | Haemoglobin |
| i.e., | that is |
| I/M | Intramuscular |
| I/V | Intravenous |
| Kg | Kilogram |
| L | Lymphocytes |
| lbs | Pounds |
| M | Monocytes |
| mg | Milligram |
| ml | Millilitre |
| mts | Minutes |
| N | Neutrophils |
| No. | Number |
| NSS | Normal Saline Solution |
| OD | Optical density |
| Pcv | Packed cell volume |
| P | Probability |
| Soln | Solution |

| | |
|---|---|
| S/C | subcutaneously |
| TLC | total leucocyte count |
| viz., | Namely |
| v/s | Versus |
| < | Lesser |
| > | Greater |
| H & E | Haematoxylin and eosin |
| % | Per cent |
| $^{o}C$ | Degrees celsius |
| $\pm$ | Plus or minus |
| ug | Microgram |

# INTRODUCTION

# CHAPTER I

## INTRODUCTION

Zinc phosphide ($Zn_3 P_2$) is a powerful rodenticide and is used extensively in houses, yards and farms to prevent the rat menace. It is a greyish black powder having distinct garlick-like odour of phosphine and is used as a 2.5 to 5.0 per cent concentrate, mixed with an appropriate bait such as meat, grainmash, flour or cooked rice. The farm animals and the domestic pets often gain access to this poison either by eating these baits or eating the destroyed pests or by the way of malacious intent (Anon, 1991) and such animals require an emergency treatment to sustain their life. Of late, Zinc phosphide poisoning in pet animals including dogs, is on increasing scale and the clinicians are unable to save their life, since neither a specific antidote nor a accrediated therapy is available against this type of poisoning.

The persual of available literature indicated that no systematic work has been carried out on the subject of poisoning in canine in general and Zinc phosphide, in particular.

Therefpre it has been propsed to study the experimental Zinc phosphide poisoning in canines along with haemato-biochemical changes and a thepapeutic trial. The

following parameters were proposed in the present investigation:

1. To induce the Zinc phosphide poisoning in experimental dogs by oral feeding and to record the clinical signs.

2. To investigate into the various haematological findings such as Total leucocyte count (TLC), Differential leucocyte count (DLC), Haemoglobin (HB), packed cell volume (PCV) and Blood clotting time.

3. To study the biochemical changes such as Blood glucose and Alanine Transaminase (ALT).

4. To workout a therapeutic regime.

5. To record the necrospy findings in animals died during the experimentation.

# REVIEW OF LITERATURE

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 CLINICAL FINDINGS

Robertson et al. (1945) while conducting the experiments on zinc phosphide poisoning in fowls, observed dullness, ruffled feathers and death without signs of struggling in them.

Mark (1947) while studying thalium posioning in dogs observed acute signs of severe vomiting, abdominal pain, respiratory distress, inappetance, thirst, temperature upto 102.5$^{o}$F and death in 3 to 5 days.

Dyrendahi et al. (1948) have described the clinical signs in a dog given rat poison experimentally. The dog died within twelve and half hours. The outstanding symptoms were dyspnoea and watery discharges from the nose immediately after the death.

Nichols et al. (1949) observed the following symptoms in two dogs given a rat poison containing sodium fluoroacetate, such as signs of extreme pain, convulsions and opisthotonus. The dogs died within four hours of poisoning and within 30 minutes of the occurence of the symptoms.

Orr (1952) observed the clinical signs of vomitings, severe abdominal pain and rigors in dogs with zinc phosphide poisoning.

Fitzaptric et al. (1955) while discussing the toxicity of rodenticide, reported that the symptoms of zinc phosphide poisoning were not specific and the general reaction being that of toxaemia.

Clarke and Clarke (1967) recorded the symptoms of marked loss of appetite, signs of abdominal pain, lethargy and coma followed by death, in case of zinc phosphide poisoning.

Buck et al. (1973) recorded the clinical signs developing in less than an hour of consumption of zinc phosphide and consisted of anorexia, lethargy, vomiting, increase in rate and depth of respiration, abdominal pain and coma followed by death in 3 to 48 hours.

Rao (1977) reported a case of rat poisoning in an Indian Sambhar which showed the signs of restlessness, copious salivation, constant shaking of head and shivering of thigh muscles with accelerated respirations and pulse. The visible mucous membranes were congested with a rectal temperature of $40^{\circ}C$.

Stowe et al. (1978) were presented 8 cases of zinc phosphide poisoning in dogs, during the period of 1974 to 1977. The common clinical signs observed by them were vomiting, running aimlessly, howling and yelping and extensor rigidity resembling strychnine convulsions. All the dogs died within six to eight hours after showing the

clinical signs. They have reported an unpleasant odour of phosphine gas in the vomitus of the dogs.

Peoples and Maddy (1979) studied the poisoning in man and animals due to the ingestion of rodent poison in California and reported the signs of nausea, vomiting, abdominal pain, chill and mental confusion, generalised Weakness, Cardiovascular collapse and respiratory failure in severe cases of poisoning.

Allen et al. (1983) carried-out the experiments on zinc toxicity in Ruminants. In the mildest form of toxicity there was inappetance and gradual loss of condition. In severely affected animals, there was also diarrhoea with dehydration or sub cutaneous oedema resulting in rapid loss of condition, profound weakness and frequently death. Jaundice was also observed in an severely affected sheep.

The clinical signs such as vomiting, diarrhoea, depression and abdominal pain were reported by Clarke (1984). According to him there were convulsions and coma some times preceding the death.

The symptoms of Zinc phosphide poisoning were nausea, Vomiting, diarrhoea and abdominal pain. There might be a symptom-free period followed by gastro-intestinal disturbances (Anon. 1985).

Papp et al. (1989) reported the symptoms of salivation, occasional vomiting and signs of muscular weakness and paralysis in dogs with narasin poisoning at Vterinary College, Budapest.

A general toxaemia with depression of appetite, dullness and increase in respiratory rate without much diagnostic signs were the findings of Blood and Rodostits, (1989) in Zinc phosphide cases in animals.

Cho et al. (1989) studied the clinical and haematological diagnosis of organophosphorus insecticide poisoning. They observed the clinical signs, such as diarrhoea, inappetance, salivation, ataxia, muscle tremors, prostration, dullness and dyspnoea.

The clinical signs of Zinc phosphide included vomition, abdominal pain, aimless running and howling, followed by depression, dyspnoea and convulsions (Anon. 1991).

Nagata (1992) in his study on toxicity of paraquate Beagle dogs observed vomiting, decreased activity and anorexia in the early stage of poisoning.

Tripathi et al. (1992) in their study of aluminium phosphide poisoning observed moderate to severe epigastric pain, burning, hausea, vomiting, diarrhoea and restlessness as the predominant presenting symptoms.

## 2.2 HAEMATOLOGICAL STUDIES

Coles (1980) reported that the normal blood coagulation time in canines as determined by capillary tube method was 4 minutes.

Benjamin (1985) recorded the following blood values of total leucocyte counts 11.8 x $10^3$, neutrophils 69 %; lymphocytes 20%; monocyte 6%; eosinophils 5% and basophils 0.5%; PCV 40-55%; haemoglobin 12 to 17.8 g/dl and the whole blood clotting time (capillary tube method) one to five minuts in normal healthy dogs.

Duncan and Prasse (1986) reported the haematological values in normal dogs as follows: WBC 6-7 x $10^3$; neutrophils 60-70%; lymphocytos 12-30%; monocytes 3-10%; eosinphils 2-10%; basophils rare; haematocrit 37-55% and haemoglobin 12-18 g/dl.

Cho (1989) reported the haemoatological changes in organophosphorous poisoning such as decrease in total number of leucocytes and mean carpuscular haemoglobin concentration and increase in haematocrit value. In differential leukocyte count the number of neutrophils and neutropil (lymphocytes) ratio increased, where as the number of lymphocytes decreased.

Graham et al. (1991) studied the changes in haemogram in zinc toxic calves. There was significant increase

in segmental neutrophils and unclassified cells and reduction in eosinophil and there was no change in band neutrophils.

Bhaumik et al. (1982) while studying the importance of haematologivcal examination in diseases of domestic animals showed the range of normal haematological values of different domestic animals. The dog had, haemoglobin 11-15 g/dl; packed cell volume 40-50%; leucocytes 7-16 x $10^3$; neutrophils 60-70%; lymphocytes 10-30%; basophils 0-2% and monocytes 3-10%.

## 2.3 BIOCHEMICAL STUDIES

Hamza et al. (1977) in their biochemical studies on the serum of buffaloes poisoned with organophosphorus compound recorded increased serum alanine amino transferease activity.

