The Impact of Dietary Cholesterol, Fatty Acid, and Low-Dose Pesticide with Saturated Fat on Blood Lipid Levels: A Comprehensive Study

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Abstract: This study aims to evaluate the impact of dietary cholesterol and fatty acids on blood lipid levels and investigate the effect of low-dose pesticide exposure in combination with saturated fat on blood lipid levels. The study utilizes male Balb/c mice divided into five treatment groups: control, fat, cholesterol, low-dose pesticide (DDVP), and DDVP combined with fat. The animals were subjected to oral administration of the respective treatments for 14 days. Various parameters, including body weight, organ body weight index, hematological parameters, and lipid profiles (total cholesterol, triglycerides, HDL, and LDL), were evaluated at the end of the exposure period.

Keywords: Dietary cholesterol, fatty acids, blood lipid levels, statistical analysis.

I. INTRODUCTION

The composition of diet exerts an important influence on the levels of serum triglycerides, cholesterol and phospholipids. Dietary fats and oils provide calories and essential fatty acids and also sources of fat-soluble vitamin A, D, E and K. In literatures it has been report that certain types of fats can increase risk of chronic CVD. Saturated fatty acids (SFA) and trans fatty acids are unhealthy whereas Monounsaturated fatty acid (MUFA) and polyunsaturated (PUFA) are considered beneficial. [1] Various types of Lipoproteins but two most are LDL (low density Lipoproteins) or bed cholesterol and HDL (good cholesterol) [2, 3]. these findings have leads to worldwide recommendations to decreases the consumption of saturation fat as well as cholesterol rich food items to decrease risk of CVD. The dietary Guidelines published by U. S [4]. Department of agriculture and USDA and USDHHS (U. S. Department of health and human services have been criticized for recommendation of cholesterol free diet [5], In addition, adipose tissue is a compartment which contains a high amount of persistent organic pollutants in organisms that are the top of food chain. Evaluate the biochemical parameters after chronic exposure [6]. The chronic exposure of DDVP (Dichlorvos) with fat or without fat consumption affected the toxicity or remains unaffected.

Materials and methods

2.1. Materials

Effect of fatty acid on blood lipid level as well as it influences in the chronic toxicity of DDVP has been tabulated in the present study. Methodology of purpose study has been given below:

2.1.1 Chemical

DDVP, (trade name Suvan) was procured from trade. all other a chemical used work of analytical grade. Vegetable oil trade name Gagan vanaspati ghee was purchased from local market. acetyl thiocholine iodide, cholesterol and other chemicals were procured from Sigma chemical Co.

2.1.2 Animals: -

Randomized in bread mail bulb c mice wing 25 se 30 were used for the study steam outlet paddy hospital was used as banding material in polypropylene cages and 4 mice house in each case the paddy has changed on alternate day the animal were kept in environmentally control room control room temperature 25 to 20 degree Celsius and work provided with palette diet and portable water and leave it on the study was approved by institutional animal ethics committee (constituted by CPCSEA, ministry of environment and forests, India)

2.2. Experimental Design: -

Five treatment groups of animals consisting 6 male Balb c mice of weight range 25-30 grams, in each group, were evaluated. Oral administration using oral feeding needle of fat, cholesterol, DDVP, and DDVP+ fat was given as treatment, while normal saline was given to the control group. Fat has been given in the form of vegetable oil at the dose of 1gram per kg per day, cholesterol at 2 gram per kg per day and ddvp at 0.6 MG per kg per day, orally was given up to 14 days. Treatment concentration of cholesterol and DDVP was prepared in normal saline (I.P.), which was given to the control group as well. In order to examine the effect of these treatments, following parameters was ebulliated 24 hours after 14 days continuous exposure

2.3 Sample collection and storage: -

At the end of the experiment period (14 days) animals were anesthetized with anesthetic ether I.P. (Narsans Pharma, India), and blood samples were collected from each treatment groups after 12 hours of fasting. blood was drawn from the orbital plexus of mice by heparinized glass micro-capillaries and stored in heparinized micro centrifuge tubes for hematology while rest of the blood was used for serum extraction for the collection of serum blood was allowed to clot at 37 degrees Celsius and the centrifuged for 10 minutes at 2700 RPM 100 microliter serum was collected and Store at

-80 degree Celsius until use. After the blood collection, each animal was dissected and the whole brain, heart, kidney, liver, spleen and lungs were removed quickly and stored at -80degree Celsius until use.

immediate release microspheres.

