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Haemato-biochemical effects of dimethoate induced sub-acute toxicity in rabbits (*Oryctolagus cuniculus*)

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Abstract
The present study was aimed to assess the subacute toxic effects of dimethoate using rabbits as mammalian model. TAFGOR (containing 35.5% dimethoate) was admistered @ 36 mg dimethoate/kg body weight per day orally for 14 days. The clinical signs of toxicity were dullness, anorexia, dehydration, excessive salivation, muscle contractions, staggering gait and loss of weight. Blood samples were collected at day 0, 7 and day 15 for hematological and biochemical analysis. The intoxicated rabbits showed a significant increase in mean monocyte, eosinophil count ($P<0.05$) and significant decrease in PCV ($P<0.05$), in comparison to control group. The mean values of Hb, TEC, MCV, MCH and MCHC, did not differ significantly ($P\leq0.05$) from corresponding control values. The mean albumin at day 7 was significantly lower ($P\leq0.05$) than corresponding control value. The mean values of ALT and creatinine in intoxicated rabbits at day 7 were significantly ($P\leq0.05$) increased as compared to the mean corresponding values of day 0 and respective values of the control group. Over the time or within the group, no significant ($P\leq0.05$) changes were observed in mean values of total protein, glucose, AST and BUN. There was 83% mortality over 14 days. In conclusion, subacute dimethoate toxicity caused marked clinical changes and mortality with noticeable hematological and biochemical alterations suggestive of hepatotoxicity and nephrotoxicity.

Keywords: Dimethoate, rabbit, subacute intoxication, hematology, biochemical changes

1. Introduction
Wide spread use and disposal of organophosphorus compounds for pest control have resulted in the release of their residue into natural water, thus inducing an environmental problem and have been widely recognized as a health hazard [1-4]. Besides fatalities, caused by high dose, exposure of animals to low dose organophosphorus insecticides has been found to cause widespread effect on body including organ specific lesions in central nervous system [5, 6], liver [7], kidneys [8] and generalized effects like immunosuppression, teratogenesis, carcinogenesis and metabolic disorders.

Organophosphorus insecticide, dimethoate, is a systemic insecticide widely used in agriculture and domestic pest control [4, 9]. It acts by interfering with the activities of cholinesterase activities and is toxic to insects, rodents, fish and humans [10-12]. Its chronic exposure has been associated with the critical increase in hepatopathy, nephropathy as well as diabetic mellitus in humans [12] and has been recognized as a possible human carcinogen [13]. Keeping in view the paucity of information regarding the toxicity of the chemical with rabbits as mammalian model, the present study was aimed at investigating clinic-pathological effects of induced sub acute dimethoate toxicity.

2. Materials and Methods
Twelve Gray giant rabbits of either sex about 1 Kg weight were maintained in cage system under standard laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee, vide No. AU/FVS/C-9/10507-12 dated 27-2-2012 and conformed to the guidelines for the Care and Use of Laboratory Animals. The rabbits were acclimatized to rearing conditions for one week. The rabbits were randomly allocated to two groups of six animals, group I (control) and group II (dimethoate intoxicated). For inducing toxicity, LD_{50} dose of TAFGOR (containing 35.5% dimethoate) was given orally in divided doses daily for 14 days i.e. 36 mg/kg body weight per day [14, 15].

The rabbits were monitored for behavioral change and mortality was recorded. Additionally, body weight and temperature was recorded at day 0, 7 and 15 in all the rabbits.
Blood samples were collected using heparin (@20 units/ml of blood) as an anticoagulant, before the start of experiment (day 0) and at days 7 and 15. The hematological investigations were carried by using MS4 multi-species hematological analyzer (MELET SCHLOESING Laboratoires-9 Chaussee Jules Cesar-Evolic 402-95520 OSNY France). The parameters recorded included Total Erythrocyte Count (TEC), Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobulin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leukocyte Count (TLC), and Differential Leucocyte Count (DLC).
For biochemical estimations plasma was collected following centrifugation of blood and stored in multiple aliquots at -20°C until used. The biochemical indices included blood glucose, total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN) and creatinine were analyzed using diagnostic kits (Aspen Laboratories Pvt. Ltd, Rapid Diagnostic Group of Companies, Karnal Road Industrial Area, Delhi, India) and semi-automatic blood chemistry analyser (model ERBA CHEM-PRO). Data were analyzed by t-test using SPSS statistical software [16].

