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INVESTIGATION THE ACUTE EFFECTS OF SULFOXAFLOR INSECTICIDE ON HEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN MALE ALBINO RAT *RATTUS NORVEGICUS*

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ABSTRACT: Sulfoxaflor (SFX), classified as a sulfoximine insecticide, has been widely utilized on a global scale, leading to permanent human exposure. The study objective was to evaluate the potential hematological and immunological alterations induced by acute toxicity of SFX insecticide in male wistar rats. To attain these objectives, twenty male rats were randomly divided into two equal groups; the 1st group was used as a control group; the other group was exposed 1100 mg/kg body weight SFX by oral gavage for 48 hours, following by 30-day recovery period. Relative liver, thymus, and spleen weights, complete blood count and histological analysis of the thymus, liver, spleen, and Peyer patches were investigated. The results showed that SFX exposure resulted in a considerable reduction in RBCs, hemoglobin, HCT, and platelets following exposure to SFX for 48 hours. However, WBCs and neutrophils increased in the SFX-intoxicated group, while lymphocytes, eosinophils, monocytes, and basophils decreased. Following a 30-day recovery period, there was a notable decrease in WBCs and neutrophils, while the number of RBCs, platelets, and hemoglobin levels increased. Histopathological examination of the thymus, liver, spleen, and Peyer patches indicated that SFX induced only a minor disruption. Nevertheless, the architecture of the bone marrow and lymph nodes was highly structured. Our data showed that the SFX's acute toxicity resulted in hemotoxic and immunotoxic alterations that were recoverable following a time of recuperation.

Key words: Hemtotoxicity, Immunotoxicity, Sulfoxaflor, Insecticides, Acute toxicity.

INTRODCUTION

Pesticides that have been used uncontrollably all over the world in agriculture exhibit an increasing threat to public health. Pesticides can affect people because they can enter their bodies through food or contact with the environment and become embedded in their tissues. (Mohany *et al.,* 2012).

A novel family of pesticide known as sulfoximines is effective against a range of sap-feeding insects, such as plant hoppers, white flies, and aphids. (Babcock *et al.,* 2011).

Sulfoxaflor (SFX) (Fig. 1) belongs to a new group named sulfoximine (Cutler *et al.,* 2013), which is widely used for the eradication of agricultural sap-feeding insects including mealybugs, scale insects, aphids and white flies. SFX is applied as an insecticide on fruits and vegetables such as tomato, pepper, aubergine, spring and winter cereals (wheat, barley, oats), cucurbits such as cucumber, watermelon, courgetteand cotton as proposed by the applicant (EFSA, 2014). SFX is a competitive modulator/agonist of the nicotinic acetylcholine receptor (nAChR) (Sparks *et al.,* 2020) that produces rapidly excitation neurotransmission in insects' central nervous systems, which leads to paralysis and ultimately death . According to Sparks *et al.,* 2013, SFX is also successful against a variety of sap-feeding insect pests that are resistant to several types of insecticides, including many that are resistant to other neonicotinoids. The Insecticide Resistance Action Committee (IRAC) has placed SFX in subgroup 4C because of its low insecticidal cross-resistance and sulfoximine structure. (IRAC, 2020).

A few of SFX's unique properties are as follows: low sorption of soil and sediment, high solubility in water, extended anaerobic half-life in both soil and water (113–120 and 103–382 days), resistance to photolysis and hydrolysis in both, and very high to high soil mobility. Consequently, SFX may pose a risk to surface and subsurface water bodies (USEPA, 2019).

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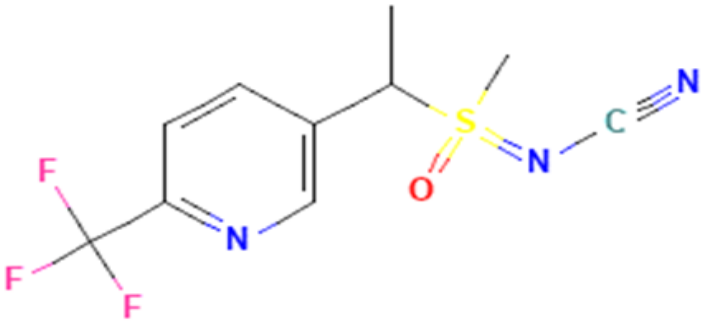


