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TOXICITY AND FATE OF THIAMETHOXAM IN ALBINO RATS *RATTUS NORVEGICUS* Bork

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ABSTRACT

The effects of thiamethoxem treatment for 28 days indicated significant increase ($P < 0.05$) in serum AST and ALT level of albino rats, exposed to 1/20, 1/30 and 1/40 of LD_{50}.On the other hand, at the same levels there was a dose-depended significant decrease in serum total protein and increasing in the createnine at groups treated with 1/20 of LD_{50}. Electrophoretic separation results revealed that presence of 14 bands in all treatment groups and there are decreases in bands volume of serum samples that treatments with 1/20 of LD_{50} in the treated groups compared with the corresponding bands in the control group. Mild pathological changes had showed in liver and kidney of rats exposed with 1/20 of LD_{50} evident that not produced any significant effects at 1/40 of LD_{50} .The concentrations of thiamethoxam residues in Liver, kidney, heart, spleen, brain, intestine, lung and muscles after four weeks from the exposure to 1/20 of LD_{50} were 15.78, 40.47, 42.18, 55.76, 35.88, 80.2, 59.21 and 13.53 mg/kg respectively. The same trend was found with1/30 and 1/40 of LD_{50}

Keywords: Rats, thiamethoxam, actara, biochemical analysis, SDS-PAGE, histopathology and residues.

INTRODUCTION

Thiamethoaxam is a relatively new class of systemic insecticides with a distinct mode of action .Newly the biological activity of neonicotinoid is ascribes to interfere with the neonecotinic acetylcholine receptors and, therefore, they exhibit specific activity against the insect nervous system. This unique mode of action makes this compound highly applicable for control the biological effect of insects in cases when these developed resistance to conventional organophosphate, carbamate and pyrethroide insecticides. They systematically control a broad spectrum of chewing and sucking pests and represent developmental insecticides that are used worldwide in a variety of agriculture crops and seed treatment (Guzsvany *et al*., 2006 and Green *et al*., 2005).

Toxicological tests during development revealed that thiomethoxam was not mutagen. But it did cause a significant increase in liver cancer in mice but not rats, in chronic dietary feeding studies (Green *et al*., 2005). Previous studies in mice have characterized a carcinogenicity mode of action that involved depletion of plasma cholesterol, cell death, both as single cell necrosis and as apoptosis , and sustained increased in cell replication rates(Meek *et al*., 2000; EPA, 2003 and Green *et al*.,2005)

The present investigation was performed to determine the hazardous effect of thiomethoxam on some clinical chemistry parameters and the residues of thiamethoxam in different organs were also investigated here.

MATERIALS AND METHODS

1-Animals

Female white rats (*Rattus norvegicus* Bork) weigh 120-140gm were obtained from the breeding animal house, Faculty of Veterinary Medicine , Zagazig University ,the rats were housed separately for 3-weeks in metal cages with free access to food (pellet ration) under normal laboratory condition the animals received a complete health ration during the experiment period.

2- Insecticides

A formulated sample of actara 25 WG under trade name of thiomethoxam,(3-{2-chloro-1,3-thiazolyl}methyl}tetra-hyro-5-methyl-N-nitro-4-h-1,3,5-oxadiazin-4-imine) was supplied by, Pesticides Laboratory Center, Dokki, Giza, Egypt

3- Animals treatment

Animals were divided into four groups of five rats each. The first groups were used as control. The second, third and fourth groups were treated with 1/20 ,1/30 and 1/40 of LD_{50} corresponding values were78.15,52.1 and 39.08 mg kg^{-1}b.w., respectively. (Thiamethoxam has an acute oral LD_{50} 1563 mg kg^{-1}b.w) for albino rats (Anonymous, 2005)**.**

All treated groups were give doses in drinking water daily for 28 days, and the dose adjusted every week according to the weight. The animals were observed daily for unusual assignments. The animal body weight was recorded at the beginning of the experiment and weekly until the end of experiment.

