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# TOXIC EFFECTS OF SHORT-TERM ORAL ADMINISTRATION OF DANITOL ON THE BLOOD AND LIVER OF RABBITS

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**Abstract**: Danitol, a synthetic pyrethroid, force fed to a group of male domesticated rabbits at a dose of 10mg/kg body weight per day for seven days, produced significant abnormal changes in the blood and liver. The red blood cell count, haemoglobin content and mean corpuscular haemoglobin were significantly decreased (15%, 14% and 31%, respectively), whereas white blood cell count increased 46%. The blood serum glutamate oxaloacetate transaminase activity and bilirubin content increased 76% and 67%, respectively after 4 days of treatment, whereas alkaline phosphatase and acid phosphatase activities, and concentrations of protein and cholesterol decreased 54%, 19%, 15% and 26%, respectively after 4 days of treatment. All other biochemical parameters including liver function tests remained unchanged. In liver only isocitrate dehydrogenase activity showed a significant increase after 7 days treatment, whereas all other hepatic biochemical components remained unaltered.

**Key words**: Danitol, insecticide toxicity, liver function tests, phosphatases, transaminases, dehydrogenases, chemical composition of blood and liver, rabbit.

## INTRODUCTION

The advancement of chemical era has posed many toxicological problems. Toxicological effects are associated either with the indirect ingestion of the insecticide through drinks, food (fatty meat, dairy products, poultry eggs) (Braun and Stanek, 1982) or through occupational exposure. The use of pesticides is unavoidable, infact necessary in certain conditions to increase agriculturel production. Pyrethroids have emerged as a complement to the organochlorine, organophosphorous and carbamates, having low toxicity to mammals (Casida *et al*., 1971; Cole *et al*., 1982; Edwards *et al*., 1987; Elliott, 1971; Elliott *et al*., 1978; Gray and Rickard, 1981; Verschoyle and Barnes 1972), rapid biodegradability (Abernathy and Casida, 1973; Abernathy *et al*., 1973; Leahey, 1979; Sharom and Solomon, 1981), little residual effects (Chambers, 1980; Malhotra *et al*., 1981; Ruscoe, 1977), and higher insecticidal activity against a wide range of insects species (Elliott *et al*., 1978; Carter *et al*., 1975) including resistant strain (Saleem and Wilkins, 1984). Pyrethroids are now being used commercially on cotton, vegetable, top fruits, and veterinary products pests and a lot of work is going on the degradation of insecticide in soil plants (Ohkawa *et al*., 1980) and animals (Hutson, 1979; Hutson and Stoydin, 1978). Many scientists all over the world are busy studying various aspects of synthetic pyrethroid, such as metabolism (Casida *et al*., 1971; Chatterjee *et al*., 1986; Ghosh, 1989; Kaneko *et al*., 1987; Sharom and Solomon), 1981), pharmacological characteristics (Eells *et al*., 1987; Staatz *et al*., 1982), ecotoxicity (Salibian and Fichera, 1981) and residual detection (Akhtar, 1982; Braun and Stanek, 1982; Crawford *et al*., 1981; David, 1982; Hutson and Stoydin, 1978); but little attention has been paid to their biochemical effects on non-target organisms (El-Sebae *et al*., 1988; Shakoori *et al*., 1988). Miyamoto (1976) has reported liver abnormalities (bile duct proliferation, infilteration and hypertrophy) in rats after

