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TOXICOLOGICAL AND HISTOPATHOLOGICAL EFFECTS OF CARBARYL ON LABORATORY RATS

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By
A.A.I. El-Kashoury

Central Agricultural Pesticides Laboratory, Agriculture Research Center, Dokki-Giza, Egypt.

ABSTRACT

A stressogenic effect of the carbamate insecticide carbaryl after administration of single oral dose 250 mg/kg b.w. to rats was followed on selected biochemical indices (plasma corticosterone and glucose levels). Also, the effects on some enzymatic activities and blood constituents were performed. A significant increase of corticosterone level associated with a significant increase in glucose level after 1, 3 and 6 hr. was observed, while, animals resumed normal values after 24 hr. The compound inhibited plasma acetylcholinesterase "AchE" significantly after 1 hr., then animals showed recovery. Fluctuated effects between decrease and increase in alanine aminotransferase "ALT" activity and total protein contents were evident. Plasma aspartate aminotransferase "AST", alkaline phosphatase "ALP" activities, urea and albumin levels were not affected at any time 1, 3, 6 and 24 hr. of the experiment. Creatinine level showed significant decrease at 1, 3 hr. after dosing with carbaryl. Histopathological lesions were not observed in acutely carbaryl exposed rats (except some changes in the liver). The laboratory data obtained showed tissue injuries after dosing, since the results are considered early indicators of adrenal lesions and primarily hepatic and muscle tissues damage.

Key words : *carbaryl, corticosterone, rats, transaminases.*

1. INTRODUCTION

Carbamate pesticides are used on crops as systemic and contact pesticides, they are specially effective in the control of softbody insects. They, are also, used in household insect sprays (Lyaniwura, 1991). Agricultural workers are often exposed to toxic levels of pesticides during applications (Hayes, 1982). Carbamate pesticides act as reversible acetylcholinesterase "AchE" inhibitors. The inhibition of AchE causes accumulation of acetylcholine at nerve endings, resulting in a cholinergic or hypersecretory syndrome (Fuortes *et al.*, 1993). The effect of carbaryl as a stressor agent and on biochemical parameters were reported by many investigators; Ray and Podder (1983) stated that acute oral administration of carbaryl to rats at doses ranging from 50 to about 500 mg/kg increased corticosterone and glucose levels. Also, Kiran *et al.*, (1985) reported that a single oral 500 mg/kg dose of carbaryl or seven oral doses of 71 mg/kg/day increased the activity of alanine aminotrasnferase "ALT", but did not affect the activity of alkaline phosphatase "ALP". In addition, administration of rams with carbaryl increased creatinine level after 48 hr. (Mount and Oehme, 1981).

This study aimed to show the effects of acute carbaryl exposure on rats using clinical, biochemical and histopathological parameters as indicators of toxicity.

2. MATERIALS AND METHODS

2.1 Experimental animals

Adult male albino rats (weighing 190±10 g, each) were obtained from the farm of General Organization of Serum and Vaccine (Helwan Farm). Starting 15 days before the beginning of the experiment, the rats were housed in groups in plastic cages, kept in room under natural conditions of illumination and temperature (22±3°C). Food and water were provided "*at libitum*".

2.2 Experimental pesticide

The carbamate insecticide "carbaryl" : 1-naphthyl methylcarbamate (85 % W.P.), was used in this investigation. The calculated LD_{50} of carbaryl (administered to rats per os.) was 339.09 mg/kg b.w., according to Weil's method (Weil, 1952).

2.3 Experimental design

Rats were divided at random into 2 groups : control (6 animals) and experimental group (10 animals). After an overnight fast, carbaryl was given in a single oral dose 250 mg/kg (suspended in water) by stomach tube. This dose represents 3/4*th* LD_{50}. The control group received a similar volume of distilled water. The signs of acute carbaryl intoxication were then observed and recorded for 24 hr. Blood samples were collected under ether anesthesia from orbital sinus vein from each of six of the surviving rats by heparinized capillary tubes at 1, 3, 6 and 24 hr. after dosing into clean, dry, and labeled eppendorf tubes (1.5 ml). The tubes contained heparin as anticoagulant (7.5 I.U./ml blood) according to Schalm (1986).

