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EFFECT OF METHOMYL AND IMIDACLOPRID ON LIVER AND KIDNEY FUNCTIONS IN MALE ALBINO RATS

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ABSTRACT

The effect of daily oral administration of Carbamate (Methomyl) ,at 0.2 , 2 and 5 mg/kg b.w and neonicotiniod (Imidacloprid), at 0.5 ,1 and 5 mg/kg b.w insecticides on male albino rats for 28 successive days, followed by 15 days recovery were studied . The most important biochemical parameters of the serum ; alanine aminotranseferase (ALT) , aspartate aminotraseferase (AST) ,alkaline phosphatase activities (ALP) , total protein , protein profile (albumin and globulin) , urea , creatinine were investigated . The activities of AST ,ALT were significantly increased at all tested times in cumulatively dose related manner .No significant changes were observed in alkaline phosphatase enzyme activity compared with the control. On the other hand serum total protein , Protein Profile (albumin and globulin) recorded highly significant decrease in all treatments with Methomyl and imidacloprid . A highly significant increases in the blood urea and creatinine with two tested insecticides in the following order ,methomyl and imidacloprid. after treatments 14 days were observed . The levels were decreased during the 21, 28 and 45 days.

Keywords : imidacloprid , methomyl , rats , Liver , Kidney

INTRODUCTION

Pesticides have been useful in agriculture pest control, there is considerable risk for human health and damage to ecosystems (Moreno *et al.*, 2007). Carbamates inhibit the enzyme acetyl cholinesterase (ACHE) which is present in erythrocyte and plasma in man (Rama and Jaga, 1991and Padilla *et al* .,2007) Carbamates affect on the human central nervous system (Hoogduijn *et al* ., 2006), also cause significant changes in total serum lipids glucose, protein levels AST, ALT, acid phosphatase and alkaline phosphatase activities in mammals (Sadek *et al.*,1989;Fayez and Kilgore, 1992 and Chevalier *et al.*,1993).They affect liver glucose 6-phosphatase and liver succinic acid dehydrogenase (Fayez and Kilgore,1992) Kidney and liver AST and ALT activities(Kiran *et al.*,1988 and Saleh, 1990a) . Carbamates have toxic symptoms and physiological changes in different animals. Toxic effects of carbamates were noticed in frogs and birds (Mullie *et al.*,1991)and suspected cause of death in ducks (Yuningshi and Dan,1985). Methomyl cause high significant increase in the blood urea ,uric acid and creatinine in rats (Zidan *et al* ., 1998) Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific areas of the nicotinic acetylcholine receptor(Anatra-Cordone and Durkin ,2005). Imidacloprid is an agonist at the nicotinic acetylcholine receptor ,and as such it is highly effective against many sucking insects (Worthing,1994;Elbert et al.,1998).This investigation aims to study the effect of Methomyl (Carbamate) and imidacloprid(neonicotinoid) insecticides on liver and kidney functions in male albino rats ,through activities of ALT ,AST, Alkaline phosphatase, total protein, Albumin, globulin, urea and creatinine in serum .

MATERIALS AND METHODS

Experimental Pesticides :

A formulated sample of Methomyl (Lannate 90 % W.P.) and Imidacloprid (Confidor 35 % S.C.) were supplied by Ministry of Agriculture , Egypt .

Experimental animals:

Forty two male adult albino rats strain of *(Rattus norvegicus)* were obtained from animals laboratory at Helwan farm , Ministry of health , Egypt . Weighing for 120 -150 gram for animal house of central Agric. Pesticides lab. They were supplied with adequate standard diet and water according to A.O.A.C (2000) given *at libitum* for one weak in the laboratory. .

Experimental Design : Animals were divided into 7 groups each of them contained 6 rats were housed in suitable cages as follows .

