ORIGINAL PROF-607

DOSE & TIME RELATED EFFECT OF HEXACHLOROCYCLOHEXANE (hch)

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ABSTRACT

Hexachlorocyclohexane (HCH) was studied for its effects on Male Oryctolagus cuniculus rabbit blood, in variable doses of 5, 10, 15 and 20 mg/kg b.w. given orally for 30 days in order to observe some haematochemical and haematoenzymic parameter changes with regard to the toxicity produced by drug and further these changes were discussed in relation to hepatic lesions produced. Dose and time related anorexia, GIT disturbance (diarrhoea), hair loss, decreased growth rate, behavioural changes and bleeding tendency were observed in HCH exposed animals. Histological changes of liver revealed granular degeneration, dilated and congested central vein, narrowed sinusoids and periportal fibrosis. These signs increased with the increase of dose. With the dose of 15mg/kg b.w, picture showed pseudonodules formation. With further increase in dose (20mg/kg), fibrosis was less marked and necrotic lesions dominated the picture pointing to the toxic nature of the chemical. Biochemical analysis showed dose and time related hypouraemia, hyperglycemia and hypercholesteremia in treated animals. Serum alkaline phosphatase and SGPT levels also increased with the increase in dose and duration. All biochemical parameters investigated had positive correlation with the hepatic lesions pointing hepatotoxic nature of the drug.

INTRODUCTION

Widespread use of pesticides has created a major problem that of acute and chronic toxicity in man following occupational exposure and environmental contamination of food; approximately 3 millions cases of acute pesticide poisoning, with 20,000 deaths, have been estimated per annum worldwide[16,17], with 99% cases occurring in the developing countries[9,10]. Among these organochlorinate pesticides are extensively used. Chronic exposure to organochlorine pesticides produced structural changes in various organs including liver[5].Wasserman et all[15] reported high level of serum cholesterol in people occupationally exposed. Accumulation of pesticides in liver was associated with distrubance of lipid metabolism and elevated serum cholesterol[16]. Carvalho[3] reported high levels of serum alkaline phosphatase, SGPT and hyperglycemia in occupationally exposed persons in Brazil. Since liver has a large capacity for metabolic conversions and

continuously exposed to xenobiotics and therapaeutic agents, its response to noxious agents is of great interest. Organochlorine pesticides like HCH are not only being metabolized through liver, but its isomers accumulate in high concentration for a longer period[7].

MATERIALS & METHODS

A total number of fifty adult male rabbit (oryctolagus cuniculus) of approximately same age were obtained and divided into five groups A, B, C , D and E each comprising of ten animals. Group A was kept as control and given empty capsules. Group B , C , D and E were given treatment. The dosage of HCH was calculated on the basis of body weight and given orally in capsules.

One capsule containing HCH with dose level 5,10,15 and 20 mg/kg body weight was given once daily for 4 weeks to B, C, D & E group animals respectively. Body weight and general conditions were recorded on weekly basis in the morning before giving feed. Blood samples from the marginal ear vein of each animal were collected weekly for biochemical analysis. At the end of experiment, liver of each animal was dissected out, weighed, grossly examined, processed and stained for histological study. Student "t" test was applied for statistical analysis of the results.

RESULTS

The growth rates of treated animals were reduced in dose and time dependent pattern and negative growth rates were observed in group C, D & E animals with significant loss of body weights in group D and E animals (table-I & Fig 1).

There was dose related increase in Relative Tissue Weight Index [(RTWI) (Fig.2)], hair loss, anorexia, diarrhoea, behavioural changes (restlessness, excitement and hypersensitivity) and bleeding tendency (noted while taking blood samples) and these signs were more marked in D and E group animals.

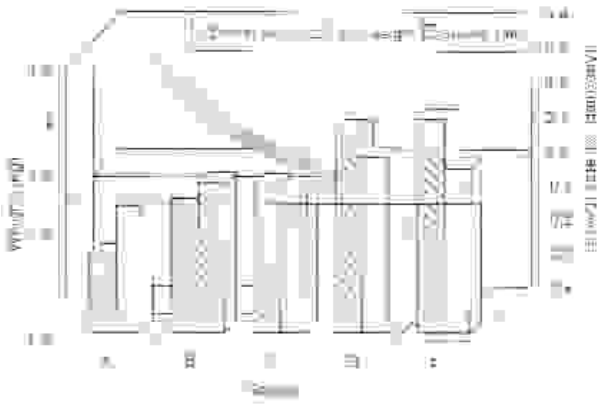

Figure-1. Graph showing the effect of Hexachlorocylohexane on the growth rate (% gain/day) of a Oryctologus cuniculus rabbit