Samples were centrifugated at 3500 rpm for 15 min, in a refrigerated centrifuge to separate plasma. Separated plasma was kept in a deep freeze at (-40°C) for selected biochemical analysis by using commercial reagent kits; corticosterone hormone in plasma was determined by radioimmunoassay (Gwosdow-Cohen *et al.*, 1982), using kits purchased from [Diagnostic Products Corporation (DPC), Los Angles, CA, USA]. Biochemical analyses were measured colourimetrically; aspartate transaminase "AST", alanine transaminase "ALT" (Reitman and Frankel, 1957); alkaline phosphatase "ALP "(Kaplan and Righetti, 1955), acetylcholinesterase "ChE" activities (Ellman *et al.*, 1961), glucose (Darham and Trinder, 1972), total protein (Weichselbaum, 1946), albumin (Dumas *et al.*, 1971), urea (Fawcett and Scott, 1960), and creatinine levels (Siest *et al.*, 1985). At the end of 24 hr. (after blood sample collection) the treated rats were sacrificed and submitted to necropsy to determine the macroscopic lesions. Brain, liver, kidney, and lung

portions were fixed in 10 % formalin saline and submitted to histopathological examination. Routine histopathological procedures were carried out according to Harris (1898).

2.4 Statistical analysis

Significance of difference was determined by the Student "t-test". P values of 0.05 or less were considered significant (Snedecor and Cochran, 1967).

3. RESULTS AND DISCUSSION

3.1 Biochemical effects

The effect of single acute oral dose of 250 mg/kg carbaryl on plasma enzyme activities (ChE, AST, ALT and ALP) and constituent level (corticosterone, glucose, total protein, albumin, urea and creatinine) are shown in Tables (1 and 2). Plasma cholinesterase activity showed an inhibition after 1 hr. But after 6 and 24 hr. of treatment, animals showed recovery followed by hyperactivity in ChE enzyme in comparison to control group. This may be due to compensation where more synthesis of ChE in the liver occurred. This suggestion is supported by the data previously reported by Cooper *et al.* (1978) and Shaker *et al.* (1988) who stated that in the recovery period, there was a fast increase in rat esterase accompanied by hyperactivity, which may probably be due to the fast reuptake of ChE to the synaptic cleft or increase in ChE synthesis. This dosing also increased corticosterone level ($0.05 > P < 0.01$) after 1, 3 and 6 hr. and glucose level, hyperglycaemia ($0.01 > P < 0.001$) but only after 1 and 6 hr. These values resumed normal levels after 24 hr. Our results agree with Ray and Podder (1983) who mentioned that acute oral administration of carbaryl to rats at doses ranging from 50 to about 500 mg/kg induced an elevation in corticosterone and glucose levels. It is established that elevation of plasma corticoid levels might be also a consequence of its increased synthesis by adrenal cortex and/or by alternations in its metabolic clearance rate (Osicka- Koprowska *et al.*, 1984). The pesticide induced hyperg-

Table (1) : Effect of carbaryl at 250 mg/kg on Cholinesterase (ChE), Corticosterone, Glucose, Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) in male albino rats.

Parameter	Time (hours)							
	1 hour		3 hours		6 hours		24 hours	
	Control	Treat-ment	Control	Treat-ment	Control	Treat-ment	Control	Treat-ment
ChE (U/L)	469.9 ±31.18	345.72 ±31.12 *	443.8 ±26.56	503.8 ±29.15	496.10 ±27.17	636.0 ±30.71 **	441.05 ±36.73	548.96 ±31.05 *
Corticoste-rone (ng/ml)	299.037 ±6.469	591.981 ±74.041 **	297.921 ±46.746	578.781 ±83.018 *	337.481 ±11.755	525.024 ±46.703 **	328.54 ±14.48	343.097 ±17.431
Glucose (mg/dL)	127.153 ±9.28	198.543 ±12.540 ***	131.44 ±4.48	136.23 ±16.86	129.7 ±9.31	171.233 ±13.110 **	139.20 ±11.00	160.16 ±12.577
AST (U/L)	198.17 ±9.325	184.70 ±8.285	202.723 ±11.339	183.79 ±12.906	212.192 ±13.00	192.005 ±12.569	211.405 ±22.305	249.946 ±19.730
ALT (U/L)	48.9 ±2.093	37.029 ±5.556	34.035 ±0.949	25.554 ±1.699 **	38.959 ±1.199	38.652 ±3.044	45.359 ±2.219	78.227 ±8.936 **
ALP (IU/L)	150.0 ±14.40	110.95 ±12.71	125.0 ±18.03	113.36 ±6.93	147.47 ±14.33	126.51 ±12.42	140.54 ±8.67	156.39 ±22.23

