Vet.Med.J.,Giza. Vol.47, No.1. (1999):117-135.

IN VIVO ACUTE EFFECTS OF PESTICIDES ON CLINICAL BIOCHEMICAL PARAMETER

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Received : 15/ 4/ 1998

Accepted : 22/ 9/ 1998

SUMMARY

One hundred thirty-two young adult male Sprague Dawley rats, averaging 60.7g initial body weight, were used in twenty-two feeding experiments to evaluate the acute effects of copper oxychloride, copper sulphate and dithane during a period of 15 and 30 days intervals. In Exp. 1, 12 rats were treated with purified basal diet (control) for consecutive 30 days, while in Exp. 2 and 3, 72 rats were randomly assigned to 12 treatments (for consecutive 30 days) supplemented with copper oxychloride (5 and 10 ppm); copper sulphate (5 and 10 ppm) and dithane (5 and 10 ppm). Whilst in Exp. 4 and 5, 48 rats were assigned to 8 treatments with copper oxychloride and dithane (2.5 + 2.5 ppm); copper sulphate and dithane (2.5 + 2.5 ppm); copper oxychloride and dithane (5+5 ppm) and copper sulphate and dithane (5+5 ppm) for consecutive 30 days. The result revealed that, the patterns on feed consumption were less clear than the growth data. All organs weight were increased in diets incorporated with dithane (except copper sulphate and dithane at 10 ppm diet) than did the corresponding rats served as control and other treatments . Also there were a significant decrease on IgA, IgG, IgM and IgD concentration after 30 days . A significant elevations on the activities of ASAT (GOT) and ALAT (GPT), $P<0.01$, during the second 15 days in all experimental rat groups, except rats fed copper oxychloride diet incorporated with dithane (2.5 +2.5 ppm) had a significant decrease on the activities of ALAT in second 15 days. Hyperglycemia and hypercholesterolaemia were recorded. Serum total protein, albumin and globulin were increased with an increase in urea level and creatinine, $P<0.01$, while uric acid recorded a significant decrease, $P<0.05$, in all experimental rats group.

Such complicated investigation to ascertain the combined effects of multiple chemicals usually requires a large number of animals and a long period of time, this is almost impossible with conventional carcinogenicity studies. The results presented in this study may be useful for the rapid detection of carcinogenic agents.

INTRODUCTION

In recent years, pesticides have been widely used in agriculture and their background levels in the environment have increased. These chemicals have been found in many natural products, in foodstuffs and in drinking water, so that exposure to pesticides has become a serious problem. They could be a source of many biochemical and physiological disturbances in animals and humans. Numerous in-vivo and in-vitro studies of possible consequences of treating animals, cell cultures or enzyme systems with these substances have been conducted (Hassan et al., 1990; Nebbia et al., 1993; Wang et al., 1993). Nevertheless, important toxicological aspects of pesticide effects in animals cells remain unknown. In addition, some pesticides such as copper sulphate and copper oxychloride have not been investigated.

So, the present investigation is conducted to study the effect of new pesticide recommended to use now such as copper sulphate, copper oxychloride and dithane (the old one) growth rate; relative organs weight, clinical biochemical parameters in serum associated with liver and kidney function and also on blood picture, blood indices and serum immunoglobulins (IgA; IgG; IgM and IgD).

MATERIALS AND METHODS

Materials:

Copper oxychloride, copper sulphate and dithane were obtained from the pesticides central laboratory (Pesticides Cosmetology Department) Cairo, Egypt.

Animals:

Twenty-two groups of six young adult male albino rats, Sprague-Dawley strain, mean weight 60.7 g were used. They were obtained from the Helwan breeding farm, Cairo, Egypt. The animals were divided into twenty-two groups and housed individually in stainless steel cages with wire mesh bottoms in a room maintained at 25-30° with about 50% relative humidity. The room was lightened on a daily photoperiod of 12 h.

Experimental diets:

The composition of the experimental diets are represented in table (1). After an accimatization period of two weeks, a consecutive 30 days feeding study was conducted, control animal group received a basic diet. Food and water were provided ad libitum. Body weight gain and food consumption were recorded periodically.

Tissue preparation:

During the setup regimen (15 and 30 days), animals were fasted for 16 hour and anesthetized with ether. Incisions were made into the abdomen and blood samples were obtained from the portal vein and left to clot and centrifuged at 1300 X g for 15 min. at 4°C to obtain serum. Liver, kidney, heart, spleen and thymus were excised, rinsed in chilled saline solution, then blotted on filter paper, weighed separately to calculate the absolute and relative organ weight.

Analytical procedures:

1- Serum glucose concentration was estimated by

enzymatic colorimetric procedures kits developed by E. Merck, 64271 Darmstadt, Germany, according to Trinder, (1969).

2- Serum total protein was determined by using colorimetric method kits No. 3327, E. Merck, Postfach 4119, D-6100 Darmstadt, Germany, according to Sunderman, et al., (1958) whereas serum albumin content was determined by using colorimetric procedures kits developed by Biocon, D-57299 Burbach/Germany, according to Webster (1974). Globulin concentration and albumin/globulin ratio (A/G) were calculated.

3- Serum total cholesterol was determined by using enzymatic colorimetric method kits developed by Biocon, D-57299 Burbach/Germany, according to Richmond, W., (1973). Serum triacylglycerol level was determined by using enzymatic colorimetric method kits developed by Biocon, D-57299 Burbach/Germany, according to Fossati and Prencipe, (1982).

4- Serum activities of glutamate oxaloacetate transaminase (ASAT) and glutamate pyruvate transaminase (ALAT) were estimated by colorimetric procedures kits supplied by Biocon, D-57299 Burbach/Germany, according to Reitman and Frankel, (1957), whilst serum level of uric acid was analyzed by enzymatic colorimetric procedures kits supplied by Diamond Diagnostic for laboratory services, Cairo, Egypt, mentioned by Henry, (1974). Serum level of creatinine was analyzed by kinetic method kits supplied by Diamond Diagnostics, described by Henry, (1974). Whereas serum level of urea was analyzed by enzymatic colorimetric method kits supplied by E. Merck, Frankfurter Str. 250, D-6100 Darmstadt 1/Germany, according to Fawcett and Scott, (1960).

5- Blood picture and blood indices were performed as follows:

a) Erythrocyte count (RBC), white blood cell (WBC) count and platelet (PLT) count, were performed according to Dace and Lewis, (1985).

b) Haematocrite value (HCT) was determined according to Boroviczeny, (1966).

c) Hemoglobin concentration (Hgb) was determined according to Van Kampen and Zijlstra, (1967).

d) Blood indices: Mean corpuscular hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were estimated according to Bessman and Johanson (1975).

6-Serum habtoglobin and serum immunoglobulins (IgG, IgA. IgM and IgD) were estimated by using single radio immunodiffusion technique, purchased from Biomidi, (Parc de La Plain-35, Avenue Marcel Dassault-F-31500 Toulouse-France), according to Verbruggen, (1975).