3. Results

Dimethoate intoxicated rabbits showed dullness, depression, anorexia, weight loss, profuse salivation, feeble respiratory movements, muscle contractions and loss of response to stimulus prior to death. The dying animals fell down suddenly, assumed lateral recumbency and exhibiting wry-neck posture. The animals rolled on its back intermittently, and death occurred within 30 to 60 minutes. Death was marked by little urination and defecation indicating relaxation of sphincters. 83% mortality was observed over the 14 day experimental period. Control rabbits were healthy and active throughout the experimental period.
The mean body weight of control rabbits increased progressively and was significantly (P≤0.05) higher at day 14 when compared with baseline value, but did not differ significantly from the day 7 value (Table 1). Dimethoate intoxicated rabbits showed progressive loss of body weight. The mean values of group II at day 7 were significantly lower (P≤0.05) than mean values of control rabbits. The mean body temperature in group II rabbits was significantly (P≤0.05) lower when compared with its baseline value and control rabbits at day 7.

Table 1: Effect of Sub acute Dimethioate toxicity on body weight and temperature

Parameter	Time Period	Groups	
		Group I (Control)	Group II (Dimethoate intoxicated)
Body Weight (Kg)	0 day	1.59 ± 0.03^{aA}	1.48 ± 0.04^{aA}
	7 day	1.73 ± 0.04^{aB}	1.34 ± 0.05^{bA}
	14 day	1.86 ± 0.04^{B}	1.1 ± 0.0*
Temperature (°F)	0 day	101.26 ± 0.26^{aA}	101.25 ± 0.48^{aA}
	7 day	101.13 ± 0.32^{aA}	98.26 ± 0.20^{bB}
	14 day	101.2 ± 0.3^{aA}	96.3 ± 0.0*

Means for each parameter in different rows bearing at least one common 'lowercase alphabet' superscript, and in different columns bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P< 0.05).*Reading from single surviving rabbit

3.1 Haematology

No significant alterations were observed in mean values of Hb in both the groups (Table 2) Significant (P≤0.05) decrease in PCV values was recorded in group II at day 7. TEC, MCV, MCH and MCHC, at day 7, did not differ significantly, from baseline values, in either of the groups.
The mean values of TLC, Heterophils, lymphocytes and Basophils at day 7 in group II did not differ significantly from that of baseline values and respective group I values. The monocytes in group II were compared with baseline means while the values increased significantly (P≤0.05) from that of the control group at day 7. Eosinophil values both at day 0 and 7 were significantly higher in dimethoate group when compared with that of control but did not reveal any significant alteration at day 7 when compared with baseline values.

Table 2: Effect of Sub acute Dimethoate toxicity on hematological indices

Parameter	Time period	Groups	
		Group I (Control)	Group II (Dimethoate intoxicated)
Hb (gm/dl)	0 day	13.46 ± 0.38^{aA}	$14.10. \pm 056^{aA}$
	7 day	12.42 ± 0.44^{aA}	12.42 ± 0.89^{aA}
	14 day	11.76 ± 0.27^{A}	15±0.0*
PCV (%)	0 day	40.33 ± 0.74^{aA}	41.30 ± 1.41^{aA}
	7 day	41.08 ± 0.86^{aA}	35.40 ± 1.52^{ab}
	14 day	41.23 ± 0.56^{A}	36.2±0*
TEC(10^6/cmm)	0day	6.10 ± 0.18^{aA}	6.63 ± 0.23^{aA}
	7 day	6.17 ± 0.21^{aA}	5.72 ± 0.31^{aA}
	14day	6.25 ± 0.14^{A}	5.4±0.0*
MCV (fL)	0day	66.28 ± 1.64^{aA}	62.38 ± 0.67^{aA}
	7 day	66.91 ± 2.35^{aA}	62.43 ± 2.09^{aA}
	14day	66.06 ± 0.96^{A}	67.2±0.0*
MCH (Pg)	0day	19.62 ± 0.52^{aA}	21.34 ± 1.02^{aA}
	7day	20.20 ± 0.91^{aA}	22.26 ± 2.35^{aA}
	14day	18.85 ± 0.25^{A}	27.7±0.0*