Fig. 1: Structural formula of SFX

Sulfoximine has received approval for use in a number of nations recently. Because they don't know enough about the sublethal effects of sulfoximines, biologists have stressed their use in threatening pollinators (Parkinson *et al.,* 2020). As a systemic insecticide, SFX may remain in plant nectar and pollen long after treatment, threatening both human and animal health. (Siviter *et al.,* 2018). Furthermore, earlier research has documented the possible danger of SFX in organisms that are not the intended targets, like bumblebee oxidative damage. (Siviter *et al.,* 2018) and insects (Wang *et al.,* 2018) in sublethal concentrations. Research on toxicokinetics demonstrated that in rats with poor metabolization, SFX is quickly absorbed and dispersed after oral administration. After treatment, SFX residue would linger in the soil, water, and plants for a few days; as a result, long-term bioaccumulation of SFX through the food chain may increase its toxicity.

Despite having more benefits than other insecticides, SFX was shown to change nicotinic acetylcholine receptors, produce neurotoxicity (Authority *et al.,* 2019), raise the newborn death rate (Ellis-Hutchings *et al.,* 2014), induce hepatotoxicity, and cause liver carcinomas in rodents (Lebaron *et al.,* 2014).

MATERIALS AND METHODS

1. Chemical

A commercially available sulfoxaflor (CAS no: 946578-00-3, [methyl (oxo) {1-[6-(trifluoromethyl) -3-pyridyl] ethyl}-λ6-sulfanylidene] cyanamide) called Closer 240 SC was obtained from Egyptian Seeds and Agricultural Chemicals Company, Tanta, Egypt.

2. Tested Animals and Husbandry

20 adult male Wistar albino rats (*Rattus norvegicus*) aged 12-14 weeks (240-297 g in weight) were obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Helwan, Egypt. The rats were maintained in the Animal House at the Department of Pesticides, Faculty of Agriculture, Menoufia University. The animals were kept under laboratory settings with a temperature of 25 ± 2 °C and a regular photoperiod (12:12 h light-dark cycle) in groups of 7 rats each in polyacrylic cages (38 cm × 23 cm 10 cm). The animals were fed standard pellets and allowed access to water *ad libitum* throughout the experimental period. They were acclimatized to laboratory conditions for two weeks before the commencement of the experiments. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Menoufia University (MUFAG/ S/ PS/ 2 / 23).

3. Experimental Design

Recovery experiment

Group I: Untreated animals (control) received tap water daily by oral gavage.

Group II: Each animal received a single dose of SFX 1100 mg/kg for 48 hours, followed by a recovery period of 1 month.

4. Body weight

The body weights of individual rats in each group were measured just before and after the completion of the treatments.

5. Blood sample collection

Blood was collected after 48 h of dosing and one day after the end of the experiments from the retro-orbital plexus under deep anesthesia after overnight starvation. The blood was collected in EDTA-treated tube for hematological analysis. The tube was carefully shaken several times to ensure thorough mixing before use for hematological analysis.

6. Complete blood picture

These parameters have been assessed in respect of complete blood count (CBC), red blood cell count (RBC's), haemoglobin (HGB), total platelet count (PLT), haematocrit (HCT), white blood cell count (WBC's), lymphocytes (LYM), neutrophil granulocytes (NEU), eosinophil granulocytes (EOS), basophil granulocytes (BAS), MON (monocytes). Haematology analyzer (MEDONIC CA620) was used to measure the CBC. (Mansour *et al.*, 2007)

7. Histopathological analysis

Tissue samples from the liver, spleen, bone marrow, thymus, Peyer's patches, and lymph nodes were fixed in 10% formalin. After gently shaking fixed tissues for 30 minutes in a succession of increasing alcohol concentrations (70, 80, 90, 95, and 100%), they were submerged in absolute ethanol for an entire night. Before the tissues were embedded in paraffin at 56 °C (30 min, 3 times), they were soaked in xylene three times for thirty minutes. Next, the tissues were divided into 4-µm slices using an Ambala microtome (Haryana, India) that was semi-automated digitally. Hematoxylin and eosin (H&E) was used for five minutes to stain the samples after they had been put on microscope slides. (Bancroft and Gamble, 2013). An Axiostar Plus (Carl Zeiss, Göttingen, Germany) microscope was used to view and take pictures of the stained slides. Zoom Browser Ex software was used to adapt the microscope to a Canon (Pc 1200 Power shot A641) digital camera.