Group 1	Control	
Group 2	0.2 mg/kg b.w.	Methomyl
Group 3	2.0 mg/kg b.w.	Methomyl
Group 4	5.0 mg/kg b.w.	Methomyl
Group 5	0.5 mg/kg b.w.	Imidacloprid
Group 6	1.0 mg/kg b.w.	Imidacloprid
Group 7	5.0 mg/kg b.w.	Imidacloprid

The effect of daily oral administration of methomyl and imidacloprid on rats for 28 days successive was Studied.

Blood samples were collected under ether anesthesia from orbital sinus vein from each of six of the surviving rats by heparinized capillary tubes at 14 ,21 ,28 and 45 days after dosing into clean ,dry ,and labeled eppendorf tubes (1.5 ml) .Samples were centrifugated at 3500 rpm for 15 min, in a refrigerated centrifuge to separate serum. Separated serum was kept in deep freeze at(-40 C^{o})for selected biochemical analysis by using commercial reagent kits . At the end of experimental period 28 days , the animals (3 rats of each group) were killed and dissected to obtain samples of the blood and four internal organs (Liver , Kidney , brain and testes)at intervals of 28 and 45 days after the insecticide administration.

Biochemical analysis:

Liver function :

Serum alkaline phosphatase activity (ALP) was determined according to the method of Kaplan and Righett (1955).The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (1957). Total protein was determined according to the method of Weichselbaum (1946) and albumin according to Doumas *et al.*(1971).

kidney function

Creatinine concentration was measured by the method adopted by Siest *et al.* (1985) .Urea concentration was measured by the method adopted by Fawcett and Scott (1960).

Statistical analysis :

Analysis of variances and mean separation was conducted using SAS (SAS institute 1986)

RESULTS AND DISCUSSION

Liver function :

Data in Tables (1,2) show the effect of methomyl and imidacloprid on liver function parameters of the tested animals. In relation to the normal group (control), AST activity showed highly significant increased with two tested insecticides, mainly by methomyl followed by imidacloprid .The same significant increase occurred with ALT activity in the same pattern. Changes occurred in alkaline phosphatase activity were not significant .These results are in agreement with Saleh (1990 a) on methomyl , Zidan *et al* .(1998) on Pyrethroids, profenofos and methomy, Aioub and Hegab (2000) on methomyl, Zaahkouk *et al.* (2000) on carbamate, Mahgoub and Medany (2001) on methomyl, El-Kashoury (2002)on Carbaryl in rats .Freedland and Karmer (1970) Suggested that enzyme levels are sensitive indicators of tissue damage , since they are liberated from cells even when the magnitude of lesions is not sufficient for morphological detection . Luckens and Phelps (1969) and Walker *et al.* (1969) recorded that the elevation in serum AST and ALT was due to degeneration and necrosis of liver cells which was accompanied by damage of cells-walls and cytolysis ,thereby pouring considerable amount of these mitochondrial enzymes in the blood stream .It has been reported that serum ALT raised only when cells of liver parenchyma are destroyed (Varley , 1969). For this reason serum ALT activity is more linked with liver disease .

Data in Tables (3,4) show the effect of methomyl and imidacloprid on total protein and albumin of the tested animals. In relation to the normal group (control), total protein showed highly significant decrease with two tested insecticides, mainly by methomyl followed by imidacloprid .The same significant decrease occurred with albumin and globulin levels in the same pattern .These results are in agreement with Saleh (1990 b) on methomyl , Zaahkouk *et al* .(2000) on carbamate , Shallan et al . (2004) on imidacloprid in rats. This decrease in total protein was interpreted by earlier authors in terms of toxic effects , including the induction of liver cell necrosis in fasted rats and chicks (Nachtomi and Alumat , 1972 ; and Broda et al ., 1976).They suggested that this depression might have been due to an alteranation in the intracellular protein synthesis mechanisms and that the oxidative enzyme changes was probably secondary in altering protein synthesis .The action of insecticides on nucleic acid and protein synthesis was investigated by Chung *et al* (1967).They found that DDT and dieldrin were able to alter rats of DNA,

RNA and protein synthesis. Moreover, Shah (1980) found that the increased lysosomal enzymatic activity was accompanied by a decrease in RNA and protein content after malathion treatment .Furthermore, Awasthi *et al.* (1984) found a reduction in the protein content of liver and kidney after the exposure to dimethoate . They suggested that this reduction could be due to adverse effects of organophosphate compounds on the lysosomal membrane which released nucleases and proteases affecting RNA and protein metabolism.

