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EFFECT OF THIAMETHOXAM AND EMAMECTIN BENZOATE ON HEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL PARAMETERS IN FEMALE RATS

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ABSTRACT

The oral toxicity of both insecticides: the neonicotinoid Thiamethoxam (TMX) and the avermectin Emamectin Benzoate (EB) was conducted daily in female rats with doses of 25, 50, 100 mg/kg/body weight (bw) for TMX and 1.25, 2.5, 5 mg/kg bw for EB for 90 successive days. Significant decrease in the body weight gain and food consumption was observed at highest dose (100 mg/kg bw) of TMX and (5 mg/kg/ bw) of EB and at necropsy the relative body weights of liver and kidney was also significantly increased at this dose level. There were no significant changes in hematological parameters for TMX, while the high dose of EB significantly decreased in all the investigated hematological parameters. In clinical chemistry parameters a significant increase ($p < 0.05$) was noted in serum AST, ALT, ALP and BUN in animals exposed to 100 mg/kg bw dose of TMX and 5 mg/kg bw of EB. On the other hand, at the same levels there was a dose-depended significant decrease in serum total protein and albumin. Oral administration of TMX and EB to female rats for 90 days resulted in 16–30% and 15–26% inhibition of serum cholinesterase (ChE) activity, respectively. Liver and kidney of rats exposed with high dose of TMX and EB had showed mild pathological changes. Based on the hematological, biochemical and histpathological studies it is evident that TMX and EB has not produced any significant effects at 20 and 1.25 mg/kg bw doses respectively but induced toxicological effects at 100 mg/kg bw for TMX and 5 mg/kg bw for EB to female rats. Hence, 25 mg/kg bw/day for TMX and 1.25 mg/kg bw/day for EB dose may be considered as No Observed Effect Level (NOEL) for female rats for both insecticides respectively.

INTRODUCTION

Thiamethoxam (TMX) is a synthetic organic insecticide included in the class of neonicotinoids, the most important new class of insecticides developed in the last three decades. Since their introduction onto the market in 1991, neonicotinoids have been the fastest growing class of insecticides, due to their expected moderate toxicity to mammals and their advantage in combating insects that are resistant to other pesticide classes (Bingham *et al.*, 2008). The annual sales of this class of chemicals reach nearly a billion dollars, corresponding to 11–15% of the total insecticide market (Tomizawa and Casida, 2003). Thiamethoxam uses for control of aphids, whitefly, thrips, ricehoppers, ricebugs, mealybugs and some lepidopterous species (Maienfisch, *et al.*, 2001). Major crops for foliar and soil treatments are leafy and fruity vegetables, potatoes, rice, cotton, deciduous fruit, citrus, tobacco and soya beans; for seed treatment use, maize, sorghum, cereals, sugar beet, oilseed rape, cotton, peas, beans, sunflowers, rice and potatoes. The insecticidal activity of neonicotinoids is primarily attributed to their action on nicotinic acetylcholine receptors (nAChRs) (Karlin, 2002; Tomizawa and Casida, 2005). Neonicotinoid acute toxicity is ascribed primarily to their action as nicotinic agonists, acting on insect and mammal nAChRs (Tomizawa and

Casida, 2003) and the toxicity induced by these substances is considered to be centrally mediated because the symptoms of poisoning are similar to those of nicotine (Tomizawa and Casida, 2005). Rodrigueset *et al.*, (2010) studied the effect of three different concentrations (25, 50 or 100 mg/kg/body weight) on rats after treatment for 7 consecutive days. They found that thiamethoxam induced an increase in the anxiety behavior at the two doses of 50 or 100 mg/kg bw of rats. Moreover, there was a significant decrease of acetylcholinesterase activity.

Emamectin benzoate (EB) is the 4″-deoxy- 4″-epi-methyl-amino benzoate salt of avermectin B, (abamectin), which is similar structurally to natural fermentation products of soil actinomycete, *Streptomyces avermitlis*. Emamectin benzoate is currently being developed for control of lepidopterous pests on a variety of vegetable crops worldwide with a very low application (Jansson and Dybas 1998). It is also extremely effective for treating and controlling sea lice infestations on Atlantic salmon, *Salmo solar* L (Armstrong R. 2000). The mechanism of action involves the blocking of γ-amino butyric acid-stimulated (GABA) chloride channels and open non-neurotransmitter-gated chloride channels (Martin, *et al.*, 2002.), causing an ion imbalance in the nervous system, resulting in paralysis and death. In 1997, Wise *et al.* conducted laboratory tests on pregnant female Sprague-Dawley rats which orally gavaged EB once daily. The results demonstrated that the high-dose of emamectin benzoate (3.5/2.5 mg/kg bw) exposure during gestation and lactation to rats produced evidence of neurotoxicity in the F1 off spring and the calculated No Observed Effect Level (NOEL) for developmental neurotoxicity of EB was determined to be 0.6 mg/kg bw/day. At the same time, groups of rats (20/sex/dose) were given emamectin (as hydrochloride salt) at 0, 0.5, 2.5 or 12.5/8/5 mg/kg bw/day in their diet for 14 weeks. The highest dose was reduced to 8 mg/kg bw/day at 3 rd week, and subsequently to 5 mg/kg bw/day at 9 th week. Significant reductions in body weight and food consumption were observed in animals receiving 12.5/8/5 mg/kg bw/day. Decreased serum glucose concentration and a slight increase in blood urea nitrogen were detected at all sampling times following treatment with 12.5/8/5 mg/kg bw/day. Neuronal cytoplasmic vacuolation and degeneration were also noted. The NOEL was 2.5 mg/kg bw/day on the basis of neurotoxicity, weight loss, and decreased food consumption in rats received high doses (JECFA, 1998).

Thiamethoxam and emamectin benzoate insecticides are currently used in great amounts in Egypt and abroad, but this can rise a problem when the possible risks of occupational and environmental contamination are taken into account. Since TMX and EB is now being considered to replace other existing pesticides, therefore the relative risk and benefits of this insecticide must be compared to the existing pesticide. Although the data about the action of TMX on nAChRs and EB on GABA receptors in insects are clear, there are a few works that demonstrate possible in vivo effects of both pesticides on the mammalian biological system. For these reasons, the present study has been carried out to evaluate 90 days oral toxicity of TMX and EB on female rats by studying hematological, biochemical and histopathological parameters.

