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GENOTOXICITY OF SYSTEMIC PESTICIDE CONFIDOR IN MALE ALBINO MICE : A- BLOOD SERUM ENZYMES AS AN INDEX

BY

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ABSTRACT

The genotoxic effect of systemic pesticide confidor was studied in male albino mice on gene expression (as key enzymes) , such as alkaline phosphatase , acetyl cholinesterase , glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT) , and total protein in blood serum.

Confidor was given orally either as a single dose (1/4 LD_{50}), sacrificing the animals 24, 48, and 69 hours post treatment, or as repeated dose using either 1/4 LD_{50} dose for four consecutive days or 1/10 LD_{50} for ten days. The calculated LD_{50} was 210 mg/kg body weight.

Serum albumin showed a gradual significant increase in quantity over 96 hr being higher in single dose treatment. Whereas globulin showed the opposite response. Confidor imposes a significant inhibitory effect on total protein biosynthesis , showing sever action at repeated doses as the time passed on. Aminotransferases enzymes (GPT and GOT) experienced biosynthesis inhibition under both scheme of treatment. Although there was a leveling up in GPT synthesis under repeated doses at 48 hr post treatment. In general,cholinesterase and alkaline phosphatases

Tested animals:

Male albino mice (*Mus musculus*),weighing 20-25 g,were obtained from the breeding unit of the Pesticide Dept., Fac. of Agric., Menofyia Univ. Animals were maintained on ad. libitum balanced diet and water.

Experimental design:

As LD_{50} dose would be high to study the genotoxic effect, we decided to use one-fourth of the LD_{50} (as single and repeated doses) and 1/10 LD_{50} as repeated dosage.

a) **1/4 LD_{50} single dose:**

Animals were randomly divided into four groups, each of 12 mice. Three of them had 1/4 LD_{50} of confidor , and the fourth received water only for control. Mice were decapitated by cervical dislocation at 14 , 48, and 96 hrs post treatment and blood serum was collected for enzyme analysis.

b) **Repeated dose treatment:**

In an attempt to clarify the confidor comulative effect , animals of this experiment were divided into four groups. Two of them were treated once a day with 1/4 LD_{50} for 1,2 or 1,2, 3 and 4 days. The third group was treated with 1/10 LD_{50} (10 doses within 20 days). The fourth group whose animals received water only in the same way was used as a control. The animals were sacrificed at 24 hrs after the respective last dose of each group.

Enzymes assays:

In order to estimate confidor genotoxicity, the activity of the following enzymes was determined : aminotransferases (GPT and GOT) using Boehringer Mannheim kits according to Reitman and Frankel(1977),alkaline phosphatase according to Kind and King (1954), cholinesterase activity was assayed according to method of Ellman(1961).

showed a highly significant toxicity appeared in gradual lower activity as the time passed on after treatment with confidor.It is evident from these results the genotoxicity of confidor on male albino mice.

INTRODUCTION

Screening of pesticides for their mutagenic potential is gaining most importance. As the presence of chemical-induced mutations occurring from environmental exposure to various pesticides. Moreover , use of pesticides in pest control seems to be indespensable to attain high agricultural productivity. Therefore, there is a growing concern about pesticides being harmful to mammals and a possible source of environmental pollution. Genotoxic effects are considered among the most serious side effects. These effects include heritable genetic deseases, carcinogenicity , and birth defects (Sherif *et al.*, 1990).

As long as human exposure to significant levels of mutagenic agents is not prevented, we have to develop our concern over genotoxicity through an awareness of the serious consequences likely to occur 100 years or more from today (Brusick, 1980).

It is often assumed that cells with enhanced ability to survive high stress (pesticides) is largely the result of altered gene expression. Therefore, expressional changes would seem to be finding a correlation between the levels of different gene product,i.e. mRNA and/or protein and the degree of survival (Singh *et al.*,1985).

In this research we intended to clarify the confidor (a new group of systemic pesticide) genotoxic effect on genes expression leading to

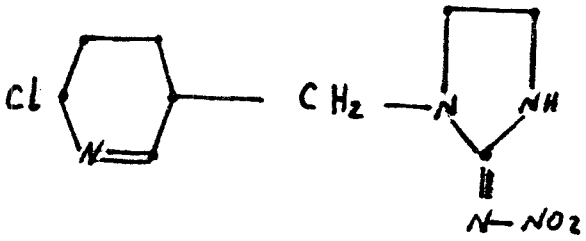
altering their levels in male albino mice as presentative to mammals.