8. Statistical analysis

Statistical analyses were conducted using SPSS software (version 21.0; SPSS Inc., Chicago, IL, United States). The data sets were compared using One-way ANOVA and Tukey's post hoc test. The data were represented as mean ±SD. Values were deemed statistically significant when $p \leq 0.05$.

Results

1. Acute toxicity of SFX and its recovery pattern

1.1. Body weight gain

In toxicological investigations, body weight is an essential criterion for assessing toxicity. Generally, a decrease or increase in body weight compared to normal may be considered a sign of toxicity, as shown in Tabel 1 during the current study. A significant increase (15.42 increase) in the body weights of control animals at the end of the study was observed compared to their initial weights. However, the weight gain of the rats intoxicated with SFX was much lower (12.14 decrease) compared to the control group ($P < 0.05$). This weight loss can be explained by a metabolic imbalance or reduced dietary intake of the rats.

1.2. Hematological parameters after exposure to SFX and recovery assessment

The findings on the impact of SFX at a dosage of 1100 mg/kg on hematological parameters are illustrated in Table 2. The highest substantial reductions of HGB (13.50 decrease), RBC count (7.54 decrease), and HCT (46.90 decrease) were spotted after 48 hours of exposure to SFX at 1100 mg/kg ($P < 0.05$).

Table 1: Impact of the single oral dose of SFX (1100 mg/kg) on body weight after 48 hours of exposure, followed by a recovery period (30 days).

Treatments	Control	SFX (1100 mg/kg)
Initial weight (gm)	295.67±5.86	242.67±10.69
Final weight (gm)	341.33±10.26	272±16.70
Net body (gain %)	15.42±1.24	12.14±2.31*

P value =<0.05
SFX: Sulfoxaflor
Net body wt. gain = [(Final weight - Initial weight) ÷ (Initial weight)] ×100

Table 2: Effect of the single oral dose of SFX (1100 mg/kg) on Complete Blood Count after 48 hours of exposure, followed by a recovery period (30 days).

Treatments	Control	SFX (1100 mg/kg) (48 hrs)	SFX (1100 mg/kg) (30 days)
HGB (G/DL)	16.33 ±0.03	13.50±0.05*	15.63±0.03*
RBC (10^6/UL)	10.00±0.50	7.54±0.04*	9.02±0.01*
HCT (%)	48.37±0.03	45.90±0.02*	47.33 ±0.03
PLT (10^3/UL)	925.00±5.00	813.67±3.00*	854.67±4.00*
WBC ($\times 10^3/MM^3$)	7.30±0.05	8.73±0.03*	8.07±0.02*
NEUT (%)	25.30±0.02	33.23±0.21*	25.00±2.04*
Lymph (%)	65.20± 0.02	60.45±1.00*	65.00±0.02*
Mono (%)	6.60±0.03	5.15±0.04*	6.45±0.01*
Eo (%)	2.70±0.70	1.07±0.38*	2.56±0.47*
Baso (%)	0.20±0.05	0.10±0.17*	0.15±0.05*

P value =<0.05
SFX: Sulfoxaflor
HGB :Hemoglobin, RBCs: Red blood cell, HCT :Hematocrit, PLT: Platelets, WBC: White blood cell, NEUT: Neutrophil, Lymph: Lymphocyte, Mono: Monocyte, Eo: Eosinophil, BASO: Basophil.

Following a 48-hour exposure to SFX, PLT (813.67 reduction) was decreased dramatically and progressively until the end of the recovery period ($P < 0.05$) (Table 2). The WBC and NEUT count increased considerably (8.73, 33.23 increase) following a 48-hour exposure to SFX (Table 2). They recovered on day 30 of the depuration procedure ($P < 0.05$). In contrast, after 48 hours of exposure to SFX, the following parameters decreased significantly: Lymph (60.45 decrease), Mono (5.15 decrease), Eo (1.07 decrease), and Baso (0.10 decrease). These parameters recovered on day 30 of the depuration process ($P < 0.05$).

