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Sulfoxaflor insecticide exhibits cytotoxic or genotoxic and apoptotic potential via oxidative stress-associated DNA damage in human blood lymphocytes cell cultures

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ABSTRACT

The need for foodstuff that emerged with the rapidly increasing world population made fertilizers and pesticides inevitable to obtain maximum efficiency from existing agricultural areas. Sulfoxaflor is currently the only member of the new sulfoximine insecticide subclass of nicotinic acetylcholine receptor agonists. In the study, it was aimed to determine the in vitro genetic, oxidative damage potential, genotoxic and apoptotic effects of three different concentrations ($10 \mu g/mL$, $20 \mu g/mL$ and $40 \mu g/mL$) of sulfoxaflor insecticide in the cultures of blood lymphocytes. In this study, the single-cell gel electrophoresis (comet), Cytokinesis Block Micronuclues Test (MN test), flow cytometry and measurement of Catalase (CAT) enzyme activity were used to determine genotoxic, apoptotic effects and oxidative damage potential, respectively. It found that there is a decrease in CPBI values and Live cell numbers. It was observed an increase in late apoptotic and necrotic cell numbers, Micronucleus frequency, and Comet analysis parameters (GDI and DCP). There is a significant difference between negative control and all concentration of insecticide for Cytokinesis Block Proliferation Index (CBPI) values and late apoptotic, necrotic and viable cell counts. An increase in CAT enzyme levels was observed at 10 and 20 µg/mL concentrations compared to control., It is found that CAT enzyme activity was inhibited at concentrations of $40 \,\mu$ g/mL. This study is crucial as it is the first study to investigate the impact of Sulfoxaflor insecticide on peripheral blood lymphocyte cells. The genotoxic, oxidative damage, and apoptotic effects of Sulfoxafluor insecticide on the results obtained and its adverse effects on other organisms raise concerns about health and safety.

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1. Introduction

The need for foodstuff that emerged with the rapidly increasing world population made the use of fertilizers and pesticides inevitable to obtain maximum efficiency from existing agricultural areas. Pesticides are generally defined as chemicals that cause the death of animals, microorganisms, insects, weeds, and other harmful creatures that damage agricultural products' production, storage, and consumption processes or reduce the damage they cause to agricultural products. Pesticides are used worldwide for agriculture, industry, public health, and domestic applications: as a result, a large proportion of the population may be exposed to these compounds (Carvalho 2017, Sabarwal *et al.* 2018).

The impact of synthetic pesticides on the environment, non-target organisms, and human health have received increasing attention. Sulfoxaflor is the ISO common name for [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}- λ 6sulfany-lidene]cyanamide. Sulfoxaflor is almost completely absorbed after oral administration and poorly metabolized; more than 93% is rapidly excreted unchanged in urine and feces (EFSA

2014). Sulfoxaflor is currently the only member of the new sulfoximine insecticide subclass (IARC subclass 4C) of nicotinic acetylcholine receptor (nAChR) agonists (Shepard 1939, Singh 2017). In experiments on fire ants (*Solenopsis invicta*), it has been observed that even at low concentrations, very severe chronic toxicity, nutritional deterioration, and colony growth maximization have been observed. Exposure of *Solenopsis invicta* to 1 and $2 \mu g/mL$ sulfoxaflor (LD₅₀ 750 mg/kg) for up to 28 days has been reported to result in colonies consuming less sugar water and collecting fewer seeds (Faroon and Harris 2002).

Today, pesticides are also known to affect non-target organisms. It has been reported to have adverse effects on non-target organisms. Many studies have reported that there is a relationship between pesticide exposure and cytogenetic damage. There are three main cytogenetic test methods (chromosomal aberrations, sister chromatid exchange and micronucleus) defined to measure cytogenetic damage. Micronucleus (MN) method is considered the most convenient and fastest (Çelik *et al.* 2003, Çelik and Akbaş 2005).

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Micronucleus assay has been used as a biomarker of DNA damage, chromosomal instability, cancer risk, and accelerated aging. The MN test has become a standard test used in genetic toxicology research because it can be applied *in vitro* and *in vivo* on all cells caused by mitosis. The increase in the number of micronuclei is considered an indirect indicator of the numerical and structural chromosomal irregularities created by various agents in cells. Agents that stimulate aneuploidy cause centromere cleavage errors and function disorders in spindle threads; clastogens contribute to the formation of MN chromosome breaks (Stopper and Müller 1997).

The Single Cell Gel Electrophoresis Method (comet assay) is a a fast, simple, sensitive quantitative method that is widely used for the last two decades on a cellular basis to identify genotoxicity in DNA and enables the measurement of DNA damage caused by various physical and chemical mutagenic factors with visual evidence (Collins *et al.* 1997, Çelik *et al.* 2013, Arisoy and Ataseven 2017). It has been published in the literature as a suitable test system to evaluate DNA damage. This method is based on the principle of conducting the DNA of single isolated cells in an electrophoretic environment in agarose gel prepared on a slide and evaluating the migrating DNA fragments with different charges and molecular weights under a fluorescent microscope after staining with DNA specific fluorescent dye according to the level of damage (Møller 2018).

In the flow cytometry, cells or particles in the liquid are passed through a chamber illuminated by laser light, and the stimuli given by the cells as they pass through the light are collected and analyzed. Here, the cells' physical properties such as size and granularity can be shown; antigenic structures on the cell surface or content can also be determined. Thus, information can be obtained about various properties of the cell, such as immune structure, DNA content, enzyme activity, cell membrane potential, and viability and apoptotic features of cells (Dunphy 2004, Atalay *et al.* 2018). It has been shown that it can analyze much more cells in a shorter time compared to light microscopy, and with this technique, an average of 10 000 cells can be evaluated in 20 seconds (Pozarowski *et al.* 2004, Toduka *et al.* 2012).

Catalase (CAT) breaks down hydrogen peroxide into molecular oxygen and water, thereby protecting the cell from damage by reactive oxygen derivatives. CAT is one of the essential enzymes which neutralize reactive oxygen forms (Glorieux and Calderon 2017). The purpose of this study was to investigate cytotoxicity, genoto-oxidative damage, and apoptotic potential of sulfoxaflor insecticide in human peripheral blood lymphocyte cells.

2. Material and method

2.1. Chemicals

Sulfoxaflor (99.97% purity) was purchased from LGC Labor GmbH (Ehrenstorfer, Germany). RPMI 1640 and Fetal Calf Serum were purchased from Sigma. Other chemicals or solvents used in this study were of cell culture, HPLC, or



Figure 1. The chemical structure of Sulfoxaflor.

analytical grade. The chemical structural formula of sulfoxaflor is shown in Figure 1.

