CHAPTER-5

COMBINATION EFFECT OF CHLORPYRIFOS AND LEAD AT THE BIOCHEMICAL LEVEL BY REPEATED SUBCHRONIC DIETARY EXPOSURE

Introduction

Chlorpyrifos, a well known OP compound, exhibits its toxicity in insects and vertebrates mainly inhibiting enzyme cholinesterase. On other hand the heavy metal lead has systemic effects on hematopoietic system, kidney, brain, gastrointestinal system, heart and immune system, which are well known. These two chemical agents are often present in food commodities as residues. Hence, it is very much essential to know their interactive effects when they are simultaneously challenged to test systems.

Clinical pathology tests being used in subchroinic studies play important role in detection of potential toxic effects on organ systems. Many tests currently being used in regulatory toxicology studies have been useful/applied in the risk assessment of chemicals. Some of the tests are very much applicable to intraspeices and interspecies extrapolation. It has been observed that there is down-regulation of hematology values in all species when the hematopoeitc system is affected. U.S. National Toxicology Program has recently evaluated the usefulness/relative sensitivity of clinical chemistry tests in identification of potential hepatic and renal toxic effects. They had evaluated 61 subchronic studies in rats for different chemicals. Liver and kidney lesions were reported in 31 and 41 % of the studies

respectively. They had observed, association between treatment related increase in ALT and SDH activities and histopathological changes in liver. There was also association between treatment related increase in BUN and creatinine and morphological changes in kidney (OECD, 2002). Considering the significance of clinical pathology tests, the present study was planned to investigate the interactive effects of a combination of chlorpyrifos and lead acetate on hematology and clinical chemistry parameters in Wistar rats on oral administration at relatively lower doses through diet for a minimum period of 90 consecutive days.

Materials and Methods

Clinical pathological tests were conducted on blood samples collected from all the animals at the end of 4^{th}, 13^{th} and 17^{th} weeks of experimental exposure. Blood samples were collected by puncturing the orbital sinus plexus with the help of a fine capillary tube under ether anaesthesia. Animals were fasted over night before blood sampling.

Around 0.5 mL of blood was collected in vials containing EDTA for hematology analysis. One drop of blood was taken on a clean glass slide, spread and stained with Leishman's stain for differential leucocyte count. For determination of clotting time, blood was allowed to flow in to a 7.5 cm capillary tube and the time required for clotting was recorded manually.

Around 1.5 mL of blood was collected from each animal in clean centrifuge tubes for serum preparation. The blood was allowed to clot at room temperature and the serum was separated by centrifugation at low speed. Approximately 0.5 mL of blood was used for RBC acetylcholine esterase estimation.

The experimental procedure is represented in Chapter 4. The parameters and procedure for hematology and clinical parameters are the same as that employed in single dose study (Chapter 2).

RESULTS

Hematology

Males

Hematological analysis of blood performed at the end of week 4, revealed significant decrease in HCT value in group 7 animals. Groups 2 and 5 animals revealed statistically significant decrease in MCV value and significant increase in platelet count. Further, MCHC value was significantly increased in groups 5 and 7 animals as compared to control group of animals (Table 1A).

After 13 weeks of exposure, significant reduction in Hb content was observed in group 4 animals as compared to control group of animals. Slight but no significant decrease in mean value of RBC, Hb and HCT were observed in animals of groups 3, 4, 6 and 7 animals. The per cent decrease in mean RBC count was 4.7, 5.8, 5.8 and 5.8 in animals of groups 3, 4, 6 and 7 animals, respectively. The mean Hb content was reduced by 3.1, 4.3, 3.7 and 3.1 per cent in groups 3, 4, 6 and 7 animals, respectively and that of HCT by 3.5, 5.5, 3.5 and 5.1 % in groups 3, 4, 6 and 7 animals, respectively. Though there were 10 fold differences between low and high dose groups, no dose dependent response was observed. Since lead causes anemic effects, this slight reduction in RBC count, Hb content and HCT value was considered to be treatment related. Statistically significant decrease in mean clotting time was observed in groups 4, 5, 6 and 7 animals (Table 1B).

At the end of recovery period, MCHC values of group 5 recovery (G5R) animals were significantly increased as compared to control recovery group animals. Significant decrease in mean clotting time was observed in animals of recovery groups 5 (G5R), 6 (G6R) and 7 (G7R) as compared to control recovery group animals (G1R) at the end of recovery period (Table 1C).

Females

The slight reduction in RBC content, Hb content and HCT value was observed in animals of groups 3, 4, 5, 6 and 7 as compared to control group of animals after 4 weeks of exposure. The RBC count was decreased by 7.7, 9.2, 6.2, 9.2 and 6.2 % in 3, 4, 5, 6 and 7 groups of animals respectively. The per cent decrease of Hb content was 2.8, 6.4, 2.8 and 2.1 in groups 3, 4, 6 and 7 respectively and that of HCT value was 7.6, 7.6, 7.6, 6.8 and 7.6 in groups 3, 4, 5, 6 and 7 respectively. Significant increase in MCHC value was observed in group 5 animals as compared to control group animals at the end of 4 weeks of exposure (Table 1D).

After 13 weeks of exposure RBC content, Hb content and HCT value of treatment group animals were comparable to the control group of animals. Statistically significant decrease in clotting time was observed in animals of groups 3, 4, 5, 6 and 7 (Table 1E).

At the end of recovery period, there were no significant variations observed between recovery animals of treatment groups and control recovery group (Table 1F).

Clinical Chemistry

Males

At the end of 4 weeks of exposure, the mean values of sodium and chloride content in groups 3, 4, 6 and 7 were significantly increased (Table 2A; Figures 3 and 5). Group 7 animals showed significant decrease in mean values of ALT and calcium as compared to control group of animals (Table 2A; Figure 9). The activity levels of serum cholinesterase were significantly decreased in groups 5 and 7 animals. RBC cholinesterase activity was reduced by 27.4 and 17.3 % respectively in groups 5 and 7 animals as compared to control group of animals after 4 weeks of exposure (Tables 2A and 3; Figure 1).

After 13 weeks of exposure, serum glucose level was significantly increased in groups 4, 5 and 7. The activity level of AST and calcium content were significantly decreased in groups 6 and 7. Group 7 animals revealed significant decrease in potassium content. The serum and RBC cholinesterases were significantly decreased in animals of groups 5 and 7 (Tables 2B and 3; Figures 1 and 10).

After 4 weeks of recovery period, group 7 recovery animals (G7R) showed significant increase in glucose level and calcium content as compared to respective control group (Table 2C; Figures 13). The levels of ALT in group 7 recovery animals (G7R), AST in recovery animals of groups 6 (G6R) and 7 (G7R) were significantly decreased as compared to control recovery (G1R) animals (Table 2C).

Females

After 4 weeks of exposure, the mean values of serum sodium content in groups 3, 4, 6 and 7, potassium content in groups 2, 3 and 6 and, chloride content in group 7 were significantly increased as compared to control animals. Further, calcium content was significantly decreased in groups 3 and 6 but lacking dose response. The reduction in activities of serum and RBC cholinesterases were observed in groups 5 and 7. The percent decrease of serum and RBC cholinesterases in groups 5 and 7 animals was 27.4 and 13.7 and 30.4 and 13.3 respectively after 4 weeks of exposure (Tables 2D and 3; Figures 2, 7 and 11).

At the end of treatment period, Group 6 animals showed decrease in calcium content as compared to control animals. The potassium content in groups 3, 6 and 7 were significantly increased. The activities of serum and RBC cholinesterases were significantly decreased in groups 5 and 7 animals (Tables 2E and 3; Figures 2 and 12).

After recovery period, level of serum glucose was significantly increased in group 7 animals. The RBC cholinesterase activity was reduced by 14.7 and 34.5.0 % in animals of groups 5 and 7 respectively (Tables 2F and 3; Figures 2 and 14).

Remaining parameters of serum of treated group animals are comparable to control group animals.

Table 1A

Hematology – Group Mean Values

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex: Male **Period: 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
WBC (10^3/µL)	Mean	13.8	13.9	17.1	12.9	12.5	12.2	15.2
	SD	3.43	1.07	3.70	3.01	1.55	1.83	5.69
RBC (10^6/µL)	Mean	6.5	6.9	6.3	6.8	6.9	6.6	6.4
	SD	0.42	0.34	0.73	0.44	0.37	0.32	0.19
Hb (g/dL)	Mean	13.8	14.2	13.3	14.3	14.7	14.4	13.9
	SD	1.45	1.03	1.45	0.99	0.45	0.58	0.40
HCT (%)	Mean	41.8	40.0	39.2	41.00	40.2	39.8	37.6
	SD	2.47	2.89	2.29	2.36	1.43	1.55	1.51
MCV (fL)	Mean	64.8	58.1*	62.9	60.6	58.1*	60.1	59.1
	SD	4.90	2.86	10.14	3.05	1.83	3.22	1.70
MCH (pg)	Mean	21.3	20.7	21.1	21.2	21.2	21.8	22.0
	SD	0.99	1.09	1.36	0.97	0.88	1.19	0.80
MCHC (g/dL)	Mean	33.1	35.6	34.1	34.9	36.5*	36.3	37.2*
	SD	3.12	0.20	4.73	1.53	0.67	0.97	0.89
Platelet (10^3/µL)	Mean	657.0	974.7*	856.3	859.5	942.8*	865.0	736.0
	SD	205.56	169.01	204.01	183.04	130.87	100.60	112.76
Clotting Time (seconds)	Mean	120.0	125.0	155.0	145.0	140.0	130.0	165.0
	SD	37.95	35.07	55.05	29.50	24.50	15.49	31.46
Lymphocyte (%)	Mean	80.7	80.3	81.3	81.3	76.1	79.1	83.4
	SD	4.89	2.58	15.36	4.50	3.71	6.88	10.53
Neutrphil (%)	Mean	17.0	17.7	16.3	16.0	20.2	22.8	14.0
	SD	5.22	2.58	5.56	5.02	2.14	6.40	6.75
Monocyte (%)	Mean	1.5	1.3	1.3	0.8	2.0	1.0	1.4
	SD	0.55	0.86	0.37	0.25	0.49	0.20	0.52
Eosinophil (%)	Mean	0.8	0.6	1.0	1.8	1.7	1.2	1.0
	SD	0.25	0.22	0.43	0.57	0.85	0.45	0.04
Basophil (%)	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Key : N= Number of observations ;

* = significant at 5% level ($p \leq 0.05$)