7- Statistical analysis: statistical analysis was done by completely randomized design in factorial arrangement (ANOVA, F-test) showed evidence of overall differences between diets, according to Snedecor and Cochran, (1980).

RESULTS

Mortality and Toxicity Observations

In general, no mortality or clinical signs of toxicity were found in rats receiving copper sulphate. Wherein, rats on dithane diet (G7), at 10 ppm had malaise; anorexia; sluggish behaviour; alopecia and skull morphological abnormalities but these observations were milder at 5 ppm. On the other hand, all rats fed copper oxychloride diet (G5) at 10 ppm died after 30 days (100% death).

Feed Intake and Body Weight Gain

Table (2) shows the weight gains of the rats fed the experimental diets for 15 and 30 days. Generally, rats consuming the diets containing either copper oxychloride (G5) or dithane (G7) at 10 ppm lost their weight than those fed the same diets at 5 ppm after 15 days. On the other hand, rats fed dithane diet (G7) at 10 ppm redress their body weight gain after 30 days, in spite of feed intake decreased by increasing the time of feeding (30 days), table (2). Rats consuming copper sulphate diet (G6) at 10 ppm, gained less weight than those fed the same diet (G3) at 5 ppm ($P<0.01$), in spite of they had almost feed intake during the first 15 days and increased in the second 15 days for rats fed doses at 10 ppm, table (2). Unexpectedly, rats fed dithane diet incorporated with copper oxychloride had higher gain weight at 10 ppm than 5 ppm after 2 weeks, but these results are diametrically opposed after 4 weeks, table (2). Adding copper sulphate to the dithane, G9 and G11. resulted in significantly decreased gains after 2 and 4 weeks.

Relative organs weights

The effect of different diets on liver, heart. Kidney, spleen and thymus weights of animals is shown in table (2). Final relative liver weight was significantly higher ($P<0.01$) for rats fed all the experimental diet after 2 and 4 weeks, except G3 and G10 had less weight after 30 days. Of dithane diets incorporated with either copper oxychloride or copper sulphate (G8; G9 and G10), had clearly elevated relative kidney weights ($P<0.05$); spleen and thymus weight ($P<0.01$) after 15 days. There was a highly increased ($P<0.01$) in absolute thymus weight in rats fed the experimental diet (G8 and G9) during both periods (2 and 4 weeks).

Blood picture and blood indices

Table (3) gives the blood erythrocytic parameters in male rats exposed to different levels of copper sulphate, copper oxychloride and dithane. There was a significant increase ($P<0.01$) in (WBC) count and (PLT) count among the groups (G2, G4, G5, G7 and G11) as compared to control rats group (G1), table (3), during the consecutive 30 days feeding study.

Table (1): Composition of the experimental diets (g/100 g diet)

Ingrediants dry base	Basic diet G_1	Copper oxychloride G_2	Copper sulphate G_3	Dithane G_4	Copper oxychlorideG$_5$	Copper sulphate G_6	Dithane G_7	Copper oxychloride + Dithane G_8	Copper sulphate + Dithane G_9	Copper oxychloride + Dithane G_{10}	Copper sulphate + Dithane G_{11}
Strach	65.2	64.7	64.7	64.7	64.2	64.2	64.2	64.7	64.7	64.2	64.2
Casein	20	20	20	20	20	20	20	20	20	20	20
Corn oil	5	5	5	5	5	5	5	5	5	5	5
Non-nutritive cellulose	5	5	5	5	5	5	5	5	5	5	5
Salt mix1	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vit. mix2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dl-Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Copper oxychloride	–	0.5[3]	–	–	1.0[4]	–	–	0.25[5]	–	0.5[3]	–
Copper sulphate	–	–	0.5[3]	–	–	1.0[4]	–	–	0.25[5]	–	0.5[3]
Dithane	–	–	–	0.5[3]	–	–	1.0[4]	0.25[5]	0.25[5]	0.5[3]	0.5[3]

1- Mineral mixture g/Kg: $CaHPO_4$, 500; Nacl, 74; $K_3C_6H_5O_7$, H_2O, 220; K_2SO_4, 52; Mgo, 24; Manganous carbonate, 3.5; Ferric citrate, 6; Zinc carbonate, 1.6; cupric carbonate, 0.3; KIO_3, 0.01; Na Se O_3. $5H_2O$, 0.01; [CrK $(SO_4)_2$.$12H_2O$], 0.55; Sucrose, finely powdered to make 1,000g, (AIN, 1977).

2- Vitamin mixture mg/kg (except as noted); Thiamin. Hcl, 600; Riboflavin, 600; Pyridoxine, Hel, 700; Nicotinic acid, 3g; D-calcium pantothenate, 1.6 g; Folic acid, 200; D-Biotin, 20; Vit. B12, 100; Retinol, 0.400 g; DL. a-Tocopheryl acetate, 5.000; Cholecalciferol, 2.5; Menaquinone 5.0; Sucrose finely powdered to make 1.000 g (AIN, 1977).

3- 0.5g/100g = 5g/kg = 5 ppm

4- 1.0 g/100g = 10 g/kg = 10 ppm.

5- 0.25 g/100 g = 2.5 g/kg = 2.5 ppm.

Table (2): Weight gain; feed consumption and tissue weights of rats maintained on either copper oxychloride; copper sulphate or dithane.

