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# Haematological and Biochemical Alterations after Difethialone Intoxication in Rattus rattus rufescens

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***Key words:*** *Difethialone, Haemoglobin, Haematocrit, RBC, Liver glycogen, SGOT, SGPT, Haemorrhage, Anaemia etc.*

## Abstract

*The toxic effects of difethialone on various haematological and biochemical aspects were studied. The changes in many parameters in Rattus rattus rufescens were recorded after administration of median lethal dose to both the sexes.*

*The results reveal that the difethialone feeding resulted in reduction of Haemoglobin concentration, RBC count and PCV percentage significantly in both the sexes. This may be the consequence of severe haemorrhage which results in the influx of cells and fluids from body stores. MCV and MCH were increased significantly and MCHC reduced. Overall blood picture shows a mixing of pernicious and megaloblastic anaemia. Highly significant decrease in liver glycogen, liver cholesterol and liver protein were recorded. Significant increase in serum glucose, SGOT and SGPT were noticed on the 5th and 8th days after treatment. Severe hyperglycemic conditions occur due to hepatic damage.*

## Introduction

Zinc phosphide has been used since 1940 with limited success because rats develop bait shyness and it is hazardous to non target species. The advent of anticoagulants in the early 1950 undoubtedly marked a new era in rodent control. Generally, first generation of anticoagulants are of multiple dose rodenticides, these are effective but require longer feeding period to achieve

effective control. Developed resistance and bait shyness are the two greatest hurdles to control the rodents by these anticoagulants.

Second generation of anticoagulants are of single dose rodenticides. These are highly potent compounds to control the rodents effectively and economically. Diafethialone is one of the latest second generation anticoagulant is therefore selected in the present investigation. Generally rodenticides affect the cardiovascular system of target species (Brewer and Haggerty, 1957, Roszkowski, 1965, Srihari and sridhara, 1979). Johnson and Voss(1952) haemorrhagic congestion followed by mononuclear infiltration in lungs in rats after Zinc phosphide treatment. Saxena and devi (1987) reported normochromic normocytic anaemia after calciferol treatment. To understand the correct mode of action haematological and biochemical studies performed after diafethialone intoxication in present investigation.

**Material And Methods**

**Determination of Median Lethal Dose**

Acclimatized healthy adult rats (Rattus rattus) of both the sexes having approximately uniform weight were selected for the determination of median lethal dose ($LD_{50}$). The experiment was carried out with a minimum range of six

concentrations (0.2 mg/kg to 0.7 mg/kg). The dose range was selected on No choice feeding tests.

Stock solution of difethialone (1.50 gm/litre) was prepared in propylene glycol. The animals were weighed, sexed and a calculated quantity of the stock solution containing active ingredient according to the dose was administered orally by stomach tube feeding method. The observations including poisoning symptoms were recorded. $LD_{50}$ was calculated by using Finney's method (1971).

The median lethal dose was computed for combined sexes of the test animals. The $LD_{50}$ value was 0.35 (0.31-0.39)mg/kg b.wt.

**Haematological and Biochemical Studies**

The toxic effects of difethialone on various haematological and biochemical parameters were studied. A median lethal dose was administered orally through stomach tube with vehicle (propylene glycol) according to body weight. The rats of both the sexes and each of treatment and vehicle control groups were sacrificed simultaneously at three autopsy intervals i.e. 2$^{nd}$, 5$^{th}$ and 8$^{th}$ days following the treatment. Blood was collected from the heart of rats by Burhoe's method (1940). Blood serum and tissue samples were collected for haematological and biochemical estimations.

**RESULTS AND DISCUSSION**

**Haematological Studies**

**Haemoglobin (Hb)**

An almost significant decrease in Haemoglobin content was observed on 2$^{nd}$ day followed by highly significant decrease on 5$^{th}$ and 8$^{th}$ day in both sexes.

**Haematocrit (PCV)**

A highly significant decrease was observed in packed cell volume on 5$^{th}$ and 8$^{th}$ day in both the sexes.