1.3. Histopathological examination

A liver histopathological examination in the control group revealed the presence of a central vein flanked by cords of hepatic hepatocytes, each containing central vesicular nuclei that were partitioned by a blood sinusoid. Although the hepatocytes in the SFX-treated group were organized in a regular pattern around a somewhat central vein coated with smooth endothelium, some hepatocytes still had vacuolated cytoplasm and blood sinusoids (Fig. 2).

Figure 3 illustrates the H&E staining of bone marrow in both the control and group administered with SFX. The bone marrow

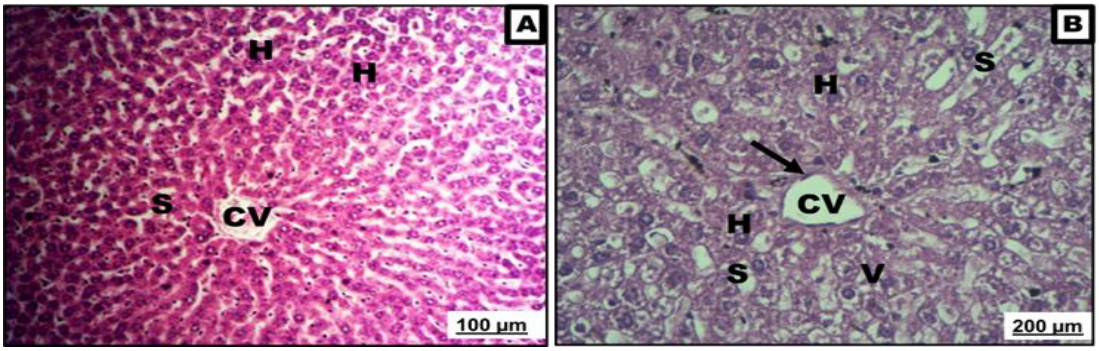
section from the control group exhibited a typical composition composed of fatty cells. Furthermore, the bone marrow section obtained from the group exposed to SFX demonstrated a well-organized structure consisting of blood cells interspersed with many fat cells.

Peyer's patches of the intestine histological observations of control rats showed lymphatic follicles covered by short villi of the intestine (Fig. 4). However, the microscopic examination of SFX-treated rat Peyer's patches revealed slightly disturbed patches.

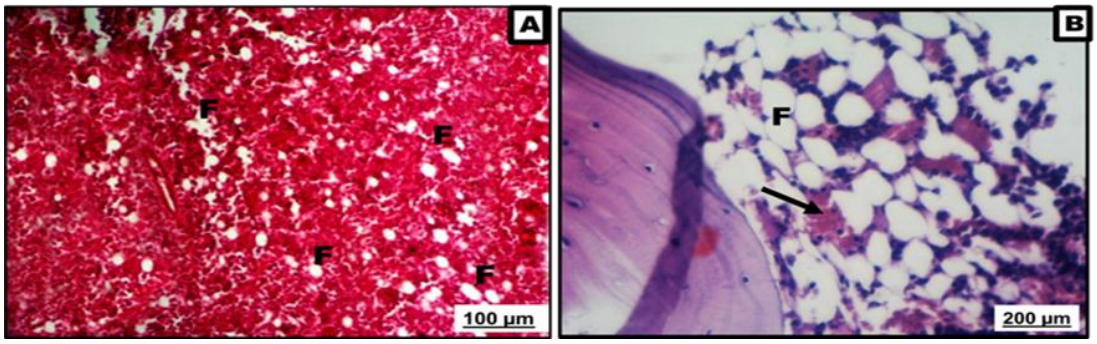
Figure 5 displays representative lymph node slide images from both the control and SFX-exposed groups. The findings from the control group demonstrated a typical architecture enveloped by a dense capsule composed of a lymphatic follicle-filled outer dark cortex and an inner medulla. In contrast, lymphatic nodules were distributed in a regular pattern, and there was some gap between the capsule and the follicle in the group that was exposed to SFX.

The histopathological impacts of SFX on the spleen of rats that were treated are displayed in Fig. 6 Spleen sections from the control group revealed the presence of lymphatic nodule-formed normal white pulp and blood sinusoid-formed red pulp. In contrast, the SFX-exposed group exhibited a marginal blurring of the line separating the white pulp from the red pulp in the spleen.