Biochemical analysis

Blood sampling

Sample was collected in clean centrifuge tubes and left at room temperature until clotting.After complete retraction of clot the samples were

centrifuged under cooling at 500rpm for 15 minutes and the serum was separated for enzyme and electrophoresis assay.

Effect on Blood Serum Chemistry

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957), whereas total protein and creatinine contents were determined according the methods described with Bradford (1976) and Henry (1974), respectively using spectrophotometric methods in blood serum.

Electrophoretic Assay of Serum Proteins

The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study serum protein pattern in different experimental groups. SDS-polyacrylamide gel electrophoresis was performed at room temperature in vertical apparatus as described by Laemmli (1970).

Protein fractions appeared as dark bands on a light background. Gels were photographed and analysed by using "Gel analyzer'' Documentation and Analysis System". The molecular weight of each protein fraction was determined by using molecular weight markers as standard. The raw volume was calculated by the software using band height and intensity, which was a measure of relative quantity of protein in each sample.

Histopathological studies

Specimens of liver and kidney were taken from each animal for the histopathological investigation. The specimens were fixed in a 10% formalin saline embedded in paraffin wax and sectioned at 5 microns thickness sections which stained with hematoxylin and eosin or microscopic examination (Lillie and Fullmer, 1976).

Residue analysis

Liver, kidney, heart, spleen, brain, intestine, lung and muscles (three samples in each) were extracted and cleaned up according to the method described by EPA (1998). The residues of thiamethoxam were determined using HPLC with the following condition: Dual delivery solvent system pump 40, UV detector 166, set at 255 nm. a C18 column was used and the mobile phase was acetonitril 100%. The flow rat 1ml/min. and retention time 3.1 min. Results were corrected using their respectively recovery rates

Statistical analysis

Co-Stat Windows software package was used for statistical analyses. Duncan and Tukey-Test at $P < 0.05$ assessed as statistical significance (Anonymous, 1986).

RESULTS AND DISCUSSION

Biochemical analysis

The blood parameters (Table 1) of rats exposed to thiamethoxam indicate that there were a significant increasing ($P < 0.05$) in AST, ALT activities and Creatinin content was noted in all treated groups after 28 days,the reduction in total protein was significant when female rats treated with 1/20 of LD_{50} for 28 days. In all cases, the treatment of rats with 1/20 of LD_{50}resulted high reduction in liver and kidney parameters comparing with untreated control groups .These results are in agreement with those of Shipra *et al*., (2010), who stated that liver function like AST and ALT were significantly increased in female rats orally administrated daily with the neonicotinoid imidacloprid at high dose 20 mg/kg bw for 13 weeks. The alteration in serum levels of alanine amino transferase (ALT) may be indication of internal organs damage especially in liver (Kaneko *et al*., 1997).

Serum protein Electrophoresis patterns .

Protein fractions were analyzed using computer software program "Gel Documentation and Analysis System". In control group protein fractions ranging in molecular weight from 36-345 kDa. The same as with female rats dietary exposed to thiamethoxam at 1/20,1/30 and1/40 of oral LD_{50} for 28 days(Figure 1 and Table 2). These results showed that the samples hade fourteen bands and their molecular weight ranged from 36 to345 kd, all treated groups were similar in number of separated protein bands and did not differentiate in rate of flow (R.F.) or molecular weight (M.W.) but it differentiated in its volumes. Volumes of the bands were increased at 345, 253, 120, 103, 77, 51, 44, 39kDs and decreased at 204, 169, 149, 63, 40, 36 kDs comparing with control and this was matched with serum biochemical analysis . This findings were consistent with previous study of Hendawy (1997), who studied the subchronic effect of an organophosphorus insecticide (profenofos) on albino rats, and found effect on serum protein profile. Certain chemicals and drugs can led to toxic hepatitis, this condition causes generalized destruction of liver cells and subsequent release of these enzymes into plasma (Hood, 1980; Anderson and Cockayne, 1989).