MATERIALS AND METHODS

Chemicals

Thiamethoxam technical 97% pure, 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5] oxadiazinan-4-ylidene-N-nitroamine) [CAS No. 153719-23-4] was obtained from Ciba-Geigy Switzerland.

Emamectin benzoate technical 98% pure is a mixture containing 90% of B1a (10 E ,14 E ,16 E ,22 Z)-(1 R ,4 S ,5′ S ,6 S ,6′ R ,8 R ,12 S ,13 S ,20 R ,21 R ,24 S)-6′-[(S)- sec -butyl] -21,24-dihydroxy-5′,11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo [15.6.1.14,8.020,24] pentacosa-10,14,16,22-tetraene-6-spiro-2′-(5′,6′-dihydro-2′ H -pyran)-12-yl 2,6-dideoxy-3- O- methyl-4- O-(2,4,6- trideoxy-3- O -methyl-4-methylamino-a-L-lyxo-hexopyranosyl)- a-L- arabino -hexopyranoside benzoate and 10% of B1b (10 E ,14 E ,16 E ,22 Z)-(1 R ,4 S ,5′ S, 6 S ,6' R ,8 R ,12 S ,13 S ,20 R ,21 R ,24 S)-21,24 - dihydroxy - 6′ - isopropyl-5′,11,13,22–tetramethyl – 2 – oxo - 3,7,19 trioxatetracyclo [15.6.1.1 4,8 .0 20,24]pentacosa-10,14,16,22-tetraene-6-spiro-2′-(5′,6′-dihydro-2′ H -pyran)-12-yl 2,6-dideoxy-3- O - methyl-4- O -(2,4,6-trideoxy-3- O -methyl-4-methylamino-a-L- lyxo -hexopyranosyl)-a-L-arabino -hexopyranoside benzoate (CAS No. 155569-91-8) obtained from Merck Limited Company USA.

Animals

Female albino rats (Sprague-Dawely), *Rattus norvegicus albinu* s, weighting 160-170 gm were purchased from the Agricultural Research Center, Giza, Egypt. Rats were kept under the laboratory conditions of 20-25 °C and 12-h dark/light cycle for two weeks as an acclimatization period. They were housed in special healthy standard cages and maintained on *ad libitum* for water and a standard rat chow diet which contains 17% protein. Animal experiments and housing procedures were performed in accordance to the animal care rules and they were approved by the authorities of the University.

Experimental design

Thirty five female rats were randomly divided into seven groups as the following;

Group (I): control group which was given daily saline as vehicle through gavage.

Group (II): the rats of this group were given daily saline containing 25 thiamethoxam mg/kg body weight.

Group (III): the rats of this group were treated daily with saline containing 50 thiamethoxam mg/kg body weight.

Group (IV): the rats in this group were administrated daily with saline containing 100 thiamethoxam mg/kg body weight.

Group (V): the rats in this group were administrated daily with 1.25 emamectin benzoate mg/kg body weight in deionized water.

Group (VI): the rats in this group were administrated daily with 2.50 emamectin benzoate mg/kg body weight in deionized water.

Group (VII): the rats in this group were administrated daily with 5 emamectin benzoate mg/kg body weight in deionized water.

Body weight, food consumption and clinical signs of toxicity were recorded through out the period of experiment. After the end of experiment rats were necropsied and blood was divided into two portions. The first portion was taken on 10% EDTA tubes (ethylenediaminetetraacetic acid) for haematological examination. The second portion was left to clot at room temperature for about 20 minutes and then centrifuged at 3000 r.p.m for 15 minutes; the supernated serum samples were drown in dry clean-capped tubes and kept in deep freezer at –20 ^{o}C until conducting the biochemical analysis.

Changes in hematological parameters

Blood collected in 10% EDTA was analyzed for red blood cells (RBCs), white blood cells (WBCs) as described by Dacie and Lewis (1984) and platelets count as described by Wu and Hoak (1974), while haemoglobin concentration (Hb) was measured according to VanKampen and Zijlstra (1961). Hematocrit value (HCT %) was determined by centrifuging blood in heparinized microhematocrit tube (capillary tubes of 1mm internal diameter and 7.5 cm length) for 5 minutes at 15,000 r.p.m (Dacie and Lewis, 1991). Mean cell volume (MCV) was calculated from the following formula:

$$\textbf{MCV, (fl) = HCT\% x 10} / \textbf{RBCs (M/mm}^3\textbf{)}$$

Changes in biochemical parameters

Liver function tests as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with colorimetric method (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) was measured calorimetrically (Belfield and Golberg, 1971). Total protein, albumin, and blood urea nitrogen (BUN) were measured according to the methods of Gornal *et al.,* (1949), Doumas *et al.*, (1971) and Fawcett (1960) respectively. Activity of cholinesterase (ChE) was calculated after 30, 60 and 90 seconds in serum at 405 nm to follow the inhibition of the enzyme activity (Unit/l) (Knedel and Bottger, 1967).

Changes in histopathological studies

Small pieces of liver and kidney of each animal of control and treated groups were fixed in 10% formal saline solution for twenty four hours. Washing was done using tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. After routine processing, paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope. (Lillie and Fullmer, 1976).

Statistical Analysis:

Means of results were calculated among 5 replicates, with their Standard Error (SE) for each group. Analysis of variance was used to make statistical comparisons (ANOVA) with Dunnett's posthoc test. SPSS computer program (SPSS, 1990) which was used to calculate the significance between groups at the same experiment at 5% probability.

RESULTS AND DISCUSSION:

Changes in body weight and food consumption:

Repeated oral administration of both thiamethoxam at 25 and 50 mg/kg bw day and emamectin benzoate at 1.25 and 2.50 mg/kg bw day did not produce any signs of toxicity and mortality during 90 days exposure. However, there was significant decrease in body weight gain of animals at highest dose (100 mg/kg bw day) of TMX and (5 mg/kg bw day) of EB comparing to control group (Table 1). There was no change in food consumption of animals exposed to 25 and 50 mg/kg bw day of TMX and 1.25 and 2.50 mg/kg bw day of EB doses. However, there was significant dose-dependent decrease in food consumption of rats exposed to (100 mg/kg bw day) of TMX and (5 mg/kg bw day) of EB at 90 days as compared with the untreated controls.

Table (1): Body weight gain (g) of female rats and food consumption in female rats after oral administration of thiamethoxam and emamectin benzoate for 90 days.