exposure to different synthetic pyrethroids such as Allethrin, Furamethrin, Permethrin etc. Yu *et al.* (1988) carried out the biochemical study about the effect of Deltamethrin on animal nerves. They reported the acute and subacute toxicity of deltamethrin has its special effects on the biochemical regulation of ionic transfer in rat brain tissue. Synthetic pyrethroids seem to evince adverse effects on nerve tissue *e.g.* Decis Sumicidin, Cymbush and Isathrin inhibited cholinesterase activity of erythrocytes, liver and brain at similar concentration as phosphamides in rats (Kagan *et al.*, 1986; Aldridge *et al.*, 1987). Six month feeding of Fenvalerate in dogs resulted in hepatic multifocal granulomas (Parker *et al.*, 1984) also resulted in increased serum cholesterol and alkaline phosphatase activity. Besides having adverse effects on nerve tissue in the body, pyrethroids also have toxic effects in other organs of body. Tang *et al* (1987) studied the effects of Deltamethrin on the cardiovascular system of rabbit. Blood pressure elevation, heart rate decrease and ECG abnormality were detected in anaesthetized rabbits after intravenous injection of Deltamethrin. Pyrethroids have also been reported to cause adverse effects on blood and blood forming organs (Qadri *et al.*, 1987). Subacute and oral doses of Permasect 25 EC affect haemoglobin and red blood cell count. Cypermethrin 92 is potent towards thrombocytes and clotting time. Azodrin 71 affects white blood cell and serum protein levels. Cypermethrin and Fenvalerate induce cytogenetic damage in cultured human lymphocytes (Puig *et al.*, 1989).

Inspite of claims of low mammalian toxicity of synthetic pyrethroids, evidence is gradually accumulating against it (Parker *et al.*, 1984a,b; Puig *et al.*, 1989; Qadri *et al.*, 1987; Radahaiah *et al.*, 1989; Shakoori *et al.*, 1988; Staatz *et al.*, 1982). In the present studies toxic effects of a recently introduced synthetic pyrethroid insecticide, Danitol, have been described in rabbit blood and liver at haematological, biochemical and histological level. These findings can then be extrapolated to human beings to assess the potential hazards in the human populations due to pyrethroid exposure.

## MATERIALS AND METHODS

A group of eight rabbits, 900-1500 gms, were maintained in the Animal House of the Department of Zoology under semi-controlled temperature conditions. They were fed on green fodder and overnight water soaked grams and provided with tap water *ad libitum*.

Fenpropathrin (α-cyano, 3-phenoxybenzyl 2,2,3,3,-tetramethylcyclopropane carboxylate) used in the present study was obtained form M/S Granulars (Pak) Ltd., 1-Shadman, Lahore, Pakistan in the form of a commercial product, Danitol 10EC. Danitol (3ml) was diluted with water upto 30 ml and was force fed with the help of a glass pipette at a dose of 1 ml/kg body weight. In this way rabbits consumed 10 mg Danitol *a.i.* per kg body weight per day. This dose was administered for a total period of seven days.

The blood samples of the rabbits were collected after 4 and 7 days of insecticide treatment from the ear veins. Serum was separated by centrifugation at 2,000 rpm at 5

°C. For haematological study EDTA was mixed with small portion of blood. At the end of stipulated period the rabbits were weighed, anaesthetized, dissected and blood samples collected directly from jugular vein by a 10 ml syringe. Livers were immediately taken out weighed and stored at -30 °C until used for biochemical analyses. A small portion of liver was fixed in Bouin's fixative and processed for histological examination.

A group of animals kept as control was also processed similarly.

*Haematological studies*

Non-coagulated blood was used for the determination of haemoglobin content according to Vankampen and Zijlstra (1961). The total red blood cell (RBC)and white blood cell (WBC) counts were performed according to routine clinical methods. These data were later utilized for the calculation of mean corpuscular haemoglobin (MCH) according to Dacie and Lewis (1977).

*Biochemical analysis of blood*

Blood serum was used for the estimation of lactate dehydrogenase (LDH; EC 1.1.1.27) activity according to Cabaud and Wroblewski (1958), isocitrate dehydrogenase (ICDH; Threo-Ds- isocitrate: NADP oxidoreductase, EC 1.1.1. 42) activity according to Bell and Baron (1960), serum glutamate oxaloacetate transaminase (SGOT; 1-aspartate 2-oxoglutarate aminotransferase, EC 2.6.1.1) and serum glutamate pyruvate transaminase (SGPT; 1-alanine 2-oxoglutarate aminotransferase, EC 2.6.1.2) activities according to Reitman and Frankel (1957), alkaline phosphatase (AkP; orthophosphoric monoester phosphorylase, alkaline optimum, EC. 3.1.3.1) activity and acid phosphatase (AcP; orthophosphoric monoester phosphohydrolase, acid optimum, EC 3.1.3.2) activity according to Bessey et al. (1964). A brief account of reaction mixture with respect to each enzyme is given below.

