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INTERACTIVE EFFECTS OF IMIDACLOPRID, PROFENOFOS AND CARBOSULFAN AT LOW CONCENTRATIONS ON HOMEOSTASIS AND HAEMATOLOGICAL INDICES IN MALE ALBINO RATS

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ABSTRACT

Toxicity data with single pesticides to test animals are far more abundant than with mixtures (Flipo *et al.*, 1992). Consequently, these data cannot be used directly to predict the effect of pesticide combinations. Three pesticides; imidacloprid, profenofos and carbosulfan, administered to rats per OS at low level dose equal 1/30 LD50 for each insecticide, which represent 111, 70 and 43 ppm, respectively on homeostasis status and haematological indices (El-Kashory & El-Said, 2001), were selected to explore their combined action of subchronic exposure studies for 90 days in adult male albino rats. Homeostasis-related parameters such as; aldosterone (Ald.), sodium ions (Na+), potassium ions (K+), total chloride ions (T.Cl-) levels, pH value and haematological indices were examined in rats after an administration with different insecticide combinations. Moreover, after withdrawal the pesticide combinations for 30 days, as a recovery period, the above mentioned parameters were evaluated, in comparison with the control group. Results showed that, pesticide combination imidacloprid/profenofos (I + P) induced significant decrease in Na+ and T.Cl- ions levels and significant increase in pH value. While, it did not alter both Ald. and K+ ions levels. Combination imidacloprid/carbosulfan (I + C) increased significantly Ald., T.Cl- ions levels and pH values. On the contrary, it reduced Na+ and K+ ions levels significantly. Combination of profenofos/carbosulfan (P + C) decreased Ald. and Na+ significantly, while, K+ and T.Cl- level and pH value did not alter. In addition, tri-combination imidacloprid/ profenofos/carbosulfan (I + P + C) increased Na+ and T.Cl- ions level and pH value; while, a marked decline in Na+ ions level was occurred, as well as, no appreciable changes in K+ ion levels were observed.

The combinations of I + P and I + C caused erythropenia (reduced RBC mass) associated with a significant decrease in PCV in I + C-treated rats. While, di-combinations P + C, I + P and I + P + C tri-combinations increased markedly of PCV and MCV. However, leukocytosis (elevated WBCS count) was observed in I + P + C-treated rats.

After the pesticides combination withdrawal, changes in some parameters returned to the normal values, in comparison with the control group; while, the others still altered. Moreover, some parameters did not exhibit any changes unless after the stop of the treatment.

In conclusion, this study supports the notion that; an interactions effects of pesticide combinations may be consider as contributor factor enhance their side effects.

Key words: **Electrolytes, Aldosterone, Haematological indices, Insecticides, Rats, Exposure, Homeostasis.**

INTRODUCTION

Since pesticides are often used alternatively or in mixture, therefore, it is expected a potential interaction between these pesticides and consequently, it may be enhanced their mammalian toxicity.

The three pesticides of different chemical classes: chloronicotinyl; imidacloprid, organophosphate; profenofos, and carbamate; carbosulfan. As a result of their wide spread use they can be a potential environmental contaminants and may cause a public health hazards (El-Kashory & El-Said, 2001). Description of the mode of

action of these pesticides were recorded (Leicht, 1996; O'Brien, 1967 and Fuortes *et al*., 1993), and they classified by WHO as moderately hazardous "Class II" (WHO, 1991).

In our earlier work, effects of the three pesticides on homeostasis-related parameters and haematological indices in rats was evaluated after an administration of each of the insecticides alone (El-Kashory & El-Said, 2001). "Homeostasis" is the maintenance of equilibrium in a biological system by means of automatic mechanisms that counteract influences tending toward disequilibrium (Tietz, 1987). It is now recognized that homeostatic mechanisms operate at all levels of organization in living systems, including the molecular, cellular, organismic and even populational levels (Tietz, 1987).

Toxicity data with single insecticide are far more abundant than with mixtures. These data cannot be used directly to predict the effects of insecticide mixtures (Flipo *et al*., 1992).

Combinations of two or three of substances aggravated or mitigated toxicity depending on the active ingredient involved (Golbs *et al*., 1978). Also, it has reported that pesticides when used in combinations could interact with each other to affect toxicity (Lyaniwura, 1990), where interactions between pesticides could take place via the absorption, distribution or elimination rates.