Kramer (1980) reported that alanine aminotransferase is present in plasma and in liver cells and an increase in plasma GPT activity in dogs and cats is associated with hepatocellular disorders. Acute hepatic diseases causing membrane damage or cell necrosis results in appreciable increases in Alanine amiro transferase activity.

Feldman (1980) reported that patients with liver cell damage tend to have elevations in alanine amino transferase (ALT), the most sensitive indicator of liver

damage. Patients with acute hepatic damage due to any cause may develop dissemination intravascular clotting (DIC), a disorder characterized by activation of the blood coagulation system which in most instances results in the generation of excess prothrombin in the systemic circulation.

Paul et al. (1984) studied the effect of quinolphos on blood ezymes and its acute toxicity in Babalus babalis, which significantly increased the activity of serum aspartate amino transferase and blood glucose concenteration

Benjamin (1985) used dextrostix and reported the normal blood glucose level ranging from 55 to 90 mg/dl of blood and the alanine amino transferase ranging from 10 to 50 units per ml of blood in canines.

Srivastava and Rampal (1989) studied the effect of quanolphos on blood urea nitrogen; glucose, protein and cholinesterase in calves and found an increase in cholinesterase, plasma proteins; glucose and blood urea nitrogen.

Lraig et al. (1991) studied senecio jacobaco toxicosis in calves and observed increase in serum glutamate dehydrogenase followed by increase in alkaline phosphatase and y-glutamyl transferase enzymes.

## 2.4 POST-MORTEM FINDINGS

Robertson et al. (1945) while conducting the experiments on Zinc phosphide posioning in fowls, observed

the autopsy findings such as venous congestion, fluid in the serous cavities and odour of phosphine in the crop of the birds.

Dyrendahi et al. (1948) have recorded the post-mortem lesions in a dog died during experimental antu rat poisoning. They found oedema in the lungs, mediastinum, and hydrothorax. In some cases there was oedema and haemorrhages in the parenchymatous organs, the gastro-inteslinal mucosa and the brain and its meninges.

Orr (1952) observed congestion of lungs with dark tarry blood and necrosis of liver was usually present in birds died due to the zinc phosphide poisoning. The carbide or acetyline odour in the crop and gizzard contents was distinct.

Fitzaptric et al. (1955) while discussing the toxicity of rodenticide (zinc phosphide) reported the systemic lesions as follows. Respiratory system, marked congestion of lungs in the majority of cases, with interlobular oedema in a few cases. There was pleural effusion of watery liquid and blood splashed on the surface of the lungs. Circulatory system-Congestion of all the parenchymatous organs, specially the liver, mammary gland and the kidney in the majority of the specimens. There was also a congestion of subcutaneous tissue in several cases and petechiae along the coronary grooves and the aortic wall in most of the animals.

Stomach - In all cases the stomach showed the lesions of gastritis. Small Intesline: In majority of cases the small intestine was empty of food. Inflammation usually occured and was intense. Enteritis was characterised by congestion of mucosa and haemorrhage into the bowel. Large intesline: No enteritis but congestion was evident. Liver: Liver was swollen and extremly congested. In some cases organ had a diffuse yellow or putty colour, in others there was patchy appearance with purple areas of congestion alternating with yellow area of degeneration. Excretory system: The kidneys were swollen and congested in the majority of animals. Other organs: The reproductive organs mesentery, peritoneum showed no significant changes.

Clarke and Clarke (1967) recorded post-mortem lesions such as venous congestion capillary break down, some interlobular lung oedema and gastro-enteritis. On opening the stomach (and more particularly, the crop and gizzard in the case of birds) an odour of carbide (acetylene) was apparent.

Hamza et al. (1977) found severe gastro-enteritis with enlrgement of mesenteric lymph nodes, spleen, liver and gallbladder. The lungs were congested and oedematous. The myocardium was congested with sub-endocardiac haemorrhages in organophosphorus poisoned cases. Thyroid and adrenal glands were also enlarged. The brain showed petechical haemorrhages all over the meninges.

Stowe et al. (1978) were presented, eight cases of zinc phosphide poisoned dogs. On autopsy, they noticed unpleasant odour of phosphine gas and the lesions included acute congestion of all internal organs including the brain.

Hatch (1978) recorded the post-mortem lesions such as pulmonary congestion and oedema, plural effusion and sub-plural haemorrhages, congestion of liver and kidney and haemorrhagic gastro-enteritis in case of zinc phosphide poisoning.

Post-mortem lesions were gastritis, oedema of the lungs and garlic-like smell of phosphine on opening the stomach, in the poisoning cases due to zinc phosphide (Clarke, 1984).

Prakash and Mathur (1987) explained that the zinc phosphide reacts with hydrochloric acid of the stomach and releases the phosphine gas, which produces necrotic lesions and kidney damage causing death from heart failure, ($Zn_3 P_2 + 6HCl = 2PH_3 + 3ZnCl_2$).

Papp et al. (1989) reported that post-mortem lesions such as moderate hypertropy and hyperemia of liver and Zenkar type degenarative changes in skeletal muscles and myoceardium in Narasin poisoned dogs.

The necropsy lesions such as congestion and haemorrhages in all organs, fatty degeneration of liver and

inflammation of small intestine were reported by Blood and Rodostits (1989) in zinc phosphide cases in pigs.

The odour of acetylene was present in vomitus and stomach contents, other lesions included were visceral congestion and pulmonary oedema (Anon, 1991).

Nagata (1992) in his study of paraquate in Beagle dogs recorded moderate thickening of alveolar wall and pleura, proliferation of fibroblast like cells and abandon fibres in the interstitium and alveoli of the lungs. In the liver there were few haemorrhages along the gall bladder.

Tripathi et al. (1992) in their study of aluminium phosphide poisoning in dogs reaveled the congestion and oedema of lungs and the inflammation of the stomach mucosa in all the cases.

## 2.5 THERAPY

The treatment of thalium poisoning in dogs involved the use of gastric lavage and emetics and when sickness continued for more than 24 hours, they needed parenteral glucose, gastric sedatives, atropine and oxygen (Mark, 1947). The British antilewisite (BAL) had no effect in this type of poisoning.

Clarke and Clarke (1967) reported that there was no known treatment for the acute zinc phosphide poisoning in canines.

Malone (1969) suggested the treatment of a suspected poisoning as follows: 1. Removal of toxicant by emesis or lavage using one per cent solution of copper sulphate, 20 to 30 ml, orally. 2. Symptomatic treatment consisting of a) treatment of central nervous and respiratory depression, using stimulants such as leptazol (50-100mg) intramuscularly Nikethamide (250-750mg) I/M. (b) Treatment for central nervous stimulation using sedatives such as pentabarbitone sodium slow intravenously 10-20 mg/kg Body weight and (3) Supportive treatment using Ringer's lactate intravenously to prevent the dehydration.

Rao (1977) reported a case of rat poisoning in an Indian Samber and treated successfully by giving largactil 2 ml I/V., atropine sulphate 5 ml I/M., dextrose saline 500 ml I/V and gastric lavage with salt water using stomach tube. Following day, the animal was found to be improved in demeanour, convulsions, shaking of head and shivering of muscles. The same line of treatment was repeated along with 500 ml of butter milk orally. On third day the animal was found to be bright and improved in condition. The body temperature was $38^{\circ}C$.

Hatch (1978) reported that there is no specific antidote for zinc phosphide ($Zn_3P_2$); or phosphine gas ($Ph_3$). The gastro-inteslinal tract should be emptied as best as possible by lavage with 5 per cent sodium bicarbonate. This solution also limits the acid hydrolysis of zinc phosphide.

Acidosis may be treated with calcium gluconate and one sixth molar sodium lactate solution. Shock may be treated by routine measures. Liver and kidney damage may be minimized by I/V 5 per cent glucose solution.

Peoples and Moddy (1979) while studying the poison in man and animals due to the ingestion of rodent poison in California, suggested the immediate treatment by giving emetic drug followed by gastric lavage and a cathartic. Niacinamide could be used as an antidote if administered within an hour of exposure (in man).

Clarke (1984) stated that there is no specific antidote for zinc phosphide poisoning, however gastric lavage might be effective if initiated in time.

The drug therapy for zinc phosphide poisoning by giving 0.2 per cent cupric sulphate solution orally to act as an emetic and gastric lavage with 1:5000 potassium permanganate solution. General supportive measures included administration of vitamin K to control haemorrhagic manifestations and morphine to control convulsions. In emergencies corticosteroid therapy is recommended (Anon, 1985).

The advised treatment in the case of zinc phosphide poisoning is by giving supportive therapy like calcium gluconate and appropriate fluid to reduce acidosis (Anon, 1991).