Groups	Initial weight (g)	Final weight (g)	Daily body weight gain (g)	Daily food intake (g/ rat) mean	Feed efficiency (%)
Group I : control	166.0±2.11^{a}	279.2±3.54^{a}	1.26±0.07^{a}	12.78	9.98±0.39^{a}
Group II: TMX 25 mg/kg bw.	166.2±2.09^{a}	253.4±4.15^{ab}	0.97±0.08^{a}	11.39	8.52±0.18^{a}
Group III: TMX 50 mg/kg bw.	166.2±2.18^{a}	248.4±2.99^{ab}	0.93±0.06^{ab}	11.17	8.38±0.25^{ab}
Group IV: TMX100 mg/kg bw.	164.4±2.31^{a}	229.4±1.98^{b}	0.71±0.03^{b}	10.24	7.19±0.17^{b}
Group V: EB 1.25 mg/kg bw.	165.4±2.62^{a}	259.0±4.11^{a}	1.05±0.08^{a}	11.77	8.93±0.32^{a}
Group VI: EB 2.5 mg/kg bw.	166.6±2.71^{a}	259.4±3.55^{a}	1.04±0.06^{a}	11.81	8.84±0.30^{a}
Group VII: EB 5 mg/kg bw.	164.6±2.14^{a}	233.0±2.15^{b}	0.77±0.04^{b}	10.41	7.42±0.21^{b}

Values across each column having the same superscript letter were not significantly different (p < 0.05). Values are mean s of five replicates ± standard error (S.E.).

Thiamethoxam was administered in 25, 50 or 100 mg/kg bw day doses, which were below the lethal value. Repeated exposure to thiamethoxam at the doses tested did not produces eizures, tremors weight loss, or other types of apparent behavioral or physiological dysfunctions (Rodrigues*, et al.* 2010). Green *et al.* (2005) stated that the mean body weight of the treated mice with different concentrations of thiamethoxam was consistently below control level at the 2500 and 5000 ppm dose levels (by 8% at 2500 and by 14% at 5000 ppm at week 50). The results of Shipra *et al.*, (2010) showed that 90 days oral exposure of high dose of the neonicotinoid imidacloprid (20 mg/kg bw) to female rats has produced significant toxic effects. There was significant decrease in the body weight of high dose exposed rats together with reduced food consumption. The weight gain in animals serves as index of growth rate (Palani *et al.,* 1999). The reduced food consumption of high dose exposed animals seems to be due to toxic potential of the neonicotinoid.

A 14 week study in Sprague-Dawely rats designed to investigate the neurotoxicity of emamectin benzoate. The rats administrated daily with EB at doses 0.25, 1, 5 mg/kg body weight. Body weight gain and food consumption

were significantly reduced at the high level dose of 5 mg/kg/day (European Agency, 1999).

Changes in relative organ weights

The relative organ weights of vital organs of female rat are shown in Table 2. The vital organs like brain and spleen did not illustrate any change in their relative organ weights at any doses of both insecticides as compared to control.

Table (2): Relative organ weight* (g%) data of female rats orally administered TMX and EB for 90 days.

Groups	Liver(%) weight	Kidney (%) weight	Brain (%) weight	Spleen(%) weight
Group I : control	3.02±0.13 ^{b}	0.55±0.01 ^{de}	0.60±0.01 ^{a}	0.29±0.01 ^{a}
Group II: TMX 25 mg/kg bw	3.26±0.15 ^{b}	0.54±0.02 ^{e}	0.58±0.01 ^{a}	0.28±0.01 ^{a}
Group III: TMX 50 mg/kg bw	3.46±0.17 ^{ab}	0.61±0.01 ^{abc}	0.60±0.03 ^{a}	0.29±0.02 ^{a}
Group IV: TMX 100 mg/kg bw	3.75±0.19 ^{a}	0.65±0.03 ^{a}	0.62±0.02 ^{a}	0.31±0.01 ^{a}
Group V: EB 1.25 mg/kg bw	3.25±0.14 ^{b}	0.57±0.02 ^{cde}	0.59±0.02 ^{a}	0.29±0.01 ^{a}
Group VI: EB 2.5 mg/kg bw	3.44±0.16 ^{ab}	0.59±0.01 ^{bcd}	0.59±0.02 ^{a}	0.30±0.02 ^{a}
Group VII: EB 5 mg/kg bw	3.68±0.21 ^{a}	0.63±0.02 ^{ab}	0.61±0.02 ^{a}	0.32±0.01 ^{a}

Values across each column having the same superscript letter were not significantly different ($p < 0.05$). Values are mean s of five replicates ± standard error (S.E.).
*** Organ weight/body weight × 100.**

However, relative weight of liver was significantly increased ($p < 0.05$) at dose (100 mg/kg bw day) for thiamethoxam and (5 mg/kg bw day) for emamectin benzoate dose. The relative weight of kidney also was significantly increased at higher dose (100 mg/kg bw day) of TMX and (5 mg/kg bw day) of EB level comparing with control group. This agreed with the study of Shipra *et al.*, (2010) where the neonicotinoid imidacloprid at a dose of 20 mg/kg bw significantly increased the relative weight of both liver and kidney. The same results were observed by Green *et al.*, (2005) who, proved that the mean relative liver weight of treated mice with TMX was increased at 2500 ppm and at 5000 ppm in different weeks when compared with control group. Eissa and Zidan (2009) indicated that administration of abamectin at both dose levels (1/10 and 1/100 of LD_{50}) resulted in a significant increase in the relative weight of treated male rat's liver and kidney in comparison with that of control group rats. Liver enlargement could be due to the accumulation of abnormal amounts of fat, predominately triglyceride, in the parenchymal cells. Triglyceride accumulation is a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation (Plaa, 1980).

Changes in hematological parameters

Data in Table 3 revealed that no significant changes were observed in RBCs, WBCs, Hb, MCV, HCT and platelets of the treated animals with thiamethoxam (25, 50, 100 mg/kg bw) for 90 days as compared to controls. The obtained results are in agreement with those found by Shipra *et al.*, (2010) who stated that the neonicotinoid imidacloprid at different doses didn't show any significant effect on the hematological parameters of treated rats compared with non-treated ones.

Table (3): Hematology data of female rats orally administered with different doses of thiamethoxam and emamectin benzoate for 90 days.

