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Experimental Study of Biochemical Effects of Insecticides in Albino Rats

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TO DETECT the acute toxicity of some insecticide rats were divided into ten treatments each one contained six rats. These rats administered a single oral dose of 1/10 LD_{50}, ¼ LD_{50} and the LD_{50} of primiphos-methyl, Chlorpyriphos-methyl and fenitrothion. After 24 hr treated rats were sacrificed. The activity of some biochemical parameters glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (Alk.PH), creatinine, urea, total protein and total cholesterol) were detected in blood serum. The results indicated that all treatments caused significant increase in the activity of GOT, GPT and (Alk.PH), as well as the level of creatinine, urea and total cholesterol as compared by control ones. While the results indicated that ¼ LD_{50} and 1/10 LD_{50} of the three tested insecticides caused significant decrease in total protein concentration. These detected differences observed on values between enzymes tested of liver and kidney function by increase or decrease it is considered a sign and pronounced that treated animals affected by oral dose through 24 hr.

Environmental contaminants, especially pesticide residues in food and water, now have a serious poisoning effect on our health and reproduction (Aioub & Hegab, 2000 and Hussein, 2000). The world economy is built on population growth. Over 600 man-made chemicals are registered with Environmental Protection Agency (EPA) as killing agents under the broad term pesticides.These chemicals are marketed in over 40,000 combinations along with 80,000 industrial chemicals. Tolerance level is defined as the level in which the chance of cancer is increased at a factor of one in a million during a lifetime of projected exposure. (So far, the (EPA) has only provided tolerance levels on 9700 pesticide compounds) This is a carcinogen rating. What about the effect caused by interruption of our hormone, immune, and nervous systems as well as brain function ?

The aim of this work is to detect the biochemical effects of exposure to toxic effects of these insecticides (primiphos-methyl, Chlorpyriphos-methyl and fenitrothion and their residues on liver and kidney functions of White Albino Rats.

Material and Methods

Insecticides used

-Primiphos-methyl 50% E.C. (Actellic): o-(2-diethylamino-6-methyl-pyrimidine-4-yl) – o,o-dimethyl phosphorothioate.

- Chlorpyrifos – methyl 50 % E.C. (Reldan): o,o-dimethyl o-(3,5,6-trichloro-2-pyridinyl) phosphorothioate.

- Fenitrothion 50% E.C. (Sumithion): o.o-dimethyl o-(3-methyl–4-nitrophenyl) phosphorothioate).

The pervious standards were obtained from Evironmental Protection Agency (EPA) and were injected in the Gas Chromatograph (G.C) equipped with Flame Photometeric Detector

Experimental animals

Sixty Adults female albino rats with an average weight of 70-85 gm were provided by the farm of experimental animals at Helwan. The animals were kept under normal laboratory conditions for 15 days before initiation of the experiment and were allowed free access to water and fed on adequate stable diet.

Samples

Blood samples were collected after scarifying of the treated rat by 1/10, ¼ LD_{50} and the LD_{50} of the three tested insecticides for 24hr and blood serum analysis were determined.

The LD_{50} of Primiphos-methyl, Chlorpyrifos-methyl and Fenitrothion were detemined: 1240, 1600 and 520 mg/kg of Body weight (Shalby , 2002).

Kits

Standard kits for the enzyme (GOT), (GPT), (ALK.PH), urea, creatinine, total protein and total cholesterol were obtained from Biomerieux laboratory Instruments Co., France.

Methods of blood analysis

GOT and GPT were determined by kits according to the method of Retiman and Frankel (1957) serum AlK.PH was determined according to the method of Kind and King (1954), serum urea was determined according to Patton and Cruoch (1977), creatinine was determined according to the method described by Husdan (1968). Serum cholesterol was determined according to the method of Siedel *et al.* (1983), Total protein was determined according to the method of Henry *et al.* (1974).

Statistical analysis

Data of liver and kidney functions were statistically analyzed according to Snedecor and Cochran (1982).