Liver and kidney histopathological examination:

The effects of thiamethoxam on liver of rats treated with 1/20 and 1/30 of LD50 are shown in Figure (2), which indicates that hepatic degenaration, necrosis, sever inflammatory cell infilteration leading to replacement of heptic tissue and also coagulative necrosis in hepatic tissue and with hemosideriosis with lenkocytic infiltration .Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzymes activities. In this respect,

Table 1. Effects of thiamethoxam treatment on the liver and kidney functions in female albino rats after 28 days of treatment

Dose (mg/kg b. wt)	Total protein	Liver function indicators		Kidney function indicators
		AST (U/L)	ALT (U/L)	Creatinin (mg/dl)
Control	10.20±0.72[a]	97±2.6 [c]	39.33±6.03[c]	0.37±0.07[c]
1/20 of LD50	8.10±1.01[b]	136.00±5.29[b]	86.00±12.12[a]	0.70±0.05[a]
1/30 of LD50	7.67±0.42[b]	118.33±7.23[b]	63.67±4.73[b]	0.66±0.06[a]
1/40 of LD50	6.53±0.50[c]	111.67±3.06[a]	50.50±9.19[bc]	0.50±0.03[b]
LSD	**1.27**	**9.12**	**15.02**	**0.09**

a Values are the mean± SE of 5 rats x 1 replicate each.

* Indicates a significant difference with the control group at the P< 0..001 by LSD test.

Table 2. Scoring sheet for the polymorphic SDS-PAGE of serum samples from female rats treated with thiamethoxam

Pand number	RF	Approx. band size in KD	Raw volumes		
			Control(A)	1/20(B)	1/30(C)
1	0.043	345	45	48	40
2	0.095	253	365	508	444
3	0.133	204	383	186	248
4	0.167	169	76	39	40
5	0.191	149	52	48	33
6	0.234	120	16	28	24
7	0.268	103	4	8	5
8	0.34	77	359	507	462
9	0.401	63	1050	708	900
10	0.486	51	717	968	900
11	0.572	44	4	5	6
12	0.644	40	215	190	154
13	0.696	39	19	44	22
14	0.878	36	1765	1181	1110

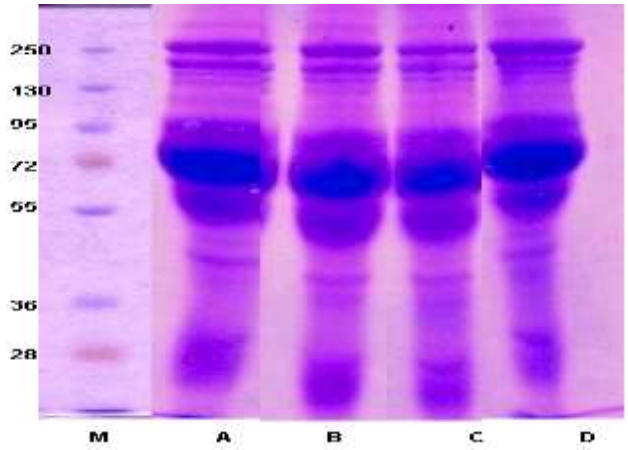

Figure 1. Serum protein profile of control (A), (B)1/20,(C) 1/30 (D) and 1/40 of LD_{50} resolved on 12% resolving Gel by SDS-PAGE. M: Protein size markers (from top to bottom): 250, 130, 95, 72, 55,36 and 28 kDa.