Tripathi et al. (1992) have treated the posioning cases by using a) potassium permanganate (1:1000) half hourly and repeated every hour till the gastric fluid was negative for $PH_3$ by silver nitrate paper test. followed by (b) sodium bicorbonate solution 60 ml after 3-4 hours (c) Intravenous fluid (4-6 litres) in first 4 hours out of which 50% was saline). (d) Dopamine infusion 8-10 ug/kg/minute. (e) Intravenous hydrocortizone 200mg every 4-6 hours and (f) Continuous oxygen administration.

# MATERIALS AND METHODS

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 SELECTION OF ANIMALS

Twenty, non-descript dogs of either sex, in the age group of 1 to 5 years, were procured locally and used in the present study. All these animals were dewormed by administering Fenbendazole (Panacur-Hoechst India Limited-Bombay) orally. Faeces of all dogs were examined daily for one week to eliminate the possibility of reinfestation. Temperature, pulse and respirations were also recorded simultaneously. The dogs were maintained on vegetarian diet and the drinking water was made available adlibitum. These animals were divided randomly into three groups of six animals in each. Dogs in group I served as healthy control.

## 3.2 INDUCTION OF POISONING

Poisoning was induced in animals of group II and III by feeding zinc phosphide ($Zn_3P_2$) at the rate of 40 mg per kg body weight, mixed in a bread mash (Clarke and Clarke, 1967). Dogs were starved for 24 hours before giving the zinc phosphide to facilitate the complete eating of the bread mash.

## 3.3 CLINICAL FINDINGS

The clinical symptoms including rectal temperature, pulse and respirations were recorded in poisoned animals.

## 3.4 HAEMATOLOGICAL STUDIES

### 3.4.1 Collection of Blood Samples

Blood samples were drawn from the cephalic/ Saphenous vein into sterile vials containing an anticoagulant Ethylene Diamino Tetracetic Acid (EDTA), added at the rate of one mg per ml of the blood. The haematological studies were carried-out within an hour of collection. The Blood samples were collected before the comencement of the experiment, (day-o), to establish the normal values and after induction of poisoning (day-1) and after the treatment (day-5). Whole blood was used for estimation of Total leucocyte count (TLC); Differential leucocyte count (DLC), Haemoglobin concenteration (HB), packed cell volume (PCV) and Blood glucose. Another set of blood samples was collected without adding any anticoagulant to collect the serum for the estimation of Alanine aminotransferase (ALT).

### 3.4.2 Total Leucocyte Count

Total leucocyte count was done as per the procedure described by Schalm et al. (1975) and the values were expressed as thousands per microliter of blood.

### 3.4.3 Differential Leucocyte Count

Differential leucocyte count was made after staining the blood smear with Leishman's stain and the values were expressed as percentage (Schalm, et al; 1975).

### 3.4.4 Haemoglobin Concentration

Haemoglobin concenteration was estimated by cyanmetheamoglobin method using a photo-electric calorimeter (Systronics) according to the procedure of Schalm et al. (1975) and the values were expressed as gm/dl.

### 3.4.5 Packed Cell Volume

Packed cell volume was estimated by microhaematocrit method as described by Schalm et al. (1975) and the values were expressed as percentage.

### 3.4.6 Coagulation Time

Coagulation time was determined by capillary tube method according to coles (1980).

## 3.5 BIOCHEMICAL STUDIES

Biochemical estimation was also made on day-o, day-1 and day-5.

### 3.5.1 Blood Glucose

Blood glucose was estimated by Follin-wu method using a diagnostic reagent-Kit (Span diagnostic Pvt. Ltd.) and the values described as mg/100ml of blood. The procedure is described in Appendix-A.

### 3.5.2 Alanine Amino Transferase (ALT)

The serum Alanine amino transferase was estimated according to the 2, 4-DNPH Method-using a diagnostic kit (Span diagnostic Pvt.Ltd.) and the values were described as units/ml by the method of Reitman and Frankel (1957). A standard curved was plotted using differential concentration of reagents for estimating the enzyme activity (unit/L) of unknown samples. The detailed procedure is shown in Appendix-B.

## 3.6 POST-MORTEM FINDINGS

Post-mortem examination was conducted on the dogs died during the experiment and the gross lesions were noted. The liver and kidney tissues were processed for histopathological studies.

## 3.7 THERAPY

Therapy was undertaken soon after the induction of poisoning in group-III animals as described below :

a) Gastric lavage was carried-out using a stomach tube and normal saline solution. In all, three lavages using 200 to 300 ml of NSS each time, were done (Fig-16 and 17).

b) Ringer's lactate solution was infused intravenously at the rate of 50 ml per kg body weight daily for four days (Fig 18).

c) Dexona (Cadila-vet) was given intravenously at the rate of 0.2 mg per kg body weight daily for four days.

d) Livobex (TTK-Vet) was given intramuscularly at the rate of 2.0 ml each dog for four days.

## 3.8 STATISTICAL ANALYSIS

The statistical analysis of the data was carried out as per the procedures described by Snedecor and Cochran (1967). The analysis of varience was done to find the significance of difference.

# RESULTS

# CHAPTER IV

# RESULTS

## 4.1 CLINICAL FINDINGS

The dogs poisoned with Zinc phosphide showed the clinical signs such as repeated vomitings (the vomitus had unpleasant garlic-like odour), salivation, restlessness with complete loss of appetite. Some dogs showed the evidence of abdominal pain, howling and yelping. There was shaking of head, rigors, muscular tremors with lethargy and dullness. Respirations were increased in rate and depth, pusle accelerated along with rise in body temperature upto $104^{o}F$ ($40_{oC}$). Visible mucous membrane were congested. At terminal stages the animals showed dyspnoea, convulsions, coma followed by death.

## 4.2 HAEMATOLOGICAL STUDIES

The results of haematological studies are presented in the Table 1 and Fig-6.

### 4.2.1 Total Leucocyte Count (TLC)

The average total leucocyte count was $11.50 \times 10^3 \pm 0.51 \times 10^3$; $8.90 \times 10^3 \pm 0.60 \times 10^3$ and $11.45 \times 10^3 \pm 0.35 \times 10^3$, per cmm of blood in the healthy control, poisoned and recovered dogs respectively. The difference of TLC before and after treatment was highly significant ($P<0.01$).

### 4.2.2 Differential Leucocyte Count (DLC)

The average per cent of differential leucocyte count in the healthy control group of dogs was as follows, Neutrophils 69 ± 0.34, Lymphocytes 21 ± 0.10, Monocytes 5.3 ± 0.05, Eosonophils 4.2 ± 0.02 and Basophils 0.5 ± 0.02. Where as the values in poisened dogs was Neutrophils, 43.0 ± 0.25, Lymphocytes 47.0 ± 0.28, Monocytes 5.2 ± 0.03, Eosenophils 4.8 ± 0.02 and Basopils nil and in recovered dogs; Neutrophils 68.5 ± 0.24, Lymphocytes 21.5 ± 0.07 Monocytes 5.2 ± 0.01, Eosinophils 4.4 ± 0.01 and Basophils 0.4 ± 0.01 (Table 1). The difference in DLC was highly significant ($P<0.01$) except monocytic cells which were insignificant ($P>0.05$).

### 4.2.3 Haemoglobin Concentration

The mean haemoglobin values were 15.5 ± 0.15, 11.0 ± 0.75 and 15.0 ± 0.36 grams per cent in healthy control group, poisined dogs and in recovered dogs respectively (Table-1). The difference of Hb before and after treatment was highly significant ($P<0.01$).

### 4.2.4 Packed Cell Volume

The mean packed cell volume was 44.0 ± 0.36; 31.0 ± 0.36 and 43.0 ± 0.36 per cent in healthy control group; poisoned dogs and in recovered dogs respectively (Table-1). The difference of PCV before and after treatment was highly significant ($P<0.01$).