Table 1 B

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex: Male **Period: 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
WBC ($10^3/\mu L$)	Mean	15.2	16.6	17.6	16.3	14.3	12.9	12.7
	SD	2.99	5.32	7.40	5.14	4.26	2.41	4.22
RBC ($10^6/\mu L$)	Mean	8.6	8.5	8.2	8.1	8.4	8.1	8.1
	SD	0.20	0.54	0.27	0.40	0.48	0.39	0.20
Hb (g/dL)	Mean	16.3	16.1	15.8	15.6*	16.3	15.7	15.8
	SD	0.71	1.31	0.40	0.46	0.76	0.58	0.59
HCT (%)	Mean	45.5	45.4	43.9	43.0	44.4	43.9	43.2
	SD	2.19	3.73	1.41	1.89	2.08	1.77	1.16
MCV (fL)	Mean	53.1	53.3	53.3	53.4	52.7	54.1	52.5
	SD	2.19	2.19	1.31	1.68	1.24	2.64	0.92
MCH (pg)	Mean	19.0	18.9	19.2	19.4	19.4	19.8	19.2
	SD	0.63	0.81	0.37	0.69	0.74	1.01	0.48
MCHC (g/dL)	Mean	35.8	35.5	36.0	36.3	36.7	36.7	36.6
	SD	0.53	0.67	0.55	0.70	1.14	0.99	0.64
Platelet ($10^3/\mu L$)	Mean	1114.5	1088.8	1004.3	970.5	1079.4	923.3	952.6
	SD	118.8	124.2	145.7	148.9	104.4	125.7	106.3
Clotting Time (seconds)	Mean	210.0	183.8	168.8	157.5*	153.8*	146.3*	131.3*
	SD	32.07	46.58	15.53	21.21	19.23	29.73	15.53
Lymphocyte (%)	Mean	83.9	84.4	82.6	86.5	85.8	84.5	81.1
	SD	2.70	4.07	8.19	5.66	2.05	5.24	3.52
Neutrphil (%)	Mean	13.8	13.0	14.8	11.3	12.5	14.0	16.3
	SD	2.25	3.55	8.36	4.71	2.20	5.48	4.50
Monocyte (%)	Mean	1.0	0.8	0.6	0.6	0.6	0.4	0.8
	SD	0.46	0.29	0.06	0.07	0.06	0.07	0.04
Eosinophil (%)	Mean	1.4	1.9	2.0	1.6	1.1	1.1	1.9
	SD	0.69	0.13	0.51	0.09	0.89	0.13	0.63
Basophil (%)	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$)

Table 1 C

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Male **Period: 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
WBC (10^3/µL)	Mean	15.6	13.1	12.3	14.2
	SD	2.69	2.79	2.60	1.32
RBC (10^6/µL)	Mean	8.6	8.4	8.6	8.6
	SD	0.38	0.34	0.56	0.26
Hb (g/dL)	Mean	15.6	15.8	16.0	15.7
	SD	0.37	0.33	0.88	0.73
HCT (%)	Mean	44.0	43.5	44.8	44.2
	SD	1.32	1.00	2.30	2.19
MCV (fL)	Mean	51.2	51.7	52.2	51.4
	SD	1.84	1.79	1.44	1.79
MCH (pg)	Mean	18.1	18.8	18.6	18.2
	SD	0.56	0.51	0.37	0.65
MCHC (g/dL)	Mean	35.4	36.3*	35.7	35.5
	SD	0.53	0.43	0.53	0.48
Platelet (10^3/µL)	Mean	1217.0	1077.8	1079.2	1056.2
	SD	111.76	106.71	107.11	117.12
Clotting Time (seconds)	Mean	150.0	115.0*	110.0*	95.0*
	SD	18.97	22.58	24.49	12.25
Lymphocyte (%)	Mean	81.5	80.7	81.8	80.5
	SD	10.05	3.93	5.78	3.78
Neutrphil (%)	Mean	16.3	15.3	15.3	15.2
	SD	8.71	3.61	3.39	1.94
Monocyte (%)	Mean	0.5	1.0	0.2	0.3
	SD	0.15	0.43	0.08	0.09
Eosinophil (%)	Mean	1.7	3.0	2.7	3.8
	SD	0.75	0.9	1.06	1.54
Basophil (%)	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0

Key : N= Number of observations, * = significant at 5% level ($p \leq 0.05$)

Table 1 D

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex: Female **Period: 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
WBC (10^3/µL)	Mean	13.2	13.2	15.2	10.9	12.8	17.1	12.8
	SD	2.16	3.50	4.29	4.30	3.59	13.17	4.65
RBC (10^6/µL)	Mean	6.5	6.3	6.0	5.9	6.1	6.0	6.1
	SD	0.45	0.54	0.59	0.52	0.40	0.78	0.45
Hb (g/dL)	Mean	14.1	14.2	13.7	13.2	14.2	13.7	13.9
	SD	0.91	1.40	0.77	1.02	0.66	1.61	1.05
HCT (%)	Mean	38.4	36.8	35.5	35.5	35.5	35.8	35.5
	SD	1.93	3.11	1.86	1.67	1.71	1.50	1.66
MCV (fL)	Mean	59.4	58.8	59.4	60.5	58.7	61.0	58.0
	SD	2.52	2.87	3.35	6.30	2.25	8.27	3.06
MCH (pg)	Mean	21.7	22.6	22.8	22.4	23.4	23.1	22.8
	SD	1.20	1.02	1.13	0.92	1.03	1.05	0.38
MCHC (g/dL)	Mean	36.7	38.5	38.5	37.3	39.9*	38.4	39.4
	SD	2.32	1.17	1.23	3.07	1.33	4.48	2.17
Platelet (10^3/µL)	Mean	758.7	973.0	871.7	757.3	757.2	800.2	948.3
	SD	93.33	97.54	90.66	66.87	85.19	74.38	99.84
Clotting Time (seconds)	Mean	125.0	125.0	115.0	125.0	125.0	140.0	150.0
	SD	35.07	44.16	44.16	12.25	35.07	30.98	26.83
Lymphocyte (%)	Mean	86.8	85.7	85.2	79.7	87.3	90.0	86.3
	SD	3.87	6.47	6.68	10.21	4.93	3.35	8.14
Neutrphil (%)	Mean	12.2	12.0	13.0	18.8	10.8	8.8	12.0
	SD	4.26	4.73	6.39	10.19	5.0	2.79	6.72
Monocyte (%)	Mean	0.5	0.7	0.5	1.0	1.0	0.0	0.5
	SD	0.15	0.25	0.24	0.35	0.35	0.0	0.15
Eosinophil (%)	Mean	0.5	1.5	1.3	1.0	0.7	1.2	1.2
	SD	0.14	0.87	0.42	0.29	0.13	0.35	0.25
Basophil (%)	Mean	0.0	0.0	0.0	0.0	0.0	00	0.0
	SD	0.00	0.0	0.00	0.00	0.0	0.00	0.00

Key : N= Number of observations ; * = significant at 5% level ($p \leq 0.05$)

Table 1 E

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period: 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
WBC (10^3/µL)	Mean	12.9	16.4	14.2	13.7	13.2	12.7	13.5
	SD	3.50	4.96	4.20	4.37	3.74	3.04	4.34
RBC (10^6/µL)	Mean	7.1	7.2	7.3	7.2	7.2	7.2	7.1
	SD	0.21	0.40	0.31	0.26	0.27	0.21	0.41
Hb (g/dL)	Mean	15.1	15.0	15.1	15.2	15.4	14.9	14.8
	SD	0.49	0.90	0.84	0.78	0.70	0.51	0.85
HCT (%)	Mean	39.3	39.6	39.3	39.8	39.9	38.9	38.5
	SD	1.46	2.23	2.06	2.10	1.45	1.52	1.89
MCV (fL)	Mean	55.1	55.1	53.9	55.5	55.9	54.0	54.2
	SD	0.87	1.56	0.92	1.64	2.49	1.54	1.47
MCH (pg)	Mean	21.2	20.8	20.8	21.2	21.6	20.8	20.9
	SD	0.35	0.38	0.42	0.53	0.99	0.47	0.56
MCHC (g/dL)	Mean	38.5	37.8*	38.5	38.2	38.6	38.4	38.6
	SD	0.52	0.53	0.27	0.71	0.45	0.50	0.50
Platelet (10^3/µL)	Mean	893.0	842.3	968.4	866.5	825.0	902.0	935.6
	SD	80.74	99.61	154.26	99.94	75.05	116.68	125.21
Clotting Time (seconds)	Mean	168.8	150.0	120.0**	108.8**	101.3**	101.3**	108.8**
	SD	15.53	22.68	16.04	22.32	15.53	15.53	15.53
Lymphocyte (%)	Mean	83.8	82.6	87.3	86.3	88.0	84.6	87.9
	SD	5.70	4.17	4.62	5.75	4.60	4.84	5.17
Neutrphil (%)	Mean	14.1	14.6	9.0	11.9	10.5	12.6	9.9
	SD	5.11	3.58	3.07	5.17	3.78	4.98	2.42
Monocyte (%)	Mean	1.0	0.3	1.4	0.6	0.5	0.6	0.5
	SD	0.26	0.16	0.40	0.24	0.29	0.22	0.29
Eosinophil (%)	Mean	1.1	2.5	2.4	1.3	1.0	2.1	0.9
	SD	0.13	0.87	0.67	0.14	0.31	0.42	0.13
Basophil (%)	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Key : N= Number of observations,

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 1 F

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period: 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
WBC (10^3/µL)	Mean	9.4	8.2	8.2	12.2
	SD	1.96	2.44	2.18	3.67
RBC (10^6/µL)	Mean	7.7	7.5	7.8	8.0
	SD	0.56	0.44	0.42	0.32
Hb (g/dL)	Mean	15.3	15.1	15.5	15.7
	SD	0.40	0.70	0.91	0.64
HCT (%)	Mean	41.5	41.0	42.6	43.1
	SD	1.60	2.12	3.10	1.83
MCV (fL)	Mean	53.9	55.2	54.6	53.8
	SD	2.20	1.49	1.62	1.30
MCH (pg)	Mean	19.9	20.3	19.9	19.6
	SD	0.96	0.50	0.44	0.66
MCHC (g/dL)	Mean	36.8	36.7	36.5	36.4
	SD	0.59	0.44	0.53	0.45
Platelet (10^3/µL)	Mean	1060.0	913.5	1021.7	763.7
	SD	118.66	164.74	102.07	172.10
Clotting Time (seconds)	Mean	95.0	85.0	100.0	100.0
	SD	12.25	22.58	15.49	15.49
Lymphocyte (%)	Mean	82.5	75.3	78.0	81.2
	SD	4.09	4.50	3.22	4.36
Neutrphil (%)	Mean	15.8	21.3	18.3	16.3
	SD	3.87	4.18	4.55	3.98
Monocyte (%)	Mean	0.6	0.7	0.7	0.8
	SD	0.18	0.22	0.22	0.24
Eosinophil (%)	Mean	1.2	2.7	3.0	1.7
	SD	0.21	0.51	0.58	0.13
Basophil (%)	Mean	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0

