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Genotoxic effects of three different tested pesticides coumatetralyl, ivermectin and imidacloprid at sublethal dose on albino rats *Rattus norvegicus albinus.*

By

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ABSTRACT

Three pesticides from three different chemical groups; coumatetralyl, imidacloprid and ivermectin were used at sublethal doses of 0.1 and 0.25 LD_{50} to perform the genototoxic effects on albino rats *Rattus norvegicus albinus* by determining the total protein and nucleic acids (RNA and DNA) in brain, kidney, liver, heart and spleen. coumatetralyl (0.1 LD_{50}) caused significant increase in DNA quantity in all tested tissues except in spleen. Whereas 0.25 LD_{50} caused highly significant increase in DNA quantity in brain and heart tissues and significant only in spleen. There were parallel results concerning RNA quantities with DNA obtained data especially upon using 0.25 LD_{50} coumatetralyl caused genotoxic effect in treated rats. This was pronounced in highly significant values for protein content that was obtained upon treatment by 0.1 and 0.25 LD_{50} in most compounds tested. The imidacloprid caused significant increase in DNA content in all tested tissues, except in the heart where it showed highly significant increase over the control value. Regarding RNA content estimated in various tissues upon

imidaclopid treatment, it showed severe reduction at both used doses, being significant in the liver, and highly significant in spleen and kidney. Total protein level showed highly significant decrease in all tested tissues under the two used concentrations. Ivermectin exerted its genotoxic effect on liver tissue, as it caused highly significant increase in DNA content under both concentrations. Whereas, RNA content showed highly significant changes being positive (increase) in heart tissue and negative (decrease) in kidney and spleen tissues upon using 0.1 LD_{50} while 0.25 LD_{50} caused highly significant decrease of RNA value in all tested tissues except liver tissue, where it showed highly significant increase in quantity.

INTRODUCTION

Pesticides are used extensively allover the world in plant protection. Egypt consumes 1 –2% of the world production, as it was reported by Amr (1990). Those pesticides as pollutants reach the human body in daily diet along food chains, deposited and accumulated in adipose tissues (Morita *et al*, 1975) and (Mori *et al*, 1983). Anticoagulants cause death by internal haemorrehage. In general, all the anticoagulant rodenticides currently available are of the two chemical types, hydroxy coumarine or indane–dione derivatives (Meehan, 1984). The toxic effect of the anticoagulant results from their commutative effect. Besides they do not include symptoms that inhibit feeding on poisons bait until a lethal dose has been ingested (Rennison, 1974). Enan (1976) reported that Warfarin caused anemia and reduced prothrombin activity. Moreover, (Park *et. al,* 1980) cleared that anticoagulants, difenacoum and brodifacoum inhibited clotting factor synthesis. Besides, sub lethal doses of warfarin and coumatetralyl lead to development of microcytic anemia early at 24 hr of treatment and followed by macrocytosis and again by the reoccurrence of microcytosis with recovery of

the treated rats (Shimaila, 1989).Confidor has a new mode ofaction. It has a flat aromatic molecule as shown in acariddine, which intercalates in the DNA base pair stack. Therefore, it could stabilize a shifted pairing in repetition sequence of bases, giving rise to genotoxic effects (Streisinger *et al*, 1966). Those genotoxic effects reconsidered among the most serous effects include heritable genetic disease, carcinogenicity and birth defects (Sherif *et. al,* 1990). Vertemic, the natural product produced by soil fungi fermentation and its mode of action in mammals is attributed to its effect on gamma amino buteric acid (GABA) receptors. Although, the most parsimonious explanation for how ivermectin works is that it specially increase membrane chloride ion permeability, there clearly other sites of action at which ivermectin affects either the host or target organism Lanakas and Gordon (1988).

The aim of this study is to assess the effect of sublethal doses of three pesticides, *Racoumin* coumatetralyl, *Vertimec* ivermectin and *Confidor* imidacloprid on total protein, RNA and DNA in different organs, liver, kidney, spleen, heart and brain in treated albino rats at sublethal doses.

MATERIALS AND METHODS

Tested pesticides: Three pesticides belonging to different groups and having different mode of action were tested. These are coumatetralyl as rodenticides, imidacloprid as insecticides and ivermectin as acaricides. They represent the main three chemical groups in large-scale use in the Egyptian environment at least for the last ten years. Sublethel dose (1/10 and ¼ LD_{50}) of these pesticides were used.