Inadequate understanding of the mechanisms of these interactions is the source of much controversy (McEwen and Stephenson, 1979). Knowledge of the various mechanisms of pesticide interactions should be utilized in predicting the human hazardous of pesticide combinations, so hazardous combinations should be avoided (Lyaniwura, 1990). Toxicological studies on the effect of pesticide combinations in animals were carried out, using different criteria, as toxicological endpoints, *i.e.* immunoresponse and haematological parameters (Akay *et al*., 1999), histopathological studies (Selmanoglu and Akay, 2000), effects on antioxidant enzymes (Panemangalore and Bebe, 2000), effects on the steroid hormone synthesis (Gray *et al*., 2001), effect on programmed cell death (apoptosis) (Abou-Donia *et al*., 2003), anti-androgenic effects (Birkhoj *et al*., 2004), neurotoxic effects (Szabo *et al*., 2005) and pharmacokinetic and pharmacodynamic interaction (Timchalk *et al*., 2005).

Moreover, no reports are available on homeostasis criterion, therefore, this study assessed if there is an interaction between combinations of three commonly used pesticides, often found as residues in food, imidacloprid, profenofos and carbosulfan, using homeostasis-related parameters and haematological indices as toxicological endpoint.

MATERIAL AND METHODS

Animals:

Male albino rats weighing about 150±10 g each were used. The animals were obtained from the Farm of General Organization of Serum and Vaccine (Helwan Farm). Animals were allowed to be acclimatized to laboratory condition for a minimum of two weeks prior to the experiment. Animals were kept on balanced diet through the experimental period.

Tested pesticides:

Three pesticides were used in this investigation and their combinations; the first: "Imidacloprid", 1-(6-chloro-3 pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine (700 g/K WP), the second: "Profenofos", O-4-bromo-2-chlorophenyl-O-ethyl S-propyl phosphorothioate (720 g/L EC) and the third: "Carbosulfan", 2,3-dihydro-2,2-dimethyl-benzofuran-7-yl (dibutyl-aminothio)-methyl carbamate (250 g/K WP). The calculated LD50 of three pesticides; imidacloprid, profenofos and carbosulfan (administered to rats per OS.) were 445, 342 and 250 mg/kg b.w., respectively, according to Weil's method (Weil, 1952).

Used dosages represent 300, 1000 and 1000-fold of acceptable daily intake (ADI) for imidacloprid, profenofos and carbosulfan, respectively, (equal 1/30 LD50 for each one of the tested pesticide).

Experimental design:

Four treatment solutions were prepared, weekly, from stock solutions and gave these solutions to animals in their drinking water. Each stock solution represent a concentration equal 1/30 LD50 for each one of the tested pesticides, the stock solutions were made every week, and diluted to provide; 111 ppm imidacloprid (I), 70 ppm profenofos (P), and 43 ppm carbosulfan (C). Rats were classified into 5 groups, each 20 male rats. One group was kept as a control group, and the remaining groups received the pesticides combinations for consecutive 90 days through drinking water as the following :

Group 1: received tap water as control group.

Group 2 (IP): received 111 ppm imidacloprid (I) + 70 ppm profenofos (P) (combination I + P).

Group 3 (IC): received 111 ppm imidacloprid (I) + 43 ppm carbosulfan (C) (combination I + C).

Group 4 (PC): received 70 ppm profenofos (P) + 43 ppm carbosulfan (C) (combination P + C).

Group 5 (IPC): received 111 ppm imidacloprid (I) + 70 ppm profenofos (P) + 43 ppm carbosulfan (C) (combination I + P + C).

Pesticide combinations were given daily through drinking water in dark glass bottles for 90 days, water consumption of rats was recorded daily, clinical behaviour was monitored daily and animals were weighed weekly through the study.

On the day 91, tested combinations were withdrawn from drinking water, and half of the rat numbers were terminated, and the remaining rats of each group were left further for 30 days, and supplied only water free from any pesticides (post-exposure), as a recovery period.