Table 1: Haematological values

| S. No. | Profile | Control group | Treated group | | |
|---|---|---|---|---|---|
| | | | Day-0 | Day-1 | Day-5 |
| 1. | Total leucocyte count (X $10_3$/UL) | 11.50 ± 0.51 | 11.50 ± 0.51 | 8.90 ± 0.60 | 11.45 ± 0.35 |
| 2. | Differential Leucocyte count (%) | | | | |
| | a) Neutrophils | 69.00 ± 0.34 | 69.00 ± 0.34 | 43.00 ± 0.25 | 68.50 ± 0.24 |
| | b) Lymphocytes | 21.00 ± 0.10 | 21.00 ± 0.10 | 47.00 ± 0.28 | 21.50 ± 0.07 |
| | c) Monocytes | 5.30 ± 0.05 | 5.30 ± 0.05 | 5.20 ± 0.03 | 5.20 ± 0.01 |
| | d) Eosionophils | 4.20 ± 0.02 | 4.20 ± 0.02 | 4.80 ± 0.03 | 4.40 ± 0.015 |
| | e) Basophils | 0.50 ± 0.01 | 0.50 ± 0.01 | - | 0.40 ± 0.01 |
| 3. | Haemoglobin (g%) | 15.50 ± 0.15 | 15.50 ± 0.15 | 11.00 ± 0.75 | 15.00 ± 0.36 |
| 4. | PCV (%) | 44.00 ± 0.36 | 44.00 ± 0.36 | 31.00 ± 0.36 | 43.00 ± 0.36 |
| 5. | Clotting time (minutes) | 3.50 ± 0.28 | 3.50 ± 0.28 | 0.45 ± 0.06 | 3.40 ± 0.07 |

Table 1(a): ANOVA of Haematological values

| S.No. | Profile | DF | SS | MSS | F ratio |
|---|---|---|---|---|---|
| 1. | Total leucocyte count | 2 | 26.53 | 13.26 | 8.68** |
| 2. | Differential Leucocyte count | | | | |
| | a) Neutrophils | 2 | 2653.00 | 1326.50 | 2763.54** |
| | b) Lymphocytes | 2 | 2653.00 | 1326.50 | 6699.49** |
| | c) Monocytes | 2 | 0.04 | 0.02 | 1.66NS |
| | d) Eosionophils | 2 | 1.12 | 0.56 | 140.00** |
| | e) Basophils | 2 | 0.84 | 0.42 | 52.50** |
| 3. | Haemoglobin | 2 | 73.00 | 36.50 | 25.42** |
| 4. | PCV | 2 | 628.00 | 314.00 | 392.50** |
| 5. | Clotting time | 2 | 129900.00 | 64950.00 | 750.58** |

** Significant (P<0.01) ::: * Significant (P<0..05) ::: NS - Not significant

Table value of F at 1% level is 6.35 and at 5% level is 3..65

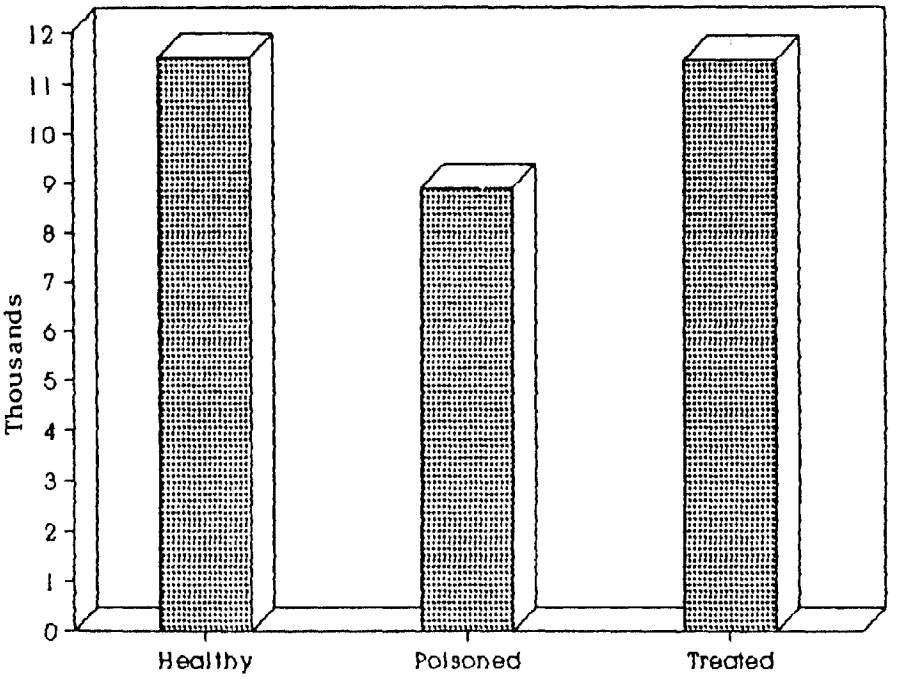


Fig.1: Histograms showing total leucocyte count in healthy, poisoned and treated dogs.

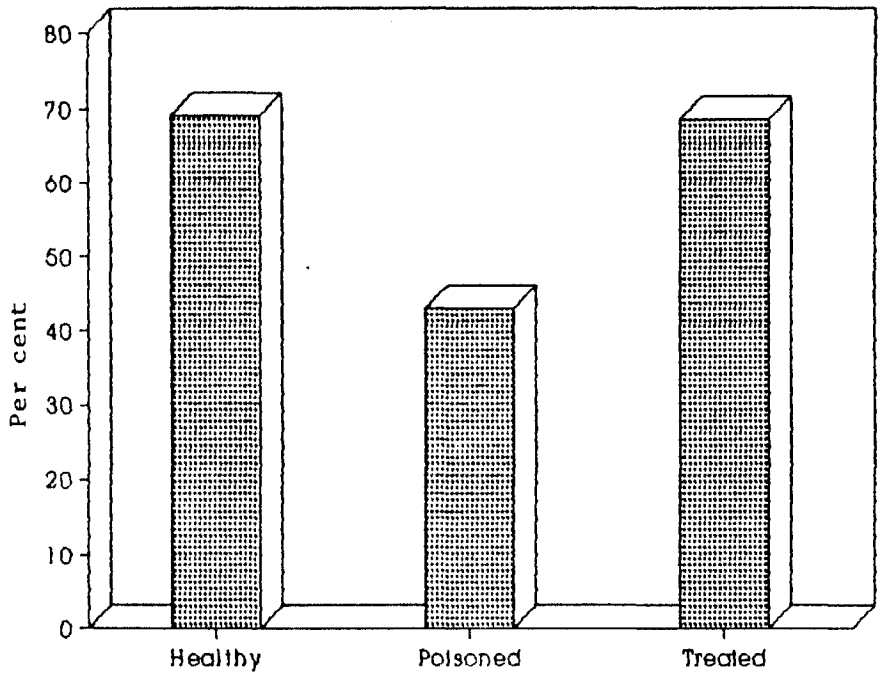


Fig.2: Histograms showing Neutrophil count in healthy, poisoned and treated dogs.

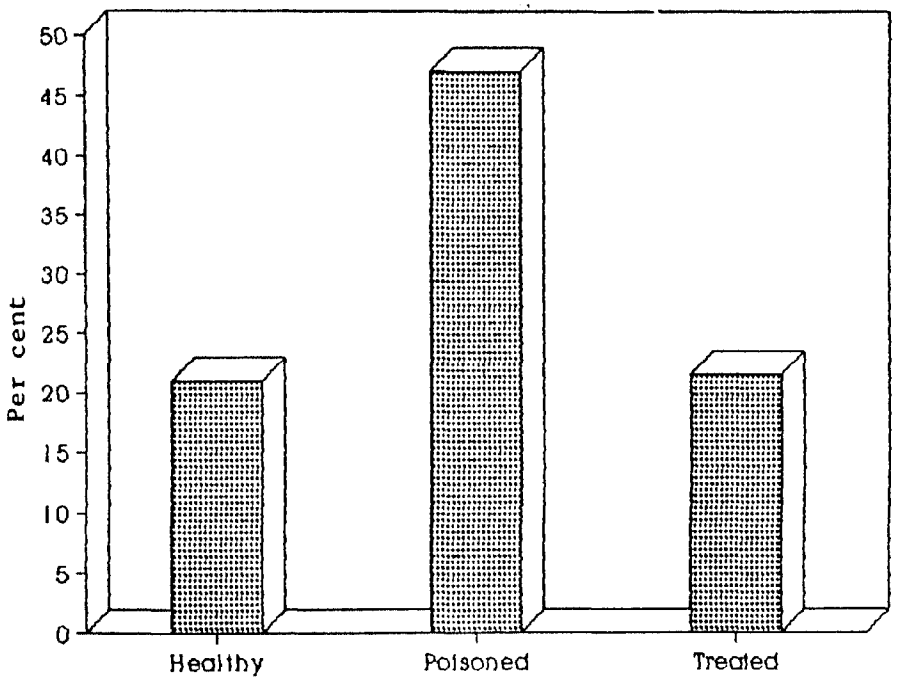


Fig.3: Histograms showing lymphocyte count in healthy, poisoned and treated dogs.