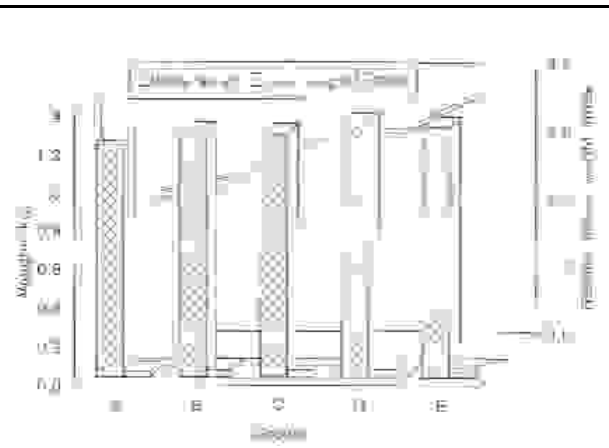

Figure-2. Graph showing the effect of Hexachlorocylohexane on the relative tissue weight index (RTWI) of a Oryctologus cuniculus rabbit

Table-I. Effect of Hexachlorocyclohexane on mean body weight, growth rate, liver weight and relative tissue weight index (RTWI) of a rabbit.

Group	Weight (kg)		Growth rate (% gain/day)	Mean liver Wt in grams	RTWI
	Initial Weight	Final Weight			
A	1.141±0.0017	1.230±0.0016	0.2966±0.0061	27.5±0.11	2.2±0.09
B	1.2445±0.0018	1.2794±0.0031	*0.1163±0.010	38.8±0.014	2.46±0.10
C	1.270±0.003	1.2651±0.0029	**-0.0163±0.005	39.77±0.26	**3.13±0.20
D	1.37±0.0035	1.32±0.0021	**-0.1666±0.006	41.5±0.17	**3.14±0.11
E	1.39±0.0033	*1.30±0.0033	**-0.3±0.01	48.7±0.01	**3.74±0.25

For statistical significance control group has been compared to treated group/strdent's t-test (p<0.01*=P<0.001**)

Table-II. Dose related effect of HCH on some hematoenzymatic parameter of rabbit

Group	Serum alkaline phosphatase U/I				Serum glutamic pyruvic transaminasa u/I			
	Ist wk	2nd wk	3rd wk	4th wk	Ist wk	2nd wk	3rd wk	4th wk
A	49.13±1.0	50.25±0.38	50.30±0.23	49.80±1.5	41.87±1.30	40.25±0.80	40.50±1.18	40.25±0.98
B	52.0±2.08	53±0.47	54.50±0.54	*57±0.63	41±0.44	43±0.82	*45±0.64	**50±1.05
C	55.0±0.86	*57±0.64	*60±1.22	**70±1.26	43±0.53	*45±1.05	**55±0.59	**60±1.22
D	*60.0±1.22	**80±1.06	**100±1.07	**110±1.26	*45±1.01	**50±0.42	**60±1.43	**69±0.39
E	*62.0±0.44	**85±0.66	**104±0.81	**114±0.73	**47±0.42	**52±0.53	**65.5±0.74	**79.5±0.42

For statistical significance control group has been compared to treated group/strdent's t-test (p<0.01*=P<0.001**)

Table-III. Dose related effect of HCH on some hematochemical parameter of Rabbit

Grp	Urea mg/dl				Glucose mg/dl				Cholesterol mg/dl			
	Ist Wk	2nd Wk	3rd Wk	4th Wk	Ist Wk	2nd Wk	3rd Wk	4th Wk	Ist Wk	2nd Wk	3rd Wk	4th Wk
A	42.25±1.01	42.5±0.81	41.87±0.59	41.75±2.16	97.25±2.72	97.5±1.84	95.5±1.21	97.75±1.23	32.3±0.8	31.87±0.42	32.37±0.94	32.0±0.88
B	41±0.71	45.5±0.01	40.5±0.42	40±0.95	97.5±1.05	97.75±1.03	*104.5±0.45	*107±0.42	33±1.3	33.5±0.86	**36.25±0.77	**38±0.51
C	40.5±0.22	40±0.29	*38±0.69	*37±0.69	99±0.59	100±0.91	*106±0.44	**109±0.63	*34±0.68	**36±0.98	**38.5±0.74	**39±0.57
D	40±.077	*39.5±.030	*35±0.69	**32±0.42	100±1.62	**107±0.55	**115±0.88	**125±0.95	*35±0.73	**37±0/76	**40±0.68	**42.5±0.5
E	39.5±0.34	*38±0.73	**30±0.61	**25±0.21	*104±0.77	**110±1.11	**130±1.11	**132.5±0.88	**36±0.69	**40±0.29	**44±0.71	**46.5±0.6

For statistical significance control group has been compared to treated group/strdent's t-test (p<0.01*=P<0.001**)

Pathological Changes

Microscopic architecture of liver revealed granular degeneration, congested sunusoids, dilated and congested central veins and mild periportal fibrosis in group B animals and these changes were more marked in group C animals. In group D animals, the liver showed marked degeneration, wide spread piece meal necrotic areas, biliary hyperplasia and marked fibrosis (Fig 3) with pseudonudule formation.