Sampling:

At the end of experiment (exposed and post-exposed periods), blood samples were collected from orbital sinus vein by heparinized capillary tubes into clean, dry eppendorf tubes contained lithium heparin as an anticoagulant (Schalm, 1986). Blood pH values were determined by Coli-Parmer meter, USA. Samples were centrifugated at 3500 rpm for 15 min. in a refrigerated centrifuge to separate plasma. Selected biochemical analysis were carried in plasma sample by using commercial reagents kits; aldosterone (Byard *et al.*, 1970), sodium, potassium and chloride ions (Trinder, 1951; Sunderman & Sunderman, 1958 and Skeggs & Hochstrasset, 1964, respectively). In addition, other blood samples were taken in dry clean tubes containing EDTA as anticoagulant (1 mg/1 ml blood). Haematological parameters as RBCs and WBCs counts made by Naeubauer haematocytometer (Schalm, 1986), Haemoglobin (Drabkin and Austin, 1935). The packed cell volume (PCV) was determined by the micro-haematocrite method (Schalm, 1986). Haematological values were then utilized for calculating erythrocyte indices, viz., the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Statistical analysis:

The data were presented as mean ± S.E. Statistical analysis was performed using student's t-test. Significances were set at $P < 0.05$, $P < 0.01$ and $P < 0.001$ (Gad and Weil, 1989).

RESULTS

Administration of pesticides combinations; imidacloprid (I) + profenofos (P), imidacloprid (I) + carbosulfan (C), profenofos (P) + carbosulfan (C) and tri-combination : imidacloprid (I) + profenofos (P) + carbosulfan (C), at the low levels of doses (14.0, 12.0 and 8.33 mg/kg body weight, respectively in drinking water) into adult male albino rats for consecutive 90 days, followed by a recovery period (30 days), did not present any clinical signs of intoxication. Plasma biochemical alterations induced by the four combinations are shown in Table (1).

1- Combination imidacloprid + profenofos (I + P):

Data in Table (1) indicated that; in the treated rats, for 90 days, sodium ions (Na+) and total chloride ions (T.Cl-) were significantly decreased ($P < 0.001$ and 0.01, respectively), while pH value significantly increased ($P < 0.001$) when compared to the corresponding group of control animals (90 days). On the contrary, aldosterone and potassium ions (K+) levels did not change significantly. After the stoppage of the treatment with combination I + P for 30 days, as a recovery period, aldosterone level, Na+ and K+ ions levels were found to be decreased significantly, whereas pH values was increased significantly when compared to the control group (120 days). While T.Cl- ions returned to the normal values in comparison with the control group.

2- Combination imidacloprid + carbosulfan (I + C):

Results illustrated in Table (1) showed that in the plasma of I + C-treated rats, the levels of Na+ and K+ ions decreased significantly, whereas, the levels of aldosterone hormone and T.Cl- were increased significantly, as well as, an elevation in the pH values was occurred when compared to the corresponding group of control animals (90 days). After withdrawal the combination I + C, aldosterone and T.Cl- ions levels exhibited significant increase; whereas, significant decrease in Na+ ions level was observed. In contrast, K+ level and pH value resumed to the normal values in comparison with the control group, 120 days (Table, 1).

3- Combination profenofos + carbosulfan (P + C):

Data represent in Table (1) showed that, the levels of K+ ions and T.Cl-, as well as, pH value, in combination P + C-treated rats did not indicate any significant changes during the experiment (90 days). However, aldosterone and Na+ ions levels were significantly decreased, as compared to the corresponding group of control animals (90 days).

After the stoppage of the treatment with the combination, K+ ions level and pH value resumed to the normal values, in comparison with the control group, while an elevation in aldosterone level accompanied with significant decrease and increase in Na+ ions and T.Cl-levels were occurred, respectively, in comparison with the control group, 120 days.

4- Combination imidacloprid + profenofos + carbosulfan (I + P + C) :

From Table (1), there was a significant increase in the levels of aldosterone and T.Cl-, as well as, in pH value in treated group with trimixture I/P/C, while K+ level did not exhibit any significant changes. In contrast, significant decrease in Na+ ions level was occurred, when compared to the control group (90 days).

After stoppage of combination treatment for 30 days, as a recovery period, Na+ and T.Cl- ions resumed to the normal values when compared with the control group (120 days), aldosterone and K+ ions levels showed a significant decrease in association with significant increase in pH value as compare with corresponding group of control animals (120 days).

The results in Table (2) showed that the combination of (I + P) caused a marked reduce in erythrocyte counts (RBCs), whereas the mean corpuscular volume (MCV) was elevated markedly in comparison with control group after 90 day of treatment. A combination of (I + C) induced a significant decrease in RBCs count and packed cell volume (PCV). As for, the combination of (P + C), the results showed a marked increase in PCV and MCV values. Also, the combination of the three pesticides, *i.e.* (I + P + C)

Table (1). Effect of imidacloprid (I), profenofos (P) and carbosulfan (C) combinations at low levels (1/30 LD_{50}) on plasma aldosterone, electrolytes (Na^+, K^+, Cl^-) and pH values in male albino rats after 90 and 30 days recovery period.