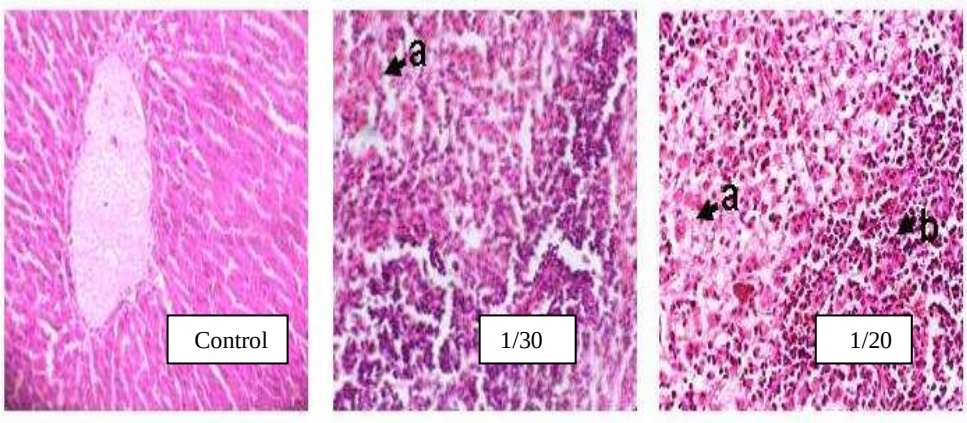

Figure 2. A photomicrograph of the liver sections of treatments with thiamethoxam (1/30 and 1/20 LD_{50}) and control rats , Hepatic degeneration a) necrosis b) sever inflammatory cell infiltration leading to replacement of hepatic tissue c).(H&E, x 100).

liver tissues, which were famous for their rich contents of aminotransferases (AST & ALT) suffer markedly from their loss under many pathological conditions (Rodwell, 1983).

Kidneys of rats administrated with 1/20 and 1/30 of LD_{50} showed cystic dilatation of renal tubules with glomerular boulation and also congested glomerular tuft, dilatation Bowmans space and coagulative necrosis in renal tubules (Figure 3). Eissa and Zidan (2009) proved that Portal tract infiltration by

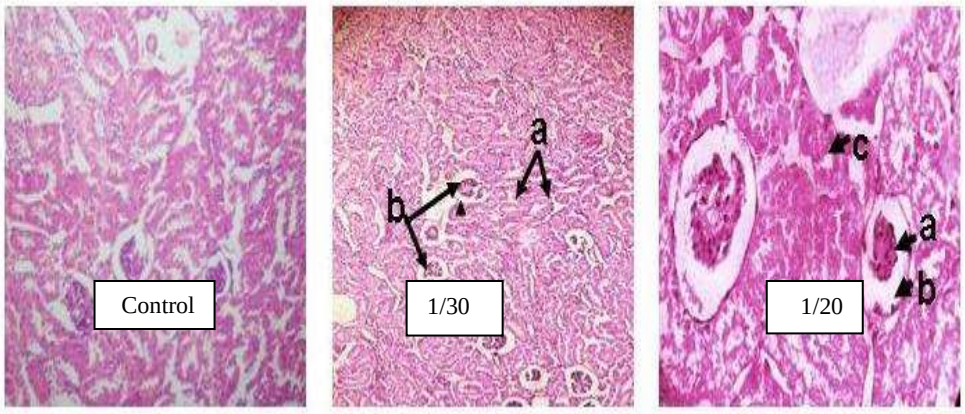

Figure -3: A photomicrograph of the kidney sections of rats treated with thiamethoxam **1/20 LD50** congested glomerular tuft, a) dilatation Bowmans space (b) degeneration (c) coagulative necrosis in renal tubules and1/30LD50 cystic dilatation of renal tubules (a) glomerular boulation (b)**. (**H&E, x 100& X300).

lymphocytes and a focus of dysplasia with cytological atypia were observed in abamectin treated male rat's liver

Effect on liver function is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987).

Residue analysis

The concentration of thiamethoxam residues after 28 days of repeated exposure to 1/20, 1/30 and 1/40 of LD_{50} are shown in Table 3, the highest residues of thiamethoxam were detected in the intestine followed descending with that in lung, spleen, heart, kidney, brain and lowest in liver. Accumulated residues depend on the inner organ, the concentration of the insecticides , and the exposure period. Such accumulation of a compound residues referred to insufficient degradation rate, facing the continuous supply with the contaminant. Despite of several investigators reported that the highest concentration from variety of toxicant were found in liver and the lowest in skeletal muscles (Afifi *et al*., 1991, Mostafa *et al*., 1994, El-Ziat,1997; Shalaby and Ayyat, 1999 and Romeh, 2006) .Thiamethoxam residues in stomach are high (2.17 and 3.78 ppm) followed by fats (1.7 and 3.03 ppm) and brain (1.6 and 2.78 ppm) after 5 and 10 days of treatment; while, residues in kidney and liver are low in albino rats treated with one-tenth of median lethal dose 1/10 of LD_{50} for ten days (Shehata EM *et al.,* 2010).