Results and Discussion

Data obtained in Table 1 indicated that all treatments caused significant increase in GOT activity as compared by that of control (52.0 u/l). A high increase of GOT activities noticed in rats, which treated by median lethal dose of pirimphos-methyl (69.0 u/L). The activities of GOT were 58.0, 57.0 and 57.0 u/L in treated rats by 1/10 LD_{50} of pirimiphos-methyl, chlorpyriphos-methyl and fenitrothion, respectively. The corresponding values were 64.0, 60.0 and 62.0 u/L in treated rats by ¼ LD_{50} of the permentioned insecticides, respectively. (GOT) activity was 61.0 and 68.0 u/L in treated rats by median lethal doses of chlorpyriphos-methyl and fenitrothion, respectively. Data obtained showed that an increase in GOT activity in treated rats by chlorpyriphos-methyl and fenitrothion (57.0, 60.0, 61.0 and 57.0, 62.0, 68.0 u/L, respectively) was found to be less than that found in treated rats by pirimiphos-methyl (58.0, 64.0 and 69.0 u/L, respectively).

TABLE 1. Biochemical parameters of blood affected by single oral tested insecticides on white a lbino rats.

Treatments / Parameters	Untreated	Primiphos-methyl			Chlorpyriphos-methyl			Fenitrothion			L.S.D 5 %
		1/10 LD_{50}	¼ LD_{50}	LD_{50}	1/10 LD_{50}	¼ LD_{50}	LD_{50}	1/10 LD_{50}	¼ LD_{50}	LD_{50}	
Glutamic oxaloacetate transaminase (u/L)	52.0 a	58.0 b	64.0 e	69.0 f	57.0 b	60.0 c	61.0 cd	57.0 b	62.0 d	68.0 f	1.615
Glutamic pyruvate transaminase (u/L)	36.0 a	39.0 b	45.0 d	49.0 e	35.0 a	50.0 e	50.0 e	38.0 b	43.0 c	45.0 d	1.523
Alk. Phosphatase (u/L)	133.0 a	136.0 b	140.0 c	153.0 e	136.0 b	146.0 d	157.0 f	140.0 c	151.0 e	169.0 g	2.085

Data obtained in the same table clearly indicated that (GPT) activities were going through the same trend as previously mentioned in case of GOT activities in treated rats with different compounds, whereas these activities were increased as compared with those found in control rats (36.00 u/L), except in case of treated rats by 1/10 LD_{50} of chlorpyriphos-methyl (35.00 u/L). The activities of GPT were 39.0, 35.0 and 38.0 u/L in treated rats by 1/10 LD_{50} of pirimiphos-methyl, chlorpyriphos-methyl and fenitrothion, respectively (Table 1). The corresponding values were 45.00, 50.00 and 43.00 u/L in case of dosing by ¼ LD_{50} of tested insecticides.

In case of dosing by LD_{50} of primiphos-methyl, chlorpyriphos-methyl and fenitrothion GPT activities was 49.0, 50.0 and 45.0 u/L, respectively. In other words, it was found that an increase in GPT activities in treated rats by ¼ LD_{50} and LD_{50} of chlorpyriphos-methyl (50.0 and 50.0uL) was found to be more than that found in treated rats by other doses.

Results in Table 1 showed that alkaline phosphates activity was significantly increased in treated rats by all doses of the previously mentioned insecticides in comparison with the control value (133.0 uL). Data in the table clearly indicated that a high increase of (ALK.PH) activity was found in treated rats by median lethal doses of pirimiphos-methyl, chloropyriphos-methyl and fenitrothion (153.0, 157.0 and 169.0 u/L, respectively). The corresponding values were 136.00, 136.00 and 140.00 u/L in case of dosing by 1/10 LD_{50} of the prementioned insecticides, respectively. (ALK.PH) activities were 140.0, 146.0 and 151.0 u/L in treated rats by ¼ LD_{50} of pirimiphos-methyl, chlorpyriphos-methyl and fenitrothion, respectively.

Similar effects were obtained by El-Harrawie *et al.* (1991) who reported that oral administration of a single dose of LD_{25} of methamidophos to mature male albino rats resulted in a significant evaluation in the plasma GOT, GPT and (ALK.PH) when blood samples at 1,3,6, 2, 24, 48 and 72 hr after administration. In the same trend, data obtained by Kandil *et al.* (1991) showed a significant increase in the activity of (GOT), (GPT) and (ALK.PH) in treated male mice by subchronic doses of cyanophos.