Values represent the mean ± S.E. (n = 6)
* P < 0.05 ** P < 0.01 *** P < 0.001 (Student's "t" test)

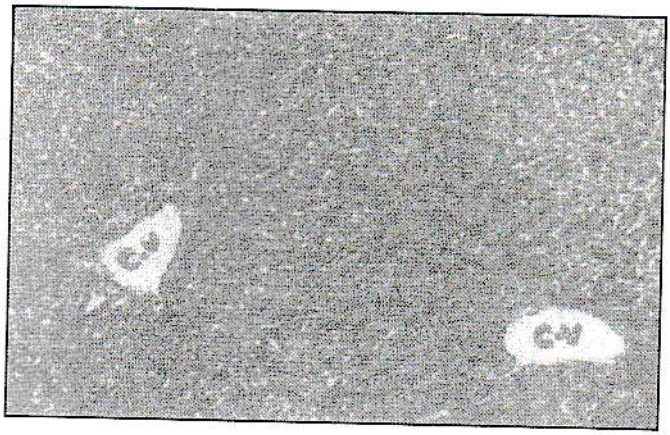
Table (2) : Effect of carbaryl at 250 mg/kg on total protein, albumin, urea and creatinine in male albino rats.

Parameter	Time (hours)							
	1 hour		3 hours		6 hours		24 hours	
	Control	Treat-ment	Control	Treat-ment	Control	Treat-ment	Control	Treat-ment
Total protein (g/dL)	7.45 ±0.41	6.27 ±0.33 *	6.63 ±0.50	7.21 ±0.39	8.08 ±0.40	7.94 ±1.07	7.79 ±0.30	9.25 ±2.97 *
Albumin (g/dL)	5.15 ±0.19	5.0 ±0.14	5.04 ±0.30	6.02 ±0.46	4.95 ±0.17	5.26 ±0.22	5.72 ±1.22	5.63 ±0.11
Urea (mg/dL)	52.83 ±1.41	60.17 ±6.00	57.08 ±2.22	56.48 ±3.97	58.110 ±2.27	63.77 ±4.69	49.21 ±3.47	48.34 ±0.19
Creatinine (mg/dL)	2.343 ±0.217	1.25 ±0.125 **	1.26 ±0.027	0.781 ±0.174 *	1.330 ±0.203	1.312 ±0.131	1.25 ±0.113	1.53 ±0.123

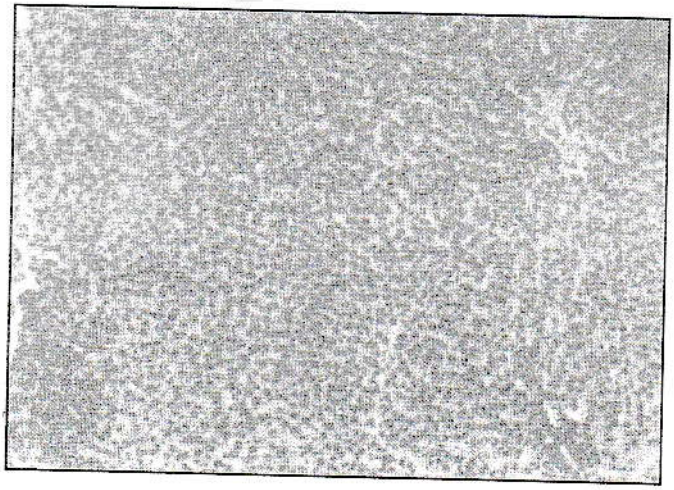
Values represent the mean ± S.E. (n = 6)
* P < 0.05 ** P < 0.01 *** P < 0.001 (Student's "t" test)

lycaemia which seems to be due to simultaneous stimulation of glycogen phosphorylase and inhibition of glycogen synthesis (Teichert-Kuliszewska and Szymezyk, 1979).