Parameters	Days	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}	G_{11}
Initial body weight, g	15 days	67.5	66	63.7	66.5	69.8	62.9	77.3	76.8	71.1	63.1	65.9
	30 days	62	59.8	55.7	59.5	58.2	55	48.5	46.9	45.7	51.3	42.5
Final body weight, g	15 days	93.3	72.6	76.3	76.7	62.8	70.1	66.5	99	107.3	105.5	89.3
	30 days	147.8	57	90.9	118.9	---	71.2	73.7	152.1	144.3	131.3	118.9
Body weight gain, g	15 days*	25.8±4.15	6.6±3.36	12.6±15.34	10.2±5.76	-7±4.74	7.2±5.12	-10.8±13.33	22.2±18.59	36.2±7.5	42.4±6.84	23.4±9.04
	30 days*	85.8±21.06	-2.8±10.03	35.2±5.76	59.4±11.37	---	16.2±16.42	25.2±14.2	105.2±34.95	98.6±12.1	80±10.2	76.4±16.38
Feed consumption/day, g	15 days*	16.6±0.28	18.3±0.42	13.15±0.35	17.8±0.35	14.4±0.57	12.6±0.28	11.3±1.06	18±0.07	16.6±0.28	16.2±0.42	16.6±0.35
	30 days*	13.6±0.22	7.6±0.14	11.02±0.04	9.8±0.23	---	14.3±0.06	8.13±0.01	7.59±0.15	11.36±0.11	9.55±0.21	12.63±0.21
Absolute liver wt.g	15 days*	3.72±0.38	3.18±0.19	3.44±0.93	3.68±0.81	2.46±0.21	2.86±0.57	3.43±0.77	4.93±0.84	4.3±0.65	4.46±0.15	3.66±0.64
	30 days*	6.44±1.31	3.08±0.43	2.84±0.29	6.02±1.01	---	2.94±0.76	4.32±1.37	6.94±1.97	6.1±0.76	5.02±0.3	5.42±1.11
Liver wt/100 g body wt.	15 days*	4.02	4.44	4.72	4.94	3.98	4.18	5.26	5.1	4.04	4.26	4.14
	30 days*	4.32	5.37	3.2	5.14	---	4.39	5.93	4.56	4.25	3.84	4.25
Absolute Kidney wt. g	15 days*	0.68±0.04	0.58±0.08	0.5±0.1	0.48±0.08	0.48±0.04	0.5±0.07	0.46±0.11	0.8±0.12	0.84±0.11	0.84±0.09	0.46±0.17
	30 days**	1.14±0.09	0.64±0.21	1.08±0.18	0.98±0.3	---	0.78±0.41	0.9±0.17	1.12±0.36	1.3±0.37	0.82±0.18	1.04±0.24
Kidney wt/100g body wt	15 days*	0.73	0.79	0.70	0.64	0.74	0.74	0.68	0.84	0.80	0.80	0.52
	30 days*	0.8	1.05	1.2	0.83	---	1.17	1.33	0.75	0.92	0.62	0.77
Absolute heart wt.g	15 days*	0.5±0.07	0.36±0.05	0.32±0.04	0.36±0.05	0.22±0.04	0.26±0.05	0.22±0.04	0.42±0.08	0.56±0.18	0.48±0.15	0.28±0.13
	30 days*	0.44±0.17	0.18±0.13	0.26±0.25	0.48±0.08	---	0.22±0.08	0.3±0.00	0.54±0.09	0.46±0.11	0.42±0.04	0.44±0.09
Heart wt/100g body wt.	15 days*	0.54	0.48	0.47	0.48	0.39	0.38	0.35	0.44	0.53	0.45	0.30
	30 days*	0.46	0.26	0.17	0.37	---	0.37	0.43	0.39	0.33	0.32	0.35
Absolute spleen wt, g	15 days*	0.44±0.05	0.3±0.07	0.28±0.08	0.26±0.05	0.3±0.00	0.18±0.04	0.1±0.004	0.56±0.17	0.64±0.09	0.56±0.15	0.36±0.23
	30 days*	0.54±0.22	0.34±0.19	0.7±0.07	0.44±0.09	---	0.18±0.04	0.26±0.11	0.52±0.15	0.38±0.11	0.28±0.16	0.46±0.11
Spleen wt/100g body wt	15 days*	0.48	0.38	0.38	0.35	0.46	0.26	0.16	0.57	0.55	0.51	0.33
	30 days*	0.37	0.49	0.79	0.38	---	0.25	0.30	0.36	0.27	0.21	0.38
Absolute thymus wt, mg	15 days*	9.8±0.84	4.6±0.89	6.4±1.52	5.8±1.1	6.2±0.45	5.8±1.9	4.8±1.3	11.8±0.84	11±1.58	9.6±0.89	8.6±1.14
	30 days**	15.8±3.11	8.4±1.14	10.8±0.84	11.4±1.52	---	8.8±1.1	9.8±0.45	20.2±4.82	17.6±1.67	11.4±1.34	10.6±0.55
Thymus wt/100g body wt.	15 days*	0.01	0.007	0.01	0.008	0.01	0.009	0.008	0.014	0.013	0.009	0.01
	30 days**	0.11	0.015	0.012	0.01	---	0.014	0.014	0.013	0.012	0.008	0.009

* P<0.01
** P<0.05

G_1. control diet
G_2. copper oxychloride diet, 5 ppm
G_3. copper sulphate diet, 5 ppm
G_4. dithane diet, 5 ppm
G_5. copper oxychloride diet, 10 ppm
G_6. copper sulphate diet, 10 ppm
G_7. dithane diet, 10 ppm
G_8. copper oxychloride + dithane diet, (2.5 + 2.5 ppm)
G_9. copper sulphate + dithane diet, (2.5 + ppm)
G_{10}. copper oxychloride + dithane diet, (5+5 ppm)
G_{11}. copper sulphate + dithane diet, (5 + 5 ppm)

Table (3): Blood erythrocytic parameters in male rats exposed to different levels of copper sulphate; copper oxychloride and dithane.

Parameters	Days	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}	G_{11}
WBC (x109/L)	15 days	9.5±0.5	20.8±0.16	6.4±0.01	3.18±0.04	27.5±0.08	6.06±0.06	10.19±0.01	8.26±0.02	13.38±0.02	7.7±0.07	11.18±0.04
	30 days	20.5±0.04	27.08±0.04	14.3±0.14	22.7±0.3	---	13.6±0.18	72.5±0.7	13±0.007	17.7±0.01	12.08±0.03	14.8±0.04
RBC (x1012/ L)	15 days	5.27±0.02	3.83±0.04	4.62±0.03	5.63±0.04	3.93±0.01	5.94±0.01	5.05±0.02	4.71±0.01	5.94±0.007	4.81±0.01	6.33±0.007
	30 days	7.41±0.01	7.58±0.02	6.51±0.01	7.1±0.01	---	8.35±0.01	6.36±0.007	7.26±0.01	6.8±0.01	7.42±0.02	7.25±0.01
Hgb (g/dL)	15 days*	9.5±0.07	7.9±0.01	11.18±0.04	9.25±2.2	3.9±5.4	9.29±0.01	8.8±0.007	8.3±0.007	10.06±0.03	8.5±0.01	11±0.00
	30 days*	13±0.007	13±0.01	15.32±0.02	12.09±0.01	---	12.7±0.01	9.9±0.007	11.8±0.01	12±0.007	12.08±0.02	13.09±0.01
Het (ratio)	15 days*	26.9±0.007	21.3±0.007	23.9±0.007	30.18±0.04	20.3±0.007	27.7±0.007	25.8±0.007	24.11±0.01	29.7±0.01	24.7±0.007	32.7±0.007
	30 days*	40.2±0.01	38.3±0.007	35.7±0.007	38.6±0.01	---	41.4±0.01	33±0.007	37.5±0.007	37.9±0.007	38.4±0.01	41.7±0.01
MCV (FL)	15 days*	50.9±0.007	51.9±0.007	51.5±0.007	53.2±0.01	51.3±0.01	46.5±0.01	51.1±0.007	51.2±0.01	49.8±0.4	51.2±0.03	51.6±0.01
	30 days*	54.1±0.007	54.5±0.01	54.7±0.01	54.3±0.01	---	49.4±0.02	52.2±0.03	51.6±0.03	55.7±0.01	51.6±0.01	57.5±0.01
MCH (Pg)	15 days*	18±0.03	20.5±0.01	24.1±0.01	19.06±0.03	19.5±0.01	15.6±0.02	17.4±0.02	17.6±0.01	17±0.01	17.6±0.007	17.4±0.01
	30 days*	17.5±0.01	16.7±0.007	23.5±0.01	17.01±0.01	---	15.2±0.03	15.6±0.02	16.2±0.007	17.6±0.01	16.3±0.02	18.1±0.11
MCHC (g/dL)	15 days*	35.3±0.01	37.1±0.03	46.9±0.01	35.8±0.01	38±0.007	33.6±0.03	34.1±0.01	34.4±0.01	34±0.01	34.4±0.02	33.6±0.01
	30 days*	32.3±0.03	33.2±0.01	43±0.01	31.3±0.02	---	30.7±0.01	30±0.06	31.5±0.007	31.7±0.01	31.5±0.007	31.4±0.01
PLT (x 10*/L)	15 days*	385±1.41	640±1.41	547±0.71	345±2.12	682±1.41	423±1.41	447±1.41	590±0.71	1051±1.41	538±1.41	885±0.71
	30 days*	1012±1.41	1078±1.41	1132±1.41	1174±1.41	---	881±0.71	1121±1.41	912±1.41	1182±1.41	832±1.41	995±2.12
Lymph (%)	15 days*	89±0.71	74±0.71	84±0.71	70±0.7	71±0.7	90±0.7	60±0.7	87±0.7	76±0.7	97±1.41	77±1.4
	30 days*	76±0.7	73±0.70	72±0.70	63±0.7	---	88±0.7	54±0.7	81±0.7	71±0.7	90±0.7	72±0.7
Neutrophils (%)	15 days*	11±0.71	25±0.71	16±0.7	22±0.7	28±1.4	4±0.7	13±0.7	5±0.7	23±1.4	3±0.7	23±0.7
	30 days*	23±0.7	26±0.7	33±0.7	27±0.7	---	9±0.7	16±0.7	15±0.7	25±0.00	10±0.7	25±0.7
Mono (%)	15 days*	1	1	1	2	1	1	---	---	---	---	---
	30 days*	1	1	1	9	---	1	---	4	4	---	3