*LDH activity*: One milliliter of $7.84 \times 10^{-4}$ M sodium pyruvate in $9.6 \times 10^{-2}$ M phosphate buffer, pH 7.5 containing $1.3 \times 10^{-3}$M NADH and 0.1ml of diluted serum (0.2:1) was incubated for 30 minutes. The reaction was stopped by 1ml of $1 \times 10^{-3}$M 2,4- dinitrophenylhydrazine in 1M HCl and $4 \times 10^{-1}$ M NaOH was used as diluent.

*ICDH activity*: In phosphate buffer, pH 7.8 containing 0.1 ml NADP (3mg/ml) and 0.1ml serum, 0.2 ml of 10 M DL- isocitrate trisodium was incubated for 30 minutes. The reaction was stopped by 0.5 ml of 1.5 mM 2,4-dinitrophenylhydrazine and 0.5 M NaOH was used as diluent.

*GOT Activity*: In 100 mM phosphate buffer with 0.2 ml serum, 0.5 ml of 100 mM L-aspartate and 2 mM 2-oxoglutarate were incubated for 30 minutes. The reaction was stopped by 0.5 ml of 1.5 mM 2-4, dinitrophenylhydrazine and 0.4M NaOH was used as diluent.

*GPT activity*: In 100 mM phosphate buffer with 0.1 ml serum, 0.5 ml of 100 mM

DL-alanine and 2 mM 2-oxoglutarate were incubated for 30 minutes. The reaction was stopped by 0.5 ml of 1.5 mM 2,4- dinitrophenylhydrazine and 0.4M NaOH was used as diluent.

*AkP activity*: 1 ml of 50mM/litre Glycine NaOH buffer (pH 10.5) which contains 0.5 mM/litre $MgCl_2$ and 5.5 mM/litre p-nitrophenyl phosphte, was mixed with 0.1 ml of serum and allowed to incubate for exactly 30 minutes. The mixture was diluted with 10 ml of 0.02N NaOH. The absorbance of the yellow coloured phenolate solution was determined at 405 nm.

*AcP activity*: 1 ml of 50mM/litre citric acid citrate buffer (pH 4.8) which contains 5.5mM/litre p-nitrophenyl phosphate was mixed with 0.2 ml of serum. The reaction mixture was allowed to incubate at 37 $^0$C for 30 minutes. Finally 10 ml of 0.02 N NaOH was added to stop the reaction. The absorbance of yellow coloured phenolate solution was determined at 405 nm.

The blood serum was analyzed to estimate protein content according to Lowry *et al.* (1951), urea content according to the diacetylmonoxime method of Natelson *et al.* (1951), cholesterol content according to Liebermann and Burchard as described by Henry and Henry (1974), glucose content according to the o-toluidine method of Hartel *et al.* (1969), bilirubin content according to Jendrassik and Grof (1938).

*Biochemical analysis of liver*

Saline extract of liver was prepared by homogenizing a weighed piece of liver (100 mg) in 5 ml of 0.89% NaCl solution with the help of motor driven Teflon glass homogenizer cooled with an ice jacket. The homogenate was centrifuged at 3000 rpm for 20 minutes. The supernatant thus obtained was used for the estimation of LDH, ICDH, GOT, GPT, AkP and AcP activities. The hepatic protein content was estimated according to Lowry *et al.* (1951).

Glycogen content was estimated according to the method described by Shibko *et al.* (1967). Nucleic acids were extracted according to the method described by Shakoori and Ahmad (1973). The DNA and RNA content were estimated according to Schmidt and Thannhauser method as described by Schneider (1957).

## RESULTS

Danitol administered at a dose of 10 mg/kg body weight per day for 7 days did not show any mortality. The growth rate was, however, significantly affected (Fig. 1). The body growth rate is drastically decreased after 2 days of insecticide feeding. The relative liver weight showed a non-significant decrease of 17% after seven days treatment.