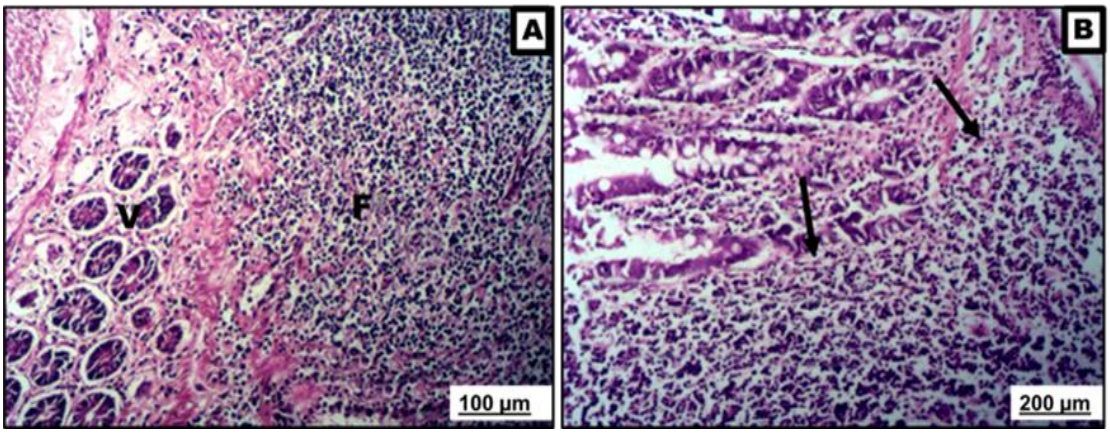
Thymus sections from the control rats exhibited normal lobule formation, with each lobule including an inner pale medulla and an outer dark cortex, as observed under a light microscope (Fig. 7). Nonetheless, the thymus in the SFX-exposed group exhibits a blurring of the boundary between the cortex and medulla.



(Fig. 2): Histopathology of the liver.



(Fig. 3): Histopathology of bone marrow.



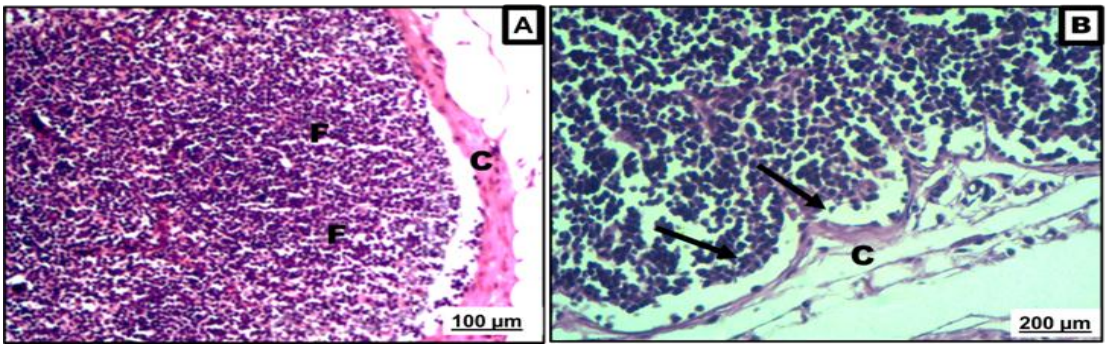
(Fig. 4): Histopathology of Peyer patches.

Fig. 2,3,4: Histopathology of the liver, bone marrow and Peyer patches.

