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TOXIC EFFECTS OF FIVE INSECTICIDES AND THEIR MIXTURE ON MALE ALBINO RATS

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ABSTRACT

Five insecticides namely; abamectin, carbosulfan, fenpropathrin, methomyl and profenofos were given by gavages to male albino rats. These insecticides were administered daily for 28 days with doses equaled 1/20 LD_{50} either singly or in a mixture of all the insecticides together. The study revealed significant decreases in body and kidneys weights, while increases in liver weights in all the treatments. Most of the treatments induced significant elevations in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while caused decreases in acetylcholinesterase (AChE) activities. Fenpropathrin and the mixture induced significant increase in total protein content of the serum, while the other treatments induced significant decreases. Creatinine concentrations recorded significant elevations in fenpropathrin and methomyl treatments, while significant decrease in case of Profenofos. Degenerative changes and granularity of hepatocytes with Kupffer cells activation were observed in the treatments with the mixture or and methomyl. Shrinking in Bowman's capsule and degenerative changes of epithelium lining renal tubules were observed in rats treated with the mixture. Moreover, necrotic changes associated with desquamation of epithelium lining tubules were shown in rats treated with the mixture, fenpropathrin and methomyl. From the biochemical data, the joint action was estimated for the mixture composed of the five insecticides. The mixture interacts antagonistically with most of the measured biochemical parameters.

Keywords: Insecticides, Biochemical parameters, Joint action, Histopathological effects, Rats

INTRODUCTION

A wide range of synthetic pesticides have been released into the rural environment through the agricultural activities to control insect pests, plant pathogens and weeds in both developed and developing countries. Presently, approximately 1,500 active ingredients have been registered as pesticides and formulators mix these compounds with one or more of some 900 inert materials to create approximately 50,000 commercial pesticide preparations registered for use. Roughly, 85% of the pesticides currently used in the world are devoted to the agricultural sector, almost 10% are dedicated to sanitary measures against vectors in public health programs, and the rest are applied in specific sites such as buildings, transport media, and residential areas (WHO, 1993).

The harmful effects of individual insecticides on a variety of experimental animals have been previously studied by many investigators (e.g. Shakoori *et al.,* 1990; Stebbins *et al.,* 2002; Mansour and Mossa, 2005).

Organophosphate (e.g. profenofos) and carbamate (e.g. carbosulfan & methomyl) insecticides inhibit acetylcholinesterase activity in the central and peripheral nervous system when ingested in acute exposure (Vidyasagar *et al*., 2004). Thus, the main target of such compounds is the central and peripheral nervous system, although many authors postulate that these compounds in both acute and chronic intoxication disturb the redox processes, changing the activities of antioxidative enzymes and causing enhancement of lipid peroxidation in many organs (Costa, 2006).

Pyrethroid insecticides (e.g. fenpropathrin) bind to voltage-sensitive sodium channels and modify their gating kinetics, thereby they disrupt nerve function and produce acute neurotoxic effects in both insects and non-target organisms (Soderlund *et al*., 2002).

While, the acaricide /insecticide compound (abamectin) inhibits the gamma-aminobutyric acid (GABA), inducing neurotransmission and paralysis in parasites (Turner and Schaeffer, 1989). The compound is also a chloride channel inhibitor, which makes it likely to affect the membrane stability (Korystov *et al.,* 1999).

In reality, humans are exposed simultaneously or sequentially to large numbers of chemicals via multiple exposure routes. In the environment, most of the chemicals exist as mixtures and their toxicity is mainly attributed to their interactions. However, assessment of the potential health hazard of chemical mixtures (e.g. pesticides) is difficult and a challenging toxicological problem, and a subject of major current concern to both the scientific and regulatory communities (Calabrese, 1991).

The changes in biochemical parameters as measured in various body fluids may often be among the more sensitive indicators of early changes in health due to exposure to pesticides in the environment (WHO, 1992), and histopathological impairment has for a long time been used as a potential marker for environmental stress (Hinton *et al.*, 1992).

The measurements changes in biochemical parameters following exposure to pesticides has become an integral part of new tests in toxicological studies; giving an early warning of signs of toxicity (Casarett, 1975; WHO, 1992). Oecsh *et al.* (1994) explored the importance of studying interactions of chemical mixtures based on enzymatic effects. Also, Mansour and Refaie (2000) added that the joint action of chemical mixtures could be estimated in a qualitative and quantitative manner based on biochemical data obtained from animals exposed to pesticide mixtures. So, the present study was conducted to evaluate and compare the toxic effects of five insecticides representing different chemical groups, and to analyze the joint action of their mixture using male albino rats.