Saleh and Zidan (1995) noticed that a highly increases in the activities of (GOT), (GPT) and (ALK.PH) enzymes in treated rats by pirimiphos-methyl.

Fouzy (1997) recorded that the administration of azinophos-methyl insecticide to lactating goats at doses LD_{10} and LD_{20} led to an increase in GOT GPT transaminases and (ALK.PH) activities in blood serum of treated goats.

Results obtained by Eissa (1999) showed that plasma GOT activity in rats was decreased after the same period of treatment with chlorpyriphos-methyl, pirimphos-methyl and Malathion. Also, plasma GPT activity was decreased after the same period of treatment with Malathion and chlorpyriphos-methyl. On the other hand, plasma alkaline phosphatase activity was significantly increased with all tested insecticides.

Data obtained in Table 2 indicated that creatinine concentration was increased in treated rats by different doses in comparison with that found in the control samples (0.75 mg/dL). It was noticed that the increase of creatinine concentration in treated rats by median lethal doses of pirimiphos-methyl (1.54 mg/dL), chlorpyriphos-methyl (1.64 mg/dL) and fenitrothion (1.92 mg/dL) was more than that found in treated rats by the other doses.

TABLE 2. Creatinine and urea parameters of blood affected by single oral doses of tested insecticides on white albino rats.

Treatments / Parameters	Untreated	Pirimiphos-methyl			Chlorpyriphos-methyl			Fenitrothion			L.S.D 5%
		1/10 LD_{50}	¼ LD_{50}	LD_{50}	1/10 LD_{50}	¼ LD_{50}	LD_{50}	1/10 LD_{50}	¼ LD_{50}	LD_{50}	
Creatinine mg/dL	0.75 a	0.87 b	1.31e	1.54f	0.95c	1.13d	1.64g	0.90bc	1.61g	1.92h	0.054
Urea mg/dL	13.11 a	15.27d	20.2 h	29.8 j	14.82 c	17.57 f	22.94 l	13.53 b	16.77e	19.5 g	0.189

Creatinine concentrations were significantly increased in treated rats by 1/10 LD_{50}, ¼ LD_{50} of fenitrothion (0.9, 1.61 and 1.92mg/dl, respectively) than those observed in treated rats by the same doses of pirimphos-methyl (0.87, 1.31 and 1.54 mg/dL) and chlorpyriphos-methyl (0.95, 1.13 and 1.64 mg/dL). On the other hands, data obtained also indicated that a single oral dose by 1/10 LD_{50} of pirimiphos-methyl,chlorpyriphos-methyl and fenitrothion increased creatinine levels from 0.75 mg/dL (control) to 0.87, 0.95 and 0.90 mg/dl, respectively after one day of treatment.

Also, creatinine concentrations were increased in case of dosing by ¼ LD_{50} of pirimiphos-methyl, chlorpyriphos-methyl and fentitrothion (1.31, 1.13 and 1.61 mg/dL respectively) than those found in treated rats by 1/10 LD_{50} of the prementioned insecticides.

Data in the same table showed the effect of different treatments on blood urea content in white albino rats which treated by single oral doses of pirimiphos-methyl, chlorpyriphos-methyl and fenitrothion insecticides (1/10 LD_{50}, ¼ LD_{50} and LD_{50}). Data in the table clearly indicated that the blood urea content was going through the same trend as previously mentioned in case of GOT, GPT, alkaline phosphatase and creatinine levels in treated rats with different compounds, *i.e.*, blood urea levels were increased as compared with those found in control rats (13.11 mg/dL).

These figures were 15.27, 20.2 and 29.8 mg/dL in case of dosing by 1/10,1/4 and LD_{50} of pirimiphos-methyl, respectively. Those values were higher than those found either in case of dosing by 1/10 LD_{50} (15.27, 14.82 and 13.53 mg/dL) or in case of using ¼ the LD_{50} (20.2, 17.57 and 16.77 mg/dL of the prementioned insecticides.

Similar effects were obtained by Asghar *et al.* (1994), who reported that urea concentration was significantly increased from 33.7 (control) to 56.7 mg/dL in treated rabbits by methyl parathion.