Key : N= Number of observations

Table 2 A

Clinical Chemistry – Group Mean Values

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 - (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
Glucose (mg/dL)	Mean	105.2	105.3	98.9	114.5	115.3	109.4	117.3
	SD	32.40	13.01	21.69	21.12	19.99	18.88	22.70
Total Protein (g/dL)	Mean	7.1	7.4	7.2	7.4	7.3	7.3	7.5
	SD	0.30	0.25	0.35	0.21	0.27	0.28	0.37
Albumin (g/dL)	Mean	3.8	4.1	3.7	4.1	4.2	4.1	4.0
	SD	0.16	0.14	0.56	0.19	0.10	0.19	0.46
Globulin (mg/dL)	Mean	3.3	3.3	3.5	3.3	3.2	3.2	3.5
	SD	0.23	0.20	0.35	0.30	0.22	0.21	0.28
Cholesterol (mg/dL)	Mean	69.5	72.8	66.1	67.8	71.1	66.2	68.0
	SD	9.32	11.50	4.65	9.84	14.61	6.25	6.35
Total Bilirubin (mg/dL)	Mean	0.23	0.17	0.18	0.18	0.16	0.16	0.18
	SD	0.048	0.028	0.076	0.039	0.023	0.015	0.028
BUN (mg/dL)	Mean	17.4	19.3	18.9	17.6	17.7	15.9	17.7
	SD	0.42	3.40	2.82	3.29	1.96	1.89	3.57
Urea (mg/dL)	Mean	37.3	41.2	40.3	37.7	37.7	34.2	37.8
	SD	1.03	7.00	5.96	6.98	4.18	3.97	7.91
Creatinine (mg/dL)	Mean	0.25	0.25	0.23	0.24	0.26	0.23	0.24
	SD	0.036	0.034	0.041	0.036	0.028	0.023	0.037
Calcium (mg/dL)	Mean	11.1	11.4	11.0	11.0	10.8	10.7	10.4**
	SD	0.47	0.25	0.41	0.34	0.40	0.26	0.38
Phosphorus (mg/dL)	Mean	9.8	10.2	10.1	10.1	10.2	10.4	10.3
	SD	0.49	0.33	0.90	0.53	0.71	0.33	0.52
Sodium (mEq/L)	Mean	153.9	156.3	161.8**	162.7**	157.9	161.1**	161.4**
	SD	2.31	1.45	2.33	2.08	2.29	0.88	0.45
Potassium (mEq/L)	Mean	5.0	5.5	5.4	5.5	5.5	5.0	5.3
	SD	0.53	0.45	0.15	0.60	0.47	0.35	0.28
Chloride (mEq/L)	Mean	114.3	115.1	117.7*	118.9**	115.4	118.4**	117.8*
	SD	1.38	1.39	1.99	1.31	1.58	1.61	1.68

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 A (continued)

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
ALT (IU/L)	Mean	48.5	47.1	51.3	47.4	45.7	39.5	38.5*
	SD	9.63	5.33	3.32	4.09	6.04	5.31	7.28
AST (IU/L)	Mean	217.0	195.9	195.8	215.3	189.8	190.8	217.6
	SD	41.4	29.0	31.7	46.1	22.6	27.7	45.5
ALP (IU/L)	Mean	306.3	282.2	281.0	321.3	299.3	297.8	333.1
	SD	85.8	98.1	86.5	80.0	83.3	50.2	52.2
Serum ChE (IU/L)	Mean	331.5	274.3	291.3	307.3	193.8**	347.7	199.3**
	SD	48.2	45.4	73.4	113.0	36.7	83.0	34.2
RBC ChE (IU/L)	Mean	1250.0	1223.0	1166.7	1316.7	908.3	1683.3	1033.3
	SD	308.2	289.4	296.1	285.9	235.4	300.7	220.6

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 B

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
Glucose	Mean	88.5	101.5	99.1	111.6*	114.4*	109.5	116.0**
	SD	9.71	7.58	13.93	13.84	18.13	18.48	15.16
Total Protein (g/dL)	Mean	7.31	7.21	7.36	7.41	7.38	7.32	7.47
	SD	0.22	0.36	0.38	0.25	0.35	0.28	0.35
Albumin (g/dL)	Mean	3.8	3.8	3.7	3.9	3.9	3.9	3.9
	SD	0.21	0.12	0.35	0.06	0.13	0.17	0.11
Globulin (mg/dL)	Mean	3.5	3.4	3.7	3.5	3.5	3.5	3.5
	SD	0.20	0.33	0.35	0.25	0.29	0.32	0.40
Cholesterol (mg/dL)	Mean	58.6	63.7	54.4	59.5	56.9	55.4	55.3
	SD	7.82	12.10	6.55	16.68	12.94	8.81	11.48
Total Bilirubin (mg/dL)	Mean	0.18	0.16	0.17	0.16	0.16	0.15	0.15
	SD	0.01	0.02	0.02	0.02	0.01	0.03	0.04
BUN (mg/dL)	Mean	20.6	19.0	18.9	19.0	18.3	18.8	18.6
	SD	1.25	2.13	2.02	1.70	2.14	2.33	1.71
Urea (mg/dL)	Mean	44.3	40.6	40.6	40.9	39.1	40.4	39.9
	SD	2.60	4.66	4.24	3.83	4.67	5.15	3.68
Creatinine (mg/dL)	Mean	0.29	0.26	0.26	0.26	0.26	0.25	0.22
	SD	0.03	0.03	0.04	0.03	0.06	0.05	0.05
Calcium (mg/dL)	Mean	10.0	10.0	9.7	9.9	9.8	9.5**	9.6**
	SD	0.30	0.32	0.35	0.16	0.32	0.18	0.34
Phosphorus (mg/dL)	Mean	7.7	7.7	7.6	7.8	7.9	7.4	7.3
	SD	0.33	0.35	0.27	0.41	0.48	0.68	0.23
Sodium (mEq/L)	Mean	150.1	150.3	149.7	150.4	150.3	148.9	148.4
	SD	5.57	4.60	1.59	2.64	2.04	6.88	5.05
Potassium (mEq/L)	Mean	5.2	5.1	5.1	5.1	5.1	5.6	4.7**
	SD	0.51	0.24	0.38	0.39	0.20	1.81	0.30
Chloride (mEq/L)	Mean	109.8	109.6	108.7	109.6	109.2	108.3	107.5
	SD	3.43	3.95	1.59	2.17	2.11	3.86	3.99

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 B (continued)

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
ALT (IU/L)	Mean	47.9	42.9	56.6	46.0	51.8	41.5	44.9
	SD	6.12	7.73	13.23	7.64	3.91	7.70	10.99
AST (IU/L)	Mean	220.2	194.2	196.0	195.1	199.1	174.6*	172.1*
	SD	27.15	30.63	24.67	24.55	21.03	42.86	28.31
ALP (IU/L)	Mean	171.7	140.8	159.0	135.7	127.9	168.5	176.3
	SD	64.23	28.52	40.11	53.04	46.33	67.48	64.50
Serum ChE (IU/L)	Mean	327.8	276.3	257.5	299.4	168.3*	331.3	168.5*
	SD	123.00	64.10	75.57	86.69	19.16	86.66	31.55
RBC ChE (IU/L)	Mean	1156.3	1101.3	1097.5	1125.0	598.3**	1100.0	543.8**
	SD	258.34	133.46	268.26	535.86	164.75	420.88	254.16

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 C

Dose: G1R - 0; G5R (CP) - 10; G6R (LA) - 500; G7R (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
Glucose	Mean	107.4	117.6	120.3	127.3*
	SD	10.45	8.50	11.26	7.72
Total Protein (g/dL)	Mean	7.5	7.7	7.5	7.6
	SD	0.29	0.35	0.19	0.22
Albumin (g/dL)	Mean	3.5	3.7	3.6	3.7
	SD	0.13	0.22	0.15	0.19
Globulin (mg/dL)	Mean	4.0	4.0	3.9	3.9
	SD	0.35	0.15	0.19	0.18
Cholesterol (mg/dL)	Mean	54.6	60.9	60.0	67.0
	SD	7.30	10.28	6.60	11.49
Total Bilirubin (mg/dL)	Mean	0.12	0.12	0.11	0.11
	SD	0.03	0.02	0.02	0.02
BUN (mg/dL)	Mean	22.4	19.6	25.1	20.5
	SD	3.29	1.64	9.71	1.47
Urea (mg/dL)	Mean	48.0	41.8	53.7	44.0
	SD	7.10	3.76	20.98	3.29
Creatinine (mg/dL)	Mean	0.32	0.30	0.31	0.29
	SD	0.01	0.03	0.09	0.04
Calcium (mg/dL)	Mean	10.5	10.9	10.8	11.0*
	SD	0.28	0.39	0.18	0.33
Phosphorus (mg/dL)	Mean	7.6	7.3	7.4	7.2
	SD	0.74	0.30	0.25	0.51
Sodium (mEq/L)	Mean	151.3	151.3	149.4	150.0
	SD	2.45	1.80	2.34	1.28
Potassium (mEq/L)	Mean	5.0	5.0	5.1	5.1
	SD	0.25	0.24	0.33	0.21
Chloride (mEq/L)	Mean	110.1	110.1	110.1	110.7
	SD	2.42	1.27	1.14	1.26

Key : N= Number of observations

Table 2 C (continued)

Dose: G1R - 0; G5R (CP) - 10; G6R (LA) - 500; G7R (CP+LA) - 10+500 ppm in diet

Sex : Male **Period : 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
ALT (IU/L)	Mean	76.3	67.2	66.1	64.9*
	SD	10.54	14.21	13.23	6.10
AST (IU/L)	Mean	212.7	221.4	167.5*	134.9**
	SD	22.94	16.02	20.49	19.09
ALP (IU/L)	Mean	220.2	139.1	180.5	168.4
	SD	69.03	34.97	15.16	57.34
Serum ChE (IU/L)	Mean	210.8	268.7	257.7	306.3
	SD	30.45	62.99	89.05	69.04
RBC ChE (IU/L)	Mean	1216.7	1120.3	1291.7	1008.3
	SD	290.6	294.4	156.3	292.7