MATREALS AND METHODS

Pesticides

Five insecticides, namely abamectin, profenofos, fenpropathrin, carbosulfan and methomyl were used in the present study. Table (1) contains some information about the above mentioned insecticides (Tomlin, 2004).

Table (1). Some information about the selected insecticides[a].

Insecticide as common name	Chemical group	Origin	Mode of action	Used formulation	Main use	Oral LD_{50} for male rat, mg/kg b.wt. (Published value for a.i.)
Abamectin	A naturally occurring soil Actinomycete isolated from fermentation of *Streptomyces avermitilis*	Syngenta AG., Ltd., Switzerland	An inhibitory neurotransmitter acts by activating $GABA_A$ receptor-gated chloride channels in vertebrate CNS	Vertimec® 1.5 % EC	acaricide and insecticide	10
Carbosulfan	carbamate	FMC Corp, USA	Cholinesterase inhibitor	Marshal® 25% EC	Insecticide	250.0
Fenpropathrin	pyrethroid	Sumitomo Chemical Co., Ltd., Japan	Acts on the nervous system of insects, and disturbs the function of neurons by interaction with the sodium channel	Danitol® 30% EC	acaricide and insecticide	70.6
Methomyl	carbamate	DuPont Crop Protection, USA	Cholinesterase inhibitor	Lannate® 90% SP	insecticide and acaricide	34.0
Profenofos	organophosphate	Syngenta, Switzerland	Cholinesterase inhibitor	Curacron® 72% EC	insecticide and acaricide	258.0

[a]Source: Tomlin (2004).
EC: Emulsfiable concentrate.
SP : Soluble powder
a.i. : Active ingredient.

Tested Animals

Adult male albino rats weighing 110 ± 10 g were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (24 ± 3 ^{o}C) on standard pellet diet and tap water *ad-libitum*. Rats were acclimatized for 1 week prior to the start of experiments.

The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, and according to the guidance for care and use of laboratory animals (NRC, 1996).

Dosing and Treatments

The tested insecticides were used at 1/20 of their LD_{50s} after modulating to the active ingredient contents (33.3, 50.0, 11.8, 1.9 and 17.9 mg kg^{-1} b.wt., of abamectin, carbosulfan, fenpropathrin, methomyl and profenofos, respectively). Seven groups of eight rats each were used; five for single treatments of each insecticide, one for the mixture of all insecticides containing 1/20 LD_{50} of each, and one served as control. Insecticide solutions of fixed volume (0.5 ml) containing the modulated concentrations of the single pesticides or the mixture were freshly prepared and given daily for 28 repetitive doses via the oral route. Rats of the control group were administered the same volume of water instead of the insecticide.

Data Recording and Sample Collection

Every week, rats were weighed and blood samples were collected from the retro-orbital venous plexus, at 10.00 a.m. to avoid variations arising from circadian rhythm. The collected blood samples were left for 20 minutes to coagulate at room temperature, and then centrifuged at 3000 rpm (600 x g) for 10 minutes to separate the sera. The sera were kept in a deep freezer (-20˚C) until analyzed. At the end of experiments, the rats were sacrificed and internal organs (liver & kidneys) were removed, cleaned and weighed. The tissue sections of liver and kidneys were prepared and stained with Haematoxylene and Eosin and examined microscopically (Carleton *et al.*, 1976).

Biochemical Analyses

The sera obtained from different treatments were subjected to certain biochemical analyses by using Shimadzu UV-VIS Recording 2401 PC. Total protein concentration was determined by the method of Weichselbaum (1946), using Stanbio Laboratory kits. Transaminases (ALT&AST) and acetyl cholinesterase (AChE) activities were determined by the methods of Reitman and Frankel (1957) and Ellman *et al.* (1961), respectively, using Qumica Clinica Aplicada kits. Creatinine concentration was determined by the method of Henry *et al.* (1974), using Stanbio Laboratory kits.

Joint Action Analysis

Binary mixtures of pesticides were previously estimated by Mansour's formula (Eq.1) according to Mansour and Refaie (2000). In the present study, this formula was modulated to evaluate mixtures composed of more than two toxicants; as mentioned below:

The Mansour's formula was used to identify the type of interaction between pairs of toxicants in terms of Interaction Index (I.I.):

$$I.I. = \frac{M+C}{A_1+A_2} \qquad \text{(Eq. 1)}$$

Where: M, C, A_1, & A_2 represent the mean values obtained from the biochemical estimation of a studied parameter: M for the mixture value; A_1 & A_2 for the values of the individual compounds in that mixture; and C for the control value.