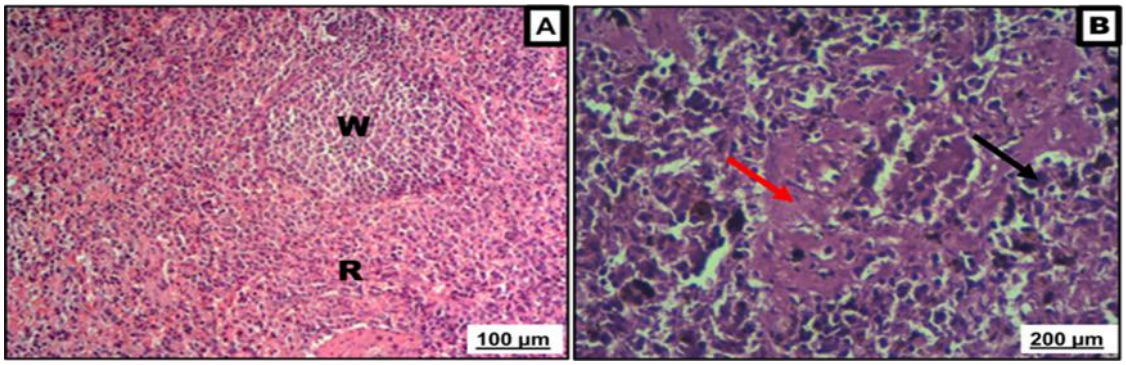
Representative micrographs of the liver, bone marrow and Peyer patches from control rats (A) and rats treated with 1100 mg/kg of SFX(B) showing: In (Fig. 2) (A) A photomicrograph of the liver of the control group showing central vein (CV) surrounded by hepatic cords of hepatocyte (H) with central vesicular nuclei, separated by blood sinusoid (S). (B) A photomicrograph of the effect of SFX at 1100 mg/kg on the liver shows regularly arranged hepatocyte (H) around a slightly central vein (CV) lined by smooth endothelium (Arrow). However, some hepatocytes still have congested blood sinusoids (S) and vacuolated cytoplasm (V). In (Fig. 3) (A) A photomicrograph of the bone marrow of the control group showing a typical structure filled with fat cells (F). (B) A photomicrograph of the effect of SFX at 1100 mg/kg on the bone marrow shows good bone marrow tissue architecture with blood cells (Arrow) and multiple fat cells in between (F). In (Fig. 4) (A) A photomicrograph of the Peyer's patches of the intestine of the control group showing lymphatic follicle (F) covered by short villi of the intestine (V). (B) A photomicrograph of the effect of SFX at 1100 mg/kg on Peyer's patches shows slightly disturbed patches (Dark arrow).

Fig. 5,6,7: Histopathology of lymph node, spleen and thymus.

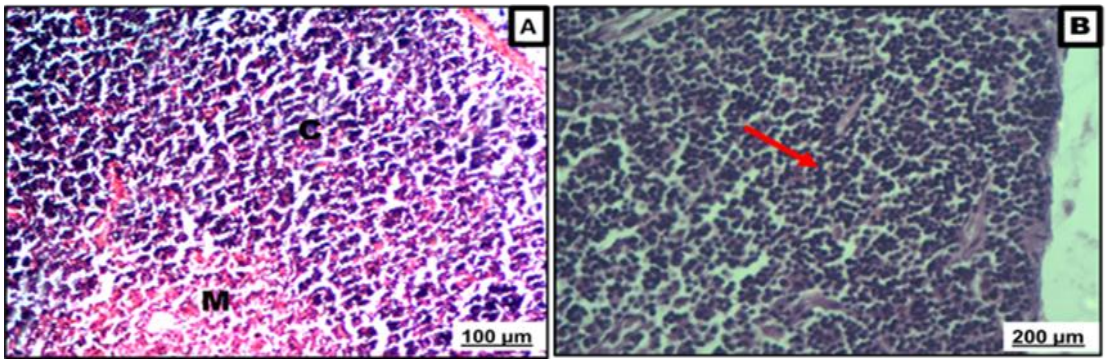
Representative micrographs of lymph nodes, spleen and thymus from control rats (A) and rats treated with 1100 mg/kg of SFX (B) showing: In (Fig. 5) (A) A photomicrograph of the lymph node of the control group showing standard structure covered by thick capsule (C) formed of outer dark cortex-filled with lymphatic follicles(F) and inner medulla. (B) A photomicrograph of the effect of SFX at 1100 mg/kg on the lymph node shows a thick capsule (C) and regularly arranged lymphatic nodule (Arrow); there is some separation between the capsule and the follicle. In (Fig. 6) (A) A photomicrograph of the spleen of the control group showing normal white pulp (W) formed of lymphatic nodules and red pulp (R) formed of blood sinusoid (S). (B) A photomicrograph of the effect of SFX at 1100 mg/kg on the spleen shows slight demarcation loss between white pulp (Dark arrow) and red pulp (Red arrow). In (Fig. 7) (A) A photomicrograph of the thymus of a control group formed of lobules, each lobule contains outer dark cortex (C) and inner pale medulla (M). (B) A photomicrograph of the effect of SFX at the dose of 1100 mg/kg on the thymus shows a loss of boundary between cortex and medulla (Dark arrow).