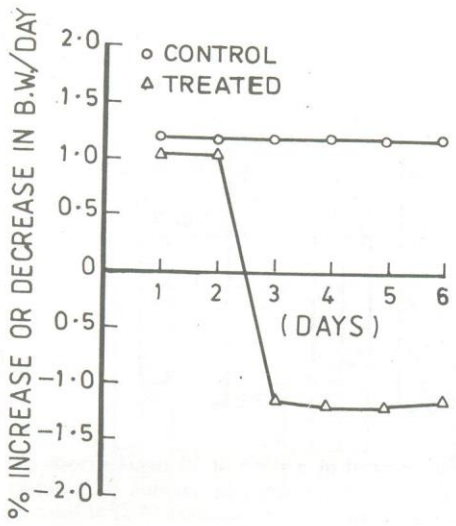


**Fig. 1**. Effect of Danitol administered at a dose of 10 mg/kg body wt/day on the body growth rate (% increase or decrease/day) of rabbits.

*Haematology*

Table 1 and Figure 2 show the effect of Danitol on haematological parameters of rabbits. RBC count in control rabbits was $5.46 \pm 0.23 X 10^6$ cells /$\mu$l of blood (n=14) which decreased 14% and 15%, respectively, after 4 and 7 days of insecticide treatment. Haemoglobin content also exhibited a significant decrease of 16% and 15%, respectively after 4 and 7 days of insecticide treatment. The WBC count however, showed 21% and 46% increase after 4 and 7 days of insecticide treatment. MCH was reduced 31% after 7 days of treatment.

**Table I: Effect of Danitol (10 mg/kg body weight/day for seven days) on haematological parameters of rabbit.**

| Parameters[a] | Control | Danitol treatment | |
|---|---|---|---|
| | 0 hour (n = 14) | 4 days (n=5) | 7 days (n=5) |
| RBC count ($x10^6$ cell/$\mu$l) | $5.46 \pm 0.23$[b] | $4.68 \pm 0.15^{**}$ | $4.66 \pm 0.17^{**}$ |
| WBC count ($x10^3$ cell/$\mu$l) | $5.23 \pm 0.34$ | $6.33 \pm 0.22^{*}$ | $7.63 \pm 0.61^{**}$ |
| Haemoglobin (g/100 ml) | $19.67 \pm 0.99$ | $15.71 \pm 0.61^{**}$ | $14.57 \pm 1.14^{**}$ |
| MCH (pg) | $36.28 \pm 1.32$ | $34.16 \pm 1.40$ | $31.30 \pm 2.29^{*}$ |

[a]Abbreviations used: MCH, mean corpuscular haemoglobin; RBC, red blood cells; WBC, white blood cells.
[b]Mean $\pm$ SEM; Student's 't' test: $^{*}P<0.05$; $^{**}P<0.01$.

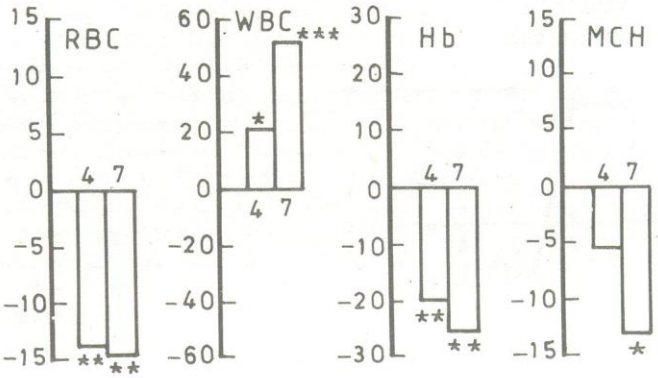


**Fig. 2.** Effect of Danitol administered at a dose of 10 mg/kg body wt/day on the various haematological parameters of rabbit. The changes in various parameters have been calculated with references to their respective controls. The numbers (4,7) at base line represent the number of days for which the rabbits were exposed to insecticide treatment.

*Blood biochemistry*

Danitol administered at a dose of 10 mg/kg body weight/day for 7 days resulted in significant changes in some enzymes and metabolites in blood serum (Table II, Fig.3).