This analysis will include, alkaline phosphatase, cholinesterase, glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT). and total protein in blood serum.

MATERIALS AND METHODS

The tested compound:

Confidor , belongs to a raw chemical group a nitromethylene analogue insecticides



Imidalloprid
(common name)

Confidor as reported by the manufacturer has a broad spectrum of activity as an acute contact and stomach poison. In addition,it has an excellent systemic properties and residual activity.

Probit mortality lines:

A series of pesticide concentrations were prepared in water and asministered to male albino mice orally using a stomach tube. Eight doses ranged from 50 to 500 mg / kg body weight were prepared, and each was replicated eight times. Eight mice were ttreated with water and used as a control. The LD_{50} value was detected after probit line was drawn and found to be 210 mg/kg B.W. (Fig.1). The data were analyzed statistically according to Finney (1971).

In addition to the above enzymes, total proteins were determined by biuret method (Alexander *et al.*,1985). Albumin content was determined using Boehringer Mannheim kits and globulin was quantified by subtracing albumin content from total proteins.

All data were analyzed statistically according to Snedecor (1962) and Duncan (1955).

RESULTS AND DISCUSSION

Confidor is a systemic pesticide recommended to be used to control various agricultural pests. As several studies pointed out pesticides damage to genetic material, of non targeting especially worm blooded animals (Abd El-Baset and El-Nahas, 1985 and Eisa and Eissa, 1992). Genetic burden for the genes available in hepatic cells that are responsible for protein biosynthesis were high lights. Obtained data for key enzymes quantity of male blood serum as a response to oral single dose of 1/4 LD_{50} of confidor (210 mg/kg B.W.) through 96 hrs are presented in Table (1).

Data clearly demonstrated the genotoxicity of pesticide confidor on genetic material expression.Total protein showed a sever inhibition in biosynthesis to give rise to 7.080 mg after 96 hrs post treatment with 1/4 LD_{50}. Whereas , untreated animals showed 0.380 mg. This might be due to damaging effect on protein biosynthesis machinery and / or inhibition to mRNA transcription.

Globulin content in blood serum showed a gradual decrease over 96 hrs period, whereas albumin quantity seems to get elevated after 24 hrs post treatment and there was no much change as the time passed till 96 hrs post treatment.

Table (1) : Genotoxic effect induced with single dose (1/4 LD_{50} value) of confidor on the blood serum enzymes and proteins of male albino mice.

Parameter	Control	Hours post-treatment		
		24 hrs	48 hrs	96 hrs
Albumin (A_1)	3.638^{b} ±0.103	4.788^{a} ±0.150	4.810^{a} ±0.238	4.310^{a} ±0.184
Globulins (G_1)	5.742	3.962	2.390	2.770
A / G Ratio	0.634^{b}	1.209^{ab}	2.013^{a}	1.556^{a}
Total protein[1]	9.380^{a} ±0.438	8.750^{a} ±0.352	7.200^{b} ±0.216	7.080^{b} ±0.205
Alkaline phosphatase[2]	10.139^{ab} ±0.942	9.196^{ab} ±1.087	12.853^{a} ±1.024	5.429^{b} ±1.893
Cholinesterase[2]	2166.750^{a} ±384.109	1114.25^{b} ±86.946	905.750^{b} ±63.928	977.000^{b} ±22.517
GOT[2]	57.250^{a} ±7.330	53.75^{a} ±10.610	26.000^{b} ±1.732	30.630^{b} ±3.462
GPT[2]	37.375^{a} ±1.179	34.500^{b} ±1.060	29.813^{c} ±0.954	32.150^{bc} ±1.231

1 : A_1 G_1 and total proteins are presented as g/100 ml serum

2 : GOT , GPT , Alk. phosphatase and Ch. E. activity are expressed U/l.

Values representing means ± S.D.. Means followed by the same letter(s) do not differ ($P < 0.01$). Duncan (1955).

Table (2) : Genotoxic effect of repeated doses (1/4 LD_{50} value) of confidor on the blood serum enzymes and proteins of male albino mice.