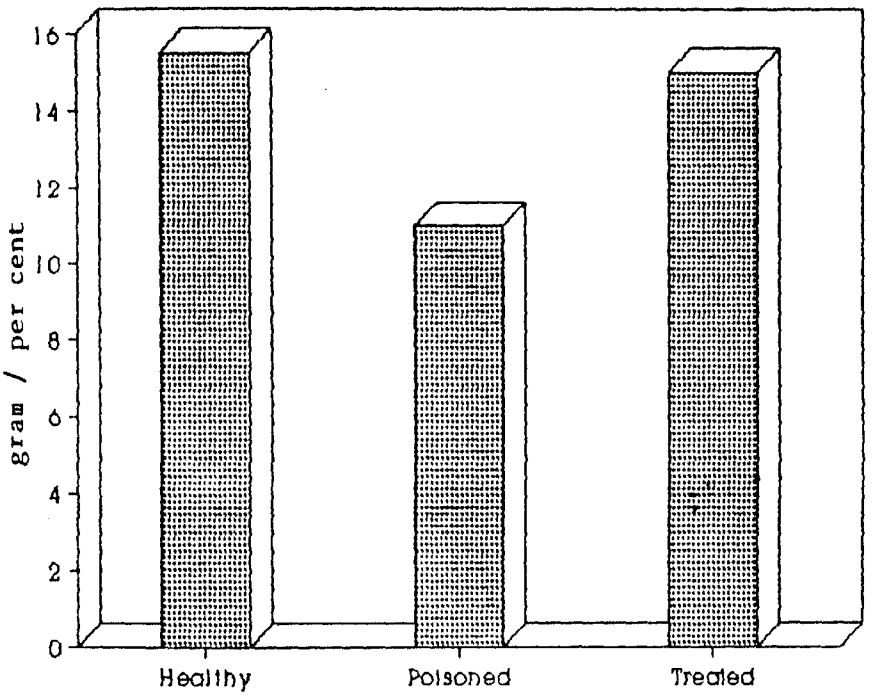


Fig.4: Histograms showing Haemoglobin content in healthy, poisoned and treated dogs.

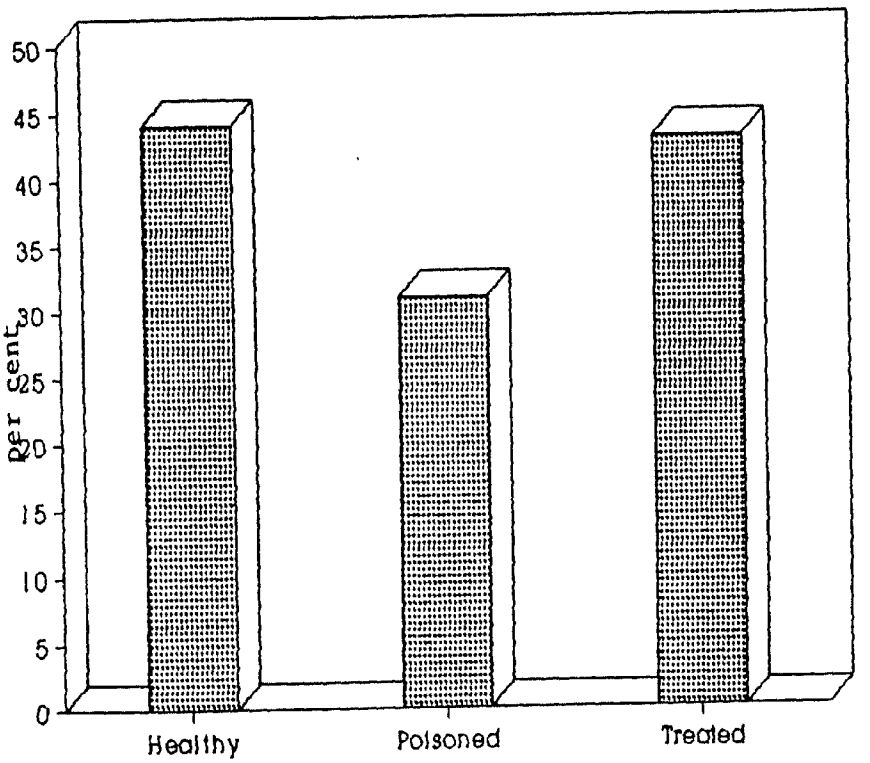


Fig.5: Histogrms showing packed cell volume in healthy, poisoned and treated dogs.

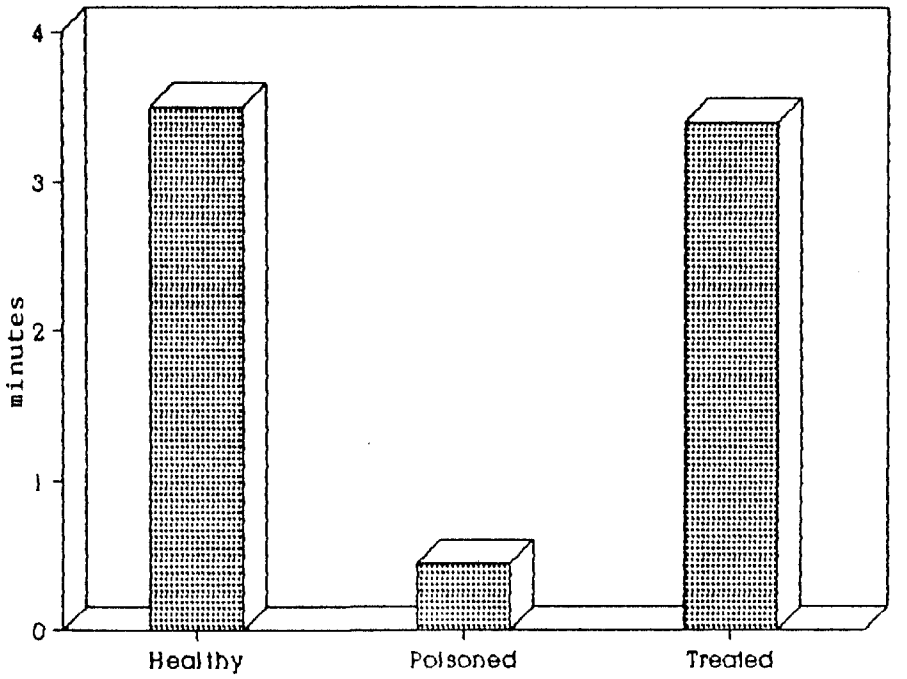


Fig.6: Histograms showing clotting time in healthy, poisoned and treated dogs.

### 4.2.5 Clotting Time

The mean value of the clotting time was 3.5 ± 0.28; 0.45 ± 0.06 and 3.4 ± 0.07 minutes in healthy control group, poisoned dogs and recovered dogs respectively (Table-1). The clotting time was significantly reduced ($P<0.01$) in poisoned dogs.

## 4.3 BIOCHEMICAL STUDIES

The biochemical estimations in dogs of control group, poisoned group and recovered group were projected in Table-2 and Fig 7-8.

### 4.3.1 Blood glucose

The mean value of the blood glucose in control group of dogs was 55.0 ± 2.24 per cent; in poisoned group it was 70.0 ± 1,73 per cent and in treated group of dogs it was 57.0 ± 2.5 per cent (Table-2). The blood glucose levels were significantly different ($P<0.01$) among the groups.

### 4.3.2 Alanine Amino Transferase (ALT)

The mean value of ALT in healthy control group of dogs was 21.0 ± 1.32 units in poisoned group it was 72.5 ± 1.12 units and in recovered animals it was 22.5 ± 1.51 units/ml (Table-2). The ALT value was more significantly ($P<0.01$) different among the groups.

Table 2: Biochemical profile

| S. No. | Profile | Control group | Treated group | | |
|---|---|---|---|---|---|
| | | | Day-0 | Day-1 | Day-5 |
| 1. | Blood glucose (mg %) | 55.00 ± 2.24 | 55.00 ± 2.24 | 70.00 ± 1.73 | 57.00 ± 2.51 |
| 2. | ALT (Units/ml) | 21.00 ± 1.32 | 21.00 ± 1.32 | 72.50 ± 1.12 | 22.50 ± 1.51 |

Table 2(a): ANOVA of Biochemical profile

| S.No. | Profile | DF | SS | MSS | F ratio |
|---|---|---|---|---|---|
| 1. | Blood glucose | 2 | 796.00 | 398.00 | 13.90** |
| 2. | ALT | 2 | 10309.00 | 5154.50 | 487.19** |