2.2. Dose selection of sulfaxoflor insecticide

The reason for choosing this insecticide is that it is a new generation insecticide and there is no study in which its effect was observed. The concentration selection was determined by taking into account the concentrations of the insecticides that belong to the same group and show toxicity in previous studies (Çavaş *et al.* 2012, Stivaktakis *et al.* 2016). Çavaş *et al.* (2012) investigated the effects at 25 μ M concentration of acetamiprid on Caco-2 cells. These concentrations were chosen to study the effects at concentrations less than 25 micromolar. The doses used by Stivaktakis *et al.* (2016) in their in vivo study were based on the analysis based on the presence of insecticide in the blood serum of rabbits exposed orally to imidacloprid.

2.3. Subjects

Three healthy male nonsmoking donors (mean age, 24.75 ± 0.82 years) provided blood samples. Donors had not been exposed to radiation or drug 6 months prior to the study. Each donor was interviewed about the possible influence of confounding factors. Questionnaires were given to each blood donor to evaluate exposure history; and informed consent forms were signed by each of them. For all the donors, hematological and biochemical parameters were analyzed and no disease was detected. Ethical Committee at Mersin University Medical Faculty approved the experiments described in this study (2022/241).

2.4. Experimental design

2.4.1. Lymphocyte isolation and cultivation of cells in culture media

Blood samples were collected from heparinized tubes from three healthy male, nonsmoker, and healthy male donors. Lymphocytes cultures were prepared according to the technique of Moorhead *et al.* (1960) with slight modifications. 5 mL of whole blood was added to 5 mL of Histopaque-1077 and centrifuged for 30 minutes at 400 g. Lymphocytes were separated from the medium with the help of a micropipette. $500 \mu L PBS + 500 \mu L$ of lymphocyte mixture was prepared. $300 \mu L$ of this mixture was placed in labeled tubes containing 5 mL of RPMI 1640 medium. At beginning of culture, solutions of sulfoxaflor insecticide ($10 \mu g/m L$, $20 \mu g/m L$ and $40 \mu g/m L$) were added to tubes and $10 \text{ mM} H_2O_2$ was used for positive control. No chemicals were used in the negative control groups. Single-cell gel electrophoresis analysis, MN test, and flow cytometry methods were applied and activity of Catalase enzyme was measured.

2.4.2. Application of single cell gel electrophoresis (comet) method

Single Cell Gel Electrophoresis was carried out with lymphocytes from three donors, as Singh et al. (1988) stated with slight modifications. Shortly, 100 µL of lymphocyte cell suspension and 100 µL of 2% low melting agarose were mixed at 37 °C and then situated on a slide precoated with a thin layer of 0.5% standard melting agarose. The cell suspension was immediately covered with a cover glass, and the slides were held for 5 min at 4 °C to solidify the agarose. After removing the cover glass, the cells were placed in a lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mMTris, 1% Triton X-100, pH 10) for 1 hour at ⁺4⁰C. After washing in distilled water, the slides were placed in a horizontal gel electrophoresis chamber filled with a cold electrophoretic buffer (1 mM EDTA, 300 mM NaOH, pH 13). Slides were kept at 4 °C for 20 min to allow the DNA to unwind. Then electrophoresis was conducted at 20°C using 25V and 300 mA for 20 min. After electrophoresis application, the slides were treated three times with a neutralization buffer (0.4 M Tris, pH 7.5). All preparative steps were conducted in the dark to prevent undesired DNA damage. The slides were stained with ethidium bromide (0.1 mg/mL, 1:4) and analyzed with a fluorescence microscope (Olympus BX 51) equipped with a CCD-4230 A video camera (Celik et al. 2014, Battal et al. 2015).

2.4.3. Slide scoring

Two slides and fifty cells per slide were evaluated per sample in order to determine the DNA damage in each experimental sample. The slides were scored blindly to prevent bias by a researcher (C.S.). The damages in cells were classified by eye into the five categories on the basis of the extent of DNA migration as undamaged (type 0), very little damage (type 1), moderate damage (type II), high damage (type III), and ultrahigh damage (type IV). In the comet assay, two different parameters were evaluated. i. Genetic damage index (GDI) ii. Damaged cell percentage (DCP), in the formula below;

Genetic damage index (GDI): 0xType 0+1xType I+2xType II + 3xType III + 4xType IV

Damaged cell percentage (DCP): Typell+Typell+TypelV (Çelik et al. 2014, Battal et al. 2015)

2.4.4. Micronucleus test

MN test was performed according to the technique described by Fenech and Morley (1986) with slight modifications. Isolated lymphocyte coats from heparinized blood samples were obtained from three healthy individuals. As a positive control, the Mitomycin C ($2 \mu g/mL$) substance was used. Sterile distilled water was used as a negative control. Isolated lymphocyte coats were prepared and numbered before hand and put into tubes containing nutrient medium.

Positive/Negative controls and three different concentrations of Sulfoxaflor insecticide (10 µg/mL, 20 µg/mL, and 40 µg/mL) solutions were prepared for each individual. Phytohemoglutunin was added into each tube. The Mitomycin-C (2 µg/mL) solution was added to positive control tubes. Culture tubes were incubated at 37 °C for 72 h. At the end of the 44th hour, Cytochalazine-B (6 µg/mL) was added to all MN tubes. At the end of 72 h. of incubation, the tubes were centrifuged at 2000 rpm. The hypotonic solution was added to the precipitate. It was kept at room temperature for 5 minutes. It was centrifuged at 2000 rpm for 10 minutes. The supernatant is discarded. The fixative solution was added and centrifuged. This process was repeated at least five times. Finally, the fixative solution was added to the precipitate and spread on slides. MN preparates were stained with Giemsa dye solution (10%). The preparates were examined under a light microscope.

2.4.5. Scoring criteria for micronuclei and cytokinesisblock proliferation index

In order to determine the frequency of binucleated cells including micronuclei (BNMN) and the total number of MN in lymphocytes (MNL), a total of 2000 binucleated cells with well-preserved cytoplasm were scored exactly for each subject and each concentration on coded slides. Three criteria were considered for micronucleus count. (i) they were separated from the prominent nuclei but included within the corresponding cytoplasm, (ii) they had a chromatin material similar to that of the primary nuclei, (iii) they were coplanar to the central nuclei (Celik 2006).

The toxic effect was evaluated in the MN study by classifying cells according to the number of nuclei. The well-known cytotoxicity index was used: an index for measuring the cell proliferation kinetics, called the cytokinesis-block proliferation index (CBPI), which was calculated following the expression: where MI–MIV represents the numbers of cells with one to four nuclei, respectively, and MIII and MIV are equally considered to be in their third cell cycle. As previously demonstrated, the CBPI is an accurate and biologically relevant index in detecting cellular toxicity or cell-cycle delay (Fenech and Morley 1986).