Parameter	Control	Hours post-treatment		
		24 hrs	48 hrs	96 hrs
Albumin (A_1)	3.638^{b} ±0.103	4.788 ±0.150	4.140 ±0.023	4.237 ±0.211
Globulins (G_1)	5.742	3.962	2.160	2.363
A / G Ratio	0.634^{b}	1.209^{ab}	1.310	1.793^{a}
Total protein[1]	9.380^{a} ±0.438	8.750^{a} ±0.352	7.300^{b} ±0.100	7.600^{b} ±0.175
Alkaline phosphatase[2]	10.138^{a} ±0.942	9.196^{ab} ±1.087	4.487^{b} ±0.942	5.478^{ab} ±1.122
Cholinesterase[2]	2166.750^{a} ±384.109	1114.25^{b} ±86.946	1017.000^{b} ±95.395	1290.750^{b} ±39.250
GOT[2]	57.250^{a} ±7.330	53.75^{a} ±10.610	31.625^{b} ±3.793	28.000^{b} ±2.380
GPT[2]	37.375^{a} ±1.179	34.560^{b} ±1.060	37.500^{c} ±0.500	29.750^{b} ±0.330

1 : A_1,G_1 and total proteins are presented as g/100 ml serum

2 : GOT , GPT , Alk. phosphatase and Ch. E. activity are expressed U/l.

Values representing means ± S.D., Means followed by the same letter(s) do not differ ($P < 0.01$), Duncan (1955).

Regarding A / G ratio , it showed the highly significant increase at all tested time intervals , being the highest at 48 hrs. The inhibitory effect of pesticides , in common , for protein biosynthesis have been decommented by various investigators (Eisa and Bayomy, 1992 ; El-Sebae, 1981 and Eisa and Eissa, 1992).

It is known that transaminases are important enzymes in all biological processes as they found mainly in the liver and their level in blood serum is a good indicator for diseased and damaged liver (Wilkinson,1970 and Garb,1971). Confidor single dose treatment caused a gradual and significant decrease in both GOT and GPT levels up to 48 hrs post treatment. Determination of both enzymes level at 96 hrs showed a significant decrease comparing with that of the control. This finding is in group accordance with Eisa and Bayomy (1992) using cyanophos pesticide in albino mice.

For the principal role of alkaline phosphatases in cellular proliferation and diferentation (Karasti,1975) and key role in regulation of gene transcription (Hwang *et al.*, 1976) we determined its levels in confidor treated mice blood serum.

Alkaline phosphatase enzyme showed a hesitated level as a response of the confidor treatment. An elevation in its activity was noticed after 48 hrs post treatment.On the other hand,at 96 hrs post treatment it decreased significantly to be 5.429 U / l comparing with its level of 12.853 U / l at 48 hrs post treatment.

Concerning the activity of cholinesterase enzyme after confidor treatment, the results clear that its activity decreased significantly after 24 hrs and till 96 hrs post treatment comparing with the control.

Table (3): Genotoxic effect induced with repeated doses (1/10 LD_{50} value) on the blood serum enzymes and proteins of male albino mice.

Parameter	Control	Treated with 1/10 LD_{50} for 10 doses
Albumin (A_1)	3.638[b] ±0.103	4.643[a] ±0.082
Globulins (G_1)	5.742	2.757
A / G Ratio	0.634	1.684
Total protein[1]	9.380[a] ±0.438	7.400[b] ±0.182
Alkaline phosphatase[2]	10.138[a] ±0.942	3.545[b] ±0.07
Cholinesterase[2]	2166.750 ±384.109	1876.75 ±193.700
GOT[2]	57.250 ±7.33	38.75 ±3.427
GPT[2]	37.375 ±1.179	35.75 ±0.87

1 : A_1 ; G_1 and total proteins are presented as g/100 ml serum.
2 : GOT, GPT, Alk. phosphatase and Ch. E. activity are expressed U/l.

Values representing means ±S.D., Means followed by the same letter do not differ ($P < 0.01$), Duncan (1955).

Table(2) illustrates the genotoxic effect of confidor repeated doses as 1/4 LD_{50} on genetic material expression as effective enzymes shown in the table.

Total protein showed a gradual and significant decrease in quantity reaching its lowest level after 96 hrs. The same trend of response was obvious for globulin. The opposite picture was noticed with albumin in blood treated male mice.