** Significant (P<0.01) ::: * Significant (P<005) ::: NS - Not significant

Table value of F at 1% level is 6.35 and at 5% level is 3.65

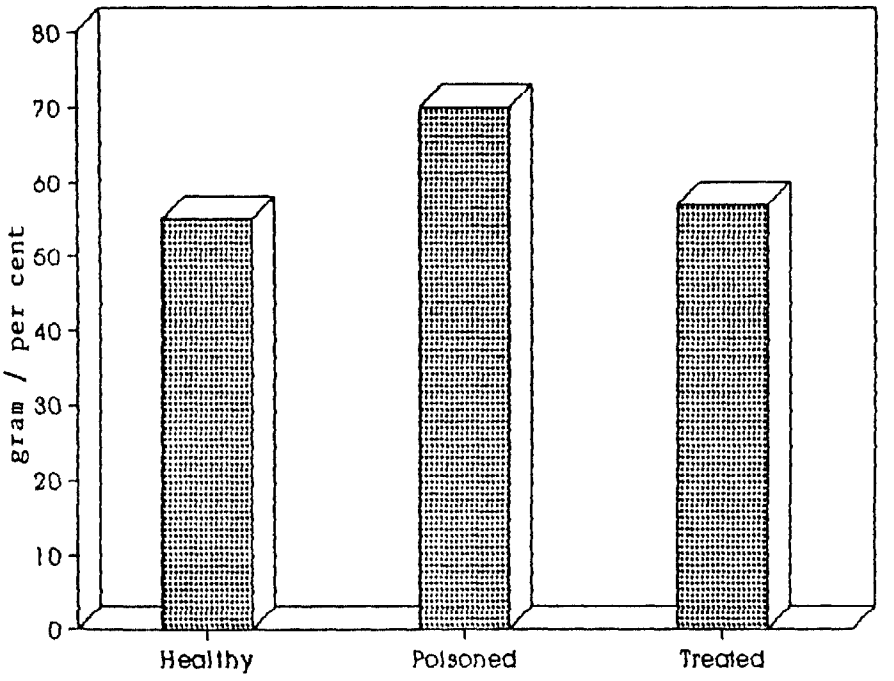


Fig.7: Histograms showing blood glucose level in healthy, poisoned and treated dogs.

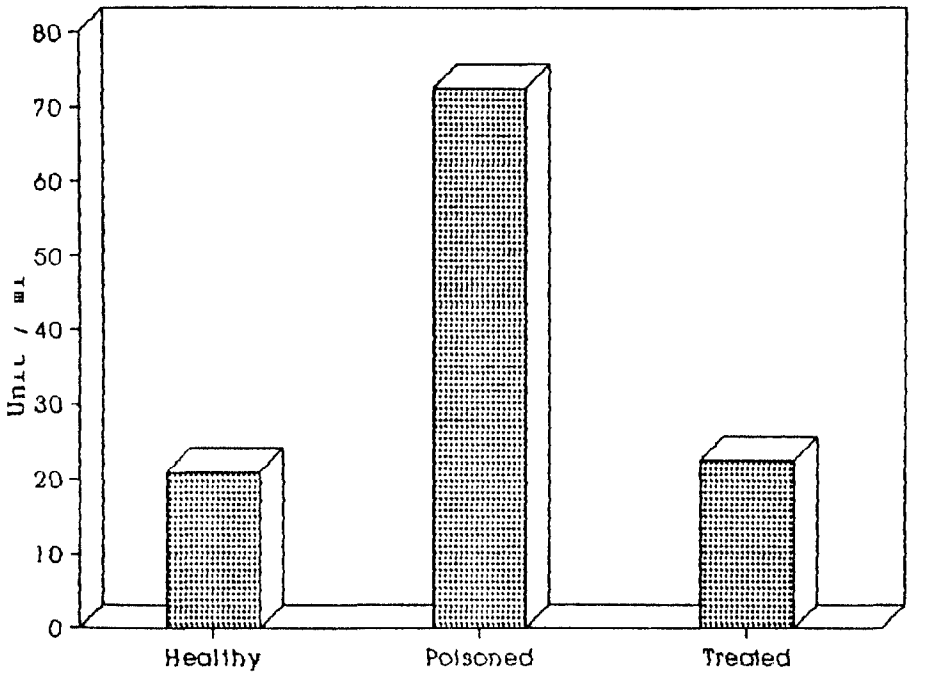


Fig.8: Histograms showing Alanine Aminotransferase in healthy, poisoned and treated dogs.

## 4.4 POST-MORTEM FINDIGNS

The autopsy conducted on dogs died during the experiment revealed the following gross lesions. The entire visera was brownish in colour indicating severe congestion of all the visceral organs (Fig-9).

**Heart:** The heart was flacid and hyperemic with enogorgement of coronary artery. The myocardium was congested with endocardiac haemonhages (Fig-10).

**Lungs:** The lungs were hyperemic and showed marked congestion (Fig-10). In few cases there was inter lobular oedema. There was pleural effusion of watery liquid and blood splashed on the surface of the lung.

**Stomach, small and Large Intestine:**

On opening the stomach there was unpleasant garlic-like odour. In all cases the stomach was empty and diffused congestion of mucous membrane was prominent (Fig-11). In majority of cases the small intestines were empty. There was evidence of congestion and haemorrhages into the bowel.

**Liver:** In all the cases the liver was dark coloured, severly congested and swollen with necrotic lesions. There was enlargement of liver and gall bladder (Fig-12). Microscopically, the liver parenchyma showed the evidence of extensive haemorrhages, along with degeneration and necrosis of hepatic cells (Fig-13).

Fig. 9: Photograph showing gross lesions of the entire viscera

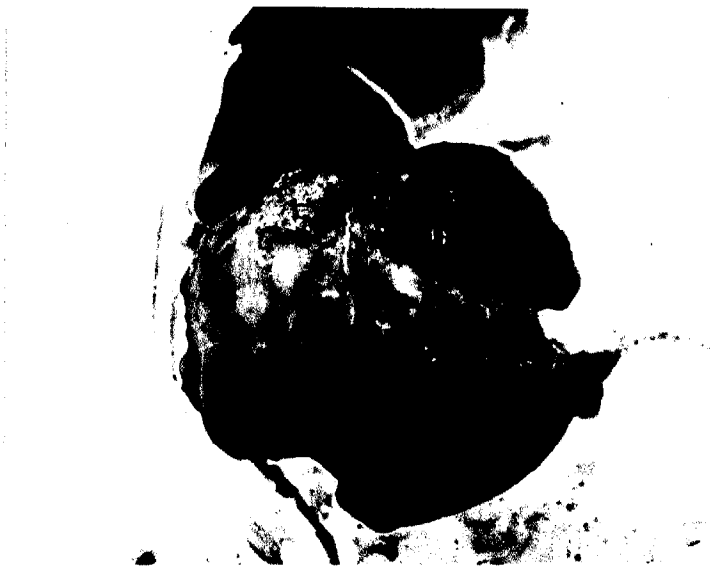

Fig. 10: Photograph showing gross lesions of heart and lungs.

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Fig. 11: Photograph showing gross lesions in the stomach.

Fig. 12: Photograph showing gross lesions on the liver.

Fig. 13: Photomicrograph of the liver (80 X H&E).

**Kidney:** The kidneys were dark, swollen and congested in majority of dogs. The bladder mucosa in few cases showed paetechial haemorrhages (Fig-14). Microscoprically, there were wide areas of necrosis of kidney parenchyma and degenerative changes of tubules (Fig-15).

**Other organs:** The reproductive organs, mesentery and peritoneum showed no significant changes.

## 4.5 THERAPY

Therapy was under taken soon after the induction of poisoning in group III animals.

1. First gastric lavage was carried-out using a stomach tube and 200 to 300 ml of normal saline solution (Fig-16 & 17). In all, three lavages were given.

2. Ringer's lactate solution was infused at the rate of 50 ml per kg. body weight, slowly, taking about 60 to 90 minutes time (Fig-18) for total infusion.

3. Dexona (Cadila-vet) was given intramuscularly at the rate 0.02 mg per kg. body weight.

4. Livobex (TTK-vet) was given intramuscularly at the rate of 2 ml each dog as a supportive therapy.

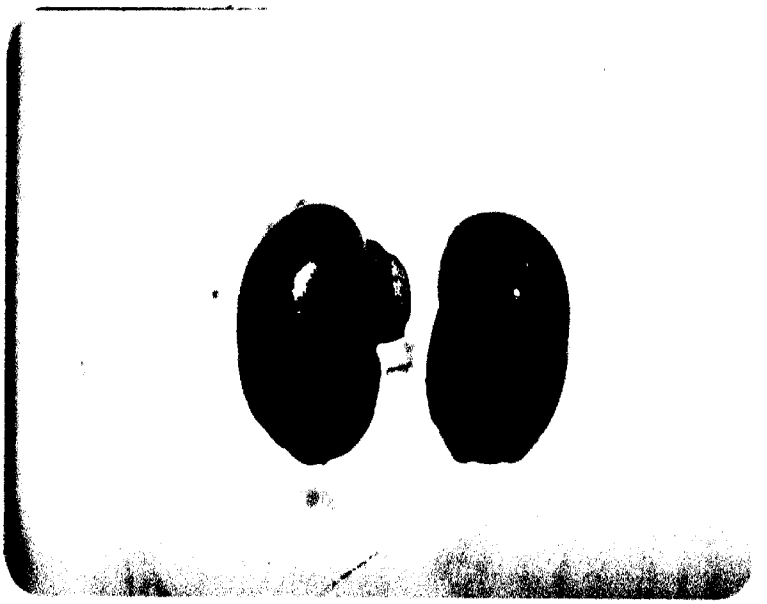

Fig. 14: Photograph showing gross lesions on the kidney.