Tested animals: The male white albino rats (*Rattus norvegicus albinus*) each weighing 100 - 175 g were used in this study. They were obtained from the animal house of the High Institute of Public Health, Alex.Univ.The animals were fed on a diet, which consisted of bread, milk and crushed maize.

Methods of testing: The animals were divided into 7 groups, each group was 4 rats. The first and second groups were treated with 0.1 and 0.25 LD_{50} of coumatetralyl, respectively. The third and fourth groups treated with 0.1 and 0.25 LD_{50} of imidacloprid. The fifth and sixth groups were treated with 0.1 and 0.25 LD_{50} of ivermectin. The last group was treated with corn oil only as control. The control and the treated animals were sacrificed, then the brain, liver, kidney, heart and spleen were removed rapidly and cleaned. The tissues were homogenized in cold distilled water to a 20%(w/v) and frozen at -4°C. One milliliter of the homogenate was added to 8 ml of tetrachloroacetic acid (TCA), 10% solution. Precipitate formed was separated from the supernatant fluid by centrifugation at 2000 rpm for 5 min. The supernatant was used for protein, RNA and DNA determination. The protein content of the tissue hydrolyzates was determined using Behring Institute Clinical Reagent kit Weichselbaum (1946). The concentration of RNA in the prepared tissue hydrolyzate was measured as described by Clowick and Kaplan (1957). The concentration of deoxyribonucleic acid (DNA) of the prepared sample was determined by using the biphenyl amine method (Chris *et. al,* 1997).

Statistical analysis: All the data were calculated as means ± SD and comparison between two groups was performed by student's t- test according to Motulsky (1987).

RESULTS AND DISCUSSION

Data presented in Table (1) clearly show the deleterious effect of coumatetralyl on nucleic acids (DNA & RNA) contents and total protein in liver, kidney, brain, heart and spleen of treated male albino rat at two doses (0.1 and 0.25 LD_{50}). Deoxyribonucleic acid content in liver tissues at 0.1 LD_{50} treatment (0.960 µg / g tissue) showed an increase in quantity over the control value (0.610 µg / g tissue). While, the concentration (0.25 LD_{50}) did not cause noticeable change compared to the untreated value (0.661 and 0.610 µg /g tissue), respectively. Despite the previous data concerning DNA content, the used rodenticide has execrated its inhibitory effects on male rat, due to its action on protein biosynthesis. Obvious values of total protein in liver tissues showed a highly significant increase over the control value. These results are direct indicator for liver function disturbance due to coumatetralyl treatment. This finding is in coherence with several previous reports (Eisa and Seleim, 1992) and (El- Sheikh *et. al*, 1993). Estimation of RNA contents in kidney, brain, heart and spleen tissues after two tested coumatetralyl doses showed significant decrease in spleen tissue at 0.1 LD_{50} and highly significant at 0.25 LD_{50} in liver, kidney and spleen tissues.

Toxic effects of ivermectin on nucleic acids and total protein in male albino rat are pronounced in all tested tissues as presented in Table (2). It is clear that DNA content in liver tissues showed higher values upon ivermectin treatment. The value is twice as much (1.26 µg /g tissue) in rat treated with 0.1 LD_{50} compared to 0.61µg / g tissue in untreated rats. This compound may act on DNA repair system and /or stimulate DNA replication. The other tissues at the above-mentioned concentrations did not show any significant variations over the control values. Higher concentrations that used (0.25 LD_{50}) did not have an impact on DNA quantity in all tested tissues except

Table (2): Genotoxic effect of ivermectin on nucleic acids (DNA & RNA) content (µg /g tissue) and total protein (mg/ g tissue) in male albino rats different organs.

Treatment	Parameters	Organ				
		Liver	Kidney	Brain	Heart	Spleen
0.1 LD_{50}	DNA RNA Total protein	1.26±0.42** 9.01±2.79** 39.2±7.85**	0.67±0.045 6.25±0.262** 41.41±3.88**	0.62±0.160 3.07±0.550 47.04±12.8**	0.88±0.070 7.36±0.79** 58.85±3.28**	0.93±0.02 3.15±0.20* 55.2±6.20**
0.25 LD_{50}	DNA RNA Total protein	0.96±0.05* 14.38±0.33** 53.37±1.27**	0.80±0.02 6.06±0.24** 66.73±16.6**	0.32±0.046 1.20±0.390** 55.68±4.80**	0.46±0.026 4.23 ±0.87 85.19±13.7**	0.59±0.330 3.95±0.74** 121.27±1.1**
Control	DNA RNA Total protein	0.61±0.04 4.56±0.75 130.65±5.6	0.55±0.03 12.15±0.98 155.5±13.5	0.644 ±0.13 3.55±0.490 152.76±6.33	0.76 ±0.14 3.37 ±0.62 108.8±8.9	1.092±0.42 9.070±1.35 111.6±1.10