From Tables (1 and 2), it is clear that no appreciable changes in plasma AST, ALP activity, albumin and urea levels were reported. While, ALT activity and total protein content exhibited fluctuating effect between decrease and increase. Results of the present work agree with those obtained by Kiran *et al.* (1985), who noted that a single oral 500 mg/kg dose of carbaryl or seven oral doses of 71 mg/kg/day increased the activity of ALT, but did not affect the activity of ALP. Freedland and Kramer (1970) suggested that enzyme levels are sensitive indicators of tissue damage, since they are liberated from cells even when the magnitude of lesions is not sufficient for morphological detection. This was confirmed by the results obtained in our study. A reduction in plasma transaminase (ALT) may be attributed to either the effect of pesticide metabolites which inhibited several endogenous enzymes particularly ALT, AST and ALP and/or to the increased rate of catabolism of these enzymes in plasma of treated animals (Kramer, 1989). Moreover, Hashem (1980) and Talcott *et al.*, (1979) reported that the depression in the activity of transaminases may be due to the formation of complex compounds with AST or ALT in the liver. While an elevation in ALT level is usually due to the leakage of damaged membranes (Götz, 1981). Because the total protein in plasma is used as a short-term marker to monitor nutritive state of rats, also, plasma protein alterations are not usually specific for particular disease condition (Tietz, 1987), therefore, significant decrease in total protein, observed in treated animals, may be due to the stress from application of the insecticide mediated through the adrenal corticosteroid hormones (Nassr *et al.*, 1996). In addition, a statistically significant decrease ($0.05 > P < 0.01$) in creatinine level after 1 and 3 hr. was observed. The size of the creatinine pool and thus the daily production of creatinine are affected by factors influencing muscle mass such as disease of muscle and, tissue wasting (Finco, 1989).



Fig(1): Liver of rat treated with a high dose of carbaryl (250 mg/kg) showing granular and vacular cytoplasm of the heptatocytes with dilated central vien (H,Ex40).



Fig(2): Liver of control rat showing the histological structure of heptatocytes, hepatic cords, central vien, portal area and heptic sinusoids (H,Ex40).

3.2 Pathological effects

A single oral dose of 250 mg/kg carbaryl caused salivation, severe tremors (15 min), lacrimation, congested eyes and protruded (exphthalmia). Finally paralysis in the back and the fore-legs and death occurred in 33 % treated rats. These effects disappeared within 4 hr., then the animals showed normal activity. Postmortem examination did not reveal any changes in internal organs (liver, kidneys, lungs and brain). Also, histopathological lesions were not observed in the different organs, except, the liver which showed granular and vascular degenerative changes in the cytoplasm of the hepatocytes beside dilation in central vein (Fig. 1). No abnormalities were observed in the control group (Fig. 2). It could be concluded that acute carbaryl intoxications seem to produce adrenal gland lesions as indicated by the increases in corticosterone level (hyperadrenocorticism) associated with increased glucose level (hyperglycaemia) observed here, which means that carbaryl acts as stressor agent. This suggestion coupled with Kassa (1987) who stated that the level of corticosterone in plasma is a more sensitive stress indicator. Also, it has become obvious that the stressogenic effect is demonstrable biochemically, even though clinical manifestations of poisoning are missing. Furthermore, our biochemical data suggest at least hepatic and muscle alterations from carbaryl exposure. It is worth mentioning that available literature revealed that there are no adequate investigations about the effect of pesticides as stressor agents, thus, comprehensive studies are essential to clarify the hazardous action of these pesticides that may be life threatening to man.

4. REFERENCES

Cooper J.P., Bloom F.M. and Roth R.H. (1978). The biochemical basis of neuropharmacology. 3rd Ed., Oxford Univ. Press, London, New York, pp. 73-101.

Darham B. and Trinder P. (1972). An improved colour reagent for determination of blood glucose by the oxidase system. Analyst., 97 : 142-145.