Fig. 15: Photomicrograph of kidney (80 X H&E).

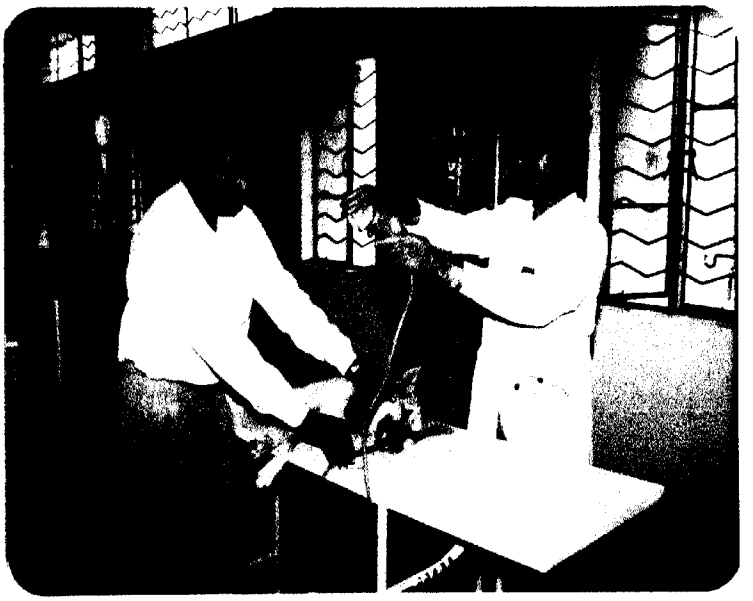

Fig. 16: Photograph showing gastric lavage - Pouring the fluid.

Fig. 17: Photograph showing removal of the gastric fluid by gravitation.

Fig. 18: Photograph showing intravenous infusion of Ringer's lactate fluid.

After first days treatment the dogs became active and were able to walk slowly, but they were anorectic and after 4 days treatment they recovered completely (Fig-19) and consumed normal diet.

Fig. 19: Photograph of a completely recovered dog.

# DISCUSSION

# CHAPTER V

# DISCUSSION

An experimental study of zinc phosphide poisoning in dogs was carried-out at the Department of Medicine, College of Veterinary Science, Rajendranagar, Hyderabad, to find out the clinical signs and haematological and biochemical changes in the posioned group of dogs. Autuopsy was conducted on the animals died during the experiment to note the post-mortem lesions. Therapeutic trial was also planned to save such cases.

During the present study of induced zinc phosphide poisoning in dogs, the animals showed clinical signs such as repeated vomitings, the vomitus had unpleasant garlic-like smell; salivation, restlessness, complete loss of appetite abdominal pain, howling and yelping, rigors, muscular tremors with lethargy and dullness. These findings corroborated with the earlier reports of Mark (1947), Nichols et al. (1949), Clarke and Clarke (1967), Rao (1977), Stowe et al. (1978) and Anon. (1991). Increased rate and depth of respirations, accelerated pulse and the rise in body temperature found in the present study were on the lines of earlier reports of Blood and Rodostits (1989) and Rao (1977). At the terminal stages, the animals showed dyspnoea, convulsions and coma followed by death as reported earlier by Clarke and Clarke (1967) and Buck et al. (1973).

The zinc phosphide when given on full stomach to monogastiric animals reacts with hydrochloric acid and liberates the phosphine gas. This gas causes gastric irritation resulting in repeated vomitings; burning sensation and abdominal pain (Prakash and Mathur 1987). The dyspnoea and increase in rate and depth of respirations might be due to lung oedema (Hamza et al; 1977; Clarke, 1984 and Tripathi et al; 1992). At the terminal stages convulsions and coma followed by death might be due to toxaemia and heart failure (Prakash and Mathur, 1987).

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In the present study, the haematological changes observed were, significant decrease ($P<0.01$) in the total Leucocyte count in the poisoned dogs as against that of healthy dogs ($8.9 \times \pm 0.6 \times 10^3$ V/S $11.50 \times 10^3 \pm 0.51 \times 10^3$. Amongst the differential leucocytic cells, the neutrophils were significantly reduced in number ($P<0.01$) in poisoned animals ($43.0 \pm 0.25$ V/S $69.0 \pm 0.34$%) and the lymphocytes were increased in count ($47.0 \pm 0.28$ V/S $21.0 \pm 0.10$%). These findings in the present study could not be explained for want of earlier data. However, Cho (1989) has reported leucopenia in cases of poisoning by organophosppho-rus compounds. The lymphocytosis seen in the present study might be due to the enlargement of lymphoid tissuess and spleen as seen in organophosphorus poisoning cases (Hamza et al., 1977).

The haemoglobin concentration was found decreased significantly ($P<0.01$) to 11.0 $\pm$ 0.75 gram per cent as against 15.5 $\pm$ 0.15 gram per cent in healthy dogs. Similarly the packed cell volume also found decreased ($P<0.01$) in experimentally poisoned dogs (31.0 $\pm$ 0.36 V/S 44.0 $\pm$ 0.36%). The decrease in haemoglobin and the packed cell volume in the present study might be due to loss of blood through paetechial haemorrhages as seen on the visceral organs (Stowe et al; 1978) at autopsy.

The clotting time was found decreased ($P<0.01$) very much in the poisoned dogs (0.45 $\pm$ 0.06 V/S 3.5 $\pm$ 0.28 minutes) when compared to healthy dogs and this might be due to that the patients with acute hepatic damage may develop disseminated intravascular clotting (DIC), a disorder characterized by activation of the blood coagulation system, which in most instances results in the generation of excess prothrombin in the systemic circulation (Feldman, 1980 and Guyton, 1991).

During the present study the changes in the biochemical profiles were also recorded. The blood glucose levels increased ($P<0.01$) to 70.0 $\pm$ 1.73 mg per cent in poisoned group of dogs as against 55.0 $\pm$ 2.24 mg per cent in the healthy dogs. These findings are in agrement with the reports of Srivastava and Rampal (1989). The increase in blood glucose level might be due to the increased stress of poison in the body releasing an hormone epinephrine

responsible for increasing plasma glucose concentration (Guyton, 1991).

The level of alanine amino transferase (ALT) also found increased ($P<0.01$) from 21.0 $\pm$ 1.32 units in the healthy dogs to 72.5 $\pm$ 1.12 units in the poisoned dogs. These findings are in agreement with the reports of Hamza et al. (1977), Kramer (1980) and Lraig et al. (1991). The increase in ALT activity might be due to the hepatic cell damage and necrosis resulting in release of ALT enzyme in the circulation (Kramer, 1980).

In the present study the autopsy was conducted on the dogs died during the experiment. The post-mortem reports revealed the following gross lesions. The entire viscera was brownish in colour indicating severe haemorrhages and congestion of all the visceral organs. The heart was flacid and hyperemic with engorgement of coranary artery. The myocardium was congested with endocardiac haemorrhages. The lungs were hyperemic and showed marked congestion with interlobular oedema. On opening the stomach, there was unpleasant garlic-like odour which was due to the release of phosphine gas. The stomach was found empty due to repeated vomitions and the mucous membrane was diffusely congested. Intestines were empty and were congested. The liver was dark in colour, congested and swollen with necrotic lesions. The kidney also was dark in colour, swollen and congested in majority of cases. The bladder

mucosa in a few cases showed paetechial haemorrhages. These post-mortum findings corroborated with the earlier reports of Dyrendahi et al. (1948), Orr (1952), Fitzaptric et al. (1955), Stowe et al. (1978), Hatch (1978), Prakash and Mathur (1987) and Tripathi et al. (1992).

As far as the treatment was concerned neither a specific antidote nor a specific treatment regime against zinc phosphide poisoning, was available. Only a symptomatic and supportive therapy was found useful in the present study. As suggested by Malone (1969), the gastric lavage was carried out in the present programme and was useful in removal of the toxic material from the stomach. Since, there were repeated vomitions, the electrotyte and fluid loss was made through the paranteral use of Ringer's lactate solution (Benjamin, 1985). In addition, the Ringer's lactate fluid was useful to reduce the acidity produced by the phosphine gas in the stomach (Hatch, 1978). Similarly the drug Dexona, a known anit-inflammatory agent used in the treatment was found useful in re-establishing the tissue perfusions and where the aggregation of platelets is involved in initiation of Disseminated intravascular coagulation, the corticosteroid might be of some value (Blood and Rodostits, 1989). The use of Livobex as a supportive therapy in the present study was justified for regeneration of hepatic cells and to protect the liver from fatty degeneration.