Groups	RBCs (M/mm^3)	WBCs (m/mm^3)	Hb (g/dl)	HCT (%)	MCV (fl)	Platelets (m/mm^3)
Group I: control	7.38±0.73^a	4.65±0.51^a	14.48±1.10^a	37.88±1.63^a	50.60±1.81	980±23.5^a
Group II: TMX 25 mg/kg bw	7.26±0.75^a	4.54±0.42^a	14.26±1.15^a	37.26±2.18^a	51.58±2.91	965±20.2^a
Group III: TMX 50 mg/kg bw	7.16±0.57^a	4.41±0.36^a	14.06±0.97^a	37.16±2.07^a	51.81±2.43	950±19.5^{ab}
Group IV: TMX 100 mg/kg bw	6.95±0.69^{ab}	4.25±0.43^a	13.85±0.89^{ab}	36.95±2.39^a	52.12±2.82	945±22.5^a
Group V: EB 1.25 mg/kg bw	6.85±0.74^{ab}	4.24±0.32^a	13.95±0.84^a	37.25±1.54^a	54.59±3.11	955±18.7^a
Group VI: EB 2.5 mg/kg bw	6.54±0.66^{ab}	4.07±0.47^{ab}	13.44±0.96^{ab}	36.44±2.16^{ab}	55.59±3.07	930±16.5^{ab}
Group VII: EB 5 mg/kg bw	6.18±o.41^b	3.63±0.24^b	13.08±0.71^b	35.68±1.91^b	57.61±2.62	910±15.8^b

Values across each column having the same superscript letter were not significantly different ($p < 0.05$). Values are mean s of five replicates ± standard error (S.E.).

Present data showed that daily oral administration of female rats with emamectin benzoate with high dose (5 mg/kg/ bw) for 13 weeks significantly decreased in dose-dependent way all the investigated hematological parameters: RBCs, WBCs, Hb, MCV, HCT and platelets. The aforementioned findings are in coincidence with those reported by Eissa and Zidan (2009) who proved that the high dose of abamectin (1/10 LD_{50}) caused significant reduction in erythrocyte counts (RBCs), leukocyte counts (WBCs) and hemoglobin concentration of treated rats. A significantly reduced amount of white blood cells could be indicative of immuno-suppression (Schroder *et al.*, 2007). The reduction in erythrocyte counts and consequently hemoglobin concentration may be attributed to more than one factor, i.e. the failure to supply the blood circulation with cells from hemohepatic tissues, since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicants.

Changes in Biochemical parameters:

Serum biochemical parameters such as AST (GOT), ALT (GPT) and ALP were not significantly changed in female rats orally administrated with TMX at doses of 25 and 50 mg/kg bw day and EB at doses of 1.25 and 2, 5 mg/kg bw day as compared to control. However, a significant increase ($p < 0.05$) was noted in serum AST, ALT and ALP in animals exposed to 100 mg/kg bw dose of TMX and 5 mg/kg bw of EB comparing with untreated control groups (Table 4).

These results are in agreement with those of Green *et al.*, (2005), who indicated that the median aspartate aminotransferase activity was increased in mice treated with thiamethioxam in diet at doses 2500 and 5000 ppm comparing to control. Alanine aminotransferase increased in a similar manner, while alkaline phosphatase activity was not affected by this treatment. Similar results were observed by 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 aforementioned results are in agreement with those reported by Hsu *et al.,* (2001) who reported that AST was elevated in abamectin-dosed rats in a dose-dependent manner. The elevation of AST activity, a cytosolic enzyme of the hepatocytes, reflects the

increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt, 1982) and this is parameter of liver damage (Klaassen and Eaton, 1991). The alteration in serum levels of alanine amino transferase (ALT) may be indication of internal organs damage especially in liver (Kaneko *et al.*, 1997).

Table (4): Changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of female rats orally administered with TMX and EB for 90 successive days.

Groups	AST (U/L)	ALT (U/L)	ALP (IU/L)
Group I : control	29.79±1.33 ^{c}	21.78±1.13 ^{c}	117.64±3.01 ^{c}
Group II: TMX 25 mg/kg bw	34.19±1.84 ^{c}	22.61±1.15 ^{c}	132.30±4.22 ^{bc}
Group III: TMX 50 mg/kg bw	50.05±2.77 ^{b}	26.26±1.17 ^{c}	156.17±5.23 ^{abc}
Group IV:TMX 100 mg/kg bw	69.83±2.25 ^{a}	37.33±2.19 ^{b}	170.33±5.52 ^{ab}
Group V: EB 1.25 mg/kg bw	35.59±1.54 ^{c}	25.81±1.16 ^{c}	118.18±3.81 ^{c}
Group VI: EB 2.5 mg/kg bw	54.02±2.10 ^{b}	33.36±2.18 ^{bc}	147.06±4.58 ^{bc}
Group VII: EB 5 mg/kg bw	76.51±3.01 ^{a}	48.78±2.21 ^{a}	193.38±5.59 ^{a}

Values across each column having the same superscript letter were not significantly different (p < 0.05). Values are mean s of five replicates ± standard error (S.E.).

Data shown in Table 5 indicated dose-depended significant decrease in serum total protein and albumin of female rats orally administrated at high doses with both insecticide, thiamethoxam (100 mg/kg/ bw) and emamectin benzoate (5 mg/kg/ bw). At the same time, there are no significant changes in the level of globulin and albumin/globulin ratio in serum of treated rats at all doses as compared with non-treated rats. Total protein, albumin, globulin, A/G ratio, were not significantly changed in animals exposed to 5 and 10 mg/kg bw day doses of the neonicotinoid imidacloprid as compared to controls. (Shipra *et al.*, 2010). Avermectin intoxication caused significant inhibition in the levels of total protein and albumin (Abdel El-Hamid and Refaie 2009). A decreased value of total protein may reflect liver or kidney disease (Sharpe *et al.*, 1996). Hypoalbuminemia (decreased albumin) is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumin (Burtis *et al.*, 1994).

Table (5): changes in concentration of total protein, albumin, glublin and blood urea nitrogen (BUN) in serum of female albino rats administrated with TMX and EB for 90 successive days.