In the same trend, Saleh and Zedan (1995), noticed that a significant increase in creatinine concentration in treated rats by 1/10 LD_{50},1/4 LD_{50} and LD_{50} of pirimiphos-methyl.

Data obtained by Zidan *et al.* (1998) showed the effect of fenvalerate profenofs and methomyl on the kidney function parameters of albbino rats. There was a high significant increase in the blood urea and uric acid with the three tested insecticides in the following order profenofos methyl and pyrethroids. The increase in creatinine levels was also highly significant but the increase with fenvalerate was more than methomyl and profenofos, respectively.

Data obtained by Aioub and Hegab (2000) indicated that the level of creatinine was significantly increased after one day in treated rats by LD_{10} and LD_{20} of methomyl.

Results presented in Table 3 indicated that ¼ LD_{50} and LD_{50} of pirimiphos-methyl decreased total plasma protein concentrations in treated rats, chlorpyriphos-methyl and fenitrothion in comparison with control value (2.9 g/dL). Total protein concentrations were 2.3, 2.1 and 2.34 g/dL in treated rats by ¼ LD_{50} of the prementioned insecticides, respectively. The corresponding values were 2.0, 1.81 and 1.9 g/dL in treated rats by median lethal doses of pirmiphos-methyl, chlorpyriphos-methyl and fenitrothion, respectively. On the other hand data at the same table clearly showed that no changes in total protein content in case of dosing by 1/10 LD_{50} of previously mentioned insecticides (2.91, 2.8 and 3.0 g/dL, respectively) as compared by that of control (2.9 g/dL).

The decrease in total protein content, caused by chorpyriphos-methyl, was more than fenitrothion and pirimiphos-methyl, respectively.

The effects of single oral doses of pirimphos-methyl, chlorpyriphos-methyl and fenitrothion on plasma total cholesterol concentrations in treated rats were showed in the same table.

The obtained results showed that, plasma total cholesterol concentrations were increased in rats treated by 1/10 LD_{50}, ¼ LD_{50} and LD_{50} of the prementioned insecticides as compared by the control value (29.1 mg /dL). Total cholesterol values were 33.67,35.5 and 42.8 mg/dL in treated rates by 1/10 LD_{50} of pirimiphos-methyl, chorpyriphos-methyl and fenitrothion, respectively. The corresponding values were 39.8, 37.48 and 45.4 mg/dL in rats, treated by ¼ LD_{50} of the prementioned insecticides, respectively. Data presented in the same table showed that the total cholesterol contents were 44.76, 48.61 and 52.3 mg/dL in treated rats by LD_{50} of the tested insecticides. The obtained results indicated that

a higher increase in total cholesterol values was accrued in treated rates by fenitrothion than that happened by the same doses of chlorpyriphos-methyl and pirimiphos-methyl.

TABLE 3. Total protein and total cholesterol parameters of blood affected by single oral doses of tested insecticides on white albino rats.

Treatments / Parameters	Untreated	Primiphos-methyl			Chlorpyriphos-methyl			Fenitrothion			L.S.D 5 %
		$^1/_{10}$ LD_{50}	¼ LD_{50}	LD_{50}	$^1/_{10}$ LD_{50}	¼ LD_{50}	LD_{50}	$^1/_{10}$ LD_{50}	¼ LD_{50}	LD_{50}	
Total protein g/dL	2.9 de	2.91 de	2.3 c	2.0 b	2.8 d	2.1 b	1.81 a	3.0 e	2.34 c	1.9 ab	0.105
Total cholesterol mg/dL	29.1 a	33.67 b	39.8 e	44.76 g	35.5 c	37.48 d	1 48.6	42.8 f	45.4 h	52.3 j	0.208

These results are in agreement with those of Asghar *et al.* (1994) who reported that total cholesterol values were elevated in male rabbits which treated by methyl parathion whereas blood total proteins decreased gradually during the trial period.

On the contrary, Saleh and Zidan (1995) found that the acute and subchronic toxicities increased the concentration of plasma protein of treated rats by pirimiphos-methyl.