*P < 0.01

On the other hand, there was a highly significant increase (P<0.01) in (Hgb) concentration; (MCH); (MCHC) and (PLT) count in group (G_3) than did the corresponding group (G_6), wherein, there was a highly significant increase (P<0.01 in RBC_5, in G6, than G3 during the experimental period (15 and 30 days). Rats fed dithane diet incorporated with copper oxychloride in both doses, had the same (MCV); (MCH); (MCHC) after 15 days and also there was a highly significant increase (P<0.01) in (PLT) count. Lymphocytosis was recorded after 15 and 30 days (P<0.01) in G6 and G10. Whereas neutrophils noted decrease (P<0.01) in G6; G8 and G10 after 15 and 30 days

Effect of feeding dietary regimen on blood biochemical parameters in rats

Table (4) gives the mean values and significance of S. total protein, albumin; globulin and (A/G) ratio, that occurred in all animals during each dietary treatment during the consecutive 30 days experimental period. After feeding rats all the experimental diets, serum total protein in G5; G8 and G_9 increased slightly (P<0.01) after 15 days, but a significant increase was observed in serum total protein of rats fed either diet (G4) or diet (G7), P<0.01, during the consecutive 30 days. Serum albumin did not differ in G3; G6 and G7 diets (3.4; 3.3 and 3.4 g/dL respectively) from control (3.1 g/dL) during the second 15 days. It Can be seen from table (3) that, there was a significant increase in globulin during the first and second period of experiment.

Table (4) depicts the changes in serum urea; serum uric acid and serum creatinine that occurred in all animals in each dietary treatment during the consecutive 30 days experimental period . Either G_4; G_6; G_7 G_9 or G11 showed higher serum uric acid concentration (P<0.01) during the first 15 days, wherein G3, G4, G10 and G11 had a significant increase in S. uric acid during the second 15 days. Overall analysis showed that S. urea concentraiton was significantly (P<0.01) higher during the consecutive 30 days in all experimental rat groups, except G4, G6 and G7 which gave lower S. urea when compared with control feeding group. Wherein S. creatinine was increased in each of G5, G10 or G11 during the first 15 days. But during the second 15 days, there was a significant increas in G4 and G11 only.

Figs. (1), (2) and (3) depicts the changes in serum glucose; total cholesterol and triacylglycerols. A significant increase (P<0.01) was observed in serum glucose concentration in G8; G9 and G10 (1.39, 1.42 and 1.99 mmol/L respectively) and a slight increase in G5 and G11, P<0.01 (1.27 and 1.29 mmol/L respectively) as compared with control rat group, G1 (1.25 mmol/L) during the first 15 days, Fig (1). But during the consecutive 30 days a significant increase (P<0.01) was observed in S. glucose in G3; G4; G7; G8; G9; G10 and G11 (1.42, 1.63, 1.55, 1.85, 1.50, 2.29 and 1.45 mmol/L respectively), Fig. (1), as compared with control rat group (1.26 mmol/L).

Table (4): The effect of feeding dietary regimen on blood biochemical parameters in rats.

Parameters	Days	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9	G_{10}	G_{11}
Total protein, g/dL	15 days*	6.38±0.11	5.91±0.06	5.3±0.18	7.24±0.17	6.6±0.21	6.2±0.07	7.7±0.06	6.5±0.22	6.5±0.23	5.6±0.25	6.14±0.08
	30 days*	7.39±0.13	6.2±0.34	6.33±0.22	7.6±0.24	---	6.4±0.2	8.3±0.41	6.7±0.32	6.6±0.16	6.6±0.28	6.38±0.15
Albumin, g/dL	15 days*	4.04±0.08	2.5±0.04	3.2±0.07	3.6±0.6	2.8±0.13	3.3±0.06	3.8±0.03	3.5±0.04	2.9±0.04	3.2±0.19	3.1±0.1
	30 days*	3.1±0.21	2.7±0.05	3.4±0.14	4.6±0.13	---	3.3±0.06	3.4±0.3	4.3±0.3	3.6±0.03	3.5±0.06	3.6±0.08
Globulin, g/dL	15 days*	2.4±0.08	3.4±0.12	2.03±0.08	3.5±0.15	3.9±0.11	2.9±0.02	4±0.05	2.9±0.11	3.6±0.08	2.7±0.11	3.04±0.08
	30 days*	4±0.03	3.4±0.13	2.8±0.18	3.2±0.01	---	3±0.08	5.3±0.04	2.9±0.01	2.9±0.05	3.3±0.13	2.6±0.18
Globuiin. ratio	15 days*	1.62±0.18	0.69±0.05	1.52±0.14	0.91±0.1	0.67±0.02	1.11±0.04	0.93±0.05	1.18±0.08	0.78±0.04	1.17±0.04	0.93±0.06
	30 days*	0.78±0.04	0.81±0.1	1.09±0.06	1.33±0.16	---	1.1±0.06	0.59±0.01	1.39±0.05	1.18±0.08	1.07±0.04	1.24±0.08
Uric acid. mg/dL	15 days**	2.51±0.72	1.34±0.57	2.58±0.66	2.92±0.45	1.5±0.00	3.08±0.44	3.52±0.45	2.50±0.50	2.70±0.06	2.43±0.25	2.84±0.62
	30 days**	1.78±0.69	1.43±0.071	2.46±0.11	2.08±0.47	---	1.43±0.19	1.10±0.11	1.38±0.21	1.84±0.12	2.06±0.14	2.47±0.23
Urea, mg/dL	15 days*	8.58±0.15	13.48±0.69	11.03±0.04	8.09±0.12	15.45±0.64	7.21±0.29	7.68±0.33	12.07±0.25	12.04±0.06	10.35±1.5	10.45±0.21
	30 days*	7.09±0.13	14.24±0.21	20.74±1.04	17.06±0.09	---	17.00±0.03	18.01±0.03	13.5±0.03	14.12±0.04	13.49±0.01	10.71±0.42
Creatinine, mg/dL	15 days*	0.79±0.06	0.63±0.04	0.72±0.04	0.55±0.04	1.03±0.05	0.41±0.06	0.93±0.05	0.92±0.02	0.86±0.05	1.12±0.10	1.07±0.06
	30 days*	0.94±0.06	0.64±0.05	0.73±0.04	1.06±0.05	---	0.83±0.04	0.90±0.01	0.82±0.04	0.76±0.04	0.93±0.007	1.01±0.021