* Significant at $p \leq 0.05$

** Highly significant at $p \leq 0.01$

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in liver. It showed a significant increase over the control value (0.61µg /g tissue) as it was 0.96 µg /g tissue. This increase in DNA content could be due to arrested and /or polyploidyin chromosomal set, especially when the quantity of estimated DNA equal twice the quantity of its correspondent in control. This conclusion was put forth as several investigations that used different pesticides to detect their influences on chromosomal aberrations (Abd El-Baset and El- Nahas, 1985 and Fayed *et. al,* 1989). Regarding the effect of ivermectin on RNA quantity of different tissues showed an overall reduction, as its synthesis is a DNA dependent process. Therefore, significant decrease in RNA followed ivermictin treatment is expected due to the decrease of DNA synthesis. Similar results for nucleic acids reduction upon pesticides treatments in different organs were recorded by EL – Elaimy and Abd El- Nabi (1990). They attributed this finding to the inhibition of the de-novo purine synthesis. Moreover, this reduction in nucleic acids content could be due to higher levels of nucleases activity as this reflects the intercede for nucleic acids catabolism. Estimated total protein in all tested tissues whether upon using 0.1 or 0.25 LD_{50} of Ivermictin came to be inhibited as it is presented in Table (2). In fact this finding is in a good agreement with Abd El- Baset and El – Nahas (1985), Eisa and Eissa, (1992) and Eissa & El – Sheikh, (1993). Severe reduction of total protein in treated tissues was in parallel with significant reduction in total estimated RNA. This finding would indicate that, Ivermictin exerts its action on RNA synthesis rather than protein biosynthesis system.These results were supported by Kim *et al* (1998).

Data presented in Table (3) clearly show the deleterious effects of Imidacloprid on nucleic acids (DNA& RNA) contents in the tested tissues at the above two doses. Estimation of DNA showed increase in all tested tissues over the control values. This increment appeared to be significant in liver, kidney and heart

Table (3): Genotoxic effect of imidacloprid on nucleic acids (DNA & RNA) content (µg /g tissue) and total protein (mg/ g tissue) in male albino rat different organs.

Treatment	Parameters	Organ				
		Liver	Kidney	Brain	Heart	Spleen
0.1 LD_{50}	DNA RNA Total protein	1.09 ±0.050 2.49±0.200* 37.19±10.1**	1.45±0.024* 6.12 ±0.6 6** 36.89±6.64**	1.15±0.270 2.93±0.22 0* 38.19±3.43**	2.10±0.6 70* 6.81±2.830** 38.59±5.53**	2.80±1.380 2.62±0.740* * 104.4±7.90**
0.25 LD_{50}	DNA RNA Total protein	1.07±0.099* 2.92±0.09 0* 52.52±9.27**	1.066±0.02* 9.460±0.58 * 55.00±16.6*	1.3±0.460 1.4 ±0.03** 48.0±840**	2.05±0.010** 5.52±0.080** 69.59±6.14**	2.600±0.98 4.580±1.07** 60.66±4.33**
Control	DNA RNA Total protein	0.61±0.040 4.56±0.750 130.65±5.6	0.55±0.030 12.15±0.98 155.5±13.5	0.644 ±0.13 3.550±0.490 152.76±6.33	0.76 ±0.140 3.37 ±0.620 108.8±8.90	1.090±0.42 9.070±1.35 111.6±1.10