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin/ globulin ratio	BUN (mg/dl)
Group I : control	7.07±0.61 ^{a}	5.31±0.43 ^{a}	1.75±0.11 ^{a}	3.01±0.22 ^{a}	11.41±1.09 ^{b}
Group II: TMX 25 mg/kg bw	6.38±0.55 ^{ab}	4.84±0.37 ^{ab}	1.52±0.09 ^{a}	3.17±0.42 ^{a}	12.63±1.15 ^{b}
Group III: TMX 50 mg/kg bw	6.09±0.62 ^{ab}	4.59±0.28 ^{ab}	1.42±0.13 ^{a}	3.23±0.31 ^{a}	14.38±1.03 ^{b}
Group IV: TMX 100 mg/kg bw	5.41±0.49 ^{b}	4.19±0.39 ^{b}	1.28±0.12 ^{a}	3.29±0.29 ^{a}	20.48±1.50 ^{a}
Group V: EB 1.25 mg/kg bw	6.50±0.80 ^{ab}	4.89±0.43 ^{ab}	1.60±0.08 ^{a}	3.04±0.43 ^{a}	12.75±0.99 ^{b}
Group VI: EB 2.5 mg/kg bw	6.16±0.62 ^{ab}	4.62±0.55 ^{ab}	1.57±0.07 ^{a}	2.96±0.28 ^{a}	14.59±1.13 ^{b}
Group VII: EB 5 mg/kg bw	5.42±0.55 ^{b}	4.10±0.25 ^{b}	1.35±0.12 ^{a}	3.04±0.35 ^{a}	23.86±1.39 ^{a}

Values across each column having the same superscript letter were not significantly different (p < 0.05). Values are mean s of five replicates ± standard error (S.E.).

On the other hand, oral administration of female rats with high dose of TMX (100 mg/kg/d) and EB (5 mg/kg/ bw) for 90 days significantly ($p < 0.05$) increased serum blood urea nitrogen (BUN) when compared with control group. Elevation of urea concentration in serum of treated female albino rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Walmsley and White, 1994).

Changes in cholinesterase activity:

Oral administration of TMX (25, 50, 100 mg/kg/ bw) and EB (1.25, 2.50, 5 mg/kg/ bw) to female rats for 90 days resulted in 16–30% and 15–26% inhibition of serum cholinesterase (ChE) activity, respectively. The inhibition of serum cholinesterase was dose-dependent but inhibition was not significant at low dose of both insecticides (20 mg/kg/d for TMX and 1.25 mg/kg bw for EB). However, significant ChE inhibition was observed at both medium and high doses (50, 100 mg/kg/ bw for TMX and 2.50, 5 mg/kg/ bw for EB) (Table 6).

Although, the results demonstrated a decrease in ChE activity induced by thiamethoxam but they do not provide any information as to whether this is due to a direct action of this insecticide on ChE or an indirect effect through an action on nAChRs. However, it is likely that the decrease in enzyme activity reflects a secondary response of the neurons to the insecticide: the thiamethoxam could be acting on the rat central nAChRs, producing an imbalance in the pattern of cholinergic neurotransmission; in this case, the cell would seek compensatory mechanisms, e.g. changes in ChE activity in order to reestablish its normal activity. Another possibility for these findings is that thiamethoxam, besides acting on nAChRs, acts also as a direct inhibitor of AChE in a similar manner to that of carbamate insecticides (Lockhart *et al.*, 2001; Tomizawa and Casida, 2005) and other cholinesterase drugs that act as nAChR and AChE inhibitors.

Table (6): Cholinesterase (ChE) concentration in serum of female rats orally administered with TMX and EB for 90 days.

Groups	(ChE) (U/L)	% of reduction
Group I : control	1358±40.18^{a}	0
Group II: TMX 25 mg/kg bw	1136±38.25^{ab}	16.34
Group III: TMX 50 mg/kg bw	1063±52.72^{b}	21.79
Group IV: TMX 100 mg/kg bw	946±29.29^{c}	30.33
Group V: EB 1.25 mg/kg bw	1152±39.58^{ab}	15.15
Group VI: EB 2.5 mg/kg bw	1086±42.12^{b}	20.02
Group VII: EB 5 mg/kg bw	1002±30.19^{bc}	26.21

Values across each column having the same superscript letter were not significantly different ($p < 0.05$). Values are mean s of five replicates ± standard error (S.E.).

Changes of liver and Kidney Histopathological Examination:

Microscopically, liver section of rat from control showed the normal hepatocytes (zigzag arrow), central vein (thin arrow), blood sinusoid with congested blood and congested blood in the central vein (thick arrow) (Fig.1 control). Meanwhile, liver of rat from group II (TMX 25 mg/kg bw) showed

hepatocytes with homogenous cytoplasm, central vein (thick arrow), central vein (thin arrows). However, liver of rat from group IV (oral administrated with 100 mg/kg bw TMX) showed congested blood (thick arrow), little number of kuppfer cells and red blood cells appeared in the liver sinusoids infiltration of white blood cells (thin arrows) (Fig 1 TMX 100 mg/kg).