2.4 Evolution parameters: -

Body weight: -

Body weight of mice was monitor daily throughout the experimental period for all the groups. Organ body weights index: -Final body weight of each live animal was recorded before the dissection. After dissection whole brain, kidneys, heart, liver spleen and lungs were stripped from fatty tissues blood vessels and dried by blotting paper and then weighted on digital balance to determine the organ body weight index

Hematology: -

Hematological parameters, such as red blood cells (RBC), white blood cell (WBC), lymphocyte and neutrophil, platelet count and PCV% determined, using bio analyzer (mindray, BC 300, Germany)

Lipid measurements: -

The profiles measured were total cholesterol, triglycerides, high density lipoproteins (HDL) and law density lipoprotein (LDL). Total cholesterol was analyzed by following the zak's method of cholesterol estimation, whereas other serum lipids like triglycerides and high-density lipoprotein (HDL) were analyzed using commercial diagnostic kits: triglycerides (Code no. AGD -TG100 from AGD clinical) and HDL (Code no. 120227 from Erba, Mannheim). LDL was analyzed using Fried Ewald's equations. the levels of the serum lipids are expressed in mg /dl.

2.5 Estimation of total cholesterol: -

Reagents

1. $FeCl_3$- CH_3COOH reagent (0.05%) - 0.05 gms of $FeCl_3$ is dissolved in 100 ml of aldehyde free CH_3COOH.

2. Concentrated H_2SO4

3. Cholesterol standard

4. Stock solution- 100 mg of cholesterol is disso

5. lved in 100 ml of acetic acid.

6. W0orking standard solution -2 ml of stock solution is dissolved in (or) dilute to 50 ml with $FeCl_3$ CH_3COOH solution. The concentration of standard 0.04 mg /ml.

Procedure

1. Pipette at 1 -5 ml of standard solution in a series of test tube.

2. The volume in each test tube is made up to 5 ml with Fecl3 CH3COOH reagent.

3. 3ml of conc. H2SO4 is added to all the test tubes and mix well.

4. standard is incubated for about 20-30min.at room temperature.

5. the intensity of standards is measured at 560nm against blank.

Blank: 5 ml of $Fecl_3$ - CH_3COOH reagent, 3 ml of H_2SO4 are taken in a test tube, mixed well and used as a blank.

Test: -

1. In the centrifuged tube 0.1 ml of serum and 10 ml of Fecl3 CH3COOH reagents are taken, mixed well for 5 minutes and then centrifuged.

2. 5 ml of supernatant is collected and added with 3 ml of H2SO4.

3. Test is incubated at room temperature to 20 -30 intensity is measured at 560nm again blank.

Calculation: -

$$\text{Cholesterol (mg/dl).} = \frac{\text{Absorbance of test} \times \text{conc. Of std.}(0.4 \text{ mg/dl})}{\text{Absorbance of standard}}$$

2.6 Estimation of triglycerides: -

The samples and reagents (Table no.1) have been brought of to room temperature prior to use. All the reagents were mix according to the following scheme and the absorbance (A) were read after 5 minutes incubation at 37°C.(Table no.2)

Calculation: -

$$\text{Triglycerides con. (mg/dL)} = \frac{\text{Absorbance of sample} \times \text{conc.of std.}}{\text{Absorbance of std.}}$$

Table No.1 Reagents

Goods buffer pH 7.2	50mmol/L
Lipase	150KU/L
Glycerol kinase	55IU/L
Glycerol phosphate oxidase	5KU/L
Peroxidase	50 IU/L
ATP	1mmol/L
Chromogens	2mmol/L

Table No.2 Quantity of chemicals

	Blank	Standard	Sample
Reagent	1ml	1ml	1ml
Standard	-	10μL	-
Sample	-	-	10μL

2.7 Estimation of high and density of proteins (HDL) :-

Reagents -1: precipitating reagent Phospho-tungstic Acid 2.4mmol/l, Magnesium chloride 40mmol/l, HDL

Cholesterol standard: 25mg/dl.Magnesium chloride 40mmol/l, HDL Cholesterol standard: 25mg/dl.