* P < 0.01

**P < 0.05

Fig.(1) *The effect of dietary regimen on serum glucose* (mmol/L) during the consecutive 30 days*

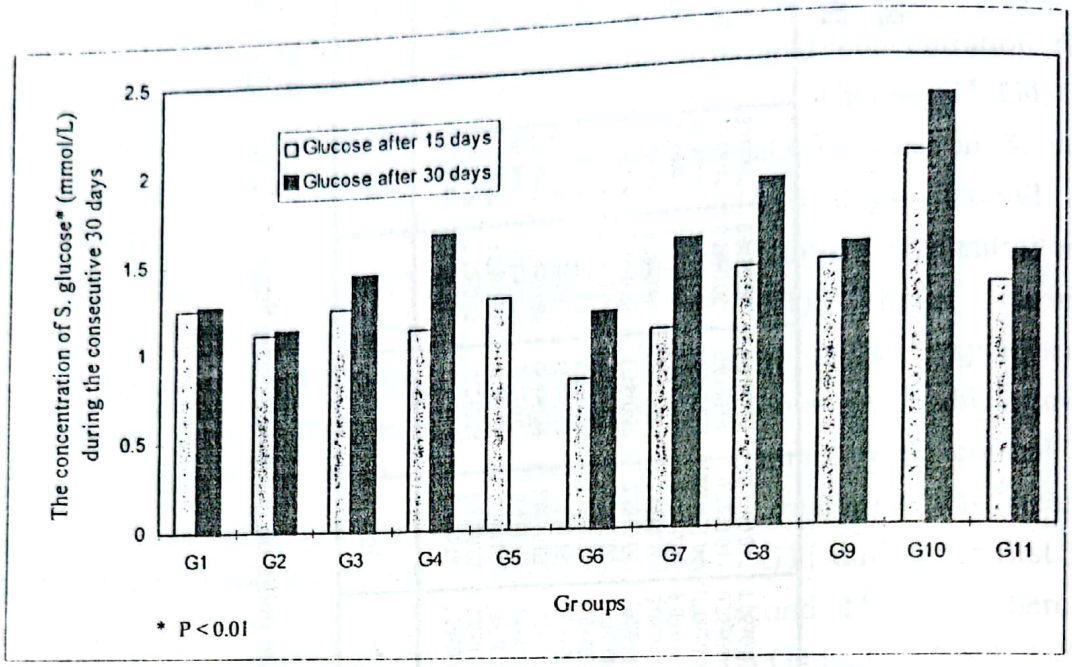


Fig.(2) *The effect of dietary regimen on serum total cholesterol* (mmol/L) during the consecutive 30 days*

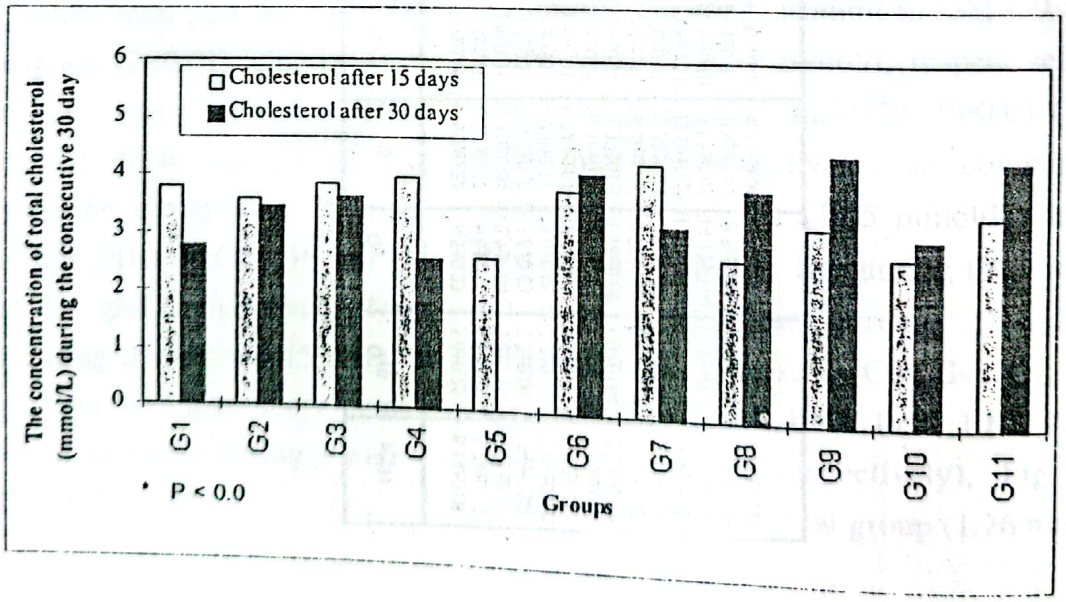


Fig.(3) *The effect of dietary regimen on serum triacylglycerols* (nmol/L) during the consecutive 30 days*