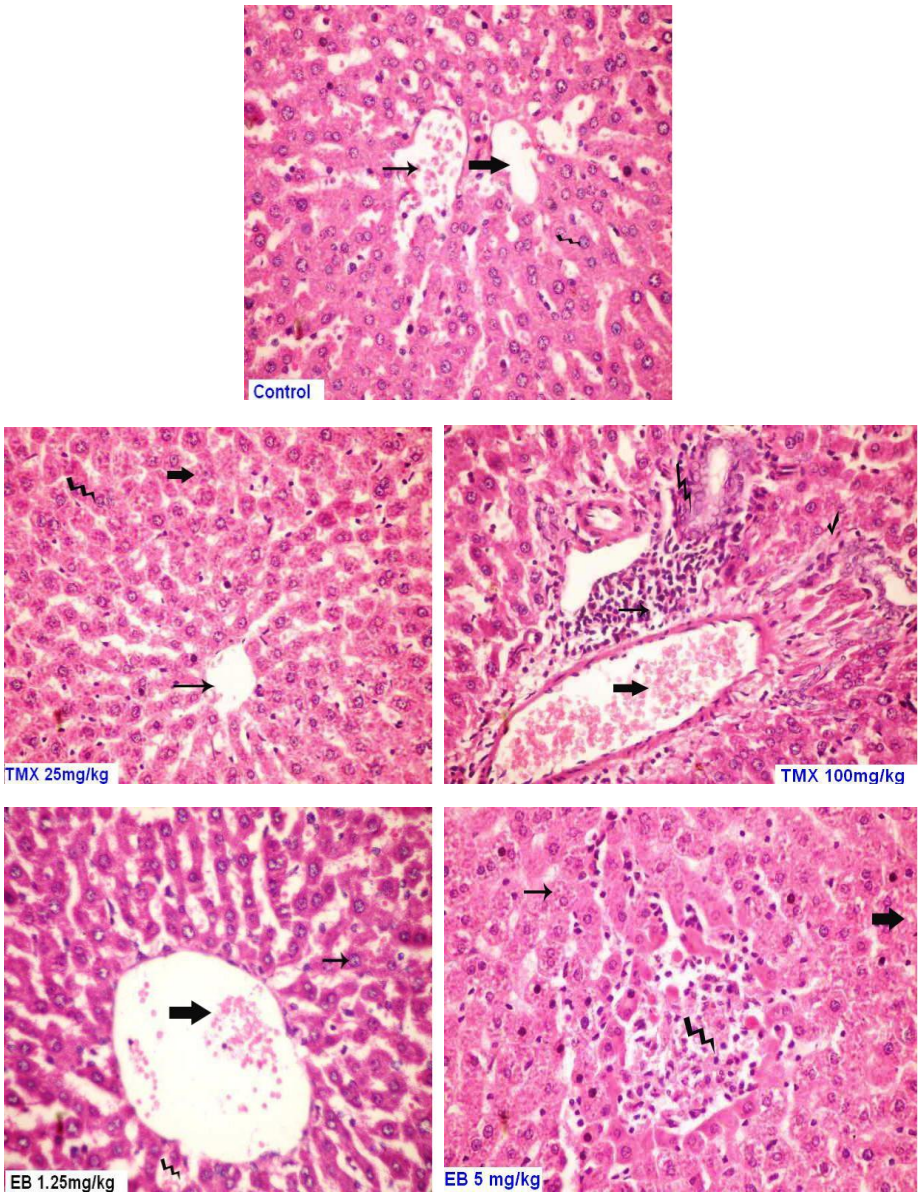

Figure (1): A photomicrograph of the liver sections of the control and oral administrated with both TMX (25 and 100 mg/kg) and EB (1.25 and 5 mg/kg) rats. (H&E, x 100).

On the other hand, there is no histological changes were noticed in liver of rat from group V where, Fig. 1 EB 1.25 mg/kg showed hepatocytes with homogenous cytoplasm, congested central vein (thick arrows), normal liver cell (thin arrows) and vaculation (zigzag arrow). Liver of rat from group VII (gavaged with EB 5 mg/kg) showed necrosis (thin arrow), vaculation (thick arrow) and kuppfer cell (zigzag arrow) (Fig. 1 EB 5 mg/kg). These results were in accordance with National Registration Authority. (2001) which revealed that in mice and rats treated with different doses of thiamethoxam, histological changes were seen in the liver which included increased cell size (hypertrophy); lymphocyte infiltration; liver cell death (necrosis) or pigmentation at or above 100 ppm in male mice, at or above 2500 ppm in female mice and in rats. At the same time Eissa and Zidan (2009) proved that Portal tract infiltration by lymphocytes and a focus of dysplasia with cytological atypia were observed in abamectin treated male rat's liver. Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzymes activities. In this respect, liver tissues which were famous for their rich contents of aminotransferases (AST & ALT) suffer markedly from their loss under many pathological conditions (Rodwell, 1983). Thus, the biochemical parameters data obtained from this investigation support this speculation in which TMX and EB-treated rats showed alteration in the activities o f aminotransferases (AST & ALT).

Microscopically, examination of kidneys of rat from control, untreated group illustrated the normal appearance of the glomerulus (thick arrow), dilatation of renal tubules (zigzag arrow), vacuolation and hemorrhage (thin arrows) (Fig. 2 control). However, kidneys of rat from TMX 25 mg/kg bw showed normal appearance of the glomerulus (thin arrow), dilatation of renal tubules (thick arrow).

Kidneys of rats orally administrated with thiamethoxam 100 mg/kg bw showed renal tubules with homogenous cytoplasm in glomerulus (zigzag arrow), highly vacuolation and hemorrhage (thick arrow) and damaged cells (thin arrow) (Fig.2 TMX 100 mg/kg). No histopathological changes were observed in kidneys of group V where, fig. 2 EB 1.25 mg/kg showed glomerulus (zigzag arrow) and damaged area (thin arrow). Meanwhile, sections from group gavaged with EB at dose 5 mg/kg bw daily for 13 weeks showed abnormal glomerulus with exception hemorrhage (zigzag arrow), and congested blood vessel (thin arrow) (fig.2 EB 5 mg/kg). The same was achieved by National Registration Authority. (2001),which indicated that in male rats at 250 ppm treated thiamethoxam and above, hyaline change (glassy appearance) of kidney cells (renal tubular epithelium) was associated with acute or chronic tubular lesions. Eissa and Zidan (2009) concerning the kidney, Vertimec (abamectin as active ingredient) at 1/10 and 1/100 of LD_{50} doses levels induced interstitial nephritis in male rat's Kidney.

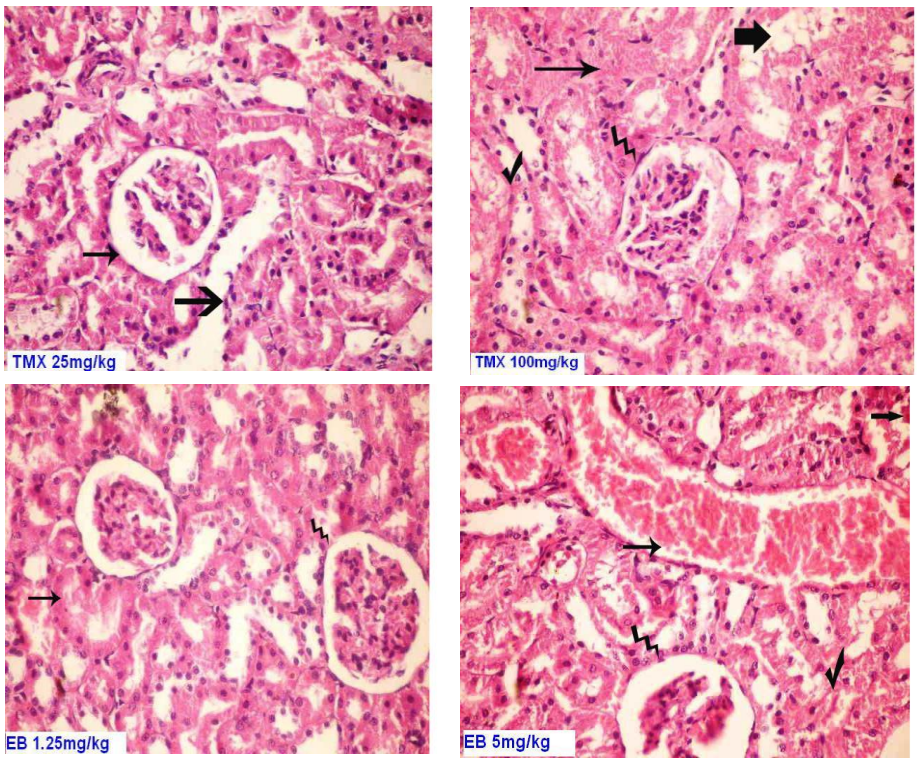

Figure (2): A photomicrograph of the kidney sections of the control and oral administrated with both TMX (25 and 100 mg/kg) and EB (1.25 and 5 mg/kg) rats. (H&E, x 100).