Procedure :-   All the reagents were mixed as per the (Table no.3) scheme and kept at 30 degree C for 10 min.for

incubation. absorbance of the standard of each test was read at 505 nm or 505 /670 nm for dichromatic analysis

against reagent blank.

Calculation

$$\text{HDL Cholesterol} = \frac{\text{Abs.of test} \times \text{conc.of std.} \times \text{dilution factor}}{\text{Abs.of std}}$$

Table No.3 Chemical and reagents

Pipette in to tubes marked	Blank	Standard	Test
Cholesterol working reagent	1000μL	1000μL	1000μL
Distilled water	50μL	-	-
HDL standard	-	50μL	-
Supernatant	-	-	50μL

2.8 Estimation of law density Lippo protein (LDL): -

LDL cholesterol was determined by using **"Friedewald formula"** which is based on measure values for total

cholesterol, HDL-C, triglyceride. VLDL cholesterol (VLDL-C) was calculated by dividing values of TG

(triglycerides) with 5. LDL-C was calculated by subtraction of combined value of HDL-C and VLDL from total

concentrations of cholesterol.

2.9 Estimation of enzyme AChE activity: -

Activity of enzyme AChE was determined following modified method described by Ellman at al. At the time

of assay, the whole brain tissue was Thawed, dilute 1:10 in 0.25M sucrose solution and homogenized using

vertical homogenizer (REMI motors, India). Homogenization was done for 100 seconds; however, after every

20 seconds of homogenization, was done for the homogenizer was deepen for 10 second into crushed ice for

cooling. The homogenates were twice centrifuged (Sigma ® Laborzentrifugen model 3-18 K, Germany) at

8,500 Xg 40 °C for 10 minutes. The supernatant was the decanted and the pellet was dilute in 0.35 M sucrose

solution for assay. The reaction was started by adding 2.6 ml phosphate buffer PH 8,100 µl of DTNB (5, 5'-

Dithio-bis (2- nitrobenzoic acid) in phosphate buffer pH 7.0 and 20µl of sample and the reaction mixture was incubated for 3 minutes 37°C. Into the reaction mixture, 20µl of ASChI, dissolved in phosphate buffer pH 7.0, was added. The blank contained the phosphate buffer in the place of substrate and the enzyme activity was read in Kinetic mode (UV VIS spectrophotometer specord ® 200 Analytik Jena AG, Germany) AChE activity was expressed as µ moles of ASChI hydrolyzed/min/ gm of brain and µmoles ASChI hydrolyzed/min/20µl.

Statistical analysis: -

The results were expressed as mean ± standard error of the mean. Individual groups were compared with control grouop using t-test. One-way ANOVA was also done to compare the groups. DDVP+VG and DDVP groups were compared separately to know the effect of vegetable oil. significant differences were indicated by p- values ≤ 0.05. All the statistical analysis was done using Sigma stat and graphs were prepared sigma plot software.

3. Results and discussion

3.1 Body weight: -

Body weight of mice was monitor daily throughout the experimental period for all the groups. Organ body weights index: -Final body weight of each live animal was recorded before the dissection. After dissection whole brain, kidneys, heart, liver spleen and lungs were stripped from fatty tissues blood vessels and dried by blotting paper and then weighted on digital balance to determine the organ body weight index.

Table 4 Result of one-way repeated measures ANOVA for body weight among the treatment groups during the experimental period:

No. of Days	(Mean Percent Body Weight ±SEM) in treatment Groups				
	C	CH	VG	DDVP+VG	DDVP
0	100 0	100 0	100 0	100 0	100 0
2	95.18 ±1.411	96.77 ±0.616	95.11 ±1.116	96.09 ±0.639	94.80 ±1.79
4	99.069 ±1.31	100.06 ±1.438	97.644 ±1.438	98.60 ±2.81	93.9. ±4.68
6	102.31 ±1.38	100.26 ±2.946	99.168 ±2.122	102.10 ±6.018	100.915 ±1.24
8	100.76 ±1.821	98.54 ±2.854	101.20 ±2.41	101.93 ±6.007	101.26 ±1.44
10	101.51 ±2.55	99.199 ±2.429	100.97 ±2.46	93.39 ±6.564	98.64 ±1.907
12	104.54 ±2.99	103.25 ±2.72	100.938 ±4.03	95.32 ±7.173	97.188 ±3.32
ANOVA					