2.4.6. Flow cytometry method/apoptosis assay

Annexin V-FITC apoptosis detection kit with PI was utilized to specify cellular apoptosis or necrosis. The kit is based on observation of the translocation of the membrane phosphatidylserine from the inner side of the plasma membrane to the cell outer side, which can be easily detected by staining with a fluorescent dye Annexin V. This protein has a high relation for phosphatidylserine, conjugated to FITC. Late and early apoptotic cells, live cells, and necrotic cells were counted in the flow cytometry analyses. The cells are grouped according to their staining properties. Approximately 4×10^5 cells were passed through flow cytometry to determine the amount of drift of the human blood lymphocyte cells exposed to three different concentrations of sulfoxaflor insecticide to the apoptotic process (Atalay *et al.* 2018).

2.4.7. Measurement of catalase enzyme level in lymphocyte cell cultures

The Measurement of the catalase (CAT) enzyme level was calculated according to the method of Velikova et al. (2000), considering the reduction of hydrogen peroxide at 240 nm. Blood was drawn into heparin tubes from three healthy male individuals, 25 years old, who did not smoke and drink alcohol. 5 mL of whole blood was added to 5 mL of Histopaque-1077 and centrifuged at 400 g for 30 minutes. Lymphocytes were separated from the environment with the help of a micro pipet. $500 \,\mu\text{L}$ PBS + $500 \,\mu\text{L}$ lymphocyte mixture was prepared. 300 µL of this mixture was placed in numbered tubes containing 5 mL of medium. The tubes were closed and left to incubate for 72 hours in a 37° C oven at a 45° angle. At the 72nd hour, Sulfoxaflor was was added in tubes at the doses of $10 \mu g/mL$, $20 \mu g/mL$, and $40 \mu g/mL$ for each individual and left to incubation for 3 hours. At the end of 3 hours, the tubes were taken out of the oven and centrifuged at 2000 rpm for 10 minutes. Washing was done with PBS 3 times. Cells were frozen and thawed. It was read with a spectrophotometer at 240 nm.

2.5. Statistical analysis

Normality control for all parameters was done with Shapiro Wilk's test. SPSS 20.0 analysis program was used to evaluate the data obtained as a result of the experimental protocols. In order to examine whether there was a statistically significant difference between the results, the applied concentrations were compared among themselves, between positive control values and negative control values. The averages of the data obtained from the experimental protocols were used for all analyzes. The differences between the groups were analyzed with the ANOVA program. The difference between multiple comparisons was acquired by the Least Significant Difference (LSD) test method. In evaluations, the p values (confidence interval) was taken as 0.05. Pearson correlation analysis was applied to determine the relationship between comet data, MN frequency, CBPI values, and flow cytometry parameters.

3. Results

3.1. Comet analysis and micronucleus assay

Table 1 represents the observed values of comet analysis and MN frequencies/CBPI values in human blood lymphocytes treated with three different concentrations of sulfoxaflor insecticide, respectively. As seen from Table 1, sulfoxaflor insecticide induced DNA damage, including values of GDI and DCP, indicating the increase in comet levels (Type 1, Type 2, Type 3, Type 4). GDI and DCP parameters increased in parallel with the sulfoxaflor insecticide concentration (Figure 2(a)). There was no significant difference between the 10 µg/mL concentration group and the negative control group. There is a statistically significant difference between the 20 and 40 µg/mL concentration groups and the negative control group (p < 0.01). Figure 2(b) presents the comet assay views. Sulfoxaflor insecticide caused the increase in MN frequency, decrease in CBPI values (Figure 3(a)). While the MN frequency was 4.30 ± 0.66 in the control group, it was 11.2 ± 2.00 in the $10 \mu g/mL$ concentration group, 9.30 ± 0.88 in the $20 \mu g/mL$ concentration group, and 12.3 ± 1.45 in the 40 µg/mL concentration group. While the CBPI value was 2.50 ± 0.01 in the control group, it was 1.77 ± 0.00 in the $10 \,\mu$ g/mL concentration group, 1.82 ± 0.00 in the $20 \,\mu$ g/mL concentration group and 1.82 ± 0.00 in the 40 µg/mL concentration group. Figure 3(b) shows MN views in binucleated cells. There is a significant difference between negative control and all concentration groups of insecticide for CBPI values (*p* < 0.001).

3.2. Flow cytometry/apoptotic assay

The data obtained as a result of flow cytometric analysis applied to lymphocyte cells exposed to sulfoxaflor insecticide are presented in Table 2. Figure 4 shows cell viability in human blood lymphocytes treated with three different concentrations of sulfoxaflor insecticide. According to flow cytometry analysis Sulfoxaflor insecticide caused an increase early/late apoptotic and necrotic cell number. While early/late

Table 1.	Distribution of	[:] damaged c	ells among th	e different	classes of	damage, g	genetic da	image index,	damaged	cell p	ercentega	and MN	l frequency,	Cytokinesis
block pro	oliferation index	Score in Re	ference to the	Genotoxic	ity Test in	the huma	n blood ly	mphocyte ce	ells treated	with	sulfoxaflor	insectic	ide <i>in vitro</i> .	

Treatment Sulfoxaflor	Repeat								GDI DCP MN		MN	CBPI	
insecticide	number	Type 0	Type I	Type II	Type III	Type IV	MN	CBPI	$Mean \pm SE$	$Mean \pm SE$	$Mean \pm SE$	Mean ± SE	
10 μg/mL	1th repeat	47	43	8	2	0	9	1,772	87.3±11.2	19.6±8.73	11.2 ± 2.00**	1.77 ± 0.00***	
	2th repeat	47	26	14	10	3	11	1,774					
	3th repeat	33	45	13	6	3	13	1,771					
20 µg/mL	1th repeat	18	44	23	11	4	8	1,822	141.6±11.0**	39.6±12.4**	$9.30 \pm 0.88^{*}$	1.82±0.00***	
	2th repeat	24	57	17	1	1	11	1,824					
	3th repeat	3	35	38	19	5	9	1,821					
40 μg/mL	1th repeat	7	44	27	18	4	12	1,826	165.3 ± 5.92**	50.3 ± 1.33**	12.3 ± 1.45**	$1.82 \pm 0.00^{***}$	
	2th repeat	23	28	29	12	8	10	1,824					
	3th repeat	8	39	32	13	8	15	1,823					
NC (SDW)	1th repeat	34	53	9	2	2	5	2,488	74.6 ± 10.8	11.3 ± 2.72	4.30 ± 0.66	2.50 ± 0.01	
	2th repeat	55	39	4	2	0	3	2,504					
	3th repeat	33	52	11	4	0	5	2,522					
PC (H_2O_2)	1th repeat	4	10	14	24	48	22	1,600	324.0±11.3***	90.6±2.40***	21.6±0.88***	1.56 ± 0.01***	
10mM (Comet)	2th repeat	0	6	8	26	60	23	1,564					
MMC (MN test)	3th repeat	2	6	8	28	56	20	1,542					

DCP: Damaged Cell Percentage; GDI: Genetic Damage Index; NC: Negative Control; PC: Positive Control; SDW: sterilled distilled water; MMC: Mytomycin C. *p < 0.05; **p < 0.01; ***p < 0.001.