**Table II: Effect of Danitol (10 mg/kg body weight/day for seven days) on the activities of various enzymes and concentration of metabolites in rabbit blood serum.**

| Parameters[a] | Control | Danitol treatment | |
|---|---|---|---|
| | 0 hour (n = 14) | 4 days (n = 5) | 7 days (n = 5) |
| LDH (IU/l) | 50.6 ± 7.73[b] | 115.2 ± 58.87 | 72.96 ± 25.47 |
| ICDH (SU/ml) | 643.24 ± 21.6 | 662.44 ± 21.58 | 626.48 ± 10.56 |
| GOT (IU/l) | 20.9 ± 3.06 | 36.9 ± 4.66* | 32.50 ± 13.69 |
| GPT (IU/l) | 31.53 ± 4.10 | 24.2 ± 3.79 | 40.60 ± 4.72 |
| AkP (IU/l) | 32.35 ± 3.27 | 14.96 ± 3.56** | 17.76 ± 3.11** |
| AcP (IU/l) | 30.56 ± 2.61 | 24.74 ± 1.30* | 33.55 ± 4.05 |
| Protein (g/100 ml) | 4.55 ± 0.17 | 3.85 ± 0.16** | 4.41 ± 0.13 |
| Urea (mg/100 ml) | 53.88 ± 6.66 | 42.58 ± 3.82 | 69.02 ± 9.21 |
| Cholesterol (mg/100 ml) | 531.42 ± 24.92 | 392.00 ± 13.61*** | 493.25 ± 20.17 |
| Glucose (mg/100 ml) | 283.42 ± 26.86 | 225.66 ± 31.51 | 387.37 ± 70.27 |
| Bilirubin (mg/100 ml) | 0.79 ± 0.13 | 1.32 ± 0.16** | 0.77 ± 0.13 |

[a]Abbreviations used: AcP, acid phosphatase; AkP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; LDH, lactate dehydrogenase.

[b]Mean SEM; Student's 't' test: *P < 0.05; **P < 0.01; ***P < 0.001.

GOT activity increased 76% after 4 days treatment, whereas AkP and AcP showed significant decrease of 54% and 19%, respectively after 4 days of treatment. The AkP activity was further decreased (45%) after seven days exposure. LDH, ICDH and GPT did not show any significant change.

From amongst the metabolites protein and cholesterol contents showed significant decrease of 15% and 26%, respectively, while bilirubin content increased 67% after 4 days insecticide treatment. The urea and glucose contents remained unaltered.