Parameters	Treatment for 90 days					Recovery period for 30 days				
	Control G1	I / P 111+70 ppm G2	I / C 111+43 ppm G3	P / C 70+43 ppm G4	I / P / C 111+70+43 ppm G5	Control G1	I / P 111+70 ppm G2	I / C 111+43 ppm G3	P + C 70+43 ppm G4	I / P / C 111+70+43 ppm G5
Aldosterone (Pg/ml)	357.54 ±20.66	403.17 ±9.58	↑ 789.41 ±31.323*** a	↓ 254.15 ±13.298** a	↑ 453.46 ±9.534** a	412.63 ±21.20	↓ 275.67 ±4.583*** bc	↑ 602.45 ±20.634*** bc	↓ 608.28 ±9.07** bc	↓ 335.75 ±22.653* bc
Na^+ (mEq/L)	136.89 ±6.27	↓ 81.15 ±1.52*** a	↓ 108.54 _3.57** a	↓ 100.48 ±2.71*** a	↓ 101.21 ±2.99*** a	125.97 ±2.72	↓ 96.56 ±10.99** c	↓ 94.99 ±5.07*** c	↓ 71.29 ±4.05*** bc	123.115 ±4.20 b
K^+ (mEq/L)	4.30 ±0.225	4.01 ±0.121	↓ 3.54 ±0.116* a	3.83 ±0.085	4.29 ±0.136	4.13 ±0.116	↓ 3.74* ±0.055 c	4.06 ±0.307	4.28 ±0.0198	↓ 3.532 ±0.081** bc
Cl^- (mmol/L)	83.47 ±2.265	↓ 75.29 ±0.336** a	↑ 96.17 ±0.459*** a	84.96 ±2.317	↑ 93.81 ±4.498*	80.40 ±1.022	88.74 ±0.160	↑ 96.41 ±0.966*** c	↑ 101.47 ±2.091*** bc	95.40 ±4.869
pH	7.407 ±0.004	↑ 7.613 ±0.26*** a	↑ 7.634 ±0.006*** a	7.419 ±0.005	↑ 7.590 ±0.024*** a	7.528 ±0.020	↑ 7.60 ±0.019* c	7.507 ±0.012	7.435 ±0.003	↑ 7.547 ±0.040** c

- Values represent the mean ± S.E. (n = 6)
a : Significant differences between treatment and control group, 90 days.
b : Significant differences between treatment (90 days) and recovery (120 days) of treatment.
c : Significant differences between recovery (120 days) of treatment and control groups.

Table (2). Effect of imidacloprid (I), profenofos (P) and carbosulfan (C) combinations at low levels (1/30 LD_{50}) on haematological indices in male albino rats after 90 and 30 days recovery period.

Parameters	Treatment for 90 days					Recovery period for 30 days				
	Control	I + P	I + C	P + C	I + P + C	Control	I + P	I + C	P + C	I + P + C
RBCs $x10^6/\mu l$	6.809 ±0.100	↓ 5.865 ±0.148*** a	↓ 6.035 ±0.262*** a	6.248 ±0.250	6.430 ±0.141	6.624 ±0.174	↓ 5.955 ±0.204* c	↓ 5.990 ±0.179* c	↓5.490 ±0.389* c	↓5.858 ±0.269* c
WBCs $x10^3/\mu l$	8.966 ±0.529	6.088 ±0.954	5.963 ±0.533	6.600 ±0.898	↑ 12.263 1.553* a	8.40 ±1.143	↓ 4.84 ±0.534* c	↓ 5.518 ±0.571* c	↓ 4.450 ±0.634* c	9.150 ±0.798 b
Hb g/dL	13.492 ±0.278	12.260 ±0.782	12.410 ±1.458	13.655 ±0.694	14.405 ±0.823	12.917 ±0.333	13.17 ±0.772	↓ 10.968 ±0.595* c	↓ 10.942 ±0.690* cb	↓ 9.125 ±0.779** cb
PCV (%)	36.40 ±0.400	34.75 ±0.661	↑ 33.50 ±0.387*** a	↑ 43.50 ±1.323** a	↑ 40.750 ±0.968** a	36.60 ±0.510	↑ 39.00 ±0.548* cb	↓ 32.00 ±1.140* c	35.50 ±0.671 b	↑ 41.50 ±0.922** c
MCV (ft)	53.419 ±1.155	↑ 62.015 ±1.243** a	55.746 ±3.140	↑ 70.235 ±3.874** a	↑ 63.485 ±1.810** a	56.320 ±1.666	↑ 65.80 ±2.025** c	53.623 ±0.447	↑ 64.306 ±2.934* c	↑ 71.505 ±3.263** c
MCH (pg)	20.387 ±0.459	20.998 ±1.520	20.435 ±2.00	21.863 ±0.787	↑ 25.215 ±1.334* a	19.516 ±0.394	↑ 22.095 ±1.043* c	18.44 ±1.304	19.99 ±0.289	21.615 ±1.319
MCHC (%)	37.160 ±0.991	35.443 ±2.579	37.288 ±4.388	34.843 ±0.962	35.263 ±1.595	35.41 ±1.204	33.835 ±2.114	34.275 ±1.565	↓ 30.823 ±0.775* b	↓ 30.383 ±1.060* c