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 D

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
Glucose	Mean	100.1	103.4	113.4	103.5	95.7	98.8	102.1
	SD	20.54	22.93	19.99	19.35	23.63	11.65	14.28
Total Protein (g/dL)	Mean	7.9	8.1	8.5	8.3	8.3	7.6	7.9
	SD	0.39	0.35	0.23	0.25	0.54	0.27	0.15
Albumin (g/dL)	Mean	4.0	4.0	4.0	3.9	4.0	4.1	4.2
	SD	0.20	0.33	0.38	0.20	0.09	0.16	0.12
Globulin (mg/dL)	Mean	3.9	4.1	4.5*	4.3*	4.3	3.5	3.7
	SD	0.30	0.58	0.46	0.21	0.52	0.30	0.13
Cholesterol (mg/dL)	Mean	80.6	87.0	91.1	79.3	89.0	81.5	98.4
	SD	4.42	8.49	19.28	11.90	18.77	13.81	13.94
Total Bilirubin (mg/dL)	Mean	0.19	0.19	0.21	0.22	0.21	0.20	0.20
	SD	0.017	0.017	0.045	0.059	0.041	0.069	0.068
BUN (mg/dL)	Mean	18.6	19.5	18.3	17.6	17.2	16.4	14.2
	SD	3.82	2.25	1.09	3.89	2.41	4.18	2.48
Urea (mg/dL)	Mean	39.7	41.8	39.0	37.7	36.8	35.3	30.3
	SD	8.14	4.88	2.19	8.38	4.92	9.07	5.32
Creatinine (mg/dL)	Mean	0.25	0.25	0.29	0.25	0.27	0.26	0.24
	SD	0.024	0.040	0.030	0.026	0.024	0.023	0.021
Calcium (mg/dL)	Mean	11.6	11.3	10.4**	11.5	11.1	10.6**	11.4
	SD	0.38	0.42	0.46	0.59	0.43	0.62	0.18
Phosphorus (mg/dL)	Mean	9.2	9.2	8.8	9.0	8.9	9.4	9.2
	SD	0.46	0.26	0.67	0.76	0.53	0.64	0.48
Sodium (mEq/L)	Mean	145.0	146.8	149.8**	149.5**	147.1	148.5**	152.6**
	SD	1.73	1.79	2.32	2.40	2.35	1.47	1.57
Potassium (mEq/L)	Mean	4.4	5.1*	5.3*	4.7	4.8	4.9*	4.6
	SD	0.37	0.30	1.10	0.50	0.75	0.21	0.13
Chloride (mEq/L)	Mean	106.9	108.3	109.2	108.5	106.5	108.3	110.7*
	SD	1.57	2.07	2.45	1.95	2.63	1.42	1.45

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 D (continued)

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 4th Week**

Parameter/Group		G1 (N=6)	G2 (N=6)	G3 (N=6)	G4 (N=6)	G5 (N=6)	G6 (N=6)	G7 (N=6)
ALT (IU/L)	Mean	43.4	40.0	47.7	42.7	41.4	40.1	35.0
	SD	7.02	4.72	5.48	10.49	4.49	5.34	7.86
AST (IU/L)	Mean	221.1	222.1	206.5	190.0	198.2	207.0	189.1
	SD	48.74	14.81	39.63	44.72	40.27	22.60	32.0
ALP (IU/L)	Mean	227.2	238.0	200.2	201.4	216.4	235.8	240.0
	SD	71.20	48.53	101.61	37.27	54.36	39.24	72.19
Serum ChE (IU/L)	Mean	518.7	502.2	701.0	661.8	376.7	532.8	447.8
	SD	215.20	290.28	271.13	256.84	26.56	395.37	125.44
RBC ChE (IU/L)	Mean	1508.3	1458.3	1383.3	1350.0	1050.0	14600.0	1308.3
	SD	247.67	302.41	276.38	316.23	298.33	281.66	243.76

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2E

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10;G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
Glucose	Mean	104.1	112.5	110.3	109.4	102.2	115.4	115.6
	SD	11.82	14.22	17.34	16.27	19.76	17.58	13.23
Total Protein (g/dL)	Mean	7.2	7.3	7.8	7.5	7.6	7.2	7.7
	SD	0.38	0.36	0.55	0.49	0.64	0.40	0.26
Albumin (g/dL)	Mean	3.9	4.0	4.1*	4.0	4..0	3.9	4.1
	SD	0.15	0.08	0.41	0.43	0.23	0.20	0.26
Globulin (mg/dL)	Mean	3.3	3.3	3.7	3.4	3.7	3.3	3.7
	SD	0.31	0.31	0.32	0.37	0.53	0.33	0.28
Cholesterol (mg/dL)	Mean	75.3	77.9	79.6	75.3	84.6	76.2	81.7
	SD	8.47	11.73	16.36	9.57	12.78	10.99	13.10
Total Bilirubin (mg/dL)	Mean	0.18	0.18	0.19	0.17	0.18	0.16	0.20
	SD	0.018	0.016	0.017	0.016	0.019	0.015	0.025
BUN (mg/dL)	Mean	17.4	18.2	20.2	17.9	18.7	19.6	19.0
	SD	2.72	2.67	2.67	3.11	2.25	3.69	2.62
Urea (mg/dL)	Mean	37.4	38.8	43.5	38.3	40.3	42.1	40.6
	SD	5.97	5.92	5.61	6.73	4.77	7.97	5.63
Creatinine (mg/dL)	Mean	0.33	0.34	0.35	0.38	0.38	0.37	0.35
	SD	0.03	0.03	0.04	0.06	0.05	0.05	0.05
Calcium (mg/dL)	Mean	10.0	10.0	10.2	9.8	9.8	9.7*	10.1
	SD	0.27	0.17	0.34	0.34	0.30	0.18	0.24
Phosphorus (mg/dL)	Mean	6.5	6.6	6.1	6.1	6.1	6.6	7.2
	SD	0.54	0.54	0.47	0.50	0.77	0.64	1.01
Sodium (mEq/L)	Mean	146.4	145.4	146.5	146.7	146.0	145.9	146.2
	SD	2.84	2.56	1.67	2.16	1.79	2.01	2.43
Potassium (mEq/L)	Mean	4.0	4.0	4.4*	4.3	4.3	5.0**	4.4*
	SD	0.29	0.22	0.29	0.27	0.19	2.03	0.31
Chloride (mEq/L)	Mean	107.7	109.7	108.2	108.2	107.4	106.9	109.4
	SD	3.72	2.03	1.80	2.48	1.95	3.71	4.91

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 E (continued)

Dose: G1- 0; G2 (CP) - 1; G3 (LA) - 50; G4 (CP+LA) - 1+50; G5 (CP) - 10; G6 (LA) - 500; G7 (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 13th Week**

Parameter/Group		G1 (N=8)	G2 (N=8)	G3 (N=8)	G4 (N=8)	G5 (N=8)	G6 (N=8)	G7 (N=8)
ALT (IU/L)	Mean	49.1	44.5	52.4	38.9	40.3	44.1	43.8
	SD	10.01	4.76	9.94	9.85	9.00	6.68	18.7
AST (IU/L)	Mean	169.1	179.7	155.2	153.5	158.4	155.6	138.2
	SD	30.26	29.88	31.20	28.35	20.86	36.32	28.13
ALP (IU/L)	Mean	145.7	161.1	135.4	135.1	107.6	125.7	153.0
	SD	79.72	62.85	81.45	66.45	51.16	32.84	55.01
Serum ChE (IU/L)	Mean	927.6	994.4	1312.8	1135.3	468.4*	1008.9	409.3*
	SD	592.44	526.85	637.11	272.56	107.84	615.25	79.69
RBC ChE (IU/L)	Mean	1437.5	1396.3	1531.3	1468.8	681.3*	1431.3	417.5**
	SD	311.23	349.94	389.76	339.72	233.74	335.80	176.78

Key : N= Number of observations

* = significant at 5% level ($p \leq 0.05$); **= significant at 1% level ($p \leq 0.01$)

Table 2 F

Dose: G1R - 0; G5R (CP) - 10; G6R (LA) - 500; G7R (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
Glucose (mg/dL)	Mean	110.0	124.7	116.0	145.0**
	SD	4.79	5.17	5.98	8.52
Total Protein (g/dL)	Mean	7.9	7.8	8.1	8.0
	SD	0.27	0.32	0.38	0.25
Albumin (g/dL)	Mean	4.2	4.2	4.2	4.2
	SD	0.14	0.16	0.20	0.24
Globulin (mg/dL)	Mean	3.8	3.7	3.9	3.8
	SD	0.18	0.23	0.26	0.31
Cholesterol (mg/dL)	Mean	87.6	86.0	83.5	83.4
	SD	6.50	11.65	5.18	8.48
Total Bilirubin (mg/dL)	Mean	0.13	0.13	0.14	0.13
	SD	0.02	0.03	0.02	0.02
BUN (mg/dL)	Mean	23.5	23.2	20.9	21.7
	SD	4.2	2.0	2.2	5.9
Urea (mg/dL)	Mean	50.2	50.0	45.0	46.3
	SD	8.93	4.50	4.84	12.88
Creatinine (mg/dL)	Mean	0.35	0.35	0.34	0.33
	SD	0.04	0.04	0.03	0.04
Calcium (mg/dL)	Mean	10.8	10.7	10.8	11.0
	SD	0.38	0.37	0.14	0.25
Phosphorus (mg/dL)	Mean	5.6	5.9	5.6	6.3
	SD	0.67	1.11	0.68	0.81
Sodium (mEq/L)	Mean	144.2	146.5	144.4	145.0
	SD	3.56	1.34	8.27	3.72
Potassium (mEq/L)	Mean	4.7	4.5	4.3	4.6
	SD	0.23	0.26	0.35	0.25
Chloride (mEq/L)	Mean	107.3	110.2	107.8	108.3
	SD	3.44	2.13	6.00	3.08

Key : N= Number of observations

**= significant at 1% level ($p \leq 0.01$)

Table 2 F (continued)