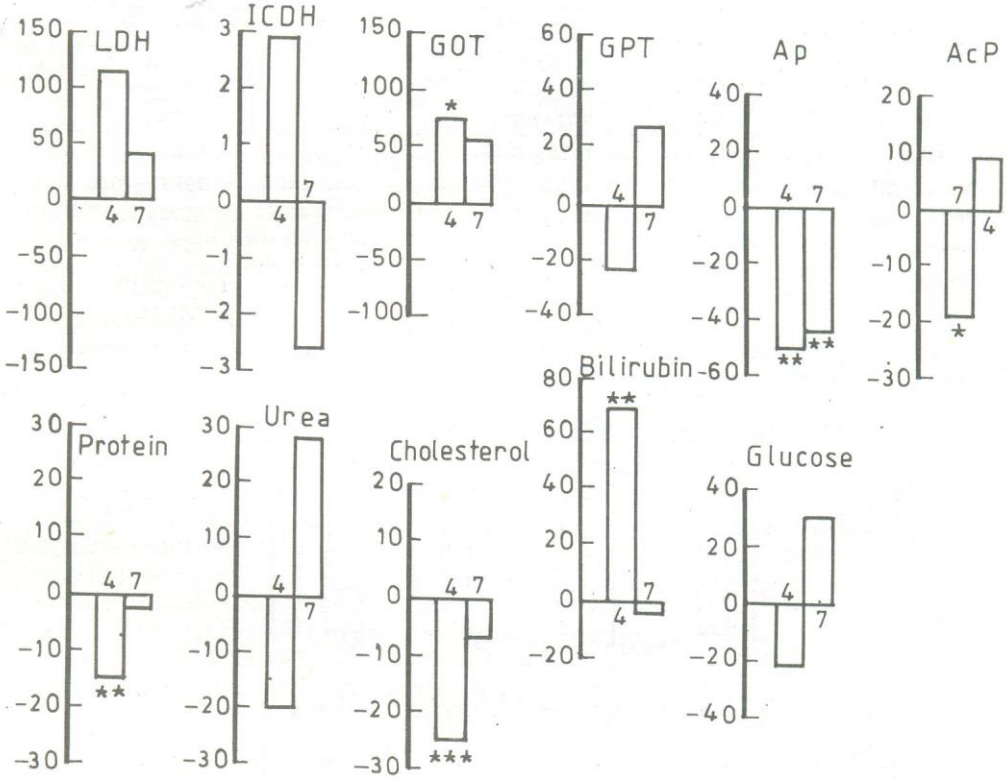


**Fig. 3.** Effect of Danitol administered at a dose of 10 mg/kg body wt/day on the activities of various enzymes and concentration of metabolites in the rabbit blood serum. The changes in various parameters have been calculated with references to their respective controls. The numbers (4,7) at base line represent the number of days for which the rabbits were exposed to insecticide treatment.

*Liver biochemistry*

Table III and Figure 4 show effect of Danitol (10 mg/kg body wt/day for 7 days) on the various hepatic enzymes and other biochemical components, which generally

remained unaltered. ICDH was the only component which showed a significant increase (8%) after 7 days of treatment.

**Table III:** EFFECT OF DANITOL (10 mg/kg body weight/day for seven days) ON ENZYMES ACTIVITIES AND CONCENTRATION OF VARIOUS METABOLITES OF RABBIT LIVER.

| Parameters[a] | Control (n=3) | Treated (n=5) |
|---|---|---|
| LDH ($X10^4$ IU/g) | 64.55±35.41 | 143.69±74.87 |
| ICDH ($X10^2$ SU/g) | 25.67±0.15 | 27.76±0.19*** |
| GOT (IU/g) | 5.74±0.84 | 6.05±0.25 |
| GPT (IU/g) | 5.72±0.33 | 5.77±0.97 |
| AP (IU/g) | 183.8±31.72 | 114.66±10.58 |
| AcP (IU/g) | 110.89±22.16 | 135.77±10.86 |
| Protein (mg/g) | 50.1±4.14 | 55.74±3.2 |
| Glycogen (mg/g) | 24.95±4.33 | 16.2±3.6 |
| DNA (mg/g) | 0.73±0.03 | 0.63±0.04 |
| RNA (mg/g) | 7.45±0.92 | 7.4±1.0 |

For abbreviations and other statistical details, see Table II.

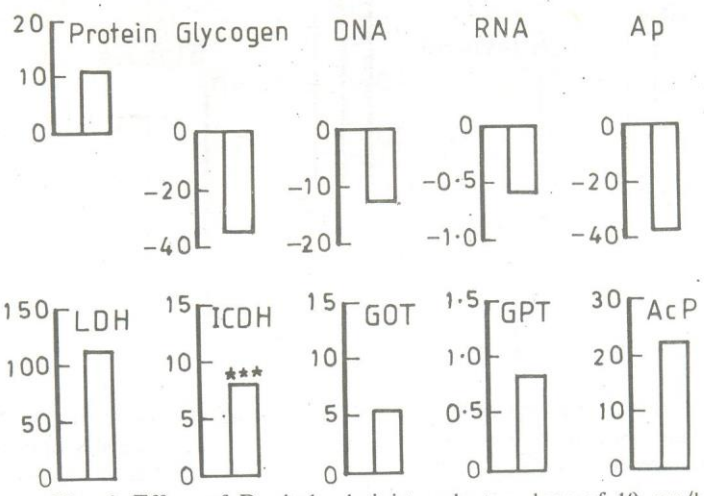


**Fig. 4.** Effect of Danitol administered at a dose of 10 mg/kg body wt/day on the activities of various enzymes and concentration of metabolites of rabbit liver. The changes in various parameters have been calculated with references to their respective controls. The numbers (4,7) at base line represent the number of days for which the rabbits were exposed to insecticide treatment.