- Values represent the mean ± S.E. (n = 6)
a : Significant differences between treatment and control group, 90 days.
b : Significant differences between treatment (90 days) and recovery (120 days) of treatment.
c : Significant differences between recovery (120 days) of treatment and control groups.

produced a significant elevation in PCV, MCV and mean corpuscular haemoglobin (MCH). However, the leukocyte count (WBCs) was elevated markedly in rats treated with the combination of the three pesticides in comparison with control rats.

During the recovery period (30 days), the results demonstrated that the combination of (I + P) caused a significant decrease in RBCs and WBCs counts, while PCV, MCV and MCH were increased markedly when compared with control group. With respect to the combination of I + C; RBCs and WBCs counts, Hb content and PCV values were reduced significantly, as well as, the combination of P + C induced a significant decrease in RBCs and WBCs counts, Hb content and mean corpuscular haemoglobin concentration (MCHC), whereas a marked elevation in MCV was observed.

As for, the combination of the three pesticides (I + P + C), the RBCs and WBCs counts and Hb content were reduced markedly, and a significant increase in PCV and MCV was detected. When compared the results after stoppage the treatment (recovery period) with that reported after treatment rats with different combinations for 90 days, the results showed that a significant reduction in WBCs count in I + P + C-treated rats and also in rats treated with combinations of P + C or I + P + C exhibited a significant decrease in Hb content; however, MCHC values were decreased markedly in rats treated with the combination of P + C.

DISCUSSION

In the present study, an administration of rats with combination I + C and combination I + P + C, at low level doses; for consecutive 90 days, induced significant increase in aldosterone level in association with decrease in sodium ions (Na+) level; meanwhile, combination P + C showed significant decrease in aldosterone level also associated with significant decrease in sodium ions (Na+) in rats for 90 days. On the contrary, rats treated with combination I + P did not show any significant changes in aldosterone level, while a marked decline in Na+ ions was observed, in comparison with the corresponding control animals (90 days). After withdrawal the four combinations; I + P, I + C, P + C, I + P + C, for 30 days, as a recovery period, animals did not recover, and both aldosterone and sodium ion levels did not resume to the normal values of the control group (120 days), except, in combination I + P + C-treated rats, sodium ions returned to normal value of the control rats.

It has been reported that, an administration of the three pesticides imidacloprid (I), profenofos (P) and carbosulfan (C) alone into male albino rats, at the same low level of the doses (14.8, 12.0 and 8.33 mg/kg body weight in drinking water, respectively) for the same time interval, 90 days, induced a significant decrease in aldosterone in association with significant increase in sodium ions rats treated with I and P. Contrary of this, an elevation in aldosterone level associated with elevation in sodium ions in rats treated with C was occurred, in comparison with the control group (90 days). After withdrawal the three pesticides, for 30 days, significant changes in aldosterone level was still occurring, while sodium ions level resumed to normal values of the control group (120 days) (El-Kashory & El-Said, 2001).

Clearly, there is a difference between the effect of the three pesticides, alone, and their combinations, at the same low levels of doses on aldosterone and sodium ions levels. That is may be due to interaction effect of these compounds, where the combinations of the two or three substances aggravated or mitigated the toxicity depending on the active ingredient involved (Golbs *et al.*, 1978). The findings presented suggest that these mixtures have the potential to induce the xenobiotic-metabolizing enzymes in the liver of mammals (Chaturvedi, 1993). Aldosterone is a mineral corticoids that promotes the conservation of sodium ions and water and the excretion of potassium ions by the kidney. It is necessary for the maintenance of a proper balance between these two electrolytes in the body (Tietz, 1987).