Dose: G1R - 0; G5R (CP) - 10; G6R (LA) - 500; G7R (CP+LA) - 10+500 ppm in diet

Sex : Female **Period : 17th Week**

Parameter/Group		G1R (N=6)	G5R (N=6)	G6R (N=6)	G7R (N=6)
ALT (IU/L)	Mean	57.0	60.8	50.7	60.0
	SD	5.37	15.04	9.79	12.69
AST (IU/L)	Mean	154.1	139.4	131.7	148.8
	SD	19.35	13.35	13.44	29.93
ALP (IU/L)	Mean	130.9	132.8	108.5	141.2
	SD	27.0	55.98	23.33	49.31
Serum ChE (IU/L)	Mean	1218.0	1225.0	1284.5	1170.3
	SD	508.60	529.39	534.55	439.61
RBC ChE (IU/L)	Mean	1450.0	1236.3	1335.3	950.0
	SD	363.85	343.03	260.13	277.49

Key : N= Number of observations

Table 3

Percent Reduction of Serum and RBC Cholinesterases

Dose: G1- 0; G5 (CP) - 10; G7 (CP+LA) - 10+500 ppm in diet

Sex : Male

Percent Reduction of Cholinesterases						
Group	**Week 4**		**Week 13**		**Week 17**	
	Serum	**RBC**	**Serum**	**RBC**	**Serum**	RBC
Group 5	41.5	27.4	48.7	48.3	-	-
Group 7	39.9	17.3	48.6	53.0	-	-
Group 5R	-	-	-	-	0	7.9
Group 7R	-	-	-	-	0	17.1

Sex : Female

Percent Reduction of Cholinesterase						
Group	**Week 4**		**Week 13**		**Week 17**	
	Serum	**RBC**	**Serum**	**RBC**	**Serum**	**RBC**
Group 5	27.4	30.4	49.5	52.6	-	-
Group 7	13.7	13.3	55.9	70.9	-	-
Group 5R	-	-	-	-	0	14.7
Group 7R	-	-	-	-	3.9	34.5

Key : - = not evaluated

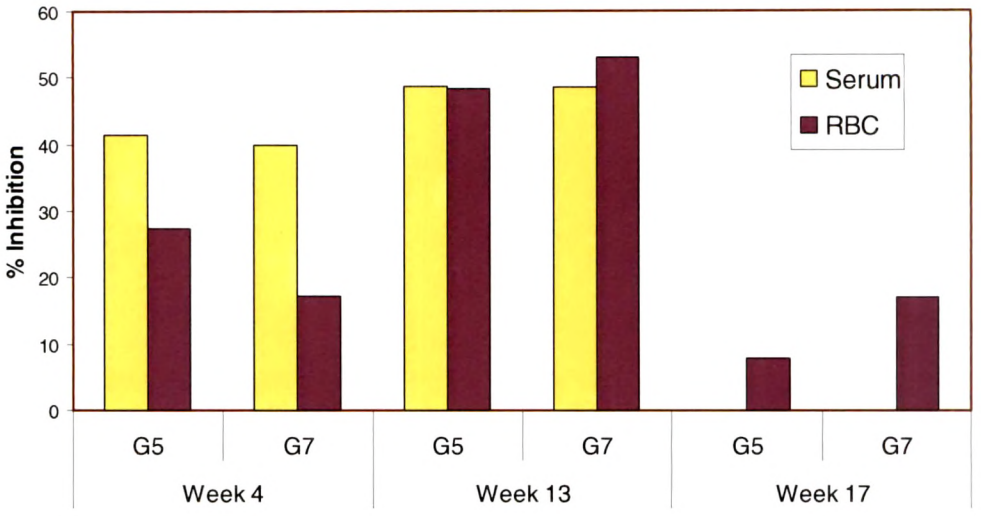

Figure 1. Percent inhibition of cholinesterases after subchronic exposure – Males

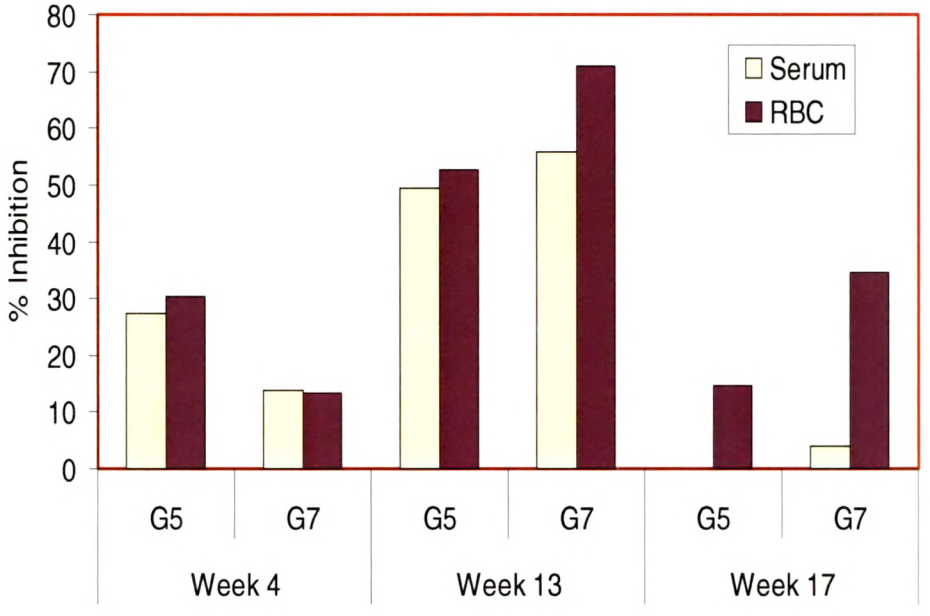

Figure 2. Percent inhibition of cholinesterases after subchronic exposure- Females

G5 (CP) - 10; **G7** (CP+LA) - 10+500 ppm in diet

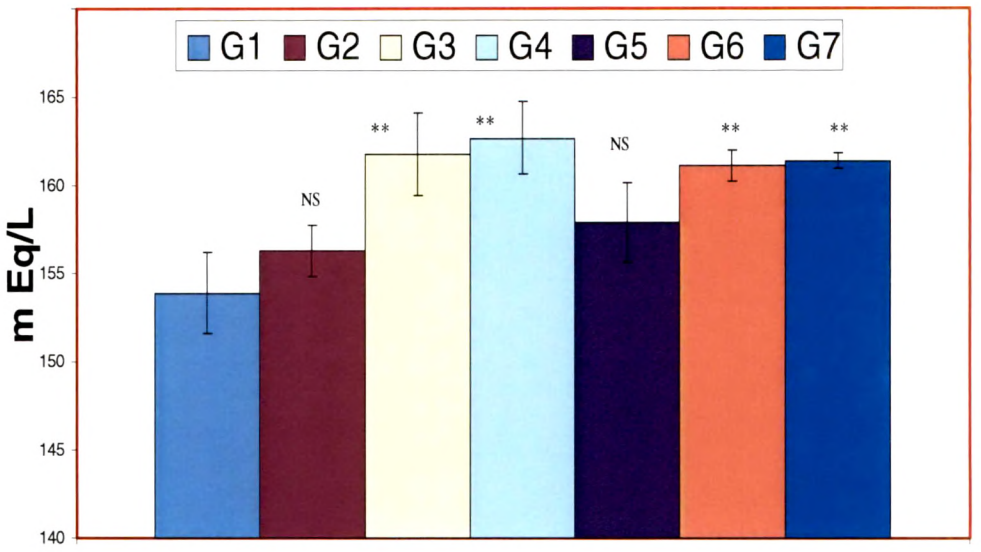

Figure 3. Sodium Values: Males – at Week 4.

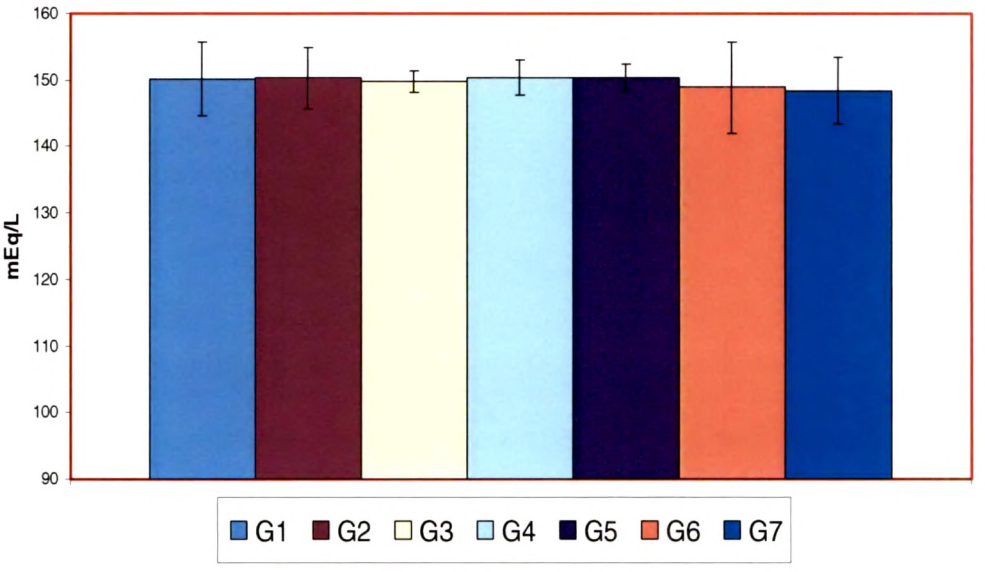

Figure 4. Sodium Values (no significant differences between control and treatment groups): Males – at Week 13.

Key : ** = significant at 1% level ($p \leq 0.01$); NS = Not statistically significant

G1- 0; **G2** (CP) - 1; **G3** (LA) - 50; **G4** (CP+LA) - 1+50; **G5** (CP) - 10; **G6** (LA) - 500; **G7** (CP+LA) - 10+500 ppm in diet

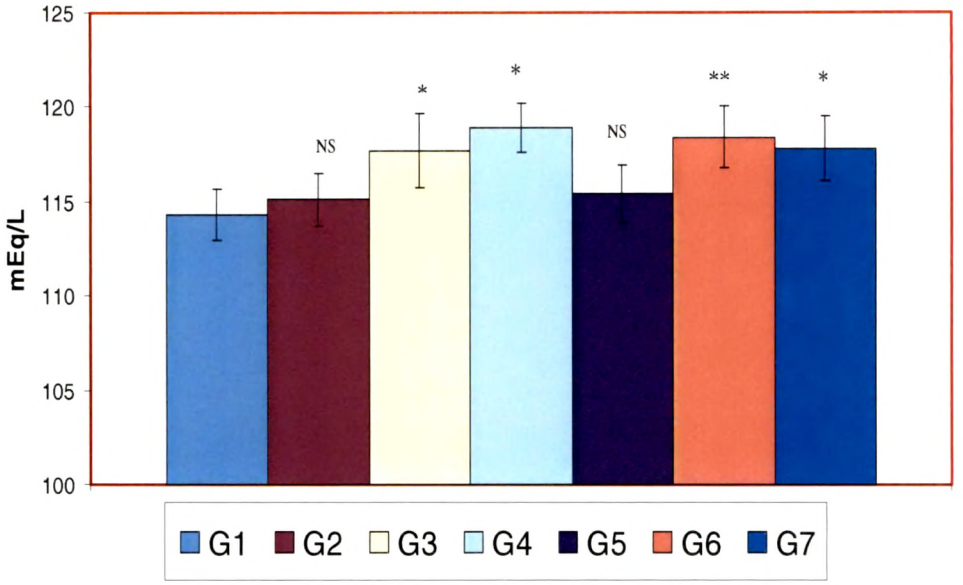

Figure 5. Chloride Values: Males – at Week 4.