Based on the above mentioned authors, the ratings of the interaction indices are as follows:

i) In case of positive effect (i.e. increase of the concerned biochemical parameters above the control values due to the effect of the individual compounds); where:

I.I. > 1 means potentiation; I.I. =1 means additive; I.I. <1 means antagonism

ii) In case of negative effect (i.e. decrease of the concerned biochemical parameters below the control values due to the effect of the individual compounds); where:

I.I. > 1 means antagonism; I.I. =1 means additive; I.I. <1 means potentiation

iii) In case of no effect (I.I.= 1.0): In such a case, it is supposed that treatment with the mixture and each of its individual compounds do not induce any change in the value of the parameters measured and thus control and treatments have nearly the same values. Here, the interaction index (I.I), if determined, will equal 1 (i.e. a result similar to that of additive effect). The prior examination of the data set of a given biochemical measurement would assist differentiation between additive and no effect cases).

For accuracy, a "safety factor" of ± 0.05 is added to the indices values when ranking the joint action. This means that additive effect will be considered for I.I. =1 ± 0.05, and the other categories ranked accordingly (Mansour and Refaie, 2000).

Here, the Mansour's formula is modified to apply to mixtures composed of more than two individual components as follows:

$$I.I. = \frac{M+C(n-1)}{\Sigma A_1+A_2+\text{------}A_n} \qquad \text{(Eq. 2)}$$

The mean values of estimated parameters are M for the mixture value; C for control value; A_1 & A_2

……A_n for the values of the individual compounds in that mixture. N = number of the individual components.

Statistical Analysis

The data are expressed as means ± S.E. The data were analyzed using SPSS (version 11.0) for windows. Paired samples *t*-test was used for comparison between the control and the treatment data.

RESULTS AND DISCUSSIONS

Sings of Toxicity and Internal Organs Weights

The results of this study revealed that most of the treatments showed signs of toxicity such as: mild tremors and, emaciation. Almost all the treatments showed signs of loss of appetite that was manifested by the decrease in body weight, throughout the experimental duration. Regardless fluctuations of body weight changes, the most important comparisons were shown by estimating the percent of weekly body weight gain. In this respect, the control value accounted to 15.51%, while the values for all treatments ranged between 7.19% to 13.95% (Table 2).

Liver and kidney weights of control rats were 5.51 g & 1.79 g for absolute weight and 2.85% & 0.93% as relative to body weight, respectively (Table 3). Comparing to the control value of the relative weight of liver (2.85%), all the tested doses demonstrated a highly significant increases and the most increase was induced by the mixture treatment which recorded 4.54%. All the tested doses demonstrated a highly significant decrease in the relative weight of kidney. The lowest value was achieved by the treatment with the mixture, which recorded 0.74% (Table 3).

Generally, the increase or decrease of body weight gain is yet a simple index of toxic effects (Lu, 1996). Several investigators reported body weight decreases in experimental animals including rabbits, mice and rats due to exposure to different insecticides (EPA, 2000; Stebbins *et al.*, 2002). Comparing to the control value of the relative weights of liver (2.85%) or kidneys (0.93%), all the tested doses demonstrated increases in relative weights of liver and decreases in those of kidneys. Such alterations have been reported by previous investigators on male rats (Mansour *et al.*, 2001) and on pregnant rats (Mansour *et al.*, 2005; Rawi *et al.*, 2005).

Table (2). Body weight changes of male rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean	% of weekly body weight gain
	0	1	2	3	4		
Control	116.25±4.51	133.25±1.74	153.75±2.34	173.38±2.74	188.38±4.00	153.0	15.51
Abamectin	120.56±2.00	132.56±3.57	142.50±3.68*	138.45±3.90**	157.65±3.12**	138.3	7.69
Carbosulfan	125.13±1.11	130.90±3.50	141.50±1.85**	151.14±1.61**	163.86±3.16**	142.5	7.74
Fenpropathrin	117.37±1.10	125.40±1.10*	133.18±1.92**	149.84±1.84**	151.14±1.61**	135.4	7.19
Profenofos	115.13±0.77	125.13±1.11**	163.25±3.66*	169.00±4.14	179.38±2.03	150.4	13.95
Methomyl	120.66±1.30	138.39±3.71	149.80±3.29	169.80±3.80	175.20±4.73*	152.8	11.30
The mixture	114.38±1.46	125.13±1.11**	139.80±2.29**	150.38±4.33**	158.39±3.71**	137.6	9.62

Each value (in gram) is a mean of 8 rat's ± SE
Statistical difference from the control: * significant at P ≤ 0.05& ** highly significant at P ≤ 0.01.
% of weekly body weight gain = [(final b.wt. - initial b.wt.) / (initial b.w. X no. of weeks)] x 100.
General mean = average reading from 0 time to 4 weeks

Table (3). Effect of repetitive doses, for 28 consecutive days, of the tested insecticides and their mixture on the weight of liver and kidney of male rats.