* Significant at $p \leq 0.05$

** Highly significant at $p \leq 0.01$

tissues. DNA values in treated rats appeared to be twice as much as those in their respective untreated rats. Regarding RNA values presented in the same Table, Imidacloprid caused highly significant decrease in RNA content in Kidney and spleen tissues when used at 0.1 LD_{50}. However, highly significant increase appeared in heart tissue. On the contrary these were significant decrease in RNA content in the rest of tested tissues. Total protein quantity in all tested tissues exposed to 0.1 LD_{50} appeared to be at the same low value of 36.9, 37.18, 38.19 and 38.59 mg/g tissue in Kidney, liver, brain and heart tissues, respectively. They all showed highly significant decrease despite higher value in spleen tissue (104.4 mg/g tissue). Higher concentration of 0.25 LD_{50} showed the same deleterious effect on protein biosynthesis hence was scoring lower level than that in control. This observation is in good agreement with Eisa and Eissa, (1992), Eissa and El – Sheikh, (1993), El Sheikh *et al,* (1993) and Chris *et al,* (1997). Lower level of RNA in treated tissues could be due to either DNA damage or toxic effects of the Imidacloprid exerted on transcripitonal enzymes. In fact, it has been reported before by Eisa and Eissa (1992) that Imidacloprid cause damage to genetic material to non-target, worm blooded animals.

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الملخص العربى

التأثيرات السامة و الوراثية لبعض مبيدات الآفات على الفأر الألبينو

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تتواجد بقايا مبيدات الآفات فى غذاء الحيوان والإنسان سواء كان عبر سلسلة الغذاء أو بطرق أخرى . ولقد ثبت تأثيراتها الضارة على صحة الإنسان والحيوان . تم اختبار ثلاثة مبيدات من ثلاثة مجموعات مختلفة – كوماتترالين (هيدروكسيد الكومارين) ، اميداكلوبراید (نيتروميثلين) و الإفيرمكتين عبارة عن أحد المشابهات للأبامكتين وهو في الأصل مركب طبيعي (ماكروسيكليك) منتج بواسطة الإستربتوميسيس . أستخدم الفأر الأبيض لدراسة التأثيرات الضارة على الأحماض النووية DNA , RNA وكمية البروتين الكلى في عدة أعضاء مختلفة هى المخ – القلب – الكبد – الكلى والطحال. وكانت النتائج كما يلى : سبب مبيد الكوماتترالين زيادة فى كمية الحمض النووي ديوكسى ريبوز فى جميع الأنسجة المختبرة فيما عدا الطحال . أدى استخدام ٢٥ر٠ الجرعة النصف مميتة إلى إحداث نقصا فى أنسجة القلب والمخ والطحال. مما يجدر الإشارة إليه توجد علاقة طردية بين كمية الحمض النووي الديوكسى ريبوز والحمض النووى الريبوزى خاصة عند استخدام ٢٥ر٠ الجرعة النصف مميتة سبب المبيد كوماتترالين ضرر للمادة الوراثية فى الفئران المعاملة به والتى أدت إلى قيم عالية المعنوية فى محتوى البروتين الكلى عند التركيز ين المستخدمين (١ر٠ ،٢٥ر٠) الجرعة النصف مميتة فى معظم الأنسجة.
ظهرت زيادة معنوية فى محتوى ال DNA فى جميع الأعضاء المختبرة ، وكانت أعلاها فى الطحال وذلك عند استخدام ١ر٠ أو ٢٥ر٠ الجرعة النصف مميتة من الإميداكلوبراید كما أن أنسجة القلب أظهرت زيادة عالية المعنوية. أدت المعاملة بمبيد الإميداكلوبراید إلى انخفاض شديد فى محتوى RNA فى جميع الأعضاء المدروسة خاصة فى الكبد والطحال . وكذا أدى إلى انخفاض كبير فى كمية البروتين الكلى. أدى استخدام المبيد إفرمكتين إلى زيادة معنوية جدا فى محتوى الحمض النووى الديوكسى ريبوز بينما الحمض النووي الريبوزى سجل زيادة فى نسيج القلب وانخفاض فى نسيج أنسجة الكلى و الطحال بعد استخدام ١ر٠ الجرعة النصف مميتة. أيضا أدى استخدام هذا المبيد بتركيز ٢٥٠ر ٠ الجرعة النصف المميتة إلى انخفاض معنوي فى قيمة الحمض النووي الريبوزى فيما عدا أنسجة الكبد التى سجلت زيادة معنوية جدا . ظهر انخفاض معنوي جدا فى كمية البروتين الكلى فى جميع الأعضاء المختبرة عند التركيز ين المستخدمين للافرميكتين فيما عدا أنسجة الطحال بعد معاملة الفأر ب ٢٥ر٠ الجرعة النصف مميتة.