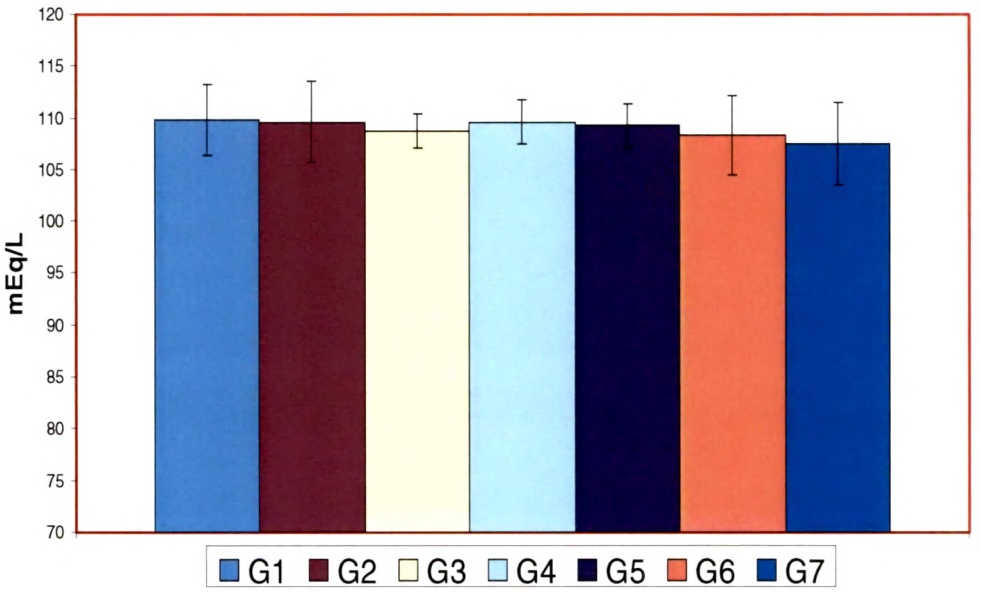

Figure 6. Chloride Values (no significant differences between control and treatment groups) : Males – at Week 13.

Key : * = significant at 5% level ($p \leq 0.05$), ** = significant at 1% level ($p \leq 0.01$), NS = Not statistically significant

G1- 0; **G2** (CP) - 1; **G3** (LA) - 50; **G4** (CP+LA) - 1+50; **G5** (CP) - 10; **G6** (LA) - 500; **G7** (CP+LA) - 10+500 ppm in diet

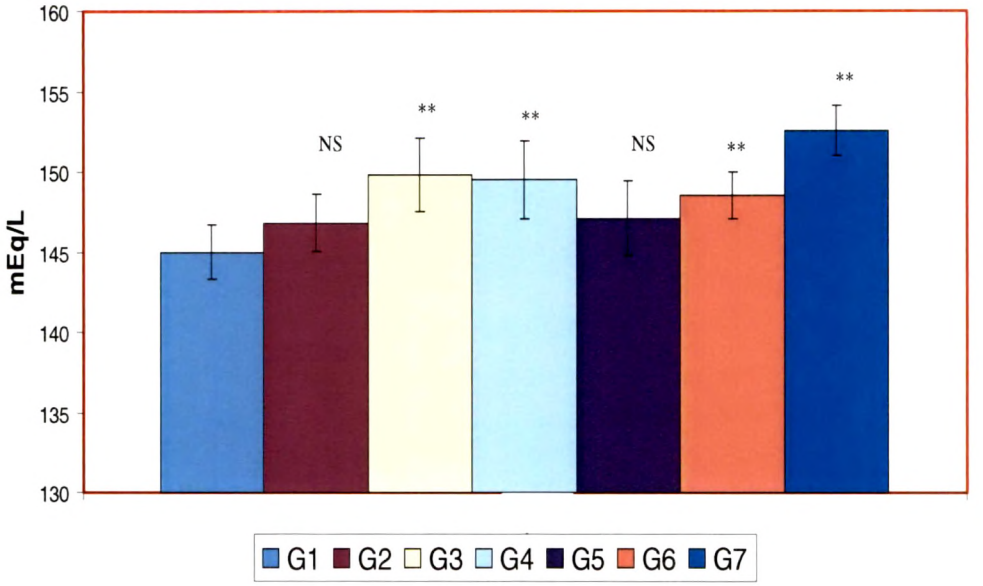

Figure 7. Sodium Values: Females – at Week 4.

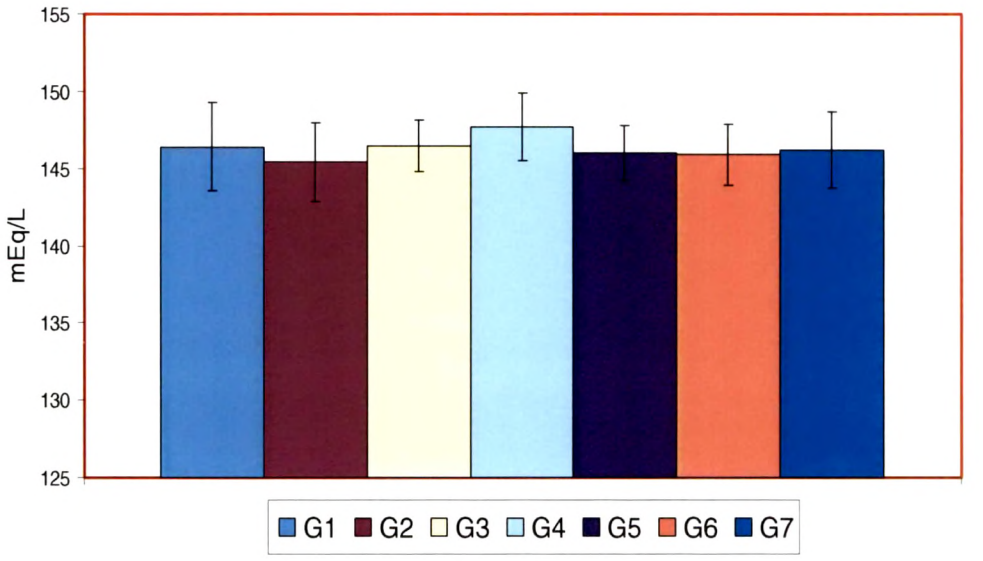

Figure 8. Sodium Values (no significant differences between control and treatment groups): Females – at Week 13.

Key : ** = significant at 1% level ($p \leq 0.01$), NS = Not statistically significant

G1- 0; **G2** (CP) - 1; **G3** (LA) - 50; **G4** (CP+LA) - 1+50; **G5** (CP) - 10; **G6** (LA) - 500; **G7** (CP+LA) - 10+500 ppm in diet

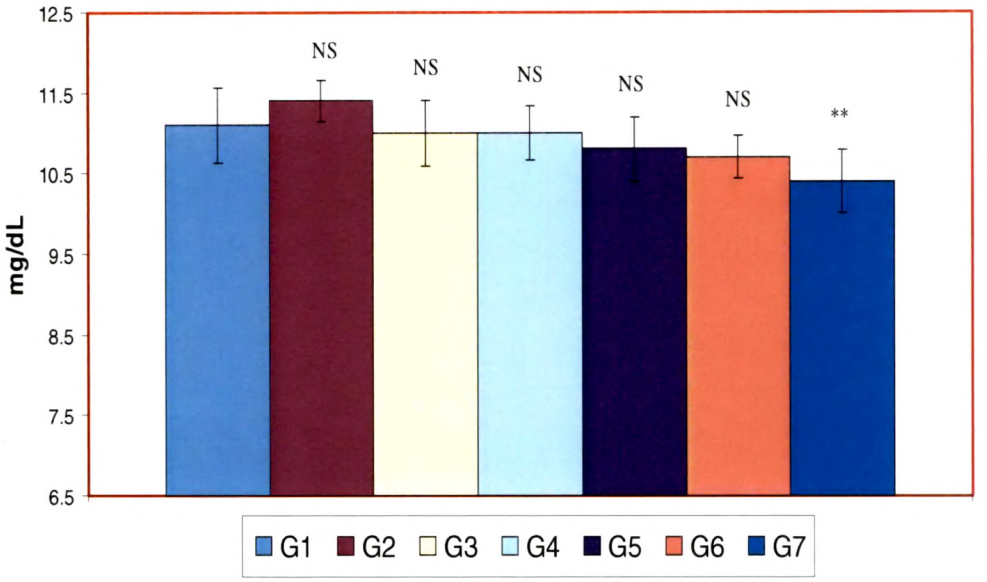

Figure 9. Calcium Values: Males – at Week 4.