Treatments	internal organs				
	Body weight	liver	liver %[a]	kidney	kidney %[a]
Control	193.32±2.46	5.51±0.02	2.85±0.04	1.79±0.03	0.93±0.01
Abamectin	157.00±1.45	6.07±0.10	3.87±0.10**	1.19±0.03	0.76±0.02**
Fenpropathrin	154.36±3.00	6.24±0.15	4.04±0.20**	1.23±0.05	0.79±0.02**
Carbosulfan	166.40±1.50	6.07±0.50	3.66±0.32**	1.48±0.03	0.89±0.01**
Profenofos	180.20±1.74	5.75±0.14	3.22±0.09**	1.41±0.01	0.78±0.00**
Methomyl	174.79±1.69	5.75±0.14	3.29±0.10**	1.41±0.01	0.80±0.00**
The mixture	156.14±1.46	7.10±0.21	4.54±0.11**	1.16±0.04	0.74±0.03**

Each value (in gram) is a mean of 5 selected rat's ± SE
Statistical difference from the control: * significant at P ≤ 0.05& ** highly significant at P ≤ 0.01.
[a]% of organ weights related to b.wt. = [liver or kidney weight/ final body weight] X 100.

Effect of Insecticides on Biochemical Parameters

Tables 4 and 5 show the activity of AST and ALT enzymes in sera of male albino rats treated with different insecticidal doses. Throughout the experimental duration, the control values showed nearly similar values for both enzymes. However, the majority of the tested doses induced increases in the activities of both enzymes compared to the control values. Focusing on the results at the end of the experiment, only the treatments with carbosulfan and fenpropathrin produced no significant changes in the activity of both tested enzymes, while most of the other treatments induced significant elevations. Several soluble enzymes of blood serum have been considered as indicators of hepatic dysfunction and damage (Shakoori *et al*., 1990). In fact, transaminases (AST& ALT) are important and critical enzymes in the biological processes. These enzymes are involved in the breakdown of amino acids into α-keto acids, which are routed for complete metabolism through the Kreb's cycle and electron transport chain. Consequently, they are considered as a specific indicator for liver damage (Harper *et al*., 1979) and responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions (Aly *et al*., 1997). In the present investigation, the activities of AST and ALT enzymes were elevated following intoxication of male albino rats with the insecticides and their mixture. This may be an indicative of initial cell injury occurring in advance of gross hepatic pathology and any condition leading to changes in membrane permeability causing a generalized release of enzymes from the cell as cited by De Bruin (1979). Mansour and Mossa (2005) found that the activities of transaminases were increased in animals after exposure to pesticides. In addition, oxidative damage is thought to be an important mechanism of damage for organophosphate pesticides (Banerjee *et al.*, 2001).

Table 6 records the activity of AChE in the sera of rats treated with different insecticidal treatments. The control values recorded 1564.5, 1539.9, 1609.7, 1593.2 and 1583.0 U/L for 0, 1, 2, 3 and 4 weeks, respectively. Throughout the experimental duration, all the insecticidal treatments induced significant decreases in the activity of the concerned enzyme. At the end of experiment, profenofos induced the highest decrease in AChE activity, accounting to 473.00 U/L. The mixture showed a similar result (473.8 U/L). The most important mode of action of insecticides especially the organophosphorus and carbamates involves the inhibition of AChE in erythrocytes, serum and brain (Vidyasagar *et al.*, 2004). While, abamectin inhibits gamma-aminobutyric acid (GABA), inducing neurotransmission, and paralysis in parasites (Turner and Schaeffer, 1998). The pyrethroid insecticides (e.g. fenpropathrin) bind to voltage-sensitive sodium channels and modify their gating kinetics, thereby they disrupt nerve function and produce acute neurotoxic effects in both insects and non-target organisms (Soderlund *et al*., 2002). It was reported that destructive changes due to the attachment of such insecticides at the ends of cholinergic nerve fibers leading to excessive production of acetylcholine and then inhibition of AChE (Narahashi, 1971). This may give an explanation for the inhibition of the activity of this enzyme recorded in the present study.