# SUMMARY

# CHAPTER V

## SUMMARY

An experimental study of the zinc phosphide poisoning in dogs was carried-out to report the clinical signs, haematological and biochemical changes, in the poisoned dogs and to note the post-mortem findings in the animals died during the experiment. A therapeutical trial was also conducted.

One group of dogs fed zinc phosphide poison showed the clinical signs such as repeated vomitions, abdominal pain, howling and yelping, salivation, loss of appetite, restlessness, muscle tremors, shaking of head, increased respirations, accelerated pulse and rise in body temperature. At the terminal stages, there was dyspnoea, convulsions, coma followed by death.

The haematological changes observed in induced zinc phosphide poisoning were reduction in total leucocytes from $11.50 \times 10^3 \pm 0.51 \times 10^3$ to $8.9 \times 10^3 \pm 0.60 \times 10^3$ cells, reduction in neutrophils from $69 \pm 0.34$ to $43 \pm 0.25$ per cent and increase in lymphocytes from $21.0 \pm 0.10$ to $47.0 \pm 0.28$ per cent. There was also decrease in haemoglobin content from $15.5 \pm 0.15$ to $11.0 \pm 0.75$ gram per cent, decreased in packed cell volume from $44 \pm 0.36$ to $31 \pm 0.36$ per cent and a significant reduction in blood clotting time from 3.5 + 0.28 to 0.45 + 0.06 minutes ($P<0.01$).

The biochemical estimations showed significant increase in whole blood glucose from 55.0 $\pm$ 2.24 to 70.0 $\pm$ 1.73 mg per cent and increase in Alanine aminotransferase (ALT) from 21.0 $\pm$ 1.32 to 72.5 $\pm$ 1.12 units/ml ($P<0.01$).

The post-mortem of dogs died during the experiment showed congestion and haemorrhages of all the visceral organs. The liver and kidneys were dark in colour, swollen and congested in majority of cases. The stomach was empty and emitted garlic-like odour. The gastric mucosa was congested and haemorrhagic. The microscopic examination of liver revealed the extensive haemorrhages in liver parenchyma and degenerative changes of the hepatic cells. Histology of the kidney showed wide areas of necrosis and intertubular haemorrhages in the kidney parenchyma.

For treatment of the poisoned dogs gastric lavage was carried out using saline solution. Infusions of Ringer's lactate fluid was useful to replace the elecrolyte and fluid loss. Dexona was used as an effective anti-inflammatory durg and for re-establishment of tissue perfusions. Livobex was used as a supportive drug for the regeneration of hepatic cells to protect the liver from fatty degeneration.

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## LITERATURE CITED


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# APPENDICES

## APPENDIX - A

## ESTIMATION OF BLOOD GLUCOSE

## (Folin-Wu Method)

Diagnostic Reagent Kit for the in vitro determination of Sugar in blood.

**Principle**

Glucose, on boiling with alkaline copper solution, reduces copper from cupric to cuprous state (cuprous oxide). The cuprous oxide so formed reduces phosphomolybdic acid to blue colored molybdenum blue, which is measured colorimetrically. The intensity of blue color is proportional to the amount of glucose.

**Special glassware required**

Folin-Wu tubes.

**Sample**

Whole blood (0.4 ml is required): Collect in Oxalate-Fluoride bulb.

**Reagents** (Supplied in the kit)

Reagent 1: Sulphuric Acid, 2/3 N

Reagent 2: Sodium Tungstate, 10% W/V

Reagent 3: Alkaline Copper Reagent

Reagent 4: Phosphomolybdate Reagent

Reagent 5: Saturated Benzoic Acid Solution

Reagent 6: Stock Glucose Standard, 1% W/V

**Preparation of working solutions**

Working standard: Dilute 1.0ml of Reagent 6 (Stock Glucose standard) upto 50 ml with Reagent 5.

All other reagents are ready for use.

**Procedure**

For Spectrophotometer & Calorimeter

**Step A** Deproteinisation of test sample: In a test tube

| | | |
|---|---|---|
| Distilled water | : | 2.6 ml |
| Blood | : | 0.4 ml |
| Reagent 1: $H_2SO_4$ 2/3N | : | 0.5 ml |
| Reagent 2: Sodium Tungstate, 10% W/V | : | 0.5 ml |

Mix well and allow to stand at room temperature for 10 minutes and centrifuge or filter through Whatman No.1 filter paper.

Step B Color Development: In Folin-Wu tubes

| | Standard (S) | Test (T) |
|---|---|---|
| Supernatant / Filtrate (From Step A) | - | 1.0 ml |
| Working Standard | 1.0 ml | - |
| Reagent 3: Alkaline Copper Reagent | 1.0 ml | 1.0 ml |
| Mix well and keep them in a boiling water bath for 10 minutes and cool them under running tap water. | | |
| Reagent 4: Phosphomolybdate Reagent | 1.0 ml | 1.0 ml |

Mix well and dilute the contents of each tube upto the 25 ml mark with distilled water. Mix by inversion and measure the O.D. of Standard (S) and Test (T) against distilled water at 620 mm on Spectrophotometer or using Red filter on Colorimeter.

Conclusions

$$\text{Blood Sugar in mg/100 ml} = \frac{\text{O.D. test}}{\text{O.D. Std.}} \times 200$$

## APPENDIX - B

## ESTIMATION OF ALANITE AMINO TRANSFERASE (ALT) (2-4-DNPH Method)

Diagnostic Reagent Kit for the in vitro determination of Serum Glutamate Pyruvate Transaminase, SGPT (Also called as Alanine Transaminase, ALT) activity by the method of Reitman & Frankel.

**Principle**

SGPT (ALT) Catelyses the following reaction:

- Ketoglutarate + L-Alanine L-Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4-Dinitrophenyl hydrazine (2, 4-DNPH) to give the corresponding hydrazone, which gives brown color in alkaline medium and this can be measured colorimetrically.

Sample

Venous blood in plain bulb: (0.1 ml Serum is required)

**Reagents** (Supplied in the kit).

Reagent 1: Buffered Alanine - KG Substrate, pH 7.4

Reagent 2: DNPH Color Reagent

Reagent 3: Sodium Hydroxide, 4N

Reagent 4: Working Pyruvate Standard, 2mM

**Preparation of working solutions**

**Solution 1:** Dilute 1 ml of reagent 3 to 10 ml with distill water, Reagent 1, 2 and 4 are ready for use.

**Procedure**

**Standard Curve:** As the reaction proceeds with time, more amounts of products are formed and since the end products inhibit the enzyme, their is more of inhibition. This is the major problem with colorimetric methods for the estimation of this enzyme. On the other hand in kinetic methods, since the enzyme activity is measured during the initial few minutes, the amount of products formed during that short time are negligible to cause any inhibition. Because of the above problem, it is necessary to standardize any colorimetric method against a standard kinetic method. In our kit, this standardization is done against the standard Karmen Unit Assay (Kinetic) and this is extrapolated to different amounts of Pyruvate and this has been thoroughly rechecked. At this point it is important to note that the standard graph of Enzyme activity (in Units/ml) on X-axis vs O.D. on Y-axis is not a linear one, which shows that O.D. increases with increase in enzyme acivity at a decreasing rate.

It is not necessary to plot standard curve everytime a test is performded. It should be plotted initially when the first test is performed. Subsequently, periodic checking c an be done by running only a couple of tubes, viz., Tubes 1 & 3 of the following table and their O.D. can be compared with the original curve.

**A. For calorimeter**

| Tube No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Enzyme activity (Units/ml) | 0 | 28 | 57 | 97 | 150 |
| Reagent 1: Buffered substrate pH 7.5 ml | 0.5 | 0.45 | 0.4 | 0.36 | 0.3 |
| Reagent 4: Working Pyruvate Standard 2mM, ml | - | 0.05 | 0.1 | 0.15 | 0.2 |
| Distilled water ml | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Reagent 2 DNPH Color Reagent, ml | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Mix well and allow to stand at RT for 20 min. | | | | | |
| Solution 1, ml | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |

Mix well by inversion, allow to stand at room temperature or 10 minutes and measure the O.D., of all the five tubes against distilled water on a colorimeter with a green filter.

Test

A For colorimeter

| | Test (T) |
|---|---|
| Reagent 1: Buffered Substrate pH 7.4 | 0.5 ml |
| | Incubate at 37°C for 5 minutes |
| Serum | 0.1 ml |
| | Mix well and incubate at 37°C for 30 minutes |
| Reagent 2: DNPH Color Reagent | 0.5 ml |
| | Mix well and allow to stand at R.T. for 20 minutes |
| Solution 1 | 5.0 ml |

Mix well and allow to stand at room temperature for 10 minutes and read the O.D. against distilled water on a colorimeter using a green filter.

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