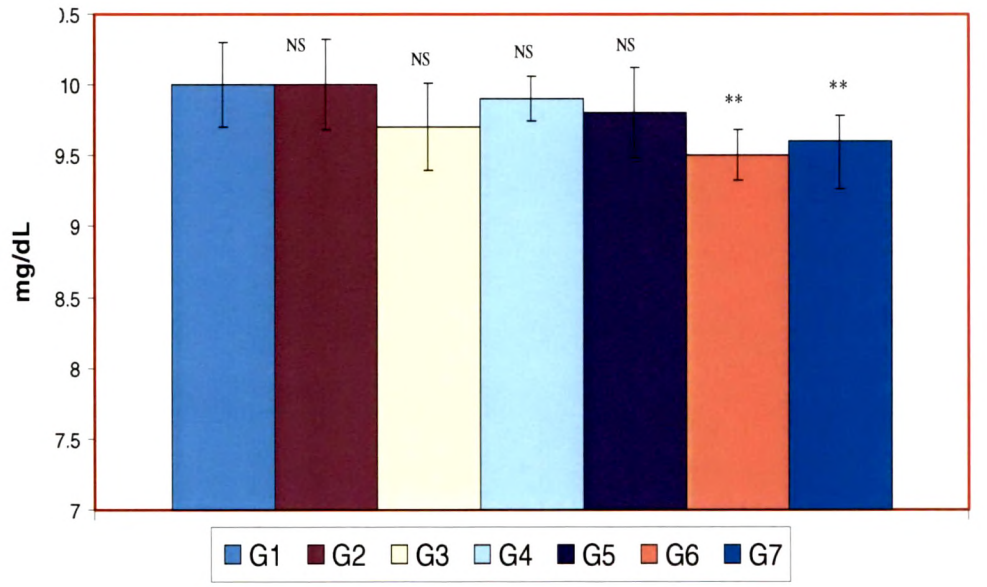

Figure 10. Calcium Values: Males – at Week 13

Key : ** = significant at 1% level ($p \leq 0.01$), NS = Not statistically significant

G1- 0; **G2** (CP) - 1; **G3** (LA) - 50; **G4** (CP+LA) - 1+50; **G5** (CP) - 10; **G6** (LA) - 500; **G7** (CP+LA) - 10+500 ppm in diet

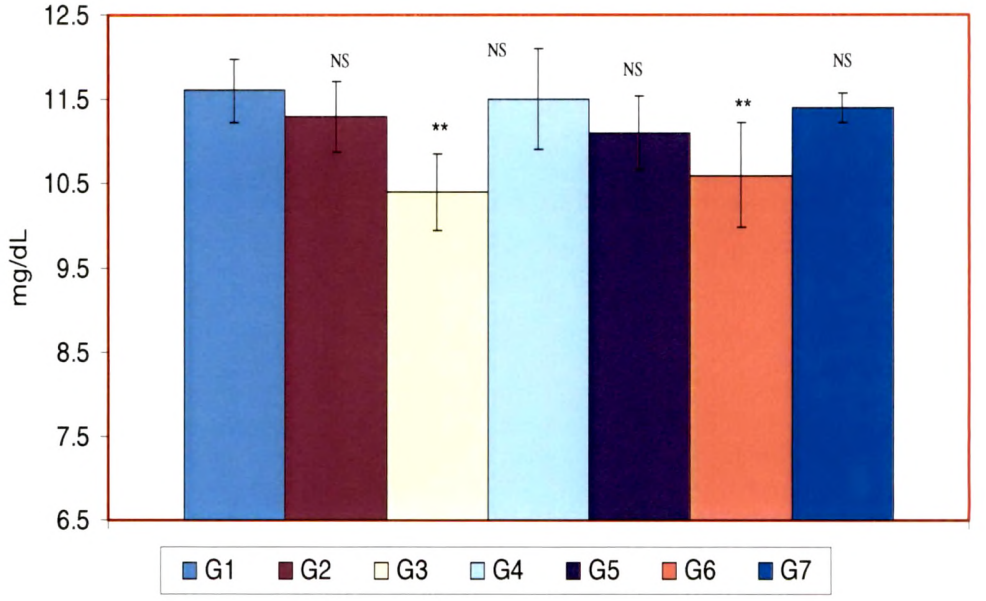

Figure 11. Calcium Values: Females – at Week 4.

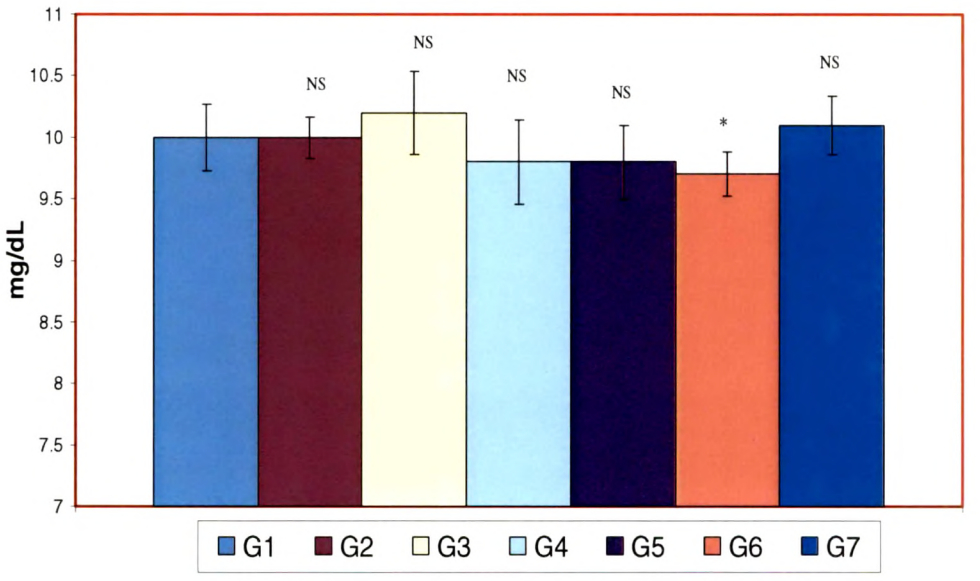

Figure 12. Calcium Values: Females – at Week 13.

Key : * = significant at 5% level ($p \leq 0.05$), ** = significant at 1% level ($p \leq 0.01$), NS = Not statistically significant

G1- 0; **G2** (CP) - 1; **G3** (LA) - 50; **G4** (CP+LA) - 1+50; **G5** (CP) - 10; **G6** (LA) - 500; **G7** (CP+LA) - 10+500 ppm in diet

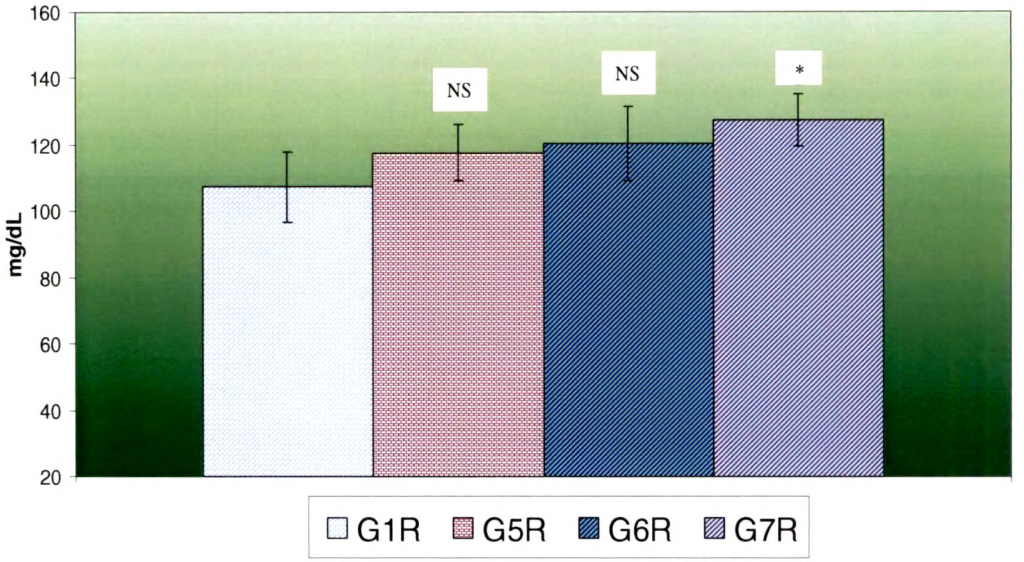

Figure 13. Glucose Values: Males – Week 17 (post exposure).

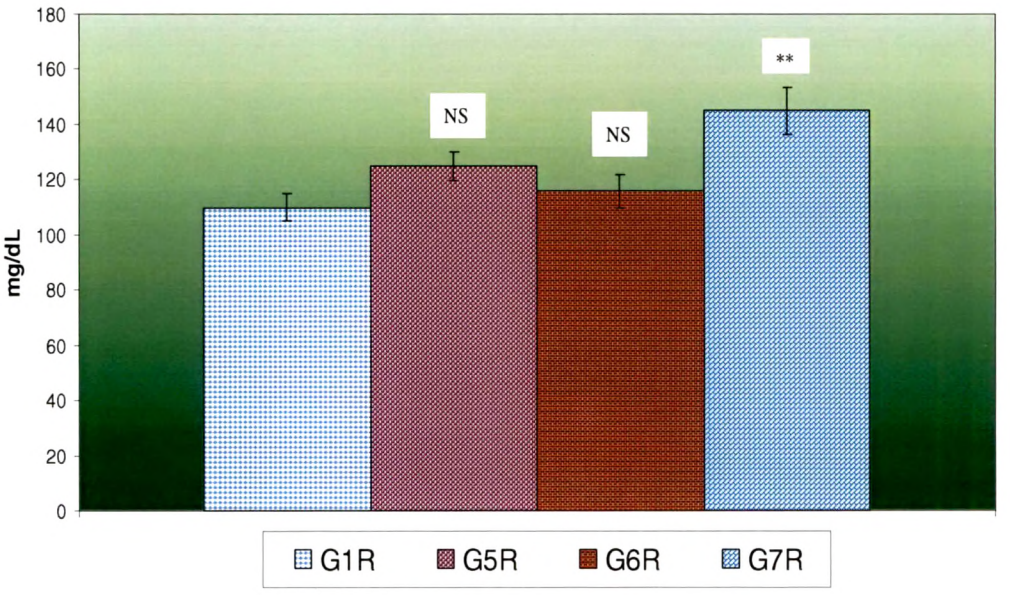

Figure 14. Glucose Values: Females – Week 17 (post exposure).

Key: * = significant at 5% level ($p \leq 0.05$), ** = significant at 1% level ($p \leq 0.01$), NS = Not statistically significant

G1R- 0; **G5R** (CP) - 10; **G6R** (LA) - 500; **G7R** (CP+LA) - 10+500 ppm in diet

DISCUSSION

The present study has evaluated the effects of repeated dietary exposure to a combination of chlorpyrifos and lead acetate on hematology and clinical chemistry parameters of blood. The evaluations have revealed some treatment related changes. The results of hematological evaluations indicate slight anaemic effects in lead treated animals at differential time scale. Clinical chemistry estimations performed at the end of weeks 4, 13 and 17 reveal treatment related changes in electrolytes, serum glucose and serum and RBC cholinesterase activity.