Kidney function: Results in Tables (5 , 6) show a high significant increase in the blood urea and creatinine with two tested insecticides in the following order methomyl and imidacloprid. after treatments 14 days . The levels were decreased during the 21 and 28 days.

Table (1): Effect of oral administration of methomyl on ALP ,ALT and AST activities in serum of male albino rats.

Treatment	ALP (U/L)				ALT(U/L)				AST (U/L)			
mg/kg b.w.	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
Control	20.0 ± 1.5	18.3 ±1.4	18.3 ±1.5	18.5 ± 5.4	27.02 ±4.1	29.2 ±1.3	30.2 ± 1.3	31.9 ± 00	34.92± 0.0	35.0±1.4	35.0±1.9	34.32 ±1.9
0.5	26.0 ± 2.6	28.0 ±5.8	30.5 ±1.90	30.0 ± 3.4	32.38± 1.7	36.84± 0.6	48.5 ±2.9	43.0±1.0	40.7±1.6	41.0 ±1.6	56.2 ±1.2	44.7 ±1.1
1	24.0 ± 2.0	28.0 ±4.9	31.2 ± 5.1	29.0 ± 4.3	33.38 ± 1.6	40.0± 0.03	47.5 ±1.9	48.9 ± 3.9	45.8 ± 1.5	52.0 ±1.6	62.0 ±2.8	45.8 ±1.1
5	25.6 ±2.3	30.0 ±0.0	31.5±5.1	30.3 ± 5.5	61.11 ±1.6	78.5 ± 0.8	77.5 ±3.6	70.0 ± 5.4	50.6 ± 5.1	55.0 ±6.2	65.2 ±2.5	61.3 ±2.5
L.S.D	7.4	10.4	14.6	14.9	5.3	6.2	13.42	10 .7	5.6	6.0	19.6	10 .6

Table (2): Effect of oral administration of Imidacloprid on ALP ,ALT and AST activities in serum of male albino rats.

	ALP (U/L)				ALT(U/L)				AST(U/L)			
Treatment mg/kg b.w	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
Control	20.0 ± 1.5	20.0±2.8	18.3± 2.8	21.3 ± 0.55	27.02 ± 1.9	29.2± 5.2	30.2 ±3.6	31.9 ± 4.3	34.92± 3.2	35.00± 5.5	35 .00± 5.5	34.92 ±4.3
0.2	21.3 ±4.8	27.0 ± 4.9	25.5 ± 2.0	24.0± 3.0	52.8± 4.5	69.84 ±6.4	78.5± 4.6	70.0 ± 5.7	43.65 ± 6.3	52.38 5.4	61.98 ± 5.2	52.38± 6.7
2	25.0 ±3.1	24.0 ± 1.1	27.6 ± 3.1	25.0± 1.0	52.38 ± 6.2	78.84± 5.3	77.5 ± 5.8	69.9± 3.9	50.21 ± 5.3	61.11 ± 5.3	52.38 ± 5.4	61.20 ± 5.3
5	23.0 ±2.7	27.0 ± 1.5	26.2 ± 4.4	26.5 ±1.5	61.11 ±7.8	96.57±4.4	77.5 ± 4.1	70.0 ± 4.6	52.32 ± 4.4	61.21± 5.9	69.80 ± 6.6	63.70 ± 3.9
L.S.D	7.4	9.8	10.11	6.9	14.3	31.3	23.4	18.7	15.8	14.2	16.8	15.2

Table (3): Effect of oral administration of methomyl in total protein ,albumin and globulin in serum of male albino rats .