Table 3. Residues of thiamethoxam in different organs of female rats after 28 days of treatment

Organs	1/20 LD50 (78.15 mg/kg)	1/30 LD50 (52.1 mg/kg)	1/40 LD50 (39.08 mg/kg)
Liver	15.78	10.99	5.13
Kidney	40.47	37.95	25.13
Heart	42.18	31.6	21.67
Spleen	55.76	43.67	37.55
Brain	35.88	28.9	19.76
Intestine	80.2	72.98	38.44
Lung	59.21	35.26	27.83
Muscles	13.53	10.77	5.29

Conclusively, the results of the hematological, biochemical, electrophoretic, histological, and residual studies indicate that the exposure rats to thiamethoxam for a duration up to 28 days is harmful, and extreme caution is needed when using thiamethoxam in agriculture applications.

REFERENCES

Afifi, N. N., A. Ramadan., I. El-Aziz,Mand E.E. Saki (1991). Influence of dimethoate on testicular and epididymal organs testosterone plasma level and their tissue residue in rats. *Deutsche-Tieraztiche-Wochenschrift*., **92** (11).419-423

Anderson, S. and S. Cockayne (1989). *Clinical Chemistry*: concepts and applications. Allston, Ann Carale editor, Philadelphia, London, 367-383.

Anonymous (1986). *Cohort Software*. Costat users manual virgin 3.03. Bekley. California, UAS.

Anonymous (2005). The e-Pesticide Manual. (B.C.P.C.) The British Crop Protection Council Software Developed by Wise and Loveys Information Services Ltd.

Balistreri, W. F. and L. M. Shaw (1987). *Liver Function*. In: Fundamentals of Clinical Chemistry. Tietz, N.W.(ed). 3 ed., W.B. Saunders Company, Philadelphia, pp. 729.

Bradford M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. **Annual Biochem**; **72**:248-54

Eissa, F.I. and N.A. Zidan (2009). Haematological, Biochemical and histopathological alterations induced by abamectin and *Bacillus thuringiensis* in male albino rats. *Australian Journal of Basic and Applied Sciences*, **3** (3): 2497-2505.

El – Ziat, A. M.(1997). Effects of contaminated feedstuffs on performance of domestic fowls. M.Sc. Thesis, Faculty of Agric. Zagazig University, Egypt

EPA (1998). Analytical method for the determination of residues of CGA 293343and metabolite CGA322704 in animal and crop substrates by high performance liquid chromatography with detection by UV and mass spectrometry, including validation data .U.S. *Environmental Protection Agency guideline*, N. 860-1340.AG.675.,Washington DC.

EPA (2003). *Draft Final Guidelines for Carcinogen Risk Assessment*, U.S. Environment Protection Agency, Washington D C.

Green, T., A. Toghill., R. Lee., F. Waechter., E .Weber., R .Peffer., Noakes, J. and M. Robinson (2005). Thiamethoxam induced mouse liver tumours and their relevance to humans. Part 2: Species Differences in Response. *Toxicological Sciences*, .86 (1), 48-55

Guzsvány,V., János C, and Ferenc, G.(2006). NMR Study of the Influence of pH on the persistence of some neonicotinoids in water. *Acta Chim. Slov.*, **53**: 52–57.

Hendawy, M. Y. I. (1997). Pesticide biotechnology for effective and safe use. M.Sc. Thesis Fac. of Agric., Zagazig University, Egypt.

Henry RJ. (1974). *Clinical Chemistry, Principles and Techniques*, 2nd Edition, Harper and Row., P. 525

Hood, W. (1980). A-*Z of Clinical Chemistry* (A guide for the trainee). Stp-Limited Falcon House, London, England, 176-256.