Results obtained by Eissa (1999) indicated that, total plasma protein concentrations were decreased in treated rats by malathion, pirimiphos-methyl, chlorpyriphos-methyl, carbosulfan and methomyl as well as their extracted residues from different parts of watermelon after 48 hr of treatments. Plasma cholesterol concentrations were decreased in treated rats by malathion, pirimiphos-methyl, carbosulfan and methomyl, while it were increased by chlorpyriphos-methyl treatment

Data obtained by Aioub and Hegab (2000) indicated that the level of total proteins in rats received the high dose (1/10 LD_{50}) of methomyl during the first three days were however similar to that of the control animals and the differences were insignificant. On the other hand, treated rats with low dose (1/20 LD_{50}) suffered from reduction in the total proteins and reduction percentages ranged from 1.0 to 7.0%.

Conclusion

Treating rats by 1/10, 1/4 of the LD_{50} of the prementioned insecticides caused bad effects on some biochemical parameters such as, GOT, GPT and Alkaline phosphates activities, creatinine, urea, total protein and total cholesterol levels. In other wards the acute and sub acute toxicities as well as the chronic toxicity of any tested compound must be taken in consideration.

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دراسة التأثيرات البيوكيميائية لمتبقيات مبيدات البريميفوس-ميثيل ، الكلوروبيرويفوس-ميثيل والفينتروثيون على فئران التجارب

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تم معاملة فئران التجارب بجرعة واحدة من ١/١٠ و ١/٤ الجرعة النصفية القاتلة ثم بالجرعة النصفية كاملة وأخذت عينات الدم بعد ٢٤ ساعة من المعاملة وذلك لتقدير بعض المعايير البيوكيميائية في فئران التجارب ، وأظهرت النتائج ما يلـــــي:

١- أدت جميع المعاملات إلى زيادة نشاط إنزيم الـــ GOT فى الفئران المعاملة عند مقارنتها بالكنترول وكانت أقصى زيادة فى نشاط الأنزيم فى الفئران المعاملة بالجرعة النصفية لمبيد البريمفوس-ميثيل .

٢- أدت جميع المعاملات إلى زيادة معنوية فى نشاط إنزيم الـــ GPT فى الفئران المعاملة عند مقارنتها بالكنترول ما عدا الفئران المعاملة بعشر الجرعة النصفية لمبيد الكلوربيريفوس-ميثيل وكانت أقصى زيادة فى نشاط الأنزيم فى الفـــئران المعاملة بربع الجـــرعة النصفية والجرعة النصفية المميتة لمبيد الكلوبيريفوس-ميثيل .

٣-أدت جميع المعاملات إلى زيادة معنوية فى نشاط إنزيم الـــ ALK.PH. فى الفئران المعاملة عند مقارنتها بالكنترول وكانت أقصى زيادة فى نشاط الأنزيم فى الفئران المعاملة بالجرعة النصفية لمبيد الفنتروثيون .

٤- أدت جميع المعاملات إلى زيادة فى قيمة الكرياتينين عند مقارنتها بالكنترول وكانت أقصى زيادة الفئران المعاملة بالجرعة النصفية لمبيد الفنتروثيون .

٥- أدت جميع المعاملات إلى زيادة تركيز اليوريا في الفئران المعاملة عند مقارنتها بالكنترول وكانت أقصى زيادة فى الفئران المعاملة بالجرعة النصفية لمبيد البريمفوس-ميثيل .

٦- لم يحدث تغير فى قيمة البروتين الكلى فى الفئران المعاملة بعشر الجرعة النصفية المميتة عند مقارنتها بالكنترول بينما حدث انخفاض معنوي فى قيمته عند معاملة الفئران بربع الجرعة النصفية المميتة للمبيدات المختبرة وكان أقصى انخفاض فى قيمة البروتين فى الفئران المعاملة بالجرعة النصفية المميتة لمبيد الكلوروبيرويفوس-ميثيل .

٧- أدت جميع المعاملات إلى حدوث زيادة معنوية فى قيمة الكوليسترول الكلى فى سيرم الفئران المعاملة عند مقارنتها بعينات المقارنة وكانت أقصى زيادة فى تركيز الكوليسترول فى الفئران المعاملة بالجرعة النصفية المميتة لمبيد الفنتروثيون.