ANALYSIS OF GENOTOXIC ACTIVITY OF PYMETROZINE IN HUMAN PERIPHERAL LYMPHOCYTES

Yıldırım Z[1], Kayraldız A[2], Dönbak L[2*], Kenger İH[2]

1. University of Gaziantep, İslahiye Vocational School, Laboratory and Veterinary Health Department, İslahiye/Gaziantep, Turkey
2. University of Kahramanmaraş Sütçü İmam, Science and Letters Faculty, Department of Biology, Kahramanmaraş, Turkey

Correspondence:
Dr Ahmet Kayraldiz
University of Kahramanmaraş Sütçü İmam, Science and Letters Faculty, Department of Biology, Kahramanmaraş, Turkey
E-mail: akayraldiz@ksu.edu.tr

Yıldırım Z, Kayraldız A, Dönbak L, Kenger İH. Analysis of genotoxicity of pymetrozine in human peripheral lymphocytes. Adv Lab Med Int. 2017; 7: 30 - 42.

ABSTRACT

Pymetrozine is an insecticide commonly used in aphid and whitefly struggle observed in citrus fruits and vegetables. In this study, possible genotoxic effects of pymetrozine were investigated in human peripheral blood lymphocytes by *in vitro* sister chromatid exchange (SCE), chromosome aberration (CA) and micronucleus (MN) tests and also cytotoxic effects of pymetrozine were determined with mitotic index (MI), proliferation index (PI), and nuclear division index (NDI) evaluations. The cells were treated with 5, 10, 20 and 40 µg/mL pymetrozine for 24 and 48 hours. SCE/Cell, CA/Cell, abnormal cell (AC), MN, and micronucleated binuclear cell (BNMN) frequencies were determined by microscopic analysis of the preparations. Obtained data were compared with the control group by using SPSS package programme (17.0). In the SCE frequency, there was a statistically significant increase only in the cells treated with the 20 µg/mL pymetrozine ($p<0.05$). Pymetrozine was markedly elevated the CA and AC incidences in the cells treated for 24 and 48 h as compared to control group ($P<0.05$). There was also a significant increase in both MN and BNMN frequencies of the cells treated with 20 µg/mL pymetrozine for 24 h and with tested doses for 48 h (except for 40 µg/mL) ($p<0,05$). Decrease in MI values was found to be significant at doses of 20 and 40 µg/mL for 24 h and 40 µg/mL for 48 h in comparison with the control ($p<0.01$). On the other hand, observed differences in PI and NBI values were found to be not significant ($p>0.05$). As a result; according to the data obtained from this study, pymetrozine had genotoxic effect and also weak cytotoxic effect in human peripheral lymphocytes.

Keywords: Pymetrozine, Genotoxicity, Cytotoxicity, Sister chromatid exchange, Chromosomal aberrations, Micronucleus

INTRODUCTION

Pesticides are some of the compounds most frequently released into the environment because of their widespread use for preventing, destroying, repelling, or mitigating pests in agriculture. Despite the beneficial effects associated with the use of pesticides, many of these chemicals may pose potential hazards to humans and to environment. Pesticides are used indiscriminately and in excessive doses, leaving residue in soil, water and air and affecting negatively human health, environment, ecological balance, and biodiversity of the nature [1 -3]. In addition to acute and chronic poisoning cases due to pesticide exposure. many of these chemicals were reported to have mutagenic effect, linked to the development of cancers or lead to developmental deficits [4 - 6].

Indiscriminate use of insecticides and other adverse effects both on environment and human health opened the new modern era of chemicals having novel mode of action with higher bioefficacy on insect control. These new group of insecticides includes avermectins, neonicotinoids, spinosyns, oxadiazines, IGR's, fiproles, pyrroles, ketoenols, benzenedicarboxamides, and pyridine azomethine, Most of these groups of pesticides play an important role in managing many arthropod pests with good bioefficacy, high selectivity, more safity to environment, and low mammalian toxicity, which make them attractive replacement for synthetic organic pesticides [7 - 8].

Pymetrozine is the only representative of the pyridine azomethines, a new dass of insecticides, and is used for control of aphids and whiteflies in field crops, vegetables, ornamentals, cotton, hop, deciduous fruit, and citrus, and of the brown planthopper. The mechanism of action of pymetrozine is biochemically unexplained, but it is known to affect nervous system [9]. It is important to evaluate the genotoxic and cytotoxic actions of these new agricultural insecticides to contribute to the toxicological data with their possible risk on the organism health. However, there is no study investigated the possible genotoxic and cytotoxic effects of pymetrozine.

Genotoxic agents have the potential to interact with DNA and may cause damage in DNA. At present, many biomarkers are used to determine the genotoxic potentials of genotoxic agents, as well as their related effects. Chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronucleus (MN) are among the cytogenetic markers widely used for the detection of early biological effects related to DNA-damaging agents [10 -13].

In this study; possible genotoxic potential of pymetrozine in human peripheral lymphocytes was investigated by using *in vitro* sister chromatid exchanges (SCEs), chromosome aberrations (CAs) and micronucleus (MN) tests and also cytotoxic effects of pymetrozine were determined with the examination of mitotic index (MI), proliferation index (PI), and nuclear division index (NDI) levels due to lack of knowledge about this properties of pymetrozine.

MATERIALS AND METHODS

Chemicals

A commercial formulation of pymetrozine, Greensun 50 WG, (containing 50% pymetrozine as active ingredient) was purchased from Syngenta (Turkey). The chemical

structure of pymetrozine are shown in Figure 1. Mitomycin-C (MMC, Kyowa, Japan) was used as a positive control and was dissolved in sterile double-distilled water. 5-Bromodeoxyuridine, colchicine, and cytochalasin B were purchased from Sigma (St. Louis, MO). Giemsa and all other chemicals were purchased from Merck (Darmstadt, Germany). All test solutions were freshly prepared before each experiment.

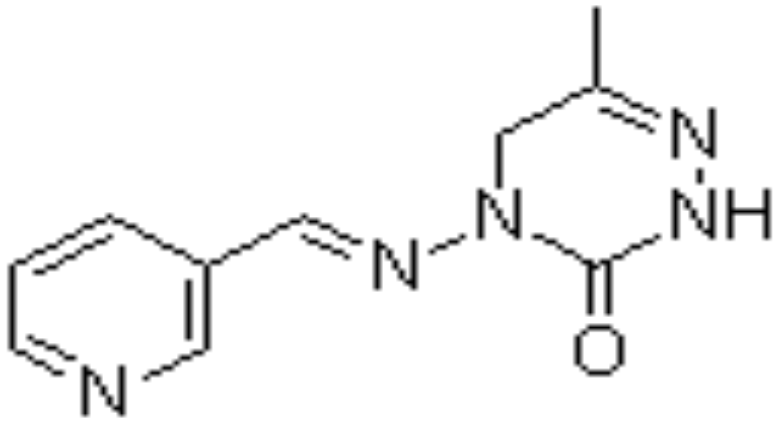

Figure 1. The chemical structure of pymetrozine.

Preparation of Test Substance

Dose