Conclusion:

The data of the present study has indicated that both thiamethoxam and emamectin benzoate have induced toxicological effects on female rats at 100 mg/kg/day and 5 mg/kg bw/day dose level respectively when exposed for a period of 90 days. However, 50 and 25 mg/kg bw/day for thiamethoxam and 2.5, 1.25 mg/kg/ bw /day for emamectin benzoate doses have caused no

adverse effects. Thus, on the basis of parameters such as development of signs of intoxication and mortality, organ body weight ratio, hematology, enzymatic changes and histopathologiacl examination of experimental rats. It may be suggested that 20 mg/kg/day for thiamethoxam and 1.25 mg/kg bw/day for emamectin benzoate produced no desinerable effects, therefore these doses may be considered as No Observed Effect Level (NOEL) for both insecticides to female rats.

REFERENCES

Abdel El-Hamid, S. R. and A. A. Refaie (2009). Ameliorative effect of *silybum marianum* extracts against avermectin induced toxicity in adult rats. J Arab. Soci. Med. Res., 4 (1):25-31.

Armstrong, R.; D. MacPhee, T. Katz and R. Endris (2000). A field efficacy evaluation of emamectin benzoatefor the control of sea lice on Atlantic salmon. Can Vet J; 4l; 607-612.

Belfield, A.; and D. Goldberg (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme, 12, 561-566.

Bingham, G.; R. V. Gunning, G. Delogu, V. Borzatta, L. M. Field and G.D. Moores (2008). Temporal synergism can enhance carbamate and neonicotinoid insecticidal activity against resistant crop pests. Pest Manage. Sci. 64, 81–85.

Burtis , C.A. and E.R. Ashwood (1994). Tietz Textbook of Clinical Chemistry. 2nd ed., W.B. Saunders Company, Philadelphia.

Dacie, J. V. and S. M Lewis (1984). Practical Haematology. 6th ed., Edinburgh: Churchill Livings tone, London.

Dacie, J. V. and S. M. Lewis (1991). Practical haematology. 7th ed., Edinburgh: Churchill Livingstone, London.

Doumas B. T.; W. A. Watson and H. G. Biggs (1971). Albumin standards and measurement of serum albumin with bromcresol green. Clin Chim Acta; 31: 87–96.

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.

European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products. (1999). Emamectin, Summary Report. Report No. EMEA/MRL/546/99-FINAL. 7 pp.

Fawectt, J. K. and J. E. Scott (1960). A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13, 156-159.

Gornall, A. G.; C. J. Bardawill and M. M. David (1949). Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751-66.

Green, T.; A. Toghill, R. Lee, F. Waechter, E. Weber and J. Noakes (2005). Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 1: Mode of action studies in the mouse. Toxicol. Sci. 86, 36–47.

Hsu, Dur-Zong, Hsu, Ching-Hung, Huang, Bu-Miin, Liu, Ming-Yie, (2001). Abamectin effects on aspartate aminotrans ferase and nitric oxide in rats. Toxicology, 165: 189–193.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1998). Eprinometin (Food Additives Series 41), World Health Organization, Geneva, Switzerland.

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

Karlin, A. (2002). Emerging structure of the nicotinic acetylcholine receptors. Nat. Rev. Neurosci. 3, 102–114.

Klaassen, C.D. and D.L. Eaton (1991). Principles of toxicology. In: Amdur, M.O., Doull, J., Klaassen, C.D. (Eds.), Toxicology, 4th ed. McGraw-Hill, New York, pp: 12.

Knedel, M. and R. Bottger (1967). A kinetic method for determination of the activity of pseudocholinesterase. Klin. Wschr., 45, 325-327.

Lillie R. D. and H. M. Fullmer (1976). Histopathologic technic and practical histochemistry, 4th ed., p. 635-636. McGraw-Hill. Book Co., New York

Lockhart, B.; M. Closier, K. Howard, C. Steward, P. Lestage (2001). Differential inhibition of [3H]-oxotremorine-M and [3H]-quinuclinidyl benzilate binding to muscarinic receptors in rat brain membranes with acetylcholinesterase inhibitors. Naunyn-Schmiedeberg's Arch. Pharmacol. 363, 429–438.

Maienfisch, P., M. Angst, F. Brandl, W. Fischer, D. Hofer, H. Kayser, W. Kobel, A. Rindlisbacher, R. Senn, A. Steinemann and H. Widmer (2001). Chemistry and biology of thiamethoxam: a second generation neonicotinoid Pest Manag. Sci., 57, 906.913

Martin, R.J.; A.P. Robertson and A.J. Wolstenholme (2002). Mode of action of the macrocyclic lactones. In: Vercruysse J, Rew RS (Eds.) Macrocyclic lactones in antiparasitic therapy. UK, Wallingford, pp: 125.

National Registration Authority for Agricultural and Veterinary Chemicals. (2001). Public release summary on evaluation of the new active thiamethoxam in the product cruiser 350 FS insecticide seed treatment. Canberra, Australia pp: 21.

Palani, V.; R. K. Senthikumaran and S. Govindawamy (1999). Biochemical evaluation of antitumor effect of *Muthu marunthu* (a herbal formulation) on experimental fibrosarcoma in rats. J. Ethnopharmacol. 65, 257–265.

Plaa, G. L. (1980). Toxic responses of the liver. In: Casarett and Doull's toxicology: the basic science of poisons. 2nd ed., J. Doull, C.D. Klaas sen and M.O. Amdur (Eds.). Macmillan, New York.