P Value	0.0005	0.40	0.227	0.893	0.149
F Value.	5.605	1.074	1.453	0.3612	1.763

Control=C; Cholesterol=CH; Vegetable oil=VG; Dichlorvos +Vegetable oil=DDVP+VEG;

Dichlorvos=DDVP,N=6

Doses: Normal saline@ 200 μl/animal/day for c group, Dichlorvos @ 0.6 mg/kg/day for DDVP group

Dichlorvos @ 0.6mg/kg/day +Vegetable oil 1g/kg/day for DDVP+VG orally,Vegetable oil @ 1g/kg/day for VG group,Cholesterol @ 2g/kg/day/for CH group

Table 5 Percent organ weights with respect to whole body weights if control and tested animal

Treated Groups	Mean body wt. before dissection (gm)	Organ body weight index of group (Mean Percent weight of organs ±SEM)					
		Lungs	**Liver**	**Kidney**	**Spleen**	**Brain**	**Heart**
C	28.98 ±1.517	0.731±0.093	6.268±0.250	1.185±0.048	0.605±0.056	1.385±0.060	0.529±0.027
CH	30.79 ±0.747	0.684 ±0.42	6.255±0.171	1.321±0.048	0.518±0.044	1.2857±0.0633	0.622*±0.030
VG	27.62 ±1.09	0.8900 ±0.265	5.879±0.292	1.4031*±0.038	0.7208±0.219	1.4152±0.036	0.5798±0.023
DDVP+VG	27.855 ±1.746	0.5810 ±0.050	5.0170*±0.337	1.299±0.070	0.4850±0.108	1.529±0.127	0.495±0.019
DDVP	29.38 ±1.528	0.5397 ±0.040	4.867*±0.257	1.391*±0.075	0.4654±0.0422	1.2846±0.119	0.588±0.034

*P≤0.05: Significant as compared to control

#P≤0.05 for heart weight when DDVP+VG and DDVP groups were compared

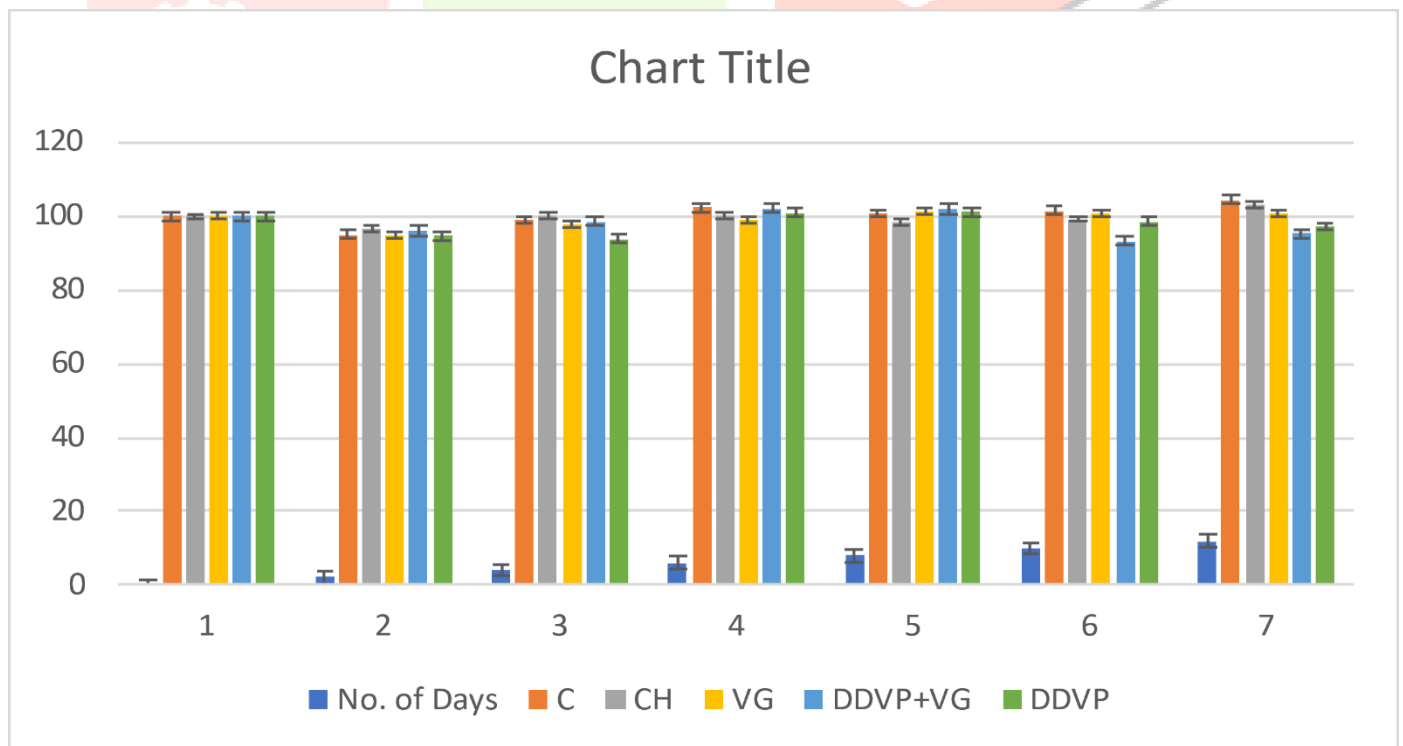#P≤0.05 for heart weight when DDVP+VG and DDVP groups were compared, n=6