**RBC Count**

It is also decreased similarly as haemoglobin. The decrease was highly significant on 5$^{th}$ and 8$^{th}$ day.

**Mean Corpuscular Haemoglobin (MCV)**

The change in MCV was highly significant in females only on 5$^{th}$ day and both male and females on 8$^{th}$ day.

**Mean Corpuscular Haemoglobin (MCH)**

An almost significant difference was noted in MCH only in females on 8$^{th}$ day.

**Mean Corpuscular Haemoglobin Concentration (MCHC)**

Similarly as MCH, an almost significant difference was noted in females on 8th day only.

Results reveal that the difethialone feeding resulted in reduction of haemoglobin concentration, RBC count and PCV percentage significantly in both the sexes. This may be the consequence of severe haemorrhage which results in the influx of cells and fluids from body stores. The increase in MCV, MCH and MCHC values implies the macrocytic amaemia in rats, which can lead to very slow production of erythroblasts in bone marrow, as a result these grow oversized with shape and have fragile membranes called megaloblasts, which is a characteristic of pernicious anaemia, can lead to megaloblastic anaemia. Moss and Hathway(1964) reported that anticoagulant may enter into the RBC's through cell membrane and destroy them. Continuous hemorrhage and dilution of blood is also responsible for that. *Helalet. al.* (1975) and Whipple (1942) also reported similar findings. In view of the above findings it can be concluded that there is a mixing of megaloblstic and pernicious type macrocytic anaemia. (Table-1)

**Biochemical Studies**

**Liver Glycogen**

A highly significant decrease was noticed on 5th and 8th day in liver glycogen level.

**Liver Cholesterol**

A non significant decrease on 2nd day followed by significant and highly significant decrease was noticed on 5th and 8th day respectively.

**Liver Protien**

Estimation of liver protein revealed an almost significant decline on the 2nd day while on the 5th and 8th day following the treatment, the decrease was highly significant.

**Serum Glucose**

An almost significant increase on 2nd day followed by highly significant increase on 5thand 8th day was observed in serum glucose level.

**Serum Glutamate Oxaloacetate Transaminase (SGOT)**

A highly significant increase was recorded in SGOT activity on all autopsy days when compared with their respective vehicle controls.

**Serum Glutamate Pyruvate Transaminase (SGPT):**

A significant increase was noticed on the 2$^{nd}$ day while a highly significant rise in SGPT levels was recorded on 5$^{th}$& 8$^{th}$ days following the treatment. (Table-2)

Result indicate considerable degeneration in liver and alteration in bio-chemical parameters due to Difethialone intoxication. Decline in liver glycogen level may be due to the glycogen breakdown and hyperglycemia results due to the mobilization of stored reserves of glycogen into the plasma. Cholesterol level also falls due to the damage of hepatocytes after anticoagulant poisoning. Similarly, the fall in liver protein may also be due to the damage to the parenchymal cells of liver.

Glucose level in blood is regulated by liver. It act as a glucostat in maintaining a constant circulating glucose level. In the present investigation hyperglycemic condition occurs due to hepatic damage caused by Difethialone, increase in SGOT and SGPT may be due to the damage caused by the anticoagulant to the liver parenchymal cells release transaminases into the blood stream which is the increased permeability of cell membranes.

Biochemical studies reveals that the reduced liver protein, cholesterol and glycogen level and increased level of serum glucose, SGOT and SGPT could be attributed to the disfunctioning of the liver paranchyma.

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**Table 1 :Haematological Alterations after Difethialone Intoxication in *Rattus rattus* rufescens**