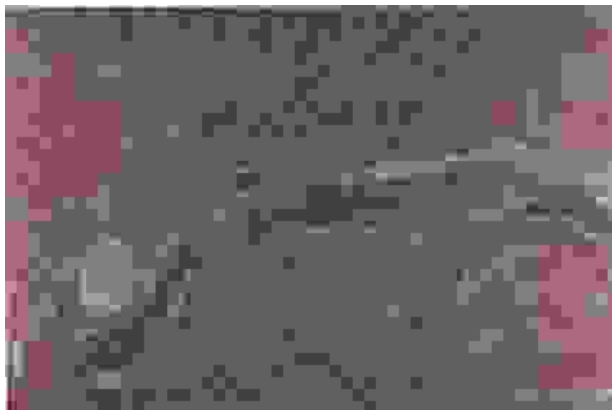

Figure-3.
i). Marked tibrosis. ii). Psedunodule

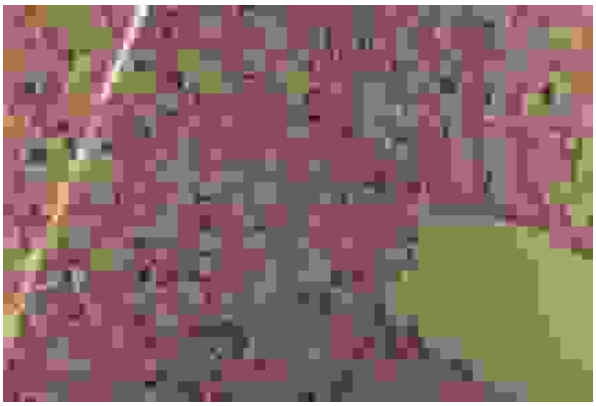

Figure-4.
i). Marked necrosis

In highest dose treated animals (group E), degeneration and necrosis were more marked and diffuse but fibrosis was far less compared to group D animals (Fig 4).

Biochemical Analysis

All blood parameters investigated remained constant in control group animals, but these varied differently in treated animals. Blood urea level gradually reduced with increase in dose. Significant decrease was noted in D and E group animals (Table-II, Fig 5-iii). Mean glucose level gradually increased with increase in dose and significant increase was observed in group D and E animal (Fig 5-iv).

Increase in serum cholesterol was recorded even earlier and with smaller doses and significant increase was noted in group C, D and E animals (table-II Fig 5-v). Serum alkaline phosphatase and SGPT levels were increased significantly even in the Ist week of treatment. Further increase followed in dose and time dependent pattern and at the end of experiment, significant increase was observed even in lowest dose treated group B animals (Table-III, Fig 5 i & ii).

DISCUSSION

Hair loss occurs with toxins deposited subcutaneously damaging directly the hair follicle[7] and since HCH has strong tendency to deposit subcutaneously[12],it would cause damage to hair follicles and thence the hair loss. Anorexia and diarrhoea in treated animals would result in low growth rate and subsequent loss of weight. Bleeding tendency could be the result of disturbance in normal hemostasis caused by partial deficiency of hemopoietic and hemostatic factors produced in organ[4]. Damaged liver would be responsible for deficient production of factors responsible for normal hemostatis, hence the bleeding tendency.

Pathological Changes

Degenerative structural changes are the outcome of metabolic disturbance, which are exhibited as fatty changes associated with water logging, hyaline degeneration ultimately leading to necrosis followed by nuclear changes in cell[13].