With time elapsed, there was a considerable fluctuations in total protein contents in the sera of albino rats either in control or in experimental groups (Table7). After 3 weeks, the treatment with abamectin achieved the maximum decrease in the total protein content, which accounted to 8.61 g/dL comparing to the corresponding control value (11.04 g/dL). The change in protein content might be due to the imbalance between the rate of protein synthesis and the rate of its degradation in the liver. The protein content in different organs was affected as a result of exposure to different insecticides (El-Bakary, 1993).

The creatinine concentrations in the sera of rats treated with different insecticides are presented in Table 8. With time elapsed, the control showed insignificant changes and recorded 1.57, 1.55, 1.59, 1.62 and 1.63 mg/dL for 0, 1, 2, 3 and 4 weeks, respectively. At the end of the experiment, only treatment with profenofos induced highly significant decrease in creatinine concentration accounting to 1.20 mg/dL. Some of the tested chemicals (e.g. fenpropathrin & methomyl) induced significant ($p \leq 0.01$) increases in creatinine concentrations; accounting to 1.84 and 1.76 mg/dL, respectively (Table 8). Creatinine is a metabolite of creatine and is excreted completely in urine via glomerular filteration. An elevation of its level in the blood is thus an indication of impaired kidney function (Lu, 1996). Yousef *et al.* (1999) found an increase in serum creatinine in rats-treated with cypermethrin.

Table (4): AST activity (IU/L) in the sera of rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean
	0	1	2	3	4	
Control	10.18±0.63	10.34±0.57	9.97±0.43	10.40±0.56	10.05±0.08	10.19
Abamectin	10.38±0.56	14.13±0.15**	12.76±0.12**	12.19±0.09*	11.94±0.09**	12.75
Carbosulfan	10.20±0.42	14.53±0.11**	12.55±0.14**	12.45±0.06**	10.19±0.15	13.00
Fenpropathrin	10.30±0.66	14.05±0.25**	13.79±0.24**	13.68±0.24**	9.74±0.34	12.43
Methomyl	10.18±0.24	15.56±0.38**	12.76±0.10**	11.15±0.16	10.48±0.21*	12.92
Profenofos	10.25±0.41	15.96±0.37**	13.78±0.06**	11.26±0.37	10.67±0.14*	12.30
The mixture	9.84±0.48	13.29±0.36**	13.51±0.34**	13.31±0.08**	11.53±0.17**	12.91

Each value is a mean ± SE. n = 8 rats
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
General mean = average reading from 1st to 4th weeks.

Table (5): ALT activity (IU/L) in the sera of rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean
	0	1	2	3	4	
Control	7.28±0.28	7.27±0.29	7.23±0.30	7.30±0.28	7.32±0.28	7.28
Abamectin	7.34±0.46	10.46±0.30**	7.40±0.28	13.37±0.34**	8.24±0.18*	9.87
Carbosulfan	7.39±0.38	10.83±0.47**	7.42±0.13	14.45±0.41**	7.17±0.38	9.97
Fenpropathrin	7.25±0.45	10.61±0.28**	7.43±0.09	13.55±0.25**	6.83±0.27	9.60
Methomyl	7.34±0.46	11.22±0.24**	7.66±0.02*	15.79±0.41**	6.68±0.14*	10.34
Profenofos	7.38±0.40	10.70±0.17**	7.75±0.16*	13.57±0.34**	7.15±0.14	9.79
The mixture	7.27±0.46	11.43±0.38**	8.39±0.34**	12.89±0.21**	8.81±0.27*	10.38

Each value is a mean ± SE. n = 8 rats
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
General mean = average reading from 1st to 4th weeks.

Table (6): AChE activity (U/L) in the sera of rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean
	0	1	2	3	4	
Control	1564.5±17.3	1539.9±75.8	1609.7±66.3	1593.2±55.1	1583.0±77.7	1581.5
Abamectin	1600.1±37.1	1269.7±67.9*	1405.4±29.2*	1225.3±98.9**	1117.1±40.5**	1254.4
Carbosulfan	1536.7±60.6	870.4±29.3**	1107.2±153.9*	985.7±62.4**	1185.0±49.3**	1037.1
Fenpropathrin	1529.0±41.7	1160.4±107.6*	1671.7±109.1*	1161.6±9.4**	1109.8±49.4**	1275.9
Methomyl	1591.9±77.4	1101.7±44.2**	818.3±73.6**	1136.5±17.5**	961.3±63.3**	1004.5
Profenofos	1573.4±45.9	1150.4±85.1*	1161.2±29.2**	914.8±80.4**	473.0±56.8**	1000.0
The mixture	1574.2±79.0	282.4±58.5**	832.6±64.9**	576.8±28.1**	473.8±35.3**	541.4

Each value is a mean ± SE. n = 8 rats
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
General mean = average reading from 1st to 4th weeks.