In the current study, "hypoaldosteronism" which accompanied by "normonatraemia" may be due to an elevation of aldosterone clearance in plasma of experimental animals (Abayasekara *et al.*, 1993). Secretion of aldosterone by the cortex of the adrenal gland is regulated by the kidney (Hood, 1980), this clearly suggests that histopathological alterations in the kidney reflect some disruption in the level of this hormone. Histopathological study on the effect of three pesticides and/or its combinations confirmed the previous hypothesis (Kandil *et al.*, 2004). Excessive production of aldosterone "hyper aldosteronism" may result in Oedema and high blood pressure (Hood, 1980). Moreover, an elevation of aldosterone level in plasma accompanied by decreasing in the sodium level (hyponatraemia) could be attributed to an increased quantities of aldosterone metabolites such as 5 α-dihydro-aldosterone and 3 α 5 β-tetrahydro-aldosterone, which show significant cross-reactivity with antisera to aldosterone are produced by the liver (Abayasekara *et al.*, 1993). Insufficient aldosterone "hypoaldosteronism" may be a result of dehydration (Hood, 1980).

Electrolytes are essential components of all living matter. Among functions of the electrolytes are maintenance of osmotic pressure and water distribution in the various body fluid compartments. Thus, abnormal levels of electrolytes may be either the cause or the consequence of a variety of disorders, and determination of electrolytes is the one of the most important functions of the clinical laboratory (Tietz, 1987).

"Hyponatraemia" is found in condition of inadequate secretion of aldosterone and renal tubular disease. Meanwhile, "hypernatraemia" is found in hyperadrenalism when there is an excess of secretion of aldosterone. In addition, the condition of severe dehydration (Bush, 1991).

Concerning the effect of pesticide combinations on potassium ions (K+); it did not exhibit any significant changes in rats dosed with combination I + P, combination P + C and combination I + P + C. While, after withdrawal the pesticide combinations, for 30 days, rats treated with combination I + P and combination I + P + C showed a significant decrease in this parameter. Similar effect was recorded after the withdrawal of imidacloprid (I) and carbosulfan (C), for 30 days, when they administered alone (El-Kashory & El-Said, 2001). Although imidacloprid and carbosulfan, alone, did not affect K+ after 90 days (El-Kashory & El-Said, 2001), combination imidacloprid/ carbosulfan (I + C) altered it significantly, which means that an aggravating or synergistic effect in toxicity was occurred. On the contrary, with combination I + P + C; these effects may be attributed to the competitive mechanisms of I and C, which act antagonistically to profenofos (Leicht, 1996), in comparison with pseticide, alone (El-Kashory & El-Said, 2001).

Potassium homeostasis involves regulation of internal balance [*i.e.*, the distribution of K+ between the external cell fluid (ECF) and internal cell fluid (ICF)], it is influenced by; change in acid-base status and catecholamine release, as well as, external balance (*i.e.* the relation of K+ input to output) (Rose, 1984 and Carlson, 1997), thus disturbance of the K+ balance has serious consequences (Tietz, 1987). Potassium depletion "hypokalaemia" most commonly develops as the result of excessive renal loss of K+ results from the action of mineralocorticoid excess, as well as, K+ depletion may be the result of a decrease in ICF volume, altered membrane potential, altered intracellular pH, and alterations of K+-dependent enzymatically mediated reactions (Carlson, 1997).

Results of the present study revealed that, combination I + C and combination I + P + C gave a marked increase in total chloride ions (T.Cl-) in rats administered for 90 days. On the contrary, combination I + P exhibited a marked decline in T.Cl- and no significant changes in rats treated with combination P + C was observed. During the recovery period (30 days), animals treated with combinations I + P and I + P + C recovered and T.Cl- resumed to normal values of the control group (120 days). Combinations effect on T.Cl- level markedly differed from that of each compound separately (El-Kashory & El-Said, 2001), in which three pesticides; imidacloprid (I), profenofos (P) and carbosulfan (C), at the same low level of doses, did not alter T.Cl- ions level significantly. This may be due to the accumulating effect of pesticides combinations that was not observed in individual pesticides (Birkhoj, *et al.*, 2004).