(Fig. 5): Histopathology of lymph node.



(Fig. 6): Histopathology of spleen.



(Fig. 7): Histopathology of thymus.

Discussion

This study showed that, after 30 days of recuperation following 48 hours of exposure to SFX, there was a considerable reduction in body weight increase. One of the most popular blood screening tests, the complete blood count (CBC), provides general health information about a person or an animal. The present study revealed that the number of RBCs, hemoglobin, HCT, and platelets suddenly declined following exposure to SFX for 48 hours. Additionally, WBCs and neutrophils increased in the SFX-intoxicated group, while lymphocytes, eosinophils, monocytes, and basophils decreased. These results are consistent with the findings of

(Sharma *et al.,* 2020), who reported that while WBC count increased after a single dosage of chlorpyrifos, RBCs, hemoglobin, and HCT decreased.

However, following a 30-day recovery period, the number of RBCs, platelets, and hemoglobin levels increased, and WBCs and neutrophils significantly decreased. A histological analysis of the thymus, liver, spleen, and Peyer patches indicated that SFX induced only a minor disruption. Nevertheless, the architecture of the bone marrow and lymph nodes was highly structured. The observed variations in most hematological and histological markers suggest that identifying these modifications may not require many days and that recuperation from pesticide exposure is fast.

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دراسة التأثيرات السمية الحادة لمبيد السلفوكسافلور الحشري على المؤشرات الدموية والمناعية في ذكور الجرذ الأبيض

إبراهيم محمود عمار، عادل غطاس يوسف، سماح سعيد عرفه، إسراء حسن عبدالوهاب

قسم مبيدات الآفات – كلية الزراعة – جامعة المنوفية

الملخص العربى

يعد مبيد سلفوكسافلور (SFX) أحد المبيدات الحشرية السلفوكسيمينية التي يتم تطبيقها على نطاق واسع في جميع أنحاء العالم، مما يؤدي إلى استمرار تعرض الإنسان لها. كان الهدف من الدراسة هو تقييم التغيرات الدموية والمناعية المحتملة الناجمة عن السمية الحادة للمبيد الحشري SFX في ذكور فئران البيضاء. ولتحقيق هذه الأهداف، تم تقسيم عشرين فأرًا ذكرًا عشوائيًا إلى مجموعتين متساويتين؛ تم استخدام المجموعة الأولى كمجموعة ضابطة؛ تم تعريض المجموعة الأخرى لـ ١١٠٠ ملجم/كجم من وزن الجسم من مبيد السلفوكسافلور عن طريق الفم لمدة ٤٨ ساعة، يليها فترة تعافي مدتها ٣٠ يومًا. تم فحص الأوزان النسبية للكبد والغدة الثيموسية والطحال صورة الدم الكاملة وتم إجراء دراسات هستوباثولوجية على الغدة الثموسيه ونخاع العظام والكبد والطحال وبقع باير. أظهرت النتائج أن التعرض لمبيد السلفوكسافلور أدى إلى انخفاض كبير في كرات الدم الحمراء والهيموجلوبين الهيماتوكريت والصفائح الدموية بعد التعرض لمبيد السلفوكسافلور لمدة ٤٨ ساعة. ومع ذلك، زادت كرات الدم البيضاء والنيتروفيل في المجموعة المعاملة بمبيد السلفوكسافلور، في حين انخفضت الخلايا الليمفاوية والايزينوفيل و المونوسيت والبازوفيل. أشار الفحص النسيجي المرضي للغدة الثيموسيه والكبد والطحال وعقد باير إلى أن مبيد السلفوكسافلور يسبب اضطرابًا بسيطًا فى الانسجة. ومع ذلك، فإن بنية النخاع العظمي والغدد الليمفاوية كانت منظمة للغاية. أظهرت نتائج الدراسة أن السمية الحادة لمبيد السلفوكسافلور أدت إلى تغيرات سامة للدم وسمية مناعية كانت قابلة للاسترداد بعد فترة من التعافي.