Table (7): Total protein concentration (g/dL) in the sera of rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean
	0	1	2	3	4	
Control	9.09±0.12	10.85±0.09	11.30±0.26	11.04±0.10	10.96±0.14	11.03
Abamectin	9.16±0.11	9.39±0.17**	9.46±0.26**	8.61±0.10**	10.31±0.18*	9.44
Carbosulfan	9.00±0.04	9.04±0.30**	12.35±0.09**	9.19±0.22**	10.07±0.19**	10.16
Fenpropathrin	9.01±0.21	9.89±0.50*	10.30±0.48*	10.87±0.48	11.17±0.28	10.56
Methomyl	9.14±0.13	9.36±0.16**	10.52±0.26*	10.15±0.23**	10.14±0.27*	10.04
Profenofos	9.12±0.11	10.71±0.31	9.14±0.17**	9.22±0.35**	10.30±0.21*	9.84
The mixture	9.05±0.13	10.15±0.12**	11.21±0.34	9.94±0.37*	11.57±0.27*	10.72

Each value is a mean ± SE. n = 8 rats
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
General mean = average reading from 1st to 4th weeks.

Table (8): Creatinine concentration (g/dL) in the sera of rats treated with the tested insecticides and their mixture.

Treatments	Time in weeks					General mean
	0	1	2	3	4	
Control	1.57±0.01	1.55±0.02	1.59±0.03	1.62±0.03	1.63±0.01	1.60
Abamectin	1.55±0.03	1.58±0.01	1.99±0.03**	1.61±0.01	1.65±0.03	1.71
Carbosulfan	1.53±0.01	1.68±0.04**	1.94±0.02**	1.38±0.01**	1.62±0.02	1.65
Fenpropathrin	1.54±0.02	1.72±0.03*	1.75±0.04**	1.89±0.03**	1.84±0.04**	1.80
Methomyl	1.52±0.02	1.74±0.02**	1.71±0.04*	1.91±0.03**	1.76±0.04**	1.78
Profenofos	1.56±0.03	1.72±0.01**	1.61±0.03	1.92±0.02**	1.20±0.01**	1.61
The mixture	1.53±0.01	1.75±0.03*	1.76±0.03**	1.72±0.02*	1.67±0.02	1.73

Each value is a mean ± SE. n = 8 rats
Statistical difference from the control: * significant at $P \leq 0.05$& ** highly significant at $P \leq 0.01$.
General mean = average reading from 1st to 4th weeks.

Table (9): Interaction Index throughout the experimental duration as well as general means, expressed by numerical values and their corresponding joint action.

Treatments	weeks				General mean
	1	2	3	4	
ALT (+ve)	0.75(An)	0.99(Ad)	0.60(An)	1.06(Po)	0.80(An)
AST (+ve)	0.74(An)	0.81(An)	0.90(An)	0.98(Ad)	0.85(An)
AChE (-ve)	1.16 (An)	1.14(An)	1.28(An)	1.32(An)	1.22(An)
Total protein (–ve)	1.11(An)	1.09(An)	1.13(An)	1.07(An)	1.10(An)
Creatinine (+ve)	0.94(An)	0.90(An)	0.94(An)	1.02(Ad)	0.95(Ad)

An=Antagonism; Ad=Additive; Po=Potentiation; An=20/25; Ad=4/25; Po=1/25

Histopathological Findings

The histopathological examination of the cross sections of liver and kidneys of untreated control group showed normal appearance. In contrast, in rats treated with the mixture, proliferation of epithelial cells of bile ducts (black arrow), degenerative changes of hepatocytes (white arrow), Kupffer cells activation (white zigzag) were observed (Plate 1A). In addition, dilatation and congestion of blood vessels (zigzag arrow), degenerative changes of hepatocytes with granularity in hepatic cells and infiltration with mononuclear cells (black arrow, Plate 1B) were demonstrated in the treatment of abamectin. Also, methomyl induced similar changes as well as an area of necrosis in parenchyma of liver tissues (Plate 1C). The intensity of the degenerative changes and the increasing of granularity of hepatocytes (Plate 1D) were also observed in rats treated with the mixture. The hepatic function tests (serum AST and ALT activities) corroborated the histopathological lesions observed in the present study. Our results regarding degeneration and vacuolation of renal or hepatic tissues go paralleled with the results of other investigators (Stebbins *et al.*, 2002; Mansour *et al.*, 2005) due to treatment with different pesticides. Also, hepatocellular necrosis and degeneration was recorded in rats treated with dursban and malathion (Mikhail *et al.*, 1979; Lox and Davis, 1983).