Figure 2. (a) Frequency of GDI and DCP in relation to Sulfoxaflor concentrations in Lymphocyte cells. (b) Comet views in human blood lymhocyte cell treated with sulfoxaflor insecticide in vitro.

apoptotic and necrotic cell number was 0.00 ± 0.00 , 0.00 ± 0.00 and 0.43 ± 0.08 in control group these data reached 0.76 ± 0.20 1.20 ± 0.05 and 3.60 ± 0.30 in $10\,\mu$ g/mL concentration group, respectively, 1.00 ± 0.28 1.20 ± 0.36 and 3.00 ± 0.55 in the $20\,\mu$ g/mL concentration group, respectively and 1.20 ± 0.20 , 1.86 ± 0.16 and 4.03 ± 0.94 in the $40\,\mu$ g/mL concentration group, respectively.

A statistically significant difference was found between all concentrations of sulfoxaflor insecticide and negative control in terms of late apoptotic, necrotic and viable cell counts. There is a statistically significant difference between the $40\,\mu\text{g/mL}$ concentration group, the highest dose group, and the control group regarding early apoptotic cell count.

3.3. Catalase enzyme activity

Table 3 exhibits CAT enzyme levels for sulfoxaflor concentrations. An increase in CAT enzyme levels was observed at 10 and $20 \,\mu$ g/mL concentrations compared to control. At concentrations of $40 \,\mu$ g/mL, CAT enzyme activity was inhibited (Figure 5). While CAT enzyme activity was 0.0697 (U/mL) in



(B)

(A)



Figure 3. (a) Frequency of Micronucleus and CBPI values in relation to Sulfoxaflor concentrations in Lymphocyte cells. (b) Micronucleated cell (a)- and mono (b)-bi (c)-trinucleated (d) cells in human peripheral blood treated with sulfoxaflor insecticide. Arrow indicates micronucleus.

the control group, it increased to 0.0735 (U/mL) in the 10 μ g/mL concentration group and to 0.0746 (U/mL) in the 20 μ g/mL concentration group. However, the 40 μ g/mL concentration group decreased to 0.0642 (U/mL) because the activity was suppressed.

3.4. Correlation analysis results

Table 4 represents the results of Pearson correlation analysis indicating the relationship between comet data, MN frequency, CBPI values, and flow cytometry parameters. A very

Table 2. Flov	v cytometry re	sults in the hum	an blood lymphocyte	e cells treated wit	h sulfoxaflor	insecticide in vitro
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Treatment		Examined				
Sulfoxaflor	Repeat	Cell				
insecticide	number	number	EAC (%)	LAC (%)	LC (%)	NEC (%)
10 μg/mL	1th repeat	10 341	0.8	1.3	94.1	3.8
	2th repeat	10 809	0.4	1.1	94.4	4
	3th repeat	11 277	1.1	1.2	94.7	3
	mean ± SE	10 809 ± 270.19	0.76 ± 0.20	$1.20 \pm 0.05^{**}$	94.40 ± 0.17 ^{**}	$3.60 \pm 0.30^{**}$
20 μg/mL	1th repeat	10 229	1	1.1	94.4	3.5
	2th repeat	9136	1.5	1.9	93	3.6
	3th repeat	10 669	0.5	0.6	96.9	1.9
	mean \pm SE	10 011.33 ± 455.72	1.00 ± 0.28	$1.20 \pm 0.36^{**}$	94.76 ± 1.14 ^{**}	$3.00 \pm 0.55^{**}$
40 μg/mL	1th repeat	7691	1.4	1.7	94	2.9
	2th repeat	7290	0.8	2.2	91.1	5.9
	3th repeat	7490	1.4	1.7	93.6	3.3
	mean ± SE	7490.33 ± 115.75	$1.20 \pm 0.20^{**}$	$1.86 \pm 0.16^{***}$	$92.90 \pm 0.90^{***}$	$4.03 \pm 0.94^{**}$
NC (SDW)	1th repeat	2588	0	0	99.7	0.3
	2th repeat	3049	0	0	99.6	0.4
	3th repeat	3062	0	0	99.4	0.6
	mean ± SE	2899.66 ± 155.87	0.00 ± 0.00	0.00 ± 0.00	99.56 ± 0.08	0.43 ± 0.08
PC (H_2O_2)	1th repeat	4681	27.8	0.6	70.3	1.1
10mM	2th repeat	4401	25.8	0.7	72.2	1.4
	3th repeat	4866	25.6	0.3	73.1	1.1
	mean ± SE	4649.33 ± 135.16	$26.40 \pm 0.70^{**}$	$\textbf{0.53}\pm\textbf{0.12}$	$71.86 \pm 0.82^{***}$	1.20 ± 0.10

EAC: Early Apoptotic Cell; LAC: Late Apoptotic Cell; LC: Live Cell; NEC: Necrotic Cell NC: Negative Control; PC: Positive Control; SDW: Sterilled Distilled Water **p < 0.01, ***p < 0.001.



Figure 4. The human lymphocyte cells treated with different concentrations of sulfoxaflor insecticide for 72 h. Cell apoptosis rate after Annexin V-FITC/PI staining was detected by flow cytometry.

Table 3. Catalase enzyme acyivity results in lymphocyte cells treated with sulfoxaflor insecticide.

Treatments (Sulfoxaflor insecticide)	Catalase enzyme activity (U/mL)
10 μg/mL	$0.0735 \pm 0.001^{*}$
20 µg/mL	$0.0746 \pm 0.0002^{**}$
40 μg/mL	$0.0642 \pm 0.002^{**}$
NC (SDW)	0.0697 ± 0.0006
	and a state of state of a

NC (SDW): Negative control (sterilled distilled water) *p < 0.05, **p < 0.01.

high correlation was found between comet analysis parameters and Micronucleus frequency. There is a very high negative correlation between CBPI value and MN frequency, whereas a moderate negative correlation value between comet parameters and CBPI draws attention. A very high correlation was found between the number of early apoptotic cells, one of the flow cytometry analysis parameters, and both the comet parameters (GDI and DCP) and the micronucleus frequency. Treatment with sulfoxaflor insecticide resulted in increased number of early-stage apoptotic cells, which reached a peak level after a dose of $40 \,\mu$ g/mL. The frequency of late apoptotic cells gradually increased with a dose and reached a peak level after a dose of $40 \,\mu$ g/mL. A very good correlation (r = 0.907) was obtained between the apoptotic indices measured by the comet assay and flow cytometry, although a higher level of apoptosis was observed by the latter method.