MCHC (%)	0day	29.73± 1.23^{aA}	34.31± 1.78^{aA}
	7 day	30.32± 1.46^{aA}	35.46± 3.12^{aA}
	14 day	28.55± 0.35^{A}	41.4±0.0*

Means for a parameter in different rows bearing at least one common 'lowercase alphabet' superscript, and in different columns bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P< 0.05).*Reading from single surviving rabbit

Table 3: Effect of Sub acute Dimethioate toxicity on Total and Differential leucocyte counts (Mean ± SE)

Parameter	Time period	Groups	
		Group I (Control)	Group II (Dimethoate intoxicated)
TLC (10^3/cmm)	0 day	8.19± 1.2^{aA}	10.26± 0.82^{aA}
	7 day	9.40± 0.24^{aA}	9.97± 1.93^{aA}
	14 day	8.99± 0.11^{A}	14.78±0.0*
Heterophill (%)	0 day	32.36± 1.79^{aA}	35.46± 3.15^{aA}
	7 day	31.93±2.88^{aA}	31.88± 2.15^{aA}
	14 day	31.33± 2.14^{A}	41.6±0.0*
Lymphocytes (%)	0 day	58.25±3.98^{aA}	47.23±4.91^{aA}
	7 day	59.40±3.47^{aA}	46.70±4.00^{aA}
	14 day	61.83±2.48^{A}	36.6±0.0*
Monocytes (%)	0 day	7.06±2.88^{aA}	11.55±2.74^{aA}
	7 day	4.95±0.62^{aA}	14.50±2.14^{aB}
	14 day	4.83±0.40^{A}	16.6±0.0*
Eosinophils (%)	0 day	1.75±0.25^{aA}	5.25±1.22^{aB}
	7 day	3.00±1.24^{aA}	6.60±1.43^{aB}
	14 day	1.50±0.22^{A}	4.3±0.0*
Basophils (%)	0 day	0.56±0.20^{aA}	0.50±0.18^{aA}
	7 day	0.72±0.32^{aA}	0.32±0.12^{aA}
	14 day	0.50±0.23^{A}	0.9±0.0*

Means for a parameter in different rows bearing at least one common 'lowercase alphabet' superscript, and in different columns bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P< 0.05).*Reading from single surviving rabbit

Table 4: Effect of Sub acute Dimethoate toxicity on Biochemical parameters in rabbits (Mean ± SE)

Parameter	Time Period	Groups	
		Group I (Control)	Group II (Dimethoate intoxicated)
Total Protein (gm/dl)	0 day	5.98±0.28aA	6.51±0.43^{aA}
	7 day	5.81±0.40^{aA}	6.23±0.44^{aA}
	14 day	5.99±0.30^{A}	3.65±0.0*
Albumin (gm/dl)	0 day	3.79±0.38^{aA}	3.41±0.21^{aA}
	7 day	3.97±0.18^{aA}	3.20±0.10^{bA}
	14 day	3.93±0.10^{A}	2.84±0.0*
Globulin (gm/dl)	0 day	2.01±0.14^{aA}	3.09±0.54^{aA}
	7 day	2.00±0.13^{aA}	3.03±0.38^{aA}
	14 day	2.05±0.24^{A}	0.81±0.0*
Glucose (mg/dl)	0 day	122.28±7.39^{aA}	104.84±2.98^{aA}
	7 day	108.83±5.06^{aA}	90.90±2.51^{aA}
	14 day	112.70±4.96^{A}	78.04±0.0*
AST (IU/L)	0 day	15.49±3.96^{aA}	17.19±2.13^{aA}
	7 day	14.71±3.54^{aA}	19.87±3.23^{aA}
	14 day	14.78±3.21^{a}	24.95±0.0*
ALT (IU/L)	0 day	16.54±4.24^{aA}	15.23±2.20^{aA}
	7 day	17.18±4.23^{aA}	28.67±3.03^{bB}
	14 day	16.66±3.52^{A}	30.94±0.0*
Creatinine (mg/dL)	0 day	0.87 ± 0.06 ^{aA}	0.89 ± 0.06 ^{aA}
	7 day	0.89 ± 0.05 ^{aA}	1.12 ± 0.06 ^{bB}
	14 day	0.82 ± 0.03^{A}	0.78±0.0*
BUN (mg/dL)	0 day	18.78±3.41^{aA}	12.61±1.53^{aA}
	7 day	20.67±2.65^{aA}	14.11±0.97^{aA}
	14 day	17.23±2.78^{A}	20.54±0.0*