Dumas B.T.,Watson W.A. and Biggs H.G. (1971). Quantitative colorimetric determination of albumin in serum or plasma. Clin. Chem. Acta, 31 : 87.

Ellman G.L., Dlane K., Courtney V. Andres J.R. and Deatherstone R.M. (1961). A new and rapid colourimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7 : 88-95.

Fawcett J.K. and Scott J.E. (1960). Determination of urea (Urease modified Berthelot reaction). J. Clin. Pathol., 13 : 156-159.

Finco D.R. (1989). Kidney function. In : Clinical Biochemistry of Domestic Animals. 4th Ed., Jiro J. Kaneko, Academic Press, pp. 496-542.

Freedland R.A. and Kramer J.W. (1970). Use of serum enzyme as aids to diagnosis. Adv. Vet. Sci. Comp. Ned., 14 : 61-103.

Fuortes L.J., Ayebo, A.D. and Kross, B.C. (1993). Cholinesterase-inhibiting insecticide toxicity (published erratum appeared in Am Fam Physican, 1993, Sep. 1; 48 (3) : 414). Am-Fam Physican, May 15; 47 (7) : 1613-1620.

Götz W. (1981). Diagnosis of hepatic disease. G-I-Tv veriag Ernst Glebeler, Germany, pp. 12-18.

Gwosdow-Cohen A., Cohen C.L. and Bosch E.L. (1982). Radio-immunoassay (RIA) of serum corticosterone in rats. Proc. Soc. Expl. Bio. Med., 170 : 29-34.

Harris H.E. (1898). Cited by Carleton M.A.; Drury, R.A.B.; Wallington E.A. and Cameron, H. (1967). 4th Ed., Oxford Univ. Press, New York, Toronto.

Hashem A. (1980). A study of the mode of action of paraquat in isolated perfused rat liver and its influencing by means of an SOD-active Cu-complex (Cu L tyr). Thesis, Tierarztliche Fakultat Ludwig Moximilians Universitat München.

Hayes A.W. (1982). Carbamate Pesticides. In : Pesticides Studied in man. Williams and Wilkins, Baltimore, MD L pp, 436-462.

Kaplan M.M. and Righetti A. (1955). Determination of ALP activity. J. Clin. Inv., 34 : 126.

Kassa J. (1987). Stressogenic effect of Dichlorvos. Vojensky Lekarsky Vyzkumny Doskolovaci, Ustav J.E. Purkyne, Praclek, 39 (9) : 406-411.

Kiran, R., Sharma M. and Bansal R.C. (1985). *In vivo* effect of carbaryl on some enzymes of rat liver, kidney and brain. Pestcides, 19 : 42-43.

Kramer J.W. (1989). Clinical Enzymology. In : Clinical Biochemistry of Domestic Animals. 4th Ed., J.J. Kaneko, Academic Press, Inc., pp. 338-363.

Lyaniwura T.T. (1991). Relative inhibition of rat plasma and erythrocyte cholinesterase by pesticide combination. Vet. Human. Toxicol., 33 (2) : 166-168.

Mount M.E. and Oehme F.W. (1981). Diagnostic criteria for carbaryl poisoning in sheep. Contam. and Toxicol., pp. 483-495.

Nassr M.Y., Nassif M.M. and Fouad F.M. (1996). Some of the clinico-biochemical effects of organophosphorus insecticides (Phoxim) in rams. Vet. Med. J., Giza, 44 (2) : 331-338.

Osicka-Koprowska A., Lipska M. and Wysocka- Paruszewska B. (1984). Effects of chlorfenvinphos on plasma corticosterone and aldosterone levels in rats. Arch. Toxicol., 55 : 68-69.

Ray S.K. and Podder M.K. (1983). Carbaryl-induced elevation of corticosterone level and cholinergic mechanism. Biosci. Rep., 3 : 973-977.

Reitman S. and Frankel S. (1957). A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28 : 57-63.

Schalm O.W. (1986). Veterinary Haematology. 4th Ed., Lea and Febiger, Philadelphia, pp. 21-86.