Transaminases ; (GOT and GPT) both of them showed an opposite response to confidor repeated dosage. Whereas GOT showed a gradual decrease in enzyme activity, GPT showed almost the same control value after 48 hrs post treatment. This may be due to building up resistancy to the pesticides (Beeman and Schmidt, 1982).

Determination of alkaline phosphatase in blood serum of male albino mice treated repeatedly with 1/4 LD_{50} dose of confidor revealed the occurence of highly significant inhibition after 48 hrs. This finding agrees with what reported by Eisa and Bayomy (1992) and contradicted with El-Elaimy *et al.*(1988).

This inhibitory effect reflects teratogenic action of confidor (Data not shown). Needless to say that repeated doses of this pesticide potentiates its genotoxic effect.

Cholinesterase enzyme activity showed a gradual significant inhibitory effect due to repeated confidor treatment by 1/4 LD_{50}(Table 2).

As there was no apparent damage to male albino mice treated with either single or repeated 1/4 LD_{50}, we intended to use 1/10 LD_{50} to acertain its genotoxic effect. This was mainly done by determining total protein and key enzymes presented in Table (3).

Data clearly showed higher albumin in treated blood serum than control (4.643 and 3.638 mg / 100 ml , respectively). On the contrary was the globulin which showed less value in treated mice, which reflects the inhibitory action of confidor either on protein biosynthesis machinery and / or mRNA transcription. Transaminase GOT showed a sever reduction due to confidor treatment , whereas GPT showed more or less slight inhibition in its activity in confidor treated males comparing to untreated (35.75 and 37.375,respectively). Both of cholinesterase and alkaline phosphatase activity were less in treated animals , with a significant variance in case of the first enzyme.

These data clearly showed the confidor inhibitory effect on tested enzymes and total protein in blood serum of male albino mice. This leave us with no doubt of its genotoxic effect even at the level of lower doses.

REFERENCES

Abd El-Baset,Soheir and El-Nahas,Soheir (1985). Effect of zomepirac sodium after acute and subacute treatments on male laboratory rats : Effects on nucleic acids. Egypt. J. Genet. Cytol. 14: 21-26.

Alexander, R.R.; J.M. Griffiths and M.L. Wikison (1985). Basic Biochemical Methods. John Wiley and Sons, New York.

Badr, E.A. ; Mousa, M. and Seehy, M.A. (1983). Cytological and biochemical alterations induced by two herbicides in the root tips of *Vicia faba*. Egypt. J. Genet. Cytol. 12: 123-136.

Beeman, R.W. and Barbara A. Schmidt (1982). Biochemical and genetic aspects of malathion specific resistance in the Indian meal moth (Lepidoptera : Pyralidae). J. Econ. Entomol. 75: 945-949.

Brusick, D.(1980).Principles of genetic toxicology. Plenum Publishing Corporation , New York, N.Y. 10011.

Cacheiro, N.L. ; L.B. Russell and N.S. Swartout (1974). Translocations , the predominant cause of total in some of mice treated with mutagens. Genetics 75: 73-91.

Duncan, O.B. (1955). Multiple range and multiple F-tests. Biometrics 11: 1-442.

Eisa, Amal and Eissa, Ragaa (1992). Genotoxic effects of cyanophos treatment on albino mice *Mus musculus* L. parents and their progenics. J. Egypt. Soc. Toxicol. 9: 17-21.

Eisa, A. Amal and M.F. Bayomy (1992). Changes in blood chemistry of albino mice due to induced intoxication with the organophosphorus pesticide cyanophos.J. Pest Control Environ. Sci. 4: 135-158.

El-Elaimy, I. ; I.L. Sharkawi and M.F. Bayomy (1988).Intoxication potentialities of oral and dermal phosphatase , alkaline phosphatase and 5-nucleotidase activities. 13th Int. Cong. Demog. Res.:149-178.

Ellman, G.I. ; K.D. Courteny ; V. Andres JR. and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Bioche. Pharmcol, 7: 88-95.

El-Sebae, A.H. (1981). The effect of pesticides on protein metabolism in some mammalian tissues. M. Sc. Thesis , High Institute of Public Health, Alex. Univ.