It is worth to mention that, imidacloprid combinations; (I + P), (I+ C) and (I + P + C) particularly, induced fluctuated changes between increase and decrease in T.Cl- ions level. This effect may be referred to the presence of imidacloprid (I) and this suggestion may supported by the results of an administration of rats with profenofos (P) and carbosulfan (C) where there is no any significant change in T.Cl- ions level either alone (El-Kashory & El-Said, 2001) or in their combination in the present study. Our results, concerning of the combinations, were markedly differed from of each compound used separately; these differences may be caused by pesticides used in combination. It could interact with each other to affect toxicity (Golbs *et al.*, 1978).

Chloride deficiency "hypochloremia" may be associated with proportional decrease in sodium ions "Na+" level as a result of overhydration (relative water excess). "Hyperchloremia" may be associated with an inhibition of carbonicunhydrase enzyme and/or in renal failure cases (Saxton and Seldin, 1986).

Also, "hyperchloremia" may be seen as a compensating response for a primary respiratory alkalosis (Saxton and Seldin, 1986).

Regarding the effect of pesticide combinations (I + P, I + C, P + C, I + P + C), on blood pH values, it was found that a significant increase "alkalosis" in its level was occurred. Except, combination P + C did not alter blood pH value, significantly, in rats. These results differed from that of pesticides, alone (El-Kashory & El-Said, 2001), where they induced significant decrease in pH value "acidosis", after administration of rats with the same low levels of doses for the same time intervals (90 days). After the recovery period, 30 days, pH values in rats treated with I + C combination resumed to normal values, while animals treated with the two combinations I + P and I + P + C did not exhibit recovery and the elevation in pH values "alkalosis" was still occurring.

It has reported that, profenofos and carbosulfan, individually, induced "acidosis" (El-Kashory & El-Said, 2001), while, when they used in combination P + C, they did not alter pH values, significantly. This may be attributed to the antagonism effect of profenofos and carbosulfan in their combination (Golbs *et al.*, 1978). Also, it means that interaction between the two pesticides could take place via the absorption, distribution or elimination rates (Lyaniwura, 1990 and Chaturvedi, 1993). The hydrogen ion concentration of the ECF is maintained within remarkably narrow limits (40 x 10-6 nmol/L) even at these extremely low concentrations, hydrogen ion have profound effects on metabolic events. Most enzymatic reactions have a narrowly defined range of pH optimum, and changes in hydrogen ion concentration have direct effects on the rates of reaction and thus, many basic biological processes (Carlson, 1997).

"Metabolic acidosis" is characterized by a decrease in pH and bicarbonate. It can occur when there is a gastrointestinal loss of bicarbonate due to diarrhea and renal failure, which may result in a decrease ability to excrete hydrogen and thus to retain bicarbonate (Carlson, 1997). Also, toxic compounds may cause "metabolic acidosis", which results in the accumulation of exogenous anions (DiBartola, 1992).

"Metabolic alkalosis" is characterized by an increase in pH and bicarbonate. Generation of metabolic alkalosis can be due to excessive hydrogen loss, bicarbonate retention, or

as a contraction alkalosis which occurs with reduction of extracellular fluid (ECF) volume due to a loss or sequestration of Na+ and Cl- containing fluid without commensurate loss of bicarbonate (Garrella *et al.*, 1975). Also, excessive renal hydrogen loss associated with mineralocorticoid excess, and low chloride intake may cause or contribute to the generation of metabolic alkalosis (Rose, 1984).

Haematological findings in rats following exposure to different combinations of selected pesticides or during the recovery period, suggest that these combinations had toxic effects or haematotoxicity during the experimental period (90 days), and the recovery period (30 days).

At 90 days, erythropenia and a significant elevation in the MCV values was recorded in rats treated with combination of the two pesticides (*i.e.* I + P). The elevation of MCV was associated with macrocytic anaemia. This may be attributed to swelling of RBCs and this, in turn, lead to the hemolysis and consequently reduced the red cell mass as shown in our results. In addition, this may be occur as a result of release of catecholamines hormones (stress hormones) and/or hypothyrodism or liver disorders (Chandrasoma and Taylor, 1991). Our studies in 2001, revealed that profenofos induced a significant reduction in RBCs and Hb content. Also, imidacloprid treatment, produced a significant decrease in Hb content.