Shaker N., Hassan, G.A., El-Nouty F.D., Abo-El-Ezz Z. and Abd-Allah G.A. (1988). *In vivo* chronic effect of dimethoate and deltamethrin on rabbits. J. Environ. Sci. Health, B23 (4) : 387-399.

Siest G., Henny J., Schiele F. and Young D.S. (1985). Kinetic determination of creatinine. Interpretation of Clinical Laboratory Tests (1985), pp. 220-234.

Snedecor G.W. and Cochran W.G. (1967). Statistical Methods. 6th Ed., Iowa State Univ. Press. Ames, Iowa, USA.

Talcott R.E., Shu H. and Wei E.T. (1979). Dissociation of microsomal oxygen reduction and lipid peroxidation with the electron acceptors, paraquat and manadione. Biochem. Pharmacol., 28 (5) : 665.

Teichert-Kuliszewska K. and Szymezyk T. (1979). Changes in rat carbohydrate after acute and chronic treatment with Dichlorovos. Toxicol. Appl. Pharmacol., 47 : 323-330.

Tietz N.W. (1987). Fundamentals of Clinical Chemistry, 3rd Ed., W.B. Sanders Co., pp. 584-595.

Weichselbaum T.E. (1946). Quantitative colorimetric determination of total protein in serum. Am. J. Clin. Pathol., 7 : 40.

Weil C.S. (1952). Tables for convenient calculation of medium effective dose (LD_{50} or ED_{50}) and instruction in their use. Biometrics, 8 : 249-263.

التأثيرات التوكسيكولوجية والتغيرات المرضية لمبيد الكاربارايل على فئران التجـــارب

عفاف عبد الحميد إبراهيم الكاشورى

المعمل المركزى للمبيدات، مركز البحوث الزراعية، الجيزة، مصر

ملخص

إستهدف هذا البحث دراسة تأثير مركب الكاربارايل – المســتخدم علــى نطاق واسع ضمن برنامج المكافحة فى الزراعة المصرية– كعامل مجهد لذكــور الفئران البيضاء وأيضا تأثيره على بعض المعايير البيوكيماوية. ويمكن تلخيــص النتائج كالاتى :

١– أعراض التسمم الحاد

أدت المعاملة بالجرعة الفمية الحادة ٢٥٠ ملليجرام/كيلوجرام مــن وزن الجسم إلى ظهور أعراض التسمم الحاد متمثلة فى الإســهال، القــئ، الدمــوع، وإحتقان وبروز العينين، ثم شلل فى الأرجل الخلفية أولا أعقبــه شــلل الأرجــل الأمامية فى الحيوانات التى حدثت لها الوفاة، إلا أن الفئران الحية عادت لطبيعتها بعد ٣ ساعات من المعاملة (إستشفاء). وقد أوضحت النتائج حدوث تثبيط عكسى لنشاط إنزيم الكولينستيريز "ChE" حيث إستعاد نشاطه بعد ثلاث ســاعات أعقبــه زيادة فى النشاط بالمقارنة بالكنترول.

٢– تأثير المركب كعامل مجهد

لوحظ إرتفاع مستوى هرمون الغدة فوق الكلويــة "الكورتيكوســتيرون" مصحوبا بإرتفاع مستوى السكر فى الدم بعد ١، ٣، ٦ساعة مــن المعاملــة، ثــم عادت القيم إلى معدلها الطبيعى بعد ٢٤ ساعة مقارنة بالكنترول.

٣– تأثير المركب على بعض المعايير الخاصة بوظائف الكبد والكلى

نتج عن المعاملة بالمبيد تغيرات متقلبة فى نشاط إنزيم النقــل الأمينــى ألانين أمينوترانسفيريز "ALT" والمحتوى البروتينى بالإضافة إلى إنخفاض فى

مستوى الكرياتينين فى الدم. وعلى العكس من ذلك لم يظهر المركب تغيرات واضحة فى نشاط إنزيم الفوسفاتيز القلوى "ALP" أو النقل الأمينى الأسبرتيت أمينو ترانسفيريز "AST" وكذلك مستوى كل من الألبيومين واليوريا.

٤- التغيرات المرضية

أظهر الفحص النسيجى وجود تغيرات تنكسية فى خلايا الكبد مصحوبة بتمدد فى الوريد الكبدى.