Histopathological examination of the kidneys revealed shrinking in Bowman's capsule (black arrow) and degenerative changes of epithelium lining renal tubules associated with occlusion of the lumen (white arrow) in rats treated with the mixture (Plate 2E), and necrotic changes associated with desquamation of epithelium lining tubules (black arrow, Plate 2F) in rats treated with the mixture, fenpropathrin and methomyl. These histopathological changes of kidney tissues in rats have been reported by previous investigators using male rats (Mansour *et al.*, 2001) as well as pregnant rats (Mansour *et al.*, 2005; Rawi *et al.*, 2005).

Joint Action

A simple way for studying the mixture toxicity is to compare its effect with the effects of its constituents at the same dose levels of their presence in that mixture. This approach requires a minimum number of experimental groups (n+1, the number of compounds in the mixture plus the mixture itself) and reflects the net effect of all compounds in the mixture (Feron and Groten, 2002). Previously, Mansour and Refaie (2000) used a simple equation to quantify the combined effects of mixtures of two pesticides (Eq 1). In the present study, this equation was modified to test mixtures of more than 2 constituents (Eq 2).

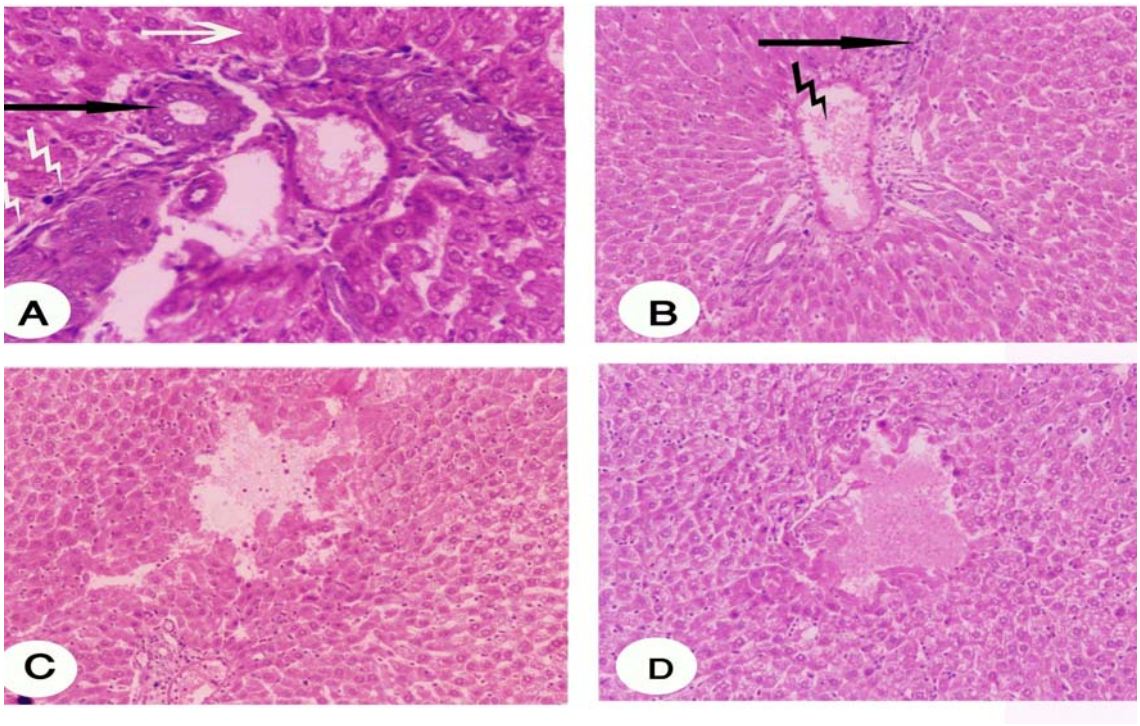

Plate 1: Histological observation in the liver of rats treated with the insecticides (abamectin, carbosulfan, fenpropathrin, methomyl, profenofos) and their mixture.