	Time (days)											
Treatment	Total protein (g/dl)				Albumin (g/dl)				Globulin (g/dl)			
(mg/kg b.w)	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
Control	6.5 ±2.1	6.5± 2.2	6.5 ±1.5	6.5 ±0.15	4.0±0.17	4±0.07	4.0± 2.3	4 .0±2.0	2.5 ±0.19	2.5 ±1.13	2.5 ±0.19	2.5± 0.27
0.2	6.5 ±2.8	6.0±2.5	5.4±2.5	5.3 ±3.0	4.0 ±1.5	3.5 ±0.12	3.6± 0.11	3.5±0.15	2.5 ±1.4	2.5 ±1.2	1.8 ±6.2	1.8 ±1.7
2	6.0±3.7	5.4 ±4.6	5.0 ±3.8	4.5 ±1.3	4.0±0.12	4.0 ± 5.1	3.5±2.0	3.3±0.15	2.0 ±2.1	1.4 ±0.29	1.5 ±2.5	1.2±1.4
5	6.0±1.8	5.6 ±3.8	4.9± 4.7	4.8 ±3.5	3.9 ±2.7	3.8±0.14	3.2 ±3.9	3.2 ± 0.5	2.1 ±0.94	1.8 ±1.14	1.7 ±1.3	1.6 ±4.2
L.S.D	0.70	0.55	0.89	0.52	0.15	0.59	0.5	0.57	0.14	0.09	0.39	0.05

Table (4) Effect of oral administration of imidacloprid in total protein ,albumin and globulin in serum of male albino rats .

Treatment (mg/kg b.w)	Time (days)											
	Total Protein (g/ dl)				Albumin (g/dl)				Globulin (g/dl)			
	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
control	6.5 ±3.1	6.5 ±1.5	6.5 ±1.2	6.4 ±1.3	4.3 ±0.17	4.2 ±0.24	4.2 ±2.4	4 ±0.05	2.2 ±1.6	2.3 ±1.19	2.3 ±1.3	2.4 ±1.2
0.5	6.4 ± 1.2	6.0 ±1.7	4.6 ± 1.1	4.5 ±1.9	3.9± 0.17	3.8 ±0.25	3.8 ±0.13	3.3 ±0.15	2.5 ±0.6	2.2± 0.98	1.4 ±0.94	1.2± 1.8
1	6.4 ±1.7	6.1 ±3.3	4.7 ±2.1	4.6 ±2.3	3.5 ±2.8	3.5 ±00	3.5 ±1.3	3.2 ±9.1	2.9 ± 1.1	2.6 ±3.3	1.2 ± 1.2	1.4± 7.5
5	6.3 ± 2.1	5.9 ±2.1	4.9 ±1.2	4.4 ±1.2	3.9 ±0.13	3.4 ±3.6	3.4 ±0.10	3.1 ±6.1	2.4 ±1.3	2.5± 1.5	1.5 ±1.10	1.3 ±5.0
L.S.D	1.8	0.45	0.89	0.52	0.15	0.59	0.50	0.57	0.15	0.59	0.50	0.57

Table (5): Effect of oral administration of methomyl on urea and creatinine concentrations in serum of male albino rats.

Treatment (mg/kg b.w)	Urea (mg/dl)				Creatinine mg/dl			
	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
Control	33.5 ± 2.5	36.5 ±1.7	33.5 ± 2.6	40.0 ± 4.0	0.65 ± 0.0	0.60 ± 1.0	0.65 ±0.01	0.65 ±1.0
0.2	36.8 ± 3.0	63.3± 3.6	74.0 ± 6.6	65.0 ±5.2	1.6± 4.4	1.9 ±0.7	1.7 ± 6.1	1.2 ± 3.0
2	63.3± 2.8	70.5± 3.8	75.0 ±4.8	61.0± 3.0	1.6± 1.3	1.8 ± 1.2	1.6 ± 1.2	1.5± 0.9
5	59.0± 6.4	79.5 ±2.9	76.6± 1.7	58.0 ±2.1	1.7± 1.8	1.7± 1.0	1.2±1.56	1.5± 2.3
L.S.D	17.8	14.4	11.3	17.3	0.25	0.41	0.43	0.84

Table (6): Effect of oral administration of imidacloprid on urea and creatinine concentrations in serum of male albino rats.