Kaneko, J.J.; J.W. Harvey and M.L. Bruss (1997). "*Clinical Biochemistry of Domestic Animals* ". 5th ed., Academic Press, San Diego, California

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature*, **227**: 680-685.

Lillie R. D. and H. M. Fullmer (1976). Histopathologic *Technic and Practical Histochemistry*, 4th ed., p. 635-636. McGraw-Hill. Book Co., New York, USA.

Meek,M.E., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-McKeeman, L.D. , Longfellow, D., Pastoor, T., Seed, J., and Patton, D.E. (2003). A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action. *Crit. Rev. Toxicol.*, **33** (6), 591-653.

Mostaf,A.M., Ahmed,Y.M. and Ahmed,M.T.(1994). Feeding cyromazine to human hens residue in tissue and effects on some biochemical constituents. *J. Egypt. Soci. Parasitology*, **24** (2)259-269.

Reitman SM, Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic transaminase, Amer. *J. Clin. Pathol*., **28**: 56-63.

Rodwell, V. W. (1983). *Conversion Of Amino Acids To Specialized Products*. In: Martin, D.W., P.A. Mayes, V.W. Rodwell, (Eds.), Harper's review of biochemis try. 19th ed. Lange Medical Publications, California, pp: 307.

Romeh,A.A.(2006). Effect of activated charcoal and rice hush ash addition on the residues of diniconazol and their effect on some blood components of catfish (*Clarias lazera*). *Zagazig Journal .Agriculture Res*.,**33**(2),267-281

Shalaby, A.A. and M. S .Ayyat (1991). Effect of natural clay addition on the residues of profenofos and monocrotophos and their effect on some blood components in hens. *Egyptian Journal of Applied Science*, **14** (6),286-300.

Shehata EM Shalaby, Abdel Razik H Farrag and Gamila SM El-Saed (2010). Toxicological potential of thiamethoxam insecticide on albino rats and its residues in some organs. *JASMR*, **5** (2): 165-172.

Shipra Bhardwaj, M.K.; U. K. Srivastava and L.P. Srivastava (2010). A 90 days oral toxicity of imidacloprid in female rats: Morphological, biochemical and histopathological evaluations. *Food And Chemical Toxicology*; **48**(5):1185-90.

سمية ومصير مبيد الثياميزوكسيم فى الفئران البيضاء

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تم دراسة التاثيرات السامة ومصير مبيد الثياميزوكسيم فى الفئران البيضاء المعرضة لمستويات مختلفة من الجرعة النصفية القاتلة LD_{50} لمدة ٢٨ يوم فى مياة الشرب .اوضحت النتائج وجود زيادة معنوية فى مستويات انزيم (ALT) وانزيم AST) والكرياتنين وانخفاض معنوى فى مستوى البروتين الكلى عند التعرض لتركيزات ١/٢٠ و١/٣٠ و١/٤٠ من قيمة الجرعة النصفية القاتلة. اوضح التفريد الكهربى لسيرم الدم وجود ١٤ حزمة من البروتينات ولم يلاحظ غياب اى منها ولكن يوجد اختلاف فى حجم هذة الحزم فى المعاملات المختلفة عند التعرض لتركيزات ١/٢٠ من قيمة الجرعة النصفية . هناك دلالات واضحة على حدوث تغيرات هستوباثولوجية فى كل من الكبد والكلى عند التعرض ل١/٢٠ و١/٣٠ من الجرعة النصفية القاتلة ولم يلاحظ تاثيرات واضحة عند الجرعة ١/٤٠ من الجرعة النصفية القاتلة.

وجد ان متبقي المبيد فى كلا من الكبد والكلى والقلب والطحال والمخ والأمعاء والرئتين والعضلات ١٥,٧٨ -٤٠,٤٧ - ٤٢,١٨ - ٥٥,٧٦- ٣٥,٨٨ - ٨٠,٢ - ٥٩,٢١ - ١٣,٥٣ ملليجرام/كجم بعد اربعة اسابيع من التعرض للتركيز ١/٢٠ من الجرعة النصفية .