Figure-1

3.2. Effect on Blood profile: -

Table 3 represents the different parameters of hematology of treated and control groups.

Among all groups values of hemoglobin was found to be least in VG group as compared to control (P≤0.05). RBC were found to be least (P=0.0017; F=6.218) in both VG and DDVP+VG group as compared to control group. Significant anova variation observed in percent values of neutrophils in VG DDVP+VG AND DDVP group while the platelet count was varying in each treatment group with respect to control. Among all groups minimum variation was observed in CH group only as compared to control in all parameters. DDVP+VG and DDVP groups exhibited significance variation in platelet

counts and % PCV when compared with each other (P≤0.05).

*P≤0.05: Significant as compared to control

#P≤0.05 for heart weight when DDVP+VG and DDVP groups were compared

#P≤0.05 for heart weight when DDVP+VG and DDVP groups were compared

Table 6 Effect of treatment on the blood profile of mice

Parameters Studies	Hematology of Treatment Groups (Mean±SEM)				
	C	CH	VG	DDVP+VG	DDVP
Haemoglobin(g/dl)	12.76±0.7004	10.82±0.92	5.30*±0.46	11.716±1.06	14±1.487
RBC(x10^9/ml)	7.456±0.513	5.91±0.592	3.947*±0.1743	4.958*±0.330	6.085±0.546
WBC(x10^6/ml)	15.66±2.106	15.966±1.44	16.550±1.49	13.86±1.29	12.4±1.04
Segnebted neutrophil (%)	12.6±0.812	13.8±1.28	28*±8.256	17.5*±1.258	16.8*±1.46
Lymphocyte (%)	81±0.894	80±1.414	63.5±9.751	76.16*±1.352	74.83*2.05
Eosinophil (%)	4.4±0.244	5±0.365	6.5±1.500	6*±0.5164	4.83±0.307
Monocyte (%)	2±0.00	1.833±0.166	2±0.00	2±0.00	2±0.00
Platelet count (x10^5/ml)	4.29±0.71	2.94±0.460	2.777±0.515	10.358±2.344	11.31*±1.89
PCV (%)	32.58±2.368	25.9*±3.225	16.42*±0.592	19.166*±1.308	24.066*#±2.291

3.1 Lipid profile of serum:

Result of estimation of lipid profile have been presented in table 4 serum triglycerides of all the groups showed significance variation among the groups *(one way ANOVA, P≤0.001)*.Value of TG was observed in decreasing order of group as C>VG>CH>DDVP+VG>DDVP (figure2). Highest value of cholesterol was found for the group which was fed with vegetable oil (VG group), in comparison of control group (C). There was no significant change was observed in high density lipoproteins (HDL) in any of experimental group. Low density lipoprotein (LDL) values were higher for all the treatment groups with respect to control group (P≤0.05).