4. Discussion

Sulfaxoflor insecticide showed statistically significant genotoxic and apoptotic effects on human peripheral blood lymphocytes cells, and this effect was dose-dependent. The micronucleus frequency and GDI and DCP values obtained from the comet analysis increased at all concentrations. Based on flow cytometry analysis, there were toxic effects on human peripheral blood lymphocytes by Sulfaxoflor insecticide, which was statistically significant. Moreover, this effect was more robust at higher concentrations of the insecticide.



Figure 5. Catalase enzyme acticity in the human lymphocyte cells treated with different concentrations of sulfoxaflor insecticide.

Flow cytometry results showed an increase in apoptotic and necrotic cell counts at all concentrations. It is found that there is a correlation between flow cytometry data and comet analysis parameters. In this study, we investigated the potential of oxidative damage of sulfoxaflor insecticide measuring CAT enzyme levels. It was observed that the catalase enzyme activity in the cells increased at $10 \,\mu$ g/mL and $20 \,\mu$ g/mL concentrations of sulfoxaflor insecticide (p < 0.05). However, it was observed that catalase enzyme activity was inhibited in cells at the highest concentration ($40 \,\mu$ g/mL) (p < 0.01).

Similar in vivo and in vitro studies using different organism types of other insecticides belonging to the neonicotinoid group have shown that they have oxidative, genotoxic, and apoptotic damage effects. In the study performed by Stivaktakis et al. (2016), genotoxic effects of Imidacloprid insecticide was investigated on rabbit lymphocytes. In their study, three male rabbits were fed with imidacloprid mixed water at 40 µg/mL doses and 80 µg/mL three times a week for four months. There was no statistically significant difference between the control and exposed groups for Micronucleus frequencies at 0 months after the first exposure. Two months after the start of imidacloprid administration, a statistically significant increase was observed in the micronucleus frequency in both groups (p < 0.001). After four months of exposure, statistically significant differences in Micronucleus frequency were reported between the control and the two exposed groups (p < 0.001). There was no statistically significant difference in the CBPI value between the control and exposed groups at any exposure time. This result highlights that imidaclorbid insecticide has no toxic effect. A statistically significant increase in MN in both groups after 2 and 4 months of exposure proved that it had a genotoxic effect. This assay is a very effectual tool for the evaluation of the genetic risk posed by exposure to chemicals, including medicine agriculture. So, this tecnique can help in understanding the mode of action of the pesticide, categorizing them as clastogens or aneugen (Bolognesi et al. 2011). Schwarzbacherová et al. (2019) investigated oxidative stress, apoptotic and genotoxic effects on bovine lymphocytes exposed to Thiakloprid for 4 hours. They observed that an increase of up to 28% in the fraction of late apoptosis cells and a parallel decrease in the fraction of viable cells (57.6%) at all concentrations of insectiside. In the analysis of Oxidative stress; Firstly, mitochondrial

Table 4. Pearson correlation results among flow cytometry, comet analysis data, micronucleus values and catalase activity.

	NEC	LC	LAC	EAC	CBPI	MN	DCP	GDI	
212**	0.216	0.017***	0.0%6	0.007***	0.662**	0 002***	0 002***	1	
-0 315**	-0.210 -0.113*	-0.917	-0.080	0.907	-0.003**	0.892	0.092	۱ ೧	
-0.059	-0.018	-0.925***	0.143*	0.869***	-0.812***	1	0.865***	0.892***	MN
-0.134*	-0.444**	0.690***	-0.498**	-0.551**	1	-0.812***	-0.700**	-0.663**	CBPI
-0.054	-0.371**	-0.977**	-0.266**	1	-0.551**	0.869***	0.835***	0.907***	EAC
-0.278**	0.910***	0.063	1	-0.266	-0.498**	0.143*	0.003	-0.086	LAC
0.178*	0.166*	1	0.063	-0.977**	0.690**	-0.925**	-0.863**	-0.917**	LC
-0.161*	1	0.166	0.910**	-0.371	-0.444**	-0.018	-0.113*	-0.216*	NEC
1	-0.161*	0.178*	-0.278*	-0.054	-0.134*	-0.059	-0.315**	-0.313**	CAT
- - - -	-0.113* -0.018 -0.444** -0.371** 0.910*** 0.166* 1 -0.161*	-0.863*** -0.925*** 0.690*** -0.977** 0.063 1 0.166 0.178*	0.003 0.143* -0.498** -0.266** 1 0.063 0.910** -0.278*	0.835*** 0.869*** -0.551** 1 -0.266 -0.977** -0.371 -0.054	-0.700** -0.812*** 1 -0.551** -0.498** 0.690** -0.444** -0.134*	0.865*** 1 -0.812*** 0.869*** 0.143* -0.925** -0.018 -0.059	1 0.865*** 0.835*** 0.003 -0.863** -0.113* -0.315**	0.985*** 0.892*** -0.663** 0.907*** -0.086 -0.917** -0.216* -0.313**	DCP MN CBPI EAC LAC LC NEC CAT

*Low correlation, **medium correlation, ***high correlation.

GDI: Genetic damage index; DCP: damaged cell percentage; MN: micronucleus; CBPI: Cytogenesis block proliferation index; EAC: early apoptosis; LAC: Late apoptosis; LC: Live cell; NEC: Necrotic cell; Cat: Catalase. superoxide levels were evaluated, and significant levels were reported at concentrations of 60, 120, and 240 µg/mL. They observed the significant increases in MN frequencies in binuclear bovine lymphocytes at 120 and 240 µg/mL concentartions,. Flow cytometry analysis obtained in this study also showed a significant increase when compared to the control group (p < 0.05). There is a parallelism between the results of both studies. A slight increase in CAT enzyme levels in the concentration group of 10 and 20 µg/mL compared to the control group indicates that Sulfoxaflor insecticide has an oxidative effect. However, the CAT enzyme was inhibited in the concentration group of 40 µg/ mL. Calderón-Segura et al. (2015) examined the genotoxic effects of the insecticide Clothianid, which belongs to the neonicotinoid group, on CD1 mice. CD1 mice were exposed to Clothianidin at doses of 20, 40, 80 mg/kg by intraperitoneal injection every three days for 21 days. Comet and MN tests were performed to examine the genotoxic effects of Clothianidin insecticide on CD1 mice on peripheral blood samples taken from mice. It has been reported that doseand time-dependent increases in micronucleus frequency are induced in erythrocytes of CD1 mice exposed to Clothianidin for 21 days. It was observed that the Clothianidin insecticide induced statistically a increase in the comet, tail length, and tail moment compared to the control group in the comet analysis. Annabi et al. (2019) investigated the genotoxic and cytotoxic effects of acetamiprid on PC12 cells by MTT and comet tests. For the MTT test, PC12 cells were treated with Aseptamiprid at doses of 100–700 μ M for 24 hours at 37 °C and 110, 220, 330 μ M for the Comet test. Induction of DNA damage was observed in PC12 cells after 24 hours of exposure to Aseptamiprid. It has been observed that acetamiprid causes an increase in DNA damage due to an increase in concentration on PC12 cells. After 24 hours of exposure to Aseptamiprid at the highest concentration, the amount of DNA damage reached approximately 4 times the negative control value. In our study, DNA damage data induced by sulfoxaflor insecticide reached 2-2.5 times that of the negative control group. Aseptamiprid insecticide has been reported to significantly reduce the viability of PC12 cells as assessed by the MTT assay. Flow cytometry results in our study and MTT data in the study of Annabi et al. support each other. Bivehed et al. (2020) investigated the DNA damage effect of Nitenpyram insecticide, which belongs to the neonicotinoid group, on U-937 cells (human lymphoma cell line). The U937 cell line was treated with Nitenpyram insecticide at concentrations of 0.5, 5, 20, 35, 50 µM for 3 hours. The Comet test was used to examine the effect of DNA damage. When tested at concentrations up to 50 µM, Nitenpiram was found to increase the level of DNA damage in U-937 cells after 3 hours of exposure without metabolic activation. When evaluated in terms of comet data, in which the damaging effects on cells were observed due to the fact that it is an insecticide belonging to the same group, it is noteworthy that the two studies support each other. Atlı Şekeroğlu et al. (2019) investigated the cytotoxic and genotoxic effects of Clothianid on human lymphocyte cultures using chromosomal aberration (CA) and Micronucleus (MN) tests.