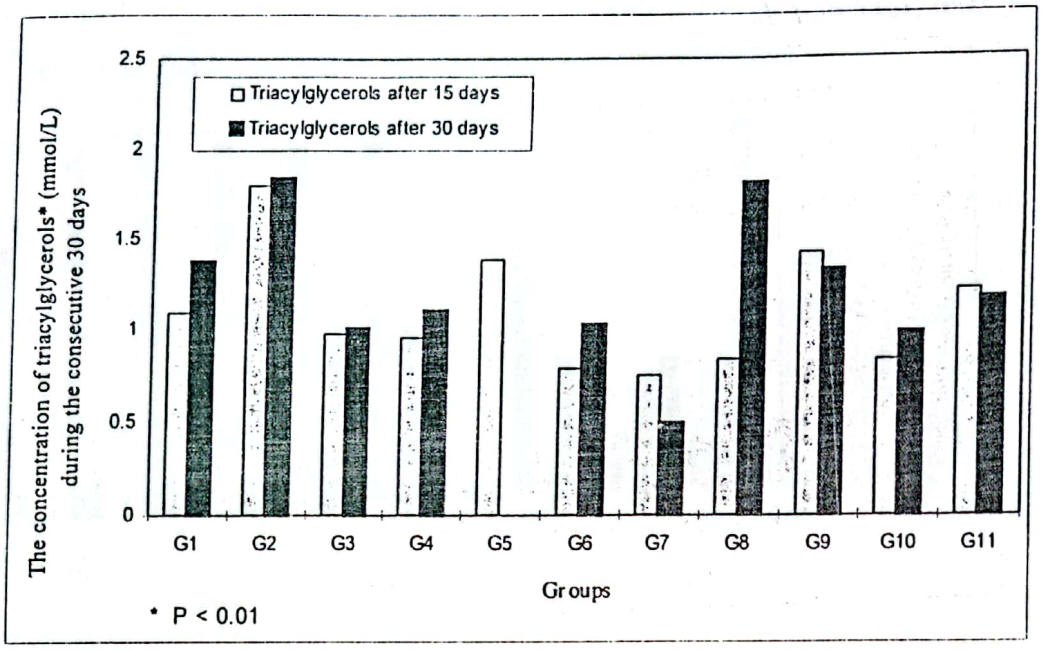


Fig.(4) *The effect of dietary regimen on ASAT* and ALAT* (µ/L) activities during the consecutive 30 days*

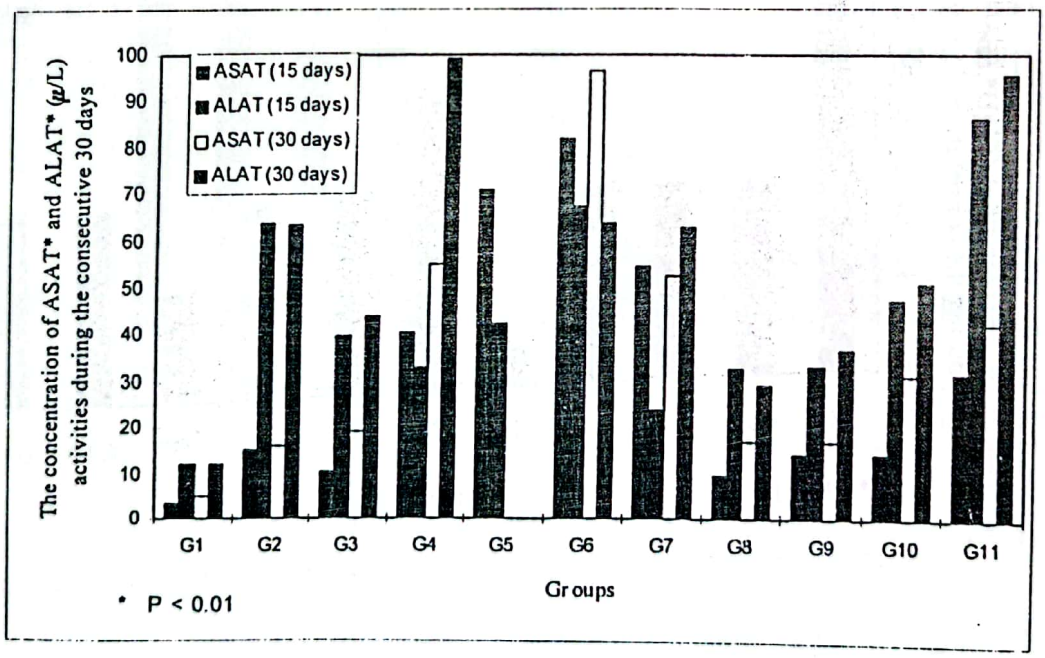
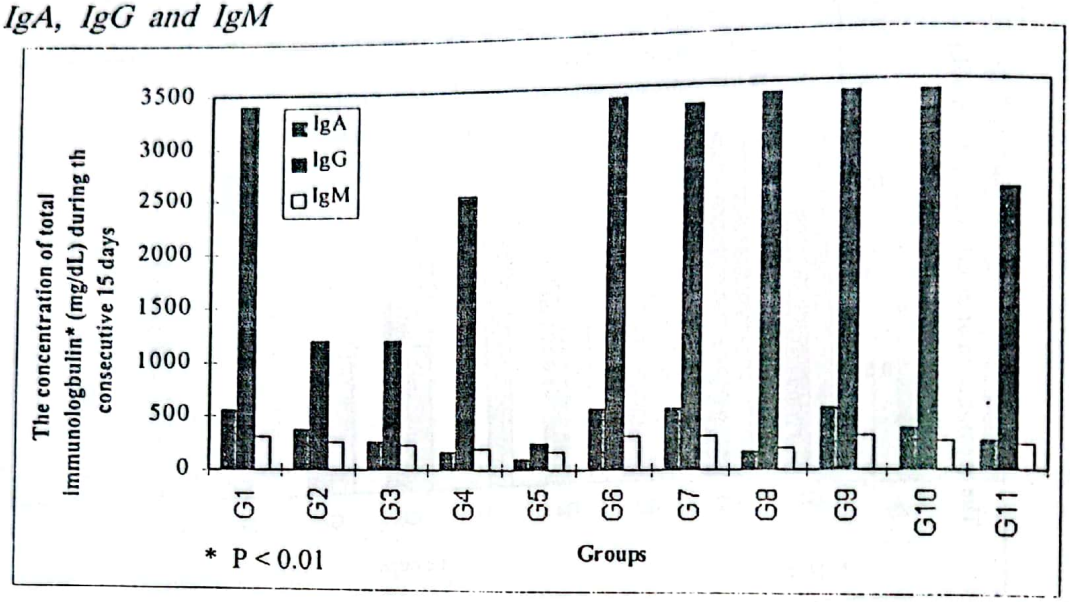


Fig.(5) *The effect of feeding dietary regimen on serum total immunoglobulin**
(a) After 15 days