Hematology

Anaemic effects were observed after 13 weeks of exposure in males and at the end of 4 weeks in females due to exposure to lead. The mechanism of lead induced anemic effect is well known. The values of RBC, hemoglobin and HCT in lead treated females were comparable to control group of animals after 13 weeks of exposure (Tables 1A – 1 F).

The hypochromic and microcytic anemia that occurs in lead poisoning results from two basic defects: shortened lifespan and impairment of heme synthesis (Goyer, 1996). Shortened life span of red blood cells is thought to be due to increased mechanical fragility of the cell membrane. Depressed heme formation is associated with failure to insert iron into protoporphyrin. The biochemical basis for this effect is not known but the effect is accompanied by inhibition of sodium and potassium dependent ATPase's (Goyer, 1996). Further, low concentrations of lead acetate can change function of receptors and channels at the plasma membrane of RBC (Slobozhanina *et al*, 2005). Slight anemic effect due to lead treatment was

observed in males at the end of week 13 and after week 4 in females. This sex related differential response with regard to differential time scale need to be studied further.

Slight decrease in mean values of RBC, Hb and HCT are observed in group 5 females (i.e., animals treated with chlorpyrifos at 10 ppm) at the end of week 4 (Table 1 A). The slight decreases in packed cell volume, red blood cells and hemoglobin due to chlorpyrifos treatment were also previously reported in conventional toxicity studies summarized by USEPA (2000) and Danish EPA (2005). However, mechanism and absence of effects after 13 weeks of exposure remained unanswered. Singh *et al.* (2004) studied in vitro effects of various organophosphate pesticides (dimethoate, chlorpyrifos, ethion and monocrotophos) on hemolysis, potassium leakage and lipid peroxidation in rat erythrocytes. All the four pesticides caused dose and time dependent increase in hemolysis and potassium leakage from erythrocytes. But they also observed decreased lipid peroxidation in erythrocyte membrane. Proton abstracting capacity of pesticides or interaction with free radicals rather than polyunsaturated fatty acids (PUFA), could be the reason for decrease in lipid peroxidation.

Groups 2 and 5 males after 4 weeks of exposure revealed significant increase in platelet count as compared to control group of males (Table 1A). However there was no dose dependent response in platelet counts. Danish EPA (2005) reported increased platelet counts after 28 and 90 days of exposure of chlorpyrifos in rats. Different toxicity studies on chlorpyrifos summarized by US EPA (2000) have reported increased platelet counts in chronic toxicity evaluations in rats but not in subchronic toxicity studies. In the present

study, variations in platelet count were observed at the end of week 4 and 13.

Variations observed in clotting time in different treatment groups i.e., both in chlorpyrifos and lead treated groups, might be associated with alterations in coagulation mechanism. Variations in platelet count because of chlorpyrifos and effects of lead on calcium, and/or effects on other clotting factors, might influence the coagulation mechanism of blood. However, like platelet counts no dose dependent variation was observed in mean values of clotting time.

Increased MCHC values observed at chlorpyrifos 10 ppm particularly after 4 weeks of exposure (in both the sexes) might be an laboratory/instrument error. Since the erythrocytes cannot be supersaturated with the Hb, the observed statistical significance might be due to increased weight of Hb in average erythrocyte (Benjamin, 2001a). Tvedten and Weiss (2000) reported that hyperchromic changes usually are not attributed to treatment with the xenobiotics. Computer graphics of automated hematology systems are more sensitive in detecting changes in cell volume or Hb concentration.

No interactive effects like synergism or potentiation were observed in any of the parameters of hematology.

Clinical Chemistry

After 4 weeks of exposure, electrolytes (sodium and chloride) in lead treated groups were significantly increased. At the end of treatment period, sodium and chloride contents of treated groups were comparable to control groups (Tables **2A, 2B, 2D and 2 E**; Figures 3, 4, 5, 6, 7 and 8). This increase might be due to minor variations in the functional capacity of nephrons due to the

action of lead on proximal tubules of kidney. Reserve functional capacity of kidney or adaptation to chemical insult might be the reason for comparable levels of electrolytes at the end of treatment period.

Kidney has remarkable ability to compensate for loss of renal function. There are number of cellular and molecular responses to a nephrotoxic insult which can ameliorate or prevent cell death. Two of the most notable responses are metallothionein induction and heat shock response. These proteins are induced during heavy metal exposure. Heat shock proteins were believed to play an important house keeping role in the maintenance of normal protein structure and/or the degradation of damaged proteins and thereby provide a defense mechanism against toxicity and/or facilitate recovery and repair. Metallothionein is a low molecular weight, cysteine rich, metal binding proteins that has a high affinity for heavy metals. Binding capacity of metallothionein to heavy metals renders heavy metals biologically inactive and thereby reduces toxicity. Metallothionein production can be induced by low, nontoxic concentrations of metals (Goldstein and Schnellmann, 1996)

In males, calcium levels were decreased in group 7 animals at weeks 4 and 13 and in group 6 animals after 13 weeks of exposure (Tables 2A and 2B; Figures 9 and 10). In females calcium levels were decreased in group 6 at the end of week 4 and 13 and in group 3 animals at the end of week 4 (Tables 2D and 2E; Figures 11 and 12). Sex specific decrease in calcium levels is inferable as seen by the decrease in males of group 7 but with no such effect in group 7 females (Tables 2D – 2F). There are reports which indicate that lead interacts with calcium content at various levels and at moderate exposure levels result in decreased serum calcium content. The competition

between lead and calcium occurs at different levels such as intestinal absorption, transport, bone deposit and mobilization and renal excretion. Disorders of intestinal calcium absorption, transport, distribution, deposition and excretion can be caused due to direct action of lead on cell membranes, and on hormones like parathhormone, osteocalcine, calcitonine, 1,25 D dihydroxyvitamine, factors which could mediate hypocalcaemic status (Ossian *et al.*,1998; ATSDR, 1999).

The mean glucose values of groups 4, 5 and 7 males after 13 weeks of exposure and, male and female animals of recovery group 7 (G7R) at the end of recovery period, were significantly increased as compared to respective control groups (Tables 2B, 2C and 2F and Figures 13 and 14). Increased kidney gluconeogenic enzymes and adenylate cyclase-cyclic AMP system were shown by Stevenson *et al.* (1976) after chronic exposure to lead in rats. They also observed suppressed insulinogenic index (the ratio of serum IRI to blood glucose concentration) in lead treated animals. This enhancement of gluconeogenesis and decreased insulin action might be the cause for lead-induced hyperglycemia observed herein.

The statistically significant increase in level of glucose was observed in group 5 (chlorpyrifos – 10 ppm) males (Table 2B) at the end of 13 weeks of exposure. Hyperglycemia due to subchronic exposure to chlorpyrifos has not been reported. However, acute hyperglycemic effect of organophosphates were reported by Satar *et al.* (2004) working on acute effects of ultrastructural changes in rat liver treated with pralidoxime following acute organophosphate poisoning in Wistar rats. Increased secretion of catecholamines from adrenal medulla due to hyperactivity of sympathetic ganglia may increase serum glucose concentration (Satar *et al.*, 2004).

Hence, role of OP chemicals in carbohydrate metabolism also can not be ruled out.

Groups 6 and 7 animals revealed reduction in mean values of ALT and AST (Tables 2A – 2F). Decreased levels of these serum enzymes at dietary concentrations of 100 and 1000 ppm of lead have been reported by Davidson (1994). Decreased Serum AST/ALT activity generally observed in toxicology studies may indicate decreased hepatocellular production or release, or an affect on the coenzyme, pyridoxal 5-phosphate (Hall, 1992).

The reduction in serum and RBC cholinesterase activities were evident at the end of week 4 and 13. The percentage reduction of cholinesterases activity reveal that the inhibition of both serum and RBC cholinesterases activities are higher in group 5 animals as compared to group 7 animals at the end of week 4. After week 13, the percent inhibitions were comparable or slightly higher in group 7 animals. After the recovery period, serum and RBC cholinesterase activities of group 7 recovery females (G7R) were decreased by 3.9% and 34.50% respectively against 0% (serum) and 14.7% (RBC) in group 5 recovery females (G5R). In case of males, 7.9 and 17.1% reduction were observed in serum cholinesterase activity of group 5 recovery (G5R) and group 7 recovery (G7R) animals, respectively (Tables 2A – 2F and 3).

The persistence of hyperglycemic condition (Tables 2C and 2F; Figures 13 and 14) and incomplete reversal to normal activities of cholinesterases in group 7 recovery animals after cessation of test substance for a period of 28 days suggesting long-lasting effects of exposure of lead and chlorpyrifos combination. The chelating complex formation properties of metal and chlorpyrifos (Pesticide Manual, 1997) might delay the biotransformation processes and hence, availability to the body for long time.

SUMMARY

The interactive/combination effects of chlorpyrifos (CPF) and lead acetate (LA) through dietary exposure on biochemical parameters were carried out in Wistar rats. The study was designed using two different dose levels of CPF and LA and grouped into seven groups; control- 0 (Group 1), CPF - 1 (Group 2), LA - 50 (Group 3), CPF -1 + LA - 50 (Group 4), CPF -10 (group 5), LA - 1000 (Group 6) and CPF -10 + LA -500 (Group 7) ppm. In addition, 4 recovery groups (G1R, G5R, G6R and G7R) were included at high dose level with control recovery, to detect persistence or delayed effects, if any. The parameters evaluated include hematology and clinical chemistry employed in conventional toxicology study and evaluated at the end of week 4 and 13 of exposure period and after 4 weeks of recovery period. There were no significant changes in hematological parameters studied except for slight anemic effects in lead treated animals. Slight anemic effects were evident in males after 13 weeks of exposure and in females after 4 weeks of exposure. Animals exposed to lead revealed increased serum electrolytes (sodium and chloride) concentration after 4 weeks of exposure and comparable levels at the end of treatment period. The reductions in serum and RBC cholinesterases activities were evident at the end of week 4 and 13 in groups 5 and 7. After the recovery period, higher inhibition of RBC cholinesterase activity was observed in recovery group of animals of LA plus CPF treated group (G7R) as compared to CPF alone treated group (G5R). The variations (increased) observed in serum glucose level at the end of exposure period (males) and its persistent in group 7 recovery animals (G7R) after with drawl of test substance for a period of 28 days could be due to higher availability of lead after cessation of treatment and its successive mechanism of enhancement of gluconeogenesis and decreased insulin action. The cholinesterase enzyme (RBC) and serum glucose level observed in group 7

recovery (G7R) animals were not comparable to group 5 recovery animals (G5R) after 28 days of recovery period. This suggests long lasting and/or persistence of effects due to CPF plus and LA treatment.