Treatment (mg/kg b.w)	urea mg/dl				Creatinine mg/dl			
	14 days	21 days	28 days	45 days	14 days	21 days	28 days	45 days
control	33.5 ± 2.5	36.5 ± 1.7	33.5 ± 2.3	40.0 ± 1.2	0.65±0.5	0.60 ±0.1	0.65 ±0.15	0.65 ± 2.0
0.5	50.5 ± 3.3	60.0± 4.4	73.0 ± 5.1	65.0 ± 2.0	1.6 ±1.0	1.8 ±0.2	1.6± 0.35	1.6 ± 4.1
1	54.5 ±6.7	57.0 ± 56.5	68.0 ±1.1	54.0 ±4.0	1.6 ±0.10	2.1± 0.10	1.9± 0.35	1.7 ±2.5
5	61.7 ± 3.9	56.5± 3.3	70.0 ± 4.6	60.0 ± 5.6	1.5 ± 0.15	1.6± 0.15	1.5 ±1.5	1.5 ± 3.8
L.S.D	13.5	15.0	15 .0	17.7	0.41	0.51	0.90	0.62

These results are in agreement with Saleh (1990 b)on methomyl , Zidan *et al* .(1998) on Pyrethroids , profenofos and methomyl, Aioub and Hegab (2000)on methomyl ,El-Kashoury (2002) carbaryl in rats . The elevation of serum urea concentration in methomyl treated rats shows an alteration in normal kidney function which might be related to the methomyl-induced renal dysfunction or my be due to hepatocellular disorder Saleh (1990b) .The levels of creatinine and total protein and albumin were also taken as parameters of toxicological adverse effects by other investigators (Enan *et al.*, 1987 ; Rajini and knishnakumari 1988 ; shaker *et al* ., 1988 ; Zidan , 1991; Cokelaere .1992 ; El-Zemity *et al* ., 1993 ; Ewies *et al* ., 1995 ; Nasr *et al* ., 1996 ; El-Said ., 1997 ; Farid 1997 and Farag , 1998) .

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تأثير مبيد المثوميل والاميداكلوبريد علي وظائف الكبد والكلي في ذكور الفئران البيضاء

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يهتم البحث بدراسة تأثير تناول مبيد الكرباميت (ميثوميل) 0.2 , 2, 5 مللي جرام / كجم من وزن الجسم و الاميداكلوبريد (نيكوتونيد) 0.5 , 1 , 5 مللي جرام / كجم من وزن الجسم عن طريق الفم للفئران البيضاء لمده ٢٨ يوم واتبعتها ١٥ يوم للاستشفاء وتم دراسة بعض المعايير الهامة في سيرم الدم و هي إنزيمات نقل الأمين AST , ALT فوسفاتيز القلوي ALP , البر تينات الكلية , ألبيومين و الجلوبيولين واليوريا و الكرياتنين . وحدث زيادة معنوية في نشاط أنزيمات AST ,ALT في كل المعاملات في حين لم تحدث تغيرات معنوية في نشاط إنزيم ALP وذلك بالمقارنة بالكنترول . كما لوحظ نقص معنوي في الألبيومين والجلوبيولين . وأظهرت النتائج تأثر وظائف الكلي وذلك بحدوث زيادة معنوية في مستوي اليوريا والكرياتنين في الدم حتى اليوم الرابع عشر ثم بدأت في التناقص حتى نهاية التجربة .