Lymphocytes were treated with Clothianidin insecticide at doses of 25, 50, and 100 µg/mL for 3 hours. Based on the results of this study, Clothianidin was reported to induce cytotoxicity and genotoxicity at high concentrations in human peripheral blood lymphocytes under these experimental conditions. The increase in genotoxic effect of clothianidin insecticide in parallel with the increase in concentration indicates that it belongs to the same group as a sulfoxaflor insecticide and has a similar effect. Ge et al. (2015) investigated effects on oxidative stress and DNA damage of Imidacloprid insecticide on Zebrafish were investigated, Catalase enzyme activity and Comet tests, respectively; DNA damage were performed in zebrafish liver tissue exposed to Imidacloprid insecticide at doses of 0.3, 1.25, and 5 mg/mL for 28 days. It is known that the extent of DNA damage is the main factor determining whether a cell is arrested or undergoes apoptosis (Dixit et al. 2012). In our study, the increase in apoptotic cells and the increase in DNA damage parameters are consistent. Significant differences in Catalase activity between fish exposed to imidacloprid and the control group were observed only during the first week of exposure. After this time point, no differences were observed between the different administration groups except for the 0.3 mg/mL Imidacloprid group on days 14 and 28. Compared to controls, significant increases in CAT activities were observed on days 14 and 28 in the 0.3 mg/ mL imidacloprid group. Fish exposed to imidacloprid was found to differ significantly from fish in the control group in terms of DNA damage. Levels of DNA damage in zebrafish have been reported to increase markedly with prolonged exposure to the Imidacloprid insecticide. This study performed by Ge et al. and our study are similar in terms of enzyme activity and comet analysis results. In our study, an increase was observed in CAT activity at 10 and 20 microgram/mL doses of sulfoxaflor insecticide, but the CAT enzyme was inhibited at 40 µg/mL doses. Loss of catalase activity is associated with increased susceptibility to oxidative stress. Benli and Celik (2021) reported that lipid peroxidation increased significantly due to oxidative stress with sulfoxaflor treatment. Fathy and Abdelkader (2021) also stated that sulfoxaflor causes oxidative stress. In our study, low-dose sulfoxaflor administration may have triggered a partial radical increase and stimulated the increase in catalase activity. However, increasing the dose of sulfoxaflor may have caused the inhibition of the catalase enzyme activity by increasing the content of intrinsic radicals to high levels. Conducting studies that show the relationship between the content of internal radicals and antioxidant enzyme activities with the treatment of sulfoxaflor may contribute to understanding this subject. We also see that the sulfoxaflor insecticide causes oxidative damage from the statistical increases in the GDI and DCP values evaluated in the comet analysis. Senyildiz et al. (2018) investigated the possible effects of neonicotinoid insecticides (Aseptamiprid, Clothianidin, Imidacloprid, Thiakloprid, and Thiamethoksam), which are widely used by A.S., on cytotoxicity and DNA damage in human neuroblastoma (SH-SY5Y) and human hepatocellular carcinoma (HepG2) cells. For the MTT test, neonicotinoid insecticides were applied to cell lines at concentrations of 0.125 mM, 0.25 mM, 0.5 mM, 1.2 mM, 4 mM, and 24 mM for 24 hours. Concentrations of 50, 100, 200, and 500 μ M were used for the comet test. In MTT test results, it was observed that neonicotinoids caused a significant decrease in cell viability in SH-SY5Y and HepG2 cells depending on the concentration. It was observed that all insecticides used at 500 mM concentrations in SHSY-5Y cells caused significant DNA damage, while only Imidacloprid, Thiamethoxam, and Thiacloprid insecticides were reported to cause some changes in HepG2 cells after 24 hours of exposure. In our study, genotoxic and apoptotic damage data obtained by Comet and flow cytometry analysis tests also show a significant increase when compared to the control group (p < 0.05). There is a parallelism between the results of both studies.

Çavaş et al. (2012) In the study in which the cytotoxicity and genotoxicity of the Aseptamiprid insecticide belonging to the neonicotinoid group on human intestinal (CaCo-2) cells were evaluated by Comet, MN, and CBPI test methods, the cells were treated with Aseptamiprid at 25, 50, 75, 150 and $300\,\mu\text{M}$ concentrations for 24 hours as a result of Aseptamiprid treatment. It was observed that the insecticide significantly increased the GDI value in human intestinal cells. A high but non-cytotoxic concentration of Aseptamiprid $(50\,\mu\text{M})$ was found to cause a two-fold increase in MN frequency. 75, 150, 300 µg/mL concentrations of other cytotoxic Aseptamiprid have also been reported to increase MN frequency significantly. In our study, genotoxic damage data obtained by Comet and Micronucleus tests also show a significant increase compared to the control group (p < 0.05). There is a parallelism between the results of both studies. Wang et al. (2016) investigated the effects of imidacloprid insecticide on the activities of various enzymes (CAT, SOD, GST) and DNA damage in earthworms. Imidacloprid insecticide at concentrations of 0.10 mg/kg, 0.50 mg/kg, and 100 mg/kg was mixed into the artificially created soil, and earthworms were left in this artificial soil for 28 days. Subchronic exposure to imidacloprid has been shown to cause DNA damage and lipid peroxidation (LPO) in the *Eisenia fetida* worm, which elicits antioxidant responses. They reported that Imidacloprid induced oxidative stress, DNA damage and SOD and GST activities were significantly influenced, and CAT activity was inhibited over most exposure ranges in Eisenia fetida. Beside high MDA levels and a significant increase in ROS levels were observed in all applications. The percentage of OTM and tail DNA increased gradually as the imidacloprid concentrations increased on day 7 of the experiment. However, at days 14, 21, and 28, the percentage of OTM and tail DNA was reported to decrease gradually with increasing Imidacloprid concentrations. Based on the current study, Imidacloprid exhibited toxicity for E. fetida. A significant increase in both ROS content and visible inhibition of CAT were observed. GST activity returned to control levels at the highest concentration (1.00 mg kg-1) in subsequent exposure periods (days 21 and 28), suggesting that SOD plays a crucial role in combating oxidative stress at this time. It has been reported that the percentage of OTM and tail DNA decreased with increasing concentrations with increasing exposure time after day 7. In