| Days of Autopsy | Sex | Haemoglobin (g/100ml) | | Haematocrit (Percent) | | R.B.C ($10^6$/cumm) | | M.C.V ($\mu^3$) | | M.C.H (Pg) | | M.C.H.C (Percent) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T | C | T | C | T |
| 2 | MALE | 14.95 ±0.69 | 13.73[a] ±0.74 | 48.2 ±3.97 | 46.24 ±3.12 | 7.55 ±0.32 | 6.85[a] ±0.17 | 63.84 ±6.54 | 67.5 ±5.87 | 19.8 ±1.99 | 20.04 ±2.16 | 31.01 ±2.35 | 29.69 ±2.99 |
| | FEMALE | 12.51 ±0.52 | 11.52[a] ±0.62 | 42 ±1.72 | 41.22 ±1.62 | 6.44 ±0.29 | 5.82[a] ±0.14 | 65.21 ±3.62 | 70.82[a] ±3.29 | 19.42 ±1.39 | 19.79 ±1.74 | 29.78 ±3.12 | 27.94 ±3.02 |
| 5 | MALE | 15.12 ±0.62 | 12.43[c] ±0.69 | 49.88 ±4.21 | 39.13[c] ±3.35 | 7.59 ±0.38 | 5.1[c] ±0.23 | 65.74 ±6.18 | 77.64 ±4.52 | 19.93 ±2.62 | 24.78 ±2.86 | 30.32 ±2.10 | 31.85 ±2.98 |
| | FEMALE | 13.11 ±0.58 | 8.69[c] ±0.56 | 44.85 ±1.19 | 36.19[c] ±1.89 | 6.55 ±0.27 | 4.34[c] ±0.24 | 68.5 ±3.15 | 84.63[c] ±3.10 | 20.01 ±2.10 | 20.14 ±2.10 | 29.23 ±2.68 | 23.97 ±2.69 |
| 8 | MALE | 15.45 ±0.66 | 10.26[c] ±0.69 | 48.85 ±3.96 | 35.55[c] ±3.67 | 7.62 ±0.39 | 3.25[c] ±0.20 | 64.1 ±3.24 | 109.38[c] ±3.86 | 20.27 ±2.86 | 31.56 ±2.67 | 31.62 ±2.35 | 28.86 ±3.01 |
| | FEMALE | 13.17 ±0.56 | 5.92[c] ±0.58 | 43.55 ±1.90 | 32.34[c] ±1.97 | 6.24 ±0.33 | 2.34[c] ±0.21 | 69.79 ±3.52 | 138.20[c] ±3.64 | 21.1 ±1.22 | 25.29[a] ±1.65 | 30.24 ±3.14 | 18.30[a] ±2.98 |

C= Control T= Treated a= P<0.05 b=P<0.01 c=P<0.001

**Table 2 : Biochemical Alterations after Difethialone Intoxication in *Rattus rattus* rufescens**

| Days of Autopsy | Groups | Liver Glycogen (mg/gm) | Liver Cholesterol (mg/gm) | Liver Protein (mg/gm) | Serum Glucose (mg%) | SGOT (units/ml) | SGPT (units/ml) |
|---|---|---|---|---|---|---|---|
| 2 | Control | 19.72 ±0.81 | 10.94 ±0.82 | 89.29 ±2.71 | 207.55 ±3.46 | 30.22 ±0.32 | 42.41 ±0.64 |
| | Treated | 15.01 ±0.48[b] | 8.96 ±1.50 | 76.15 ±2.01[a] | 220.15 ±1.54[a] | 110.25 ±0.95[c] | 47.25 ±0.52[b] |
| 5 | Control | 22.91 ±0.72 | 11.66 ±0.92 | 89.01 ±2.50 | 209.42 ±3.12 | 29.55 ±0.31 | 41.45 ±0.35 |
| | Treated | 8.25 ±0.42[c] | 6.10 ±0.71[b] | 52.51 ±1.15[c] | 358.18 ±3.10[c] | 157.45 ±0.38[c] | 97.25 ±0.91[c] |
| 8 | Control | 22.02 ±0.95 | 11.15 ±1.52 | 91.25 ±1.56 | 220.19 ±1.77 | 30.18 ±0.42 | 42.25 ±0.36 |
| | Treated | 2.10 ±0.24[c] | 3.10 ±0.78[c] | 29.35 ±1.46[c] | 263.11 ±3.61[c] | 215.59 ±0.62[c] | 138.72 ±0.42[c] |

C= Control T= Treated a= $P<0.05$ b=$P<0.01$ c=$P<0.001$

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