Fayed, A.H. ; A.S. Mandour; R.M. Sherif and S.H. Shawky (1989). Genotoxic effects of the Amitraz and Deltamethrin (RUP-987) insecticides on root-tip cells of *Vicia faba*. Egypt. J. Genet. Cytol. 18: 107-119.

Finney, D.J. (1971). Probit Analysis , 3rd ed. Cambridge Univ. Press, Cambridge, England, 333 pp.

Garb, S. (1971). Laboratory tests in common use. Springer Publ. Co. Inc., New York.

Hwang, K.P. and T.A. Cough (1976). IARC Sci. Publi. 20: 309.

Karaskis, S. (1975). Cell proliferation and subcellular localization of alkaline phosphatase activity in rat liver parenchajna during 920 carcinogensis.Cancer Res.35:482.

Kind, P.R.N. and E.J. King (1954). Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. J. Clin. Pathol. 7: 322.

Moustafa, Y. (1987). Induction of chromosomal aberration and sister chromatid exchanges in human lymphocytes *in vitro* with pyrethroid insecticides. Egypt. J. Genet. Cytol. 16: 391-400.

Reitman, S. and Frankel,S. (1957). A colormetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28: 56-63.

Sherif, R.M.; Fayed, A.H.; Mandour,A.S. and A.A. El-Fishawi (1990). Mutagenic and teratogenic activity of amitraz and deltamethrin on albino rats (*Rattus narvegicus*). Egypt. J. Appl. Sci. 5: 81-93.

Singh, N.K. ; A.K. Handa; P.M. Hasegawa and R.A. Bressoan (1985). Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol. 79: 126-137.

Snedecor, G.W. (1962). Statistical methods applied to experiments in agriculture and biology. The Iowa State Univ.,Press, Ames. Iowa, U.S.A., p.1523.

Westlake, G.E. ; J.B. Peter ; A.D. Martin ; P.I. Stanley and Linda,C.S.(1981 a).Organophosphorus poisoning effects of selected organophosphorus pesticides plasma enzymes and brain esterases of Japanese quill. J. Agric. Food Chem. 29: 772-997.

---------- (1981 b). Carbamate poisoning effects of selected carbamate pesticides plasma enzymes and brain esterases of Japanese quill. J. Agric. Food Chem. 29: 772-785.

Wilkinson, J.H. (1970). Clinical significance of enzyme activity measurements. Clin. Chem. 16: 882-891.

الملخص العربي

التاثير السام للمبيد الجهازى كونفيدور على المادة الوراثية لذكور الفئران البيضاء

١- تحليل بعض الانزيمات الهامة فى الدم كمؤشر

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تم دراسة التاثير السام للمبيد الجهازى الجديد كونفيدور على التعبير الجينى لبعض الجينات المتخصصة فى التعبير عن انزيمات الالكالين فوسفات و الاستيل كولين أستر ، ترابرامين (جلوتاموليبروفات)جلوتاميك اوكسال استيل و البروتين الكلى فى دم ذكور الفئران البيضاء.

أعطى المبيد عن طريق الفم بجرعتين مختلفتين (ربع ، عشر التركيز المطلوب لقتل ٥٠% من الفئران) ، كذلك كجرعة واحدة او جرعة مكررة لمدة ٤ أيام او ١٠ أيام.

تم أخذ العينات بعد ٢٤ ساعة من اعطاء الجرعة سواء كانت جرعة واحدة اومكررة. اتضح ان الالبومين يزيد تدريجيا زيادة معنوية تحت اى معاملة ولكنها اعلى فى الجرعة الواحدة و الجلوبيولين أظهر عكس هذه النتيجة.

اظهرت كمية البروتين مدى التاثير الضار للمبيد على المادة الوراثية حيث انخفضت كميته معنويا خاصة عند تكرار الجرعة.

انزيمات نقل مجموعة الامين كانت اكثر حساسية للتثبيط بالمبيد باى جرعة (ربع وعشر التركيز المميت النصفى).

انزيمات الكولين استراز و الالكالين فوسفاتيز ايضا ظهر التاثير العام للمبيد خفض كمية انزيمات الكولين استراز و الالكالين فوسفاتيز. مما يرجح تاثيره الضار اما على النسخ لتكون RNA الرسول أو التخليق الحيوى للبروتين أو كلاهما معا.