Plate 1A: Histological observation in the liver of rats treated with the mixture showing proliferation of epithelial cells of bile ducts (black arrow), degenerative changes of hepatocytes (white arrow), Kupffer cells activation (white zigzag)

Plate 1B: Histological findings in the liver of rats treated with abamectin showing dilatation and congestion of blood vessels (zigzag arrow), degenerative changes of hepatocytes with granularity in hepatic cells and infiltration with mononuclear cells (black arrow).

Plate 1C: Histological findings in the liver of rats treated with methomyl showing an area of necrosis in parenchyma of liver tissues.

Plate 1D: Histological findings in the liver of rats treated with the mixture showing intensity degree of degenerative changes and increasing of granularity of hepatocytes.

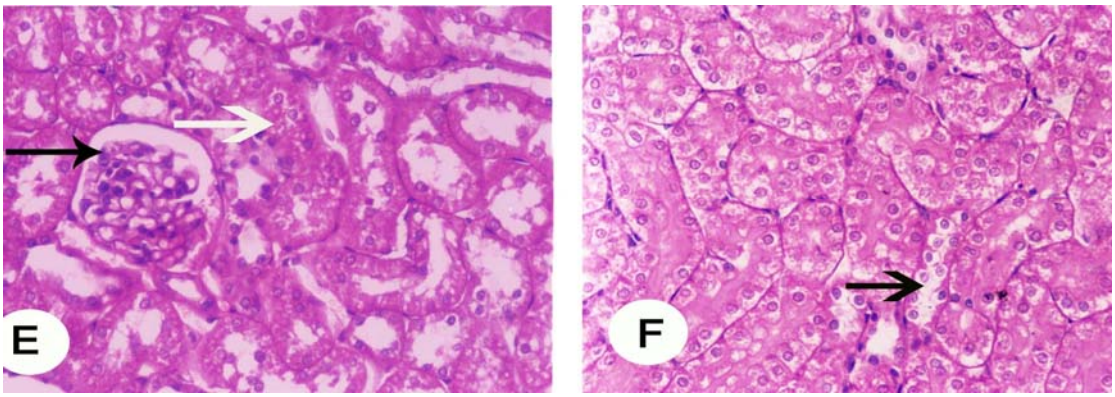

Plate 2: Histological observation in the kidneys of rats treated with the insecticides (abamectin, carbosulfan, fenpropathrin, methomyl, profenofos) and their mixture.

Plate 2E: Histological observation in the kidneys of rats treated with the mixture showing shrinking in Bowman's capsule (black arrow) and degenerative changes of epithelium lining renal tubules associated with occlusion of the lumen (white arrow).

Plate 2F: Histological observation in the kidneys of rats treated with fenpropathrin showing necrotic changes associated with desquamation of epithelium lining tubules (black arrow).

$$I.I. = \frac{M + C \times (n-1)}{\Sigma A_1 + A_2 + \text{------} A_n}$$

By applying the equation for example on ALT after 4 weeks (refers to Table 5)

$$I.I. = \frac{8.81 + 7.32 \times 4}{8.24 + 7.17 + 6.83 + 6.68 + 7.15} = 1.06\ (Po) > 1.05$$

The data of biochemical measurements presented in Tables 4-8 reveal that the mixture comprised the five insecticides affected the activity of the concerned enzymes and other determined parameters in a degree mostly different than those of the individual agents, which means occurrence of interaction between the mixture components. Focusing on the results of each parameter, as a general mean, it appears that the single compounds induced higher values than the corresponding controls, especially in case of AST, ALT and creatinine, while induced lower values in case of AChE and total protein. Accordingly when analyzing the joint action of the former case it will be treated as "positive effect", while the later case as "negative effect". The data shown in Table 9 demonstrate that the joint action of the mixture based on ALT was accounted to antagonism, additive, antagonism, and potentiation after 1, 2, 3 and 4 weeks of exposure, respectively. The general mean for the interaction index (I.I.) throughout the experimental duration was accounted to 0.80 which ranked as antagonism. The mixture components interacted antagonistically also with AST, AChE and total protein, while interacted additively with creatinine (general mean of I.I. = 0.95).

The predomination of antagonistic effect obtained in the present study are in harmony with the findings obtained by Krishnan *et al.* (1994) and Mansour and Heikal (2001). However, the interaction between pesticides can be expected, either because they have common cellular targets (Rashatwar and Matsumura, 1985) or because they have common metabolic pathways (Tardif *et al.*, 1993). For these reasons, the interaction between xenobiotics based on effects on enzymes (e.g. inhibition, induction, shift of routes of metabolism, and posttranslational modification of enzymes) could be very profound (Oesch *et al.*, 1994).

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