Plaa, G. L. and W. R. Hewitt (1982). Detection and evaluation of chemically induced liver injury. In: Hayes, W. (Ed.), Principles and Methods of Toxicology. Raven Press, New York, pp: 407.

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

Rodrigues, K.J.; M.B. Santana, J.L. Do Nascimento, D. L. Picanço-Diniz, L.A. Maués, S.N. Santos, V.M. Ferreira, M. Alfonso, R. Durán and L.R. Faro (2010). Behavioral and biochemical effects of neonicotinoid thiamethoxam on the cholinergic system in rats. Ecotoxicol. Environ. Saf. 73, 101–107.

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.

Schroder, M.; M. Poulsen, A. Wilcks, S. Kroghsbo, A. Miller, T. Frenzel, J. Danier, M. Rychlik, K. Emami, A. Gatehouse, Q. Shu, K. Engel, I. Altosaar and I. Knudsen (2007). A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. Food Chem Toxicol, 45: 339–349.

Sharpe, P.C.; R. McBride, and G.P. Archbold (1996). Biochemical Markers of Alcohol Abuse. QJM; 89: 137-144.

Shipra Bhardwaj, M.K.; U. K. Srivastava, 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.

SPSS (1990). SPSS/PC for the IBM PC/X1. Inc. Chicago, IL. USA.

Tomizawa, M. and J. E. Casida (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48, 339–364.

Tomizawa, M. and J. E. Casida (2005). Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45, 247–268.

VanKampen, E. J. and W. G. Zijlstra (1961). Standardization of hemoglobinometry. II. The hemiglobincyanide method. Clin Chim Acta., 6: 538–544.

Walmsley, R. N. and G. H. White (1994). A Guide to Diagnostic Clinical Chemistry. 3rd ed., Blackwell Scientific Publication, London.

Wise, L. D.; H. L. Allen, C. M. Hoe, D. R. Verbeke and R. J. Gerson (1997). Developmental neurotoxicity evaluation of the avermectin pesticide, emamectin benzoate in Sprague-Dawley rats. Neurotoxicology and Teratology: 19:315 326.

Wu, K. K. and J. C. Hoak (1974). A new method for quantitative detection of platelet aggregates in patients with arterial insufficiency. Lancet, 11: 924-926.

تأثير كل من المبيدات الحشرية ثياميثوكسام و امامكتن بنزوات علي مكونات الدم و الصفات البيوكيميائية و الهستولوجية لاناث الجرذان البيضاء

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أجريت الدراسة الحالية لتوضيح تأثير سمية المبيدات الحشرية عن طريق الفم لكل من مبيد النيكوتينويد ثياميثوكسام و الافرميكتين امامكتن بنزوات علي إناث الجرذان البيضاء بجرعات من ٢٥ و ٥٠ و ١٠٠ مجم / كجم / من وزن الجسم يومياً بالنسبة لثياميثوكسام و ١.٢٥ و ٢.٥ و ٥ مجم / كجم / من وزن الجسم يومياً لامامكتن بنزوات لمدة ٩٠ يوما متتالية. و كان من اهم نتائج الدراسة وجود انخفاض معنوي في وزن الجسم المكتسب واستهلاك الغذاء عند تناول أعلى جرعة من المبيدين (١٠٠ مجم / كجم/ من وزن الجسم يومياً) من الثياميثوكسام و (٥ مجم / كجم/من وزن الجسم يومياً) من امامكتن بنزوات وعند تشريح جثث الجرذان لوحظ ان هناك زيادة في الأوزان النسبية للكبد والكلى بشكل معنوي أيضًا عند هذا المستوى من الجرعة. لم تكن هناك تغييرات معنوية في قياسات صورة الدم بالنسبة لمبيد الثيوميثوكسام ، في حين ان الجرعة العالية من امامكتين بنزوات ادت الي حدوث انخفاض بشكل ملحوظ في كافة قياسات صورة الدم التي تم دراستها. بخصوص القياسات البيوكيميائية وجد أنه توجد زيادة معنوية (عند P > ٠.٠٥) في مستوى إنزيمات الكبد (الألانين أمينوترانسفيريز، الأسبرتات أمينو ترانسفيريز والالكالين فوسفاتيز) و كذلك اليوريا في سيرم الدم للحيوانات المعرضة ل ١٠٠ مجم / كجم/ من وزن الجسم يومياً من الثياميثوكسام و ٥ مجم / كجم/ من وزن الجسم يومياً من امامكتن بنزوات. من ناحية أخرى ، على نفس المستويات من الجرعة العالية وجد انخفاض معنوي في البروتين الكلي والألبومين في مصل الفئران المعاملة مقارنة بالعينة الضابطة. التناول عن طريق الفم لكل من المبيدين لإناث الفئران لمدة ٩٠ يوما أسفر عن تثبيط أنزيم الكولينستيريز في مصل الدم بمعدل ١٦-٣٠ ٪ بالنسبة لمبيد الثيوميثوكسام ، و بمعدل ١٥-٢٦ ٪ بالنسبة لمبيد امامكتن بنزوات على التوالي. الفحص الهستولوجي لأنسجة الكبد والكلى من الفئران التي عرضت لجرعات عالية من ثياميثوكسام و امامكتن بنزوات أظهرت تغيرات مرضية خفيفة. واستنادًا إلى الدراسات التى اجريت علي صورة الدم ، و كذلك البيوكيميائية والهستولوجية التي أوضحت أن كلا المبيدين ثياميثوكسام و امامكتن بنزوات لم ينتجا أي آثار سامة كبيرة عند تناول اناث الجرذان لهم بمعدل ٢٠ و ١.٢٥ ملجم / كجم/يومياً على التوالي ولكن الجرعات العالية من كلا المبيدين (١٠٠ مجم / كجم/ من وزن الجسم يومياً من الثياميثوكسام و ٥ مجم / كجم/ من وزن الجسم يومياً من امامكتن بنزوات) احدثت تأثيراً سامأ علي اناث الجرذان. وبالتالي فأنه يمكن اعتبار جرعة ٢٥ مجم / كجم/من وزن الجسم يومياً من الثياميثوكسام و ١.٢٥ مجم / كجم/ من وزن الجسم يومياً من امامكتن بنزوات هي الجرعة التي لا يلاحظ عندها اي اضرار سامة بالنسبة لإناث الجرذان البيضاء على التوالي لكل من المبيدين الحشريين.

قام بتحكيم البحث

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