our study, the genotoxic damage data obtained with the Comet test and the suppression of CAT activity at high sulfoxaflor concentration show that the insecticide has oxidative damage potential. Yao et al. (2022) studied the acute toxicity, enantioselective behavior, and enzymatic activities of rac-sulfoxaflor in zebrafish in the bioaccumulation experiment. They demonstrated that the bioconcentration of sulfoxaflor in zebrafish was enantioselective, and the four enantiomers accumulated in zebrafish in the order (+)-2S,3S-sulfoxaflor > (-)-2R,3R-sulfoxaflor > (+)-2R,3Ssulfoxaflor > (–)-2S,3R-sulfoxaflor. The effect of racsulfoxaflor on the enzymatic activities of zebrafish showed that superoxide dismutase and glutathione-S-transferase activities and malondialdehyde content were significantly increased as compared to those in control, whereas acetylcholinesterase was significantly decreased in the sulfoxaflor exposure treatment (p < 0.05), indicating that sulfoxaflor caused oxidative damages and induced enzymatic activity in zebrafish. This study provides important information on the enantioselective action and toxic effects of sulfoxaflor, which can help assess the potential ecological risk of chiral pesticides to aquatic organisms. Azpiazu et al. (2021) investigated assessed the acute oral toxicity of sulfoxafor alone and in combination with a single dose of fuxapyroxad, a succinate dehydrogenase inhibitor (SDHI) fungicide, in three bee species: Apis mellifera, Bombus terrestris and Osmia bicornis. They demonstrate that sulfoxafor is somewhat less toxic than the recently banned neonicotinoids imidacloprid, thiamethoxam and clothianidin, but much more toxic than other neonicotinoids (acetamiprid, thiacloprid) still in use in the EU at the time this study was conducted in their laboratory results. They indicated that sulfoxaflor is relatively new and is regarded as a likely substitute for the neonicotinoid insecticides recently banned in the EU. Zhang et al. (2020) performed a life risk assessment of sulfoxaflor on soil invertebrate earthworms under effective concentrations. They reported that sulfoxaflor had significant influence on the toxicity at different exposure times and doses and degraded quickly in artificial soil with a degradation rate of 0.002-0.017 mg/(kg·d) and a half-life of 12.0-15.4 d. At 0.5 mg/kg and 1.0 mg/kg, the ·OH – content, antioxidant enzyme activeities, thiobarbituric acid reactive substances (TBARS) and 8-OHdG content had significant differences compared to those in the control group. On the 56th day, significant differences were only observed in the Glutathione S-transferase enzyme (GST) activity and 8-OHdG content at 1.0 mg/kg dose compared to those in the control group due to the degradation of sulfoxaflor. When an organism is exposed to toxic products, it produces and accumulates reactive oxygen species (ROS), which play seriously important roles in redox homeostasis and cell antioxidant signal transduction, and excess ROS can harm proteins, lipids and nucleic acids (Feng et al. 2015). ROS is the general name given to various molecules, including free radicals such as superoxide anion (O2-), hydroxyl radical (OH-), hydrogen peroxide (H_2O_2) , singlet oxygen. In our study, the increase in DNA damage parameters such as GDI and DCP, which occurred in parallel with the dose increase, and the

suppression of CAT enzyme inhibition, show that it may be due to the increase in the amount of free radicals.

5. Conclusion

The results obtained from the Comet analysis indicated that the Sulfoxaflor insecticide caused statistically significant genotoxic damage on the human peripheral blood lymphocytes cells in a dose-dependent manner. High correlation rates between GDI and DCP parameters were obtained by Comet analysis, It was determined that Sulfoxaflor insecticide caused statistically significant early, late apoptotic effects and necrotic effects in a dose-dependent fashion by the flow cytometry analysis. Among the parameters studied in the flow cytometry analysis, a solid positive correlation suggested that Sulfoxaflor insecticide had an apoptotic effect on the human peripheral blood lymphocyte cells in in vitro . In the comet analysis, Type 4 comet images indicating the GDI value primarily support the apoptotic activity of Sulfoxaflor insecticide. The presence of a high correlation rate between the comet analysis and the flow cytometry parameters suggests that the two methods complement each other. This study supports that Sulfoxaflor insecticide has apoptotic and genotoxic effects on human peripheral blood lymphocyte cells. The in vitro data we obtained in this study are of concern about health and safety. Therefore, it is inevitable that more in-depth and in vivo studies will be needed in the future.

Disclosure statement

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References

- Annabi, E., Ben Salem, I., and Abid-Essefi, S., 2019. Acetamiprid, a neonicotinoid insecticide, induced cytotoxicity and genotoxicity in PC12 cells. *Toxicology Mechanisms and Methods*, 29 (8), 580–586.
- Arisoy, H., and Ataseven, Y., 2017. Evaluation of environmental consciousness level of farmers in agricultural practices in Turkey: the case of province of Konya, Turkey. *Journal Environmental Protection and Ecology*, 18 (4), 1592–1604.
- Atalay, H., Çelik, A., and Ayaz, F., 2018. Investigation of genotoxic and apoptotic effects of zirconium oxide nanoparticles (20 nm) on L929 mouse fibroblast cell line. *Chemico-Biological Interactions*, 296, 98–104.
- Atlı Şekeroğlu, Z., et al., 2019. Cytotoxicity and genotoxicity of clothianidin in human lymphocytes with or without metabolic activation system. Drug and Chemical Toxicology, 42 (4), 364–370.