Table:7 Results of estimation of lipid profile of each group, after treatment

Parameters for lipid Profile (mg/dl)	Treatment Groups (Mean ± SEM)				
	C	CH	VG	DDVP+VG	DDVP
Total Cholesterol P Value	177.37±17.663	245.95±18.24 0.119	256.55±17.76 0.03	225.18±4.7 0.057	225.71±20.42 0.425
Triglycerides P Value	235.53±48.25	90.15±8.96 0.037	128.2±16.025 0.22	44.19±6.3 0.02	20.86±2.9 0.01

HDL P Value	23.59±0.9847	25.54±1.607 0.8	26.2±0.39 0.225	23.86±1.17 0.885	22.3±1.21 0.77
LDL P V0.43alue	98.85±1.3	203.03±27.95 0.004	223.99±17.42 0.0026	194.5±5.36 0.001	220.17±45.3 0.0098

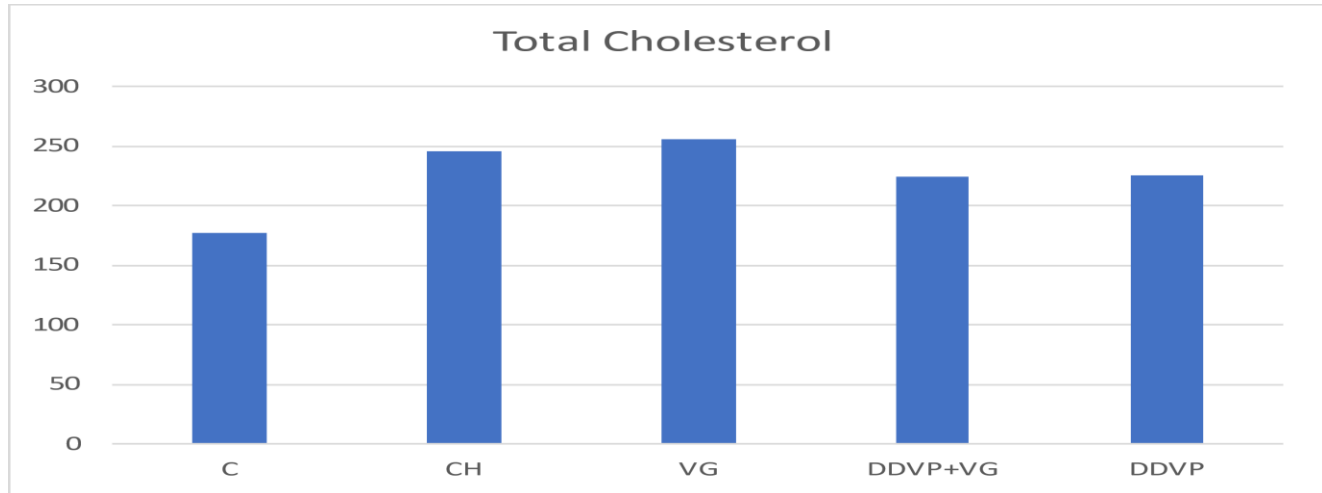Individual Treatment groups were compared with control group using 't' test:n=6

Figure 2 Effect of cholesterol

3.4 Estimation of AChE: -

Estimation of reactivated of brain AChE activity has been presented in table 5. No significant difference was observed in the activity of AChE in brain of all treatment groups expect VG w hich showed least % of AChE inhibition than control.Highest inhibition was found in serum samoles of DDVP group as compared to control and other groups.This value was also significantly higher then DDVP+VG group when it was compare with DDVP(P=0.04).

Table 8 AChE estimate in serum and brain of mice after treatment

	C	CH	VG	DDVP+VG	DDVP	P VALUE	F VALUE
	AChE in treatment group One way ANOVA (Mean± SEM) in μ mols/gm						
Brain	2.22± 0.108	2.3300± 0.213	2.09± 0.24	2.205± 0.195	1.77± 0.088	0.298	1.31
Serum	5.29± 0.40	4.75± 0.089	4.19*± 0.13	4.57± 0.27	4.106*± 0.43	0.094	1.129

P≤0.05 compared to control group n=6

Result of the present study suggest that cholesterol and fat rich diet treatments do not significantly affect the body mass and cholesterol level in blood can be increased by taking fat and cholesterol rich diets.From this study this is also evident that high cholesterol and fat as diatary element does not affect the blood cholesterol level.

4. Conclusion

In this study exposure of low doses of pesticide may not show any pronounced effect of toxicity but reduce serum AChE levels suggest the if it is exposed for long duration; accumulation of these pesticide can cause adverse effect on health.

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