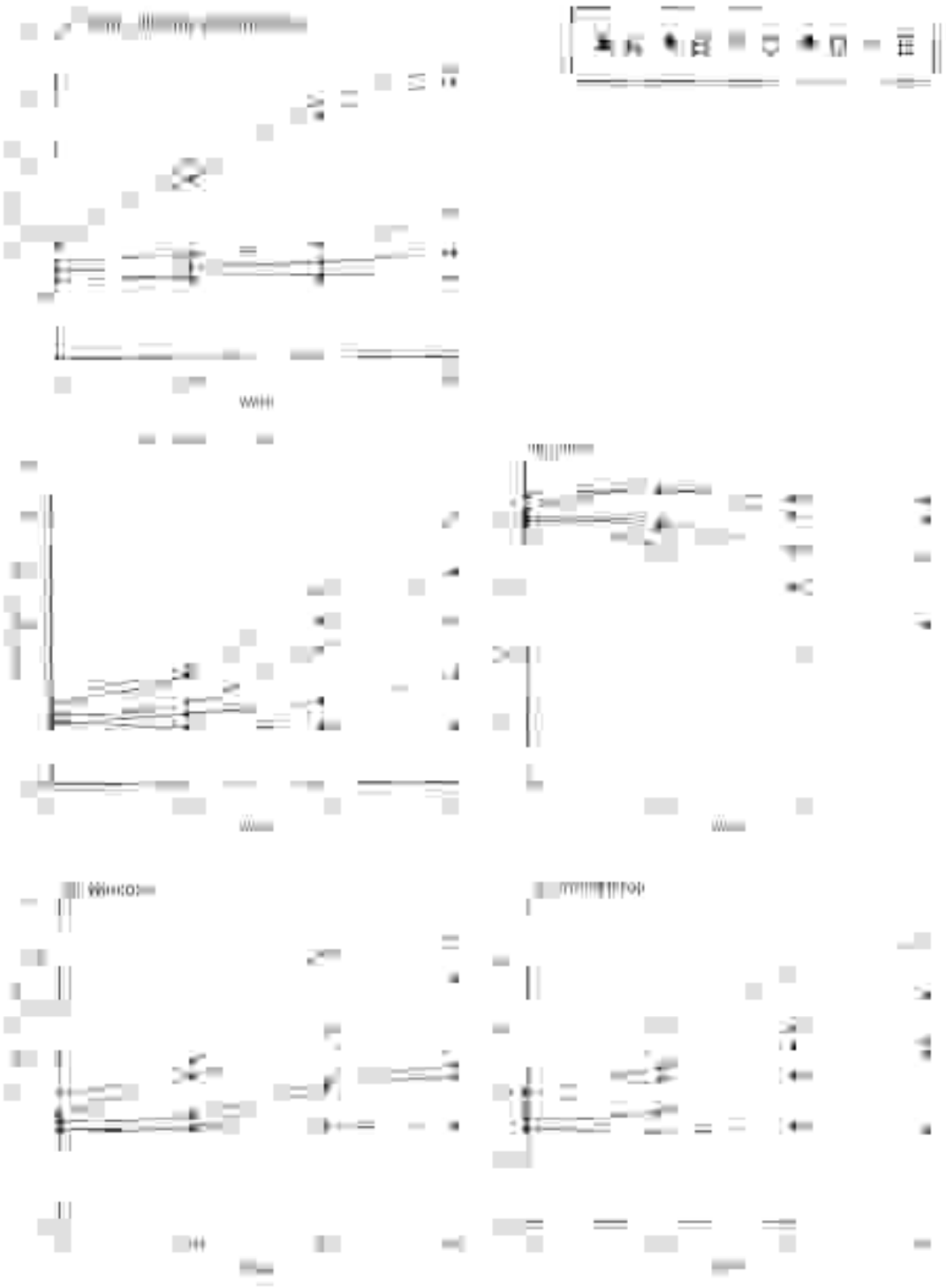

Figure-5. Graph showing dose related effect of hexachlorocyclohexane on some haematochemical and haematoenzymic parameter of rabbit

Biochemical Analysis

Urea is formed in the liver and represents the principal end product of protein catabolism[6]. Decreased blood urea concentration in this could be the result of decreased synthesis of urea in liver due to liver damage as evidenced by pathological findings.

Accumulation of some toxicant in blood and liver causes glycogenolysis in liver, hence hyperglycemia[8]. Liver is especially important in maintaining normal blood glucose concentration, the glucose which is extra or more than the need of body is converted into glycogen and stored[6]. Necrosis of liver causes hindrance in smooth functioning, glucose cannot be converted into glycogen with the result the glucose concentration rises[13]. Increased glycogenolysis due to accumulative property of toxicant and decreased glycogenesis due to necrotic lesions of liver could be responsible for hyperglycemia.

Hyperglycemia stimulates the increased secretion of insulin which binds to specific receptors of muscle and fat cells and causes increased synthesis of triglycerides and cholesterol from glucose and decreased enzymatic hydrolysis of lipids[11]. Further, fatty change in liver is always accompanied hypercholesteremia[14]. Hyperglycemia and fatty changes in liver produced by drug (HCH) could be responsible for hypercholesteremia.

The level of serum alkaline phosphatase is always considered to be an index for obstructive and degenerative hepatic disease[4]. Serum alkaline phosphatase increase may be attributed to necrosis and degenerative hepatic lesions observed in this case.

Increased serum levels of transaminases (SGPT & SGOT) have been described to be associated with cell necrosis of many different tissues and especially the increased SGPT value is considered to be specific for acute liver necrosis[4, 13]. Necrotic lesions in this study could be responsible for enzymatic changes, which in turn effect the other blood parameters, and these effects are statistically highly significant.

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