- Azpiazu, C., *et al.*, 2021. Toxicity of the insecticide sulfoxafor alone and in combination with the fungicide fuxapyroxad in three bee species. *Scientifc Reports*, 11, 6821.
- Battal, D., et al., 2015. SiO2 Nanoparticule-induced size-dependent genotoxicity – an in vitro study using sister chromatid exchange, micronucleus and comet assay. Drug and Chemical Toxicology, 38 (2), 196–204.
- Benli, P., and Çelik, M., 2021. Glutathione and its dependent enzymes' modulatory responses to neonicotinoid insecticide sulfoxaflor induced oxidative damage in zebrafish in vivo. *Science Progress*, 104 (2), 368504211028361.
- Bivehed, E., et al., 2020. Evaluation of potential DNA-damaging effects of nitenpyram and imidacloprid in human U937-cells using a new statistical approach to analyse comet data. Exposure and Health, 12 (3), 547–554.
- Bolognesi, C., et al., 2011. Micronuclei and pesticide exposure. Mutagenesis, 26 (1), 19–26.
- Calderón-Segura, M.E., *et al.*, 2015. Genotoxicity of the neonicotinoid insecticide Poncho (clothianidin) on CD1 mice based on alkaline comet and micronucleus assays. *Toxicity and Hazard of Agrochemicals*, 113, 61174.
- Carvalho, F.P., 2017. Pesticides, environment, and food safety. *Food and Energy Security*, 6 (2), 48–60.
- Collins, A., et al., 1997. Comet assay in human biomonitoring studies: reliability, validation, and applications. *Environmental and Molecular Mutagenesis*, 30 (2), 139–146.
- Çavaş, T., et al., 2012. In vitro genotoxicity evaluation of acetamiprid in CaCo-2 cells using the micronucleus, comet and γH2AX foci assays. *Pesticide Biochemistry and Physiology*, 104 (3), 212–217.
- Çelik, A., et al., 2003. Cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 539 (1–2), 91–97.
- Çelik, A., and Akbaş, E., 2005. Evaluation of sister chromatid Exchange and chromosomal aberration frequencies in peripheral blood lymphocytes of gasoline station attendants. *Ecotoxicology and Environmental Safety*, 60 (1), 106–112.
- Çelik, A., 2006. The assessment of genotoxicityof carbamazepine using Cytokinesis-Block (CB)Micronucleus assay in cultured human blood lymphocytes. Drug and Chemical Toxicology, 29 (2), 227–236.
- Çelik, A., et al., 2013. The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: single cell gel electrophoresis and micronucleus test. Food and Chemical Toxicology, 53, 249–255.
- Çelik, A., et al., 2014. In vitro genotoxicity of fipronil sister chromatid exchange, cytokinesis block micronucleus test, and comet assay. DNA and Cell Biology, 33 (3), 148–154.
- Dunphy, C.H., 2004. Applications of flow cytometry and immunohistochemistry to diagnostic hematopathology. *Archives of Pathology & Laboratory Medicine*, 128 (9), 1004–1022.
- Dixit, D., et al., 2012. Inhibition of casein kinase-2 induces p53-dependent cell cycle arrest and sensitizes glioblastoma cells to tumor necrosis factor (TNF alpha)-induced apoptosis through SIRT1 inhibition. Cell Death & Disease, 3 (2), e271–e271.
- European Food Safety Authority (EFSA). 2014. Conclusion on pesticide peer review conclusion on the peer review of the pesticide risk assessment of the active substance sulfoxaflor. *EFSA Journal*, 12 (5), 3692.
- Faroon, O., and Harris, M.O. 2002. Toxicological profile for DDT, DDE, and DDD. U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry.
- Fathy, S.M., and Abdelkader, I.Y., 2021. Effect of resveratrol on the inflammatory status and oxidative stress in thymus gland and spleen of sulfoxaflor-treated rats. *Environmental Toxicology*, 36 (7), 1326–1337.
- Fenech, M., and Morley, A.A., 1986. Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation. *Mutation Research*, 161 (2), 193–198.
- Feng, L., et al., 2015. Inhibition and recovery of biomarkers of earthworm Eisenia fetida after exposure to thiacloprid. Environmental Science and Pollution Research International, 22 (12), 9475–9482.
- Ge, W., et al., 2015. Oxidative stress and DNA damage induced by imidacloprid in zebrafish (Danio rerio). *Journal of Agricultural and Food Chemistry*, 63 (6), 1856–1862.

Glorieux, C., and Calderon, P.B., 2017. Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. *Biological Chemistry*, 398 (10), 1095–1108.

Møller, P., 2018. The comet assay: ready for 30 more years. *Mutagenesis*, 33 (1), 1–7.

Moorhead, P.S., et al., 1960. Chromosome preparations of leucocytes cultured from peripheral blood. Experimental Cell Research, 20, 613–616.

Pozarowski, P., Grabarek, J., and Darzynkiewicz, Z., 2004. Flow cytometry of apoptosis. *Current. Protocol. Cell Biology*, 18 (18.8), 18.8.1–18.8.33.

Sabarwal, A., Kumar, K., and Singh, R.P., 2018. Hazardous effects of chemical pesticides on human health–Cancer and other associated disorders. *Environmental Toxicology and Pharmacology*, 63, 103–114.

Schwarzbacherová, V., et al., 2019. In vitro exposure to thiacloprid-based insecticide formulation promotes oxidative stress, apoptosis and genetic instability in bovine lymphocytes. *Toxicology in Vitro*, 61, 104654.

Shepard, H.E., 1939. The *chemistry and toxicology of insecticides*. Minneapolis: Burgess Publishing Co.

Singh, M.D., 2017. Nano-fertilizers is a new way to increase nutrients use efficiency in crop production. *International Journal of Agriculture Sciences*, 9, 3831–3833.

Singh, N.P., *et al.*, 1988. Simple technique for quantitation of low levels of DNA damage in individuals cell. *Experimental Cell Research*, 175 (1), 184–191.

Stivaktakis, P.D., et al., 2016. Long-term exposure of rabbits to imidaclorpid as quantified in blood induces genotoxic effect. Chemosphere, 149, 108–113.

Stopper, H., and Müller, S., 1997. Micronuclei as a biological endpoint for genotoxicity: a minireview. *Toxicology in Vitro*, 11 (5), 661–667.

Şenyildiz, M., Kilinc, A., and Ozden, S., 2018. Investigation of the genotoxic and cytotoxic effects of widely used neonicotinoid insecticides in HepG2 and SH-SY5Y cells. *Toxicology and Industrial Health*, 34 (6), 375–383.

Toduka, Y., Toyooka, T., and Ibuki, Y., 2012. Flow cytometric evaluation of nanoparticles using side-scattered light and reactive oxygen speciesmediated fluorescence-correlation with genotoxicity. *Environmental Science & Technology*, 46 (14), 7629–7636.

Velikova, V., Yordanov, I., and Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science*, 151 (1), 59–66.

Wang, J., et al., 2016. DNA damage and oxidative stress induced by imidacloprid exposure in the earthworm Eisenia fetida. Chemosphere, 144, 510–517.

Yao, D., et al., 2022. Enantioselective bioaccumulation and toxicity of racsulfoxaflor in zebrafish (Danio rerio). Science of the Total Environment, 817, 153007.

Zhang, X., et al., 2020. The toxic effects of sulfoxaflor induced in earthworms (Eisenia fetida) under effective concentrations. International Journal of Environmental Research and Public Health, 17 (5), 1740.