Moreover, severe oxidative stress can lead to haemolysis. In principle, the erythrocyte is very sensitive to peroxidative reactions. Under normal conditions, the erythrocytes is very well protected against peroxidative reactions by the presence of catalase and glutathione. This balance, however, can be disturbed by different chemicals. Oxidative damage can result indirect injury to the cell membrane due to lipid peroxidation and bring about changes in membrane proteins (*i.e.* enzymes) and consequently, the permeability of such membranes of any erythrocyte (Bush, 1991). The combination of I + C caused erythropenia and a reduction in the PCV values. These results suggest to exist of absolute anaemia as a result of hemolytic or depression anaemia (Jain, 1993). As for carbosulfan treatment, there was no significant changes in haematological findings after 90 day of exposure (El-Kashory & El-Said, 2001).

However, a relative polycythemia and macrocytic anaemia were detected in rats exposed to the combinations of the two pesticides (*i.e.* P + C) or the three pesticides (*i.e.* I + P + C).

The relative polycythemia associated with elevation of PCV values without increasing of red cell mass and this may be attributed to dehydration and/or splenic contraction (Feldman *et al.*, 2000).

No recovery observed in haematological findings after stoppage of treatment for 30 day. Where, the combinations of the two pesticides (*i.e.* I + P, I + C and P + C) induced erythropenia accompanied by decreasing in Hb content and PCV values and also leukopenia was observed. This possibly due to the decrease of red cell production, as the result of failure of production or suppression or destruction of steam cells in the bone marrow, which lead to decrease generally erythrocytes and leukocytes. Therefore, the bone marrow shows a marked decrease in cellularity (Chandrasoma and Taylor, 1991).

However, leukopenia may be occur as a result of glucocorticoids, which produced lymphopenia, eosinopenia and monocytopenia.

During the recovery period (30 day), a significant decrease in Hb content was continued in profenofos-treated rats. Meanwhile, imidacloprid-treated rats showed a significant decrease in RBCs count, Hb content and MCHC values. A significant elevation in MCV and MCH values in carbosulfan-treated rats was noticed. No significant changes in leukocytes count throughout the experimental period either after exposure to the selected pesticides individually or following stoppage the treatment within the recovery period (El-Kashory & El-Said, 2001).

In addition, erythropenia and macrocytic-hypochronic anaemia was noticed in rats exposed to the combination of the three pesticides (I + P + C). These results suggest to the presence of depression anaemia and hemolysis of RBCs, which associated with elevation of MCV. On the contrary, leukocytosis was detected in rats exposed to combination of the three pesticides, (*i.e.* I + P + C). This may be due to release of catecholamine (*i.e.* Ipinephrin) as a stress hormone, which causes demargination of neutrophils, is often accompanied by lymphocytosis and sometimes by monocytosis and eosinophilia (Jain, 1993).

In the present study, some biochemical and haematological parameters did not change significantly after an administration with pesticide combinations for 90 days. But, when the treatment was stopped for 30 days, as recovery period, an alteration in these parameters was appeared. The difficulty of explanation of this "phenomena" may due to our inadequate understanding of the mechanisms of these interaction (McEwen and Stephenson, 1979), or how combinations of pesticides might act on the mammalian physiology, there are potentially many possible interactions between these agricultural chemicals and animals (Porter *et al.*, 1993). Interaction between pesticides could take place via the absorption, distribution or elimination rates. In addition, induction and inhibition of the liver microsomal enzymes (*i.e.*, cytochrom P450) could also play significant roles in pesticides interaction and toxicity (Lyaniwura, 1990).

Concerning the appearance of toxic effects of pesticide combinations, after stopping of the treatment, many explanations are possible; redistribution of toxic residues (traces) in different organs or biological system of pesticides-treated rats over the recovery period, and hence, release of lipid peroxidative substances from damaged tissues by mixture of pesticides used into blood circulation, however an exposure might have led to the activation of detoxifying pathways.

CONCLUSION

In the present study, pesticide combinations altered Ald. level, it means that; there is an effect on both adrenal gland and kidney function. That is worth to say, these combinations have an endocrine disrupting effect which leads, consequently, to impairment in electrolyte balance. These results were discussed in light of the possible interaction between two or three pesticides and provide first hand information on the effect of mixtures, of three pesticides often found as residues in food, on homeostasis-related parameters and haematological indices. However, identification of the pesticide(s) responsible for these changes would require further studies of the individually pesticides as well as various combinations of the pesticides.

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