IgA, IgG and IgM



IgD

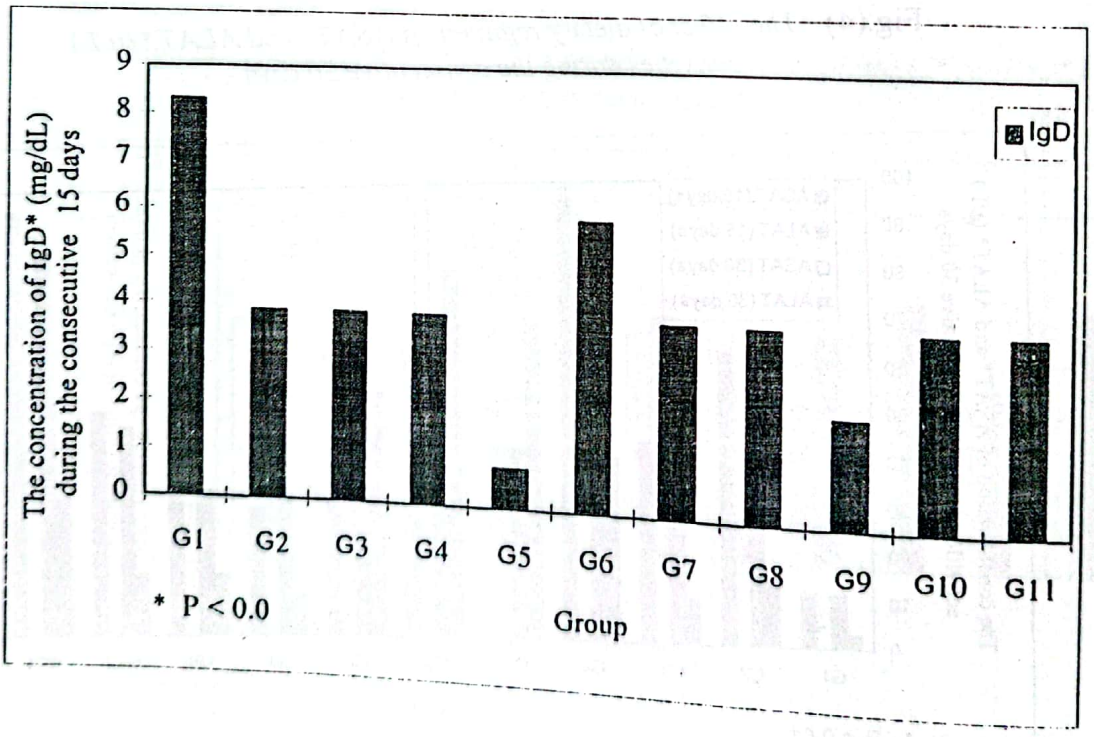
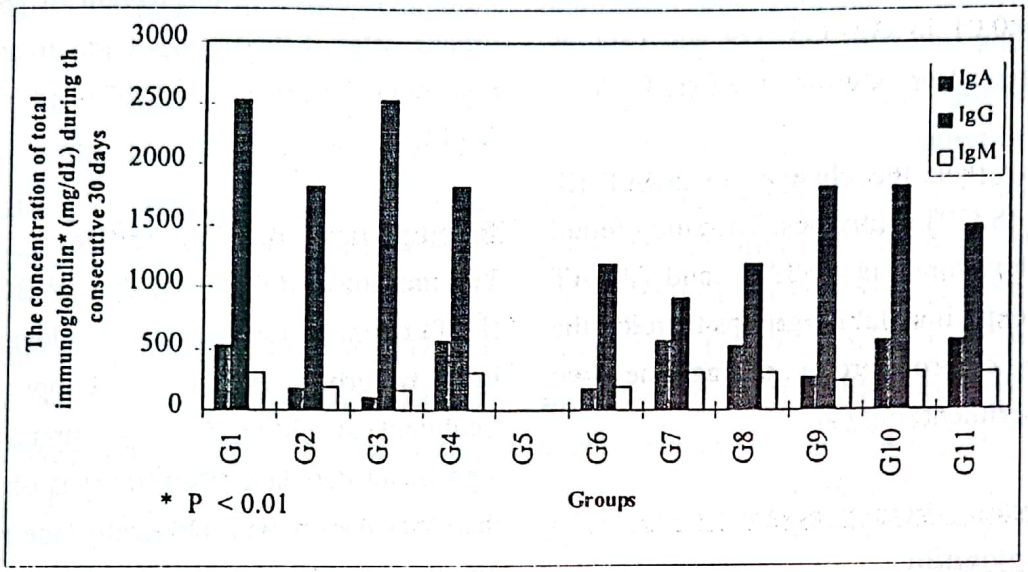
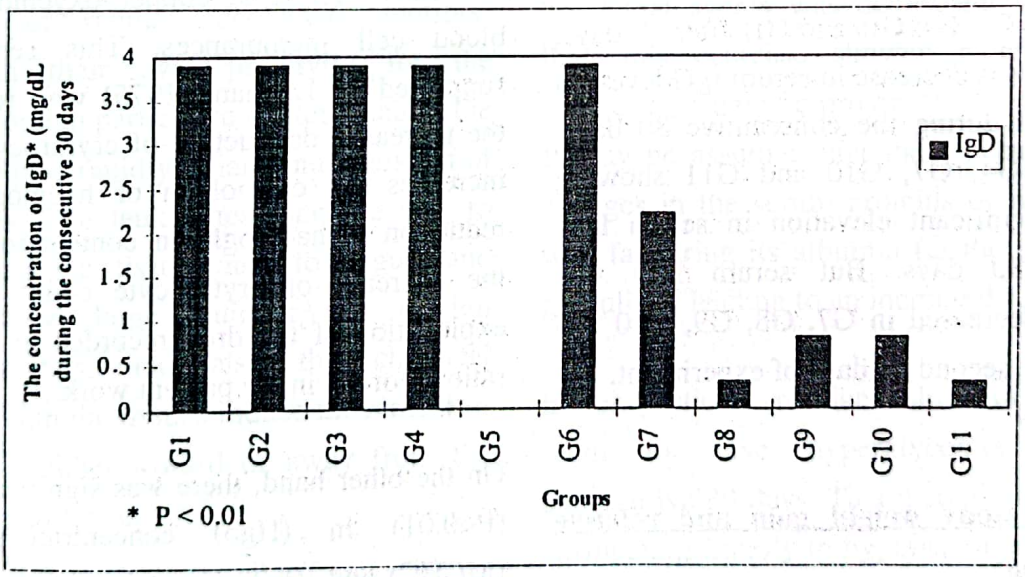


Fig.(5) *The effect of feeding dietary regimen on serum total immunoglobulin**
(b) After 30 days

IgA, IgG and IgM



IgD



On the other hand, there was a highly significant increase (P<0.01) in serum cholesterol in all treated groups (except G4) during the second 15 days, Fig (2).

Whereas mean serum triacylglycerol was increased. P <0.01 in G2; G5; G9 and G11 as compared with control rat during the first 15 days.

Fig (4), shows that, the changes in ASAT (S. GOT), ALAT (S.GPT) activities. The study found significant elevations in ASAT and ALAT activity in all experimental rat groups than did the corresponding control group during the two periods of experiment, Fig (4).

Effect of feeding dietary regimen on serum total immunoglobulin

Serum total IgA; IgG; IgM and IgD are summarized in Fig. (5). Serum IgA levels was greatest in G4; G7; G9; G10 and G11 after 30 days, wherein there was decrease in serum IgG levels in all treated rats during the consecutive 30 days. With studies G4; G7; G10 and G11 showing statistically significant elevation in serum IgM levels after 30 days. But serum IgD was significantly decreased in G7, G8, G9, G10 and G11 during the second 15 days of experiment.