## DISCUSSION

Almost all the haematological parameters were drastically affected after sublethal Danitol treatment. The RBC count, haemoglobin content and MCH decreased significantly. The decrease in red blood cell count and haemoglobin lowered the oxygen supply to different tissues thus resulting in low energy production. The significant decrease in RCB count can be explained on the basis of inhibitory effect of Danitol on histogenesis. Decrease in haemoglobin content and MCH can be explained due to decreased size of RBC or impaired biosynthesis of heme in bone marrow. Danitol may also have inhibitory effects on delta-aminolevulinic acid dehydratase which play a major role in haemosynthetic pathways as reported by Chiba and Kikuchi (1983). Schlegel and Kufner (1979) and Ohi *et al.* (1980) also reported inhibition of this enzyme resulting in the blockage of haemosynthetic system after treatment with lead. Danitol may be exerting some sort of inhibition on the haemosynthetic pathway. The significant increase in WBC count indicated the activation of defence mechanism and immune system of rabbit. This induction of white blood cells is a positive response for survival due to cell mediated immune response of animals (Kollar and Roan, 1980). The significant increase in WBC can also be correlated with the persistant lymphopenia and neutrophilia. Previous studies from this lab with other insecticides (*i.e.* organophophorous and organochlorines) have shown significant decrease in the haemoglobin content, red blood cell count and PCV, while a prominent increase in WBC count has always been recorded (Shakoori and Ali, 1986; Ali and Shakoori, 1981; Shakoori *et al.*, 1982,1984).

Liver is the centre of biotransformation of foreign compounds and is most vulnerable to the chemical assaults (Kulkarni and Hodgson, 1980). Varions enzymes are prone to the effect of insecticides and its metabolites. In most of the cases these enzymes leak out from necrotic hepatocytes into the blood stream in abnormal amounts. Under pathological conditions the parenchymal cells of the hepatic lobules fail to carry out vital functions resulting in disturbances in intermediary metabolism. Several of the soluble enzymes of blood stream have been considered as indicator of hepatic dysfunction and damage (Kulkarni and Hodgson, 1980). In the present study LDH, ICDH, GOT, GPT, AkP and AcP, protein, urea, cholesterol, glucose and bilirubin have been considered as indicators of liver damage by Danitol. No significant changes were observed in the levels of LDH, ICDH and GPT. GOT exhibited significant increase after 4 days treatment. GPT also increased but the increase was not significant. Increased transaminase activities have also been observed in response to other insecticides too (Lane and Scura, 1970). AkP was significantly inhibited after 7 days treatment. AcP also exhibited significant decrease after 4 days exposure and then it returned to the normal levels. All these enzymes are concerned with energy processes of body and hence decrease in their activities may be taken as indication of the impaired energy processes of the cell. AkP is found primarily in cell membrane. Decrease in its activity may be taken as an index of parenchymal damage. These findings are corroborated by the work of Onikienko (1963). Various alcohol phosphate ions, I-cysteine etc inhibit the activity of alkaline phosphatase (Lojda *et al.*, 1979). The alcohol moieties, which are metabolic products of Danitol, may be involved in enzyme inhibition. AcP is used to estimate the interference with catabolic and autophagic processes in the liver. The decrease in AcP acitvity due to Danitol

treatment could be due to decreased rate of synthesis or an increased rate of degradation of lysosomal enzymes. Roux *et al.* (1976) reported AcP inhibition together with marked induction of delta-amino laevulinate synthetase one of the rate limiting enzyme of the heme biosynthesis (Omura *et al.*, 1965; Cooper *et al.*, 1965). The increase in heme synthesis results in increase in cytochrome P-450, which enhances the detoxification of insecticide. All these events may contribute to the hypertrophy of endoplasmic reticulum and increased amount of drug metabolizing enzymes associated with drug treatment (Remmer and Merker, 1965).

Amongst metabolites, proteins and cholesterol decreased significantly after 4 days of treatment but tend to return to the normal level after seven days. The increase in protein may be due to increased rate of translation of proteins. The cholesterol decreased significantly, which may be due to decreased rate of biosynthesis of cholesterol. The increased bilirubin content may be attributable to excessive hepatocyte damage. Glucose and urea contents were unaffected.

Hepatic enzymes and metabolites did not show any significant change except ICDH which showed a prominent increase. The increased ICDH activity should imply accelerated activity of Kreb's cycle enzymes and thus enhance aerobic respiration or it may show greater activity of hexose monophosphate shunt to generate NADPH equivalent for various P-450 oxygenations.

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