Means for each parameter in different rows bearing at least one common 'lowercase alphabet' superscript, and in different columns bearing at least one common 'uppercase alphabet' superscript does not differ significantly (P< 0.05). *Reading from single surviving rabbit

3.2 Serum Biochemistry

Total protein in Group II at day 7 was comparable to the base line and control values. The mean albumin at day 7 was comparable to base line mean but significantly lower (P≤0.05) than that of control value. No changes were observed in means of globulin, glucose, AST and BUN over the time or within the groups. Mean ALT at day 7 was significantly (P≤0.05) increased in Group II as compared to day 0 and respective mean value of Group I. The mean Creatinine was significantly increased (P≤0.05) at day 7 when compared with baseline values and with that of corresponding control mean.

4. Discussion

Although dimethoate is a non-persistent organophosphorus group, its large-scale use and decomposition rates in the environment cause accumulation of these compounds in soil,

from where they enter groundwater and rivers [17]. From the perusal of available literature it is evident that the abundant use of pesticides in our orchards is a warning signal that we should reduce the chemical use and adopt biological methods of controlling agricultural enemies before our environment is contaminated. In this study, the Dimethoate toxicity was experimentally induced in rabbits. The Dose of Dimethoate was selected on the basis of lethal dose of the insecticide in the rabbits.

The clinical signs observed in present study intoxicated rabbits have been reported earlier in albino rats [18]. Mortality of 83% was observed over the 14 day experimental period following dimethoate intoxication. Dimethoate has been observed to be highly toxic to different species of animals with acute LD_{50} of 500mg/Kg for dimethoate [14]. The nervous signs including lateral recumbency and intermittent rolling on back observed prior to death might be due to acetylcholinesterase inhibition [15, 19].

In this study no significant alterations were observed in Hb concentration in rabbits which were intoxicated. However, one study has observed decrease in Hb concentration due to a direct effect on bone marrow as reported in rabbits [12]. The discrepancy might be attributed to short period of study with intact compensatory mechanisms. Significantly decreased PCV might be due to malabsorption of nutrients or the hyperactivity of the animal [20]. The present observation was in accordance with previous workers [20] who observed no effect on the basophil values following dimethoate administration in mice. However, decrease in TLC and lymphocyte values following dimethoate administration has been reported in *Oncorhynchus mykiss* and mice [20, 21].

The decreased albumin may be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver [10, 12]. Significant decrease in albumin, but no overall effect on the total protein, in the present study, might be suggestive of the role of other protein fractions in the blood.

In the present study dimethoate intoxication in rabbits showed elevated ALT values indicating severe hepatotoxicity. The observations were in accordance with the reports from earlier workers in different species [10, 12, 22]. Significant increase in creatinine values may be attributed to dimethoate induced nephrotoxicity resulting in reduced creatinine clearance, an indicator of glomerular dysfunction [10, 12].

5. Conclusion

It was concluded that Dimethoate was markedly toxic to rabbits, resulting in severe clinical toxicosis and even death with repeated exposures, thus presenting Dimethoate as an important environmental contaminant and a health hazard to the humans and animals. Marked hematological alterations were noted among rabbits after the administration of sub-acute exposures. The blood biochemical alterations were suggestive of hepatotoxicity and nephrotoxicity, which may be used as a guide to diagnosis and severity of intoxication.

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