DISCUSSION

Feed intake, Body weight gain and relative organs weights

The cancer-promoting effect of copper oxychloride and dithane in rats was associated with a toxic effect, as evidenced by a significant (P<0.01) reduction in food consumption and weight gain during the 2-4 week promoting treatment.

Organs of treated animals may reveal a specific target organs response Hayes, (1989); changes in body weight and in the weight of some selected organs after different pesticide treatments were reported by Akay et al., (1992) and Yaboah et al., (1992).

Blood picture and blood indices

The present study showed that (WBC) count and (PLT) count were significantly elevated (P<0.01) in all rat groups treated with copper oxychloride treatment at either 5 or 10 ppm. Wherein, a significant decrease (P<0.01) was observed in all the other parameters. Generally, the reductions in blood corpuscles may be attributed to the destruction of haematopoietic tissues and/or the destructive effect of copper oxychloride to red blood cell membrances. This seems to be supported by Linman, (1975) who reported that the increased destruction of erythrocytes directly increases the catabolism of haemoglobin. The reduction in haemoglobin content together with the decrease of erythrocyte count add to the explanation of the drop recorded in haematocrit ratio recorded in the present work.

On the other hand, there was significant increase (P<0.01) in (Hgb) concentration; (MCH); (MCHC) and (PLT) count in copper sulphate (5 ppm) regimen group (G3) than did the corresponding copper sulphate (10 ppm) regimen group (G6), but, there was increase in RBC5, and HCt (ratio); decrease in corpuscular indices

(MCV, MCHC, MCH) in G6 than G3. Also the results revealed that, there was normal (Hgb) levels in G6 than G3, table (3), this usually indicates that red cell are being lost or destroyed with compensatory increased red cell production in the bone marrow, Sacher and Mc Pherson, (1991). The present results are in agreement with that obtained by Ibrahim (1992) who revealed similar haematological effect on fish exposed to some industerial pollutant included flouride.

While, both dithane regimen diets G4 and G7 had announced very highly significant increase ($P<0.01$) in (WBC) count and (PLT) count during the second period.

These increases may be due to, the white blood cells, in particular the neutrophils and monocytes, are capable of rapid, independent motility, coupled with their avid proclivity for the ingestion of foreign particulate matter, enables the white cells to pass rapidly in large numbers out of the capillaries. The leukocytes then are free to wander among the tissues and to engulf and thereby destroy large number of foreign particulate matter, and also the chemical substances within the tissues induce movement of the leukocytes either toward or away from the source of the substance.

Effect of feeding dietary regimen on blood biochemical parameters in rats

Total serum proteins and its fractions are affected by various environmental factors Coles, (1988) level of total serum proteins in affected rats in this study were evident to show a significant increase or decrease in comparison with the control group of rats, table (4). This could be attributed to either inhibitory or stimulatory effect of copper oxychloride; copper sulphate and dithane on protein synthesis, Albumin, globulin ratio has been used to aid in interpretation of total protein values. The ratio will remain normal if both fractions are uniformly altered and be abnormal if an alteration in one fraction predominates Robert and Keith, (1986). "A/G" ratio was increased significantly during the second 15 days of experiment in G3; G4; G6; G8; G9; G10 and G11, since the albumin remained almost constant, while total globulins were decreased. In contrast, El-Hennawy, et al., (1980), reported that A/G ratio in the serum of rats given 3 types of herbicidal agents was decreased. They reported that, this decrease showed a change in the synthesis of hepatic protein due to liver damage. It may be assumed that there occurred adaptive changes in the scrum proteins of rats, in such a way favouring its albumin (at the expense of its globulins), leading to an increased "A/G" ratio.

Either pesticide residues alone or incorporated seem to have hyperglycemia during the consecutive 30 days. This increase may be due to attributed primarily to hypoinsulinemia leading to rising blood glucose.

Exposure to copper oxychloride, copper sulphate and dithane administration induced hypercholesterolaemia. The serum cholesterol

profile in G2, G3, G6, G7, G8, G9, G10 and G11 treated rats showed, there was a significant increase up to the end of the fourth week. The copper oxychloride, copper sulphate and dithane pesticides can, thus, be considered as cholesterologenic factors either by stimulating synthesis or impairing catabolism of cholesterol. On the other hand, the dietary regimen diets have no effect on triacylglycerols of rats where the mean values of triacylglycerols level of treated rats showed significant change and remained more or less close to the corresponding control group.

The measured activity of the serum enzyme aspartate and alanine transferase among the treated and normal control rats along the whole experiment showed much discrepancy with high level of ALT in respect to AST. Discrepancy among values of ALT and/or AST of rats treated with different pesticides had been reported by Enan et al., (1982); Husain et al., (1987) and Neskovic et al., (1992). In the present investigation, enzyme ALT cytoplasmic, liver specific enzyme. Friedel et al., (1979) level among the control and treated rats at different doses and time intervals increased significantly, while the activity of AST (liver, cardiac, muscle..) increased with the higher doses (10 ppm, G5, G6 G7) at all time intervals. Such findings most probably denote liver cell affection (toxic hepatitis). And also this increase was attributed to the destruction of the cells of the liver, heart and other tissues.

5 and 10 ppm of pesticides residues (single or mixture) under investigation, seem to have effect on the kidney function of rats where the mean values of creatinine and blood urea levels of treated rats showed significant change, $P< 0.01$ and remained more or less close to the corresponding control group. However, other studies with pesticides residues showed significant increase in blood urea, Mostafa et al., (1992 a and b). Wherein, serum uric acid levels was significantly decreased ($P<0.05$) in the second period of experiment in all treated rats.

Effect of feeding dietary regimen on serum total immunoglobulin

On the basis of the data reported here, elevated serum IgA in G4; G7; G9; G10 and G11 after 30 days and IgM (its dominates the primary antibody response) might be more readily explained to be the result of increased production. In the gut, isotype switching, activation and differentiation of IgA progenitors upon antigen exposure are regulated by accessory cells, T helper and T suppressor cells Elson et al., (1979); Kiyono et al., (1982); Kawanashi et al., (1983); Mestecky and Mc Ghee, (1987), that are localized in the Peyer's patches. Recently, a third level of regulation for IgA synthesis has been described that involves putative contrasuppress or cells, Suzuki et al., (1986). It is now widely accepted that B cells originating at Peyer's patches migrate via the systemic compartment (including lymph nodes and spleen) to distant mucosal sites such as the lamina propria.

Mehta, (1991) stated that immunoglobulins in serum and cerebrospinal fluid (CSF) are derived

from both intrathecal synthesis and/or transudation from serum. Moreover release of immunoglobulins especially IgM by malignant cells could be present Ernerudh et al., (1987).

On the other hand, there was significant decrease, $P<0.01$, in serum IgG (it is the one formed in the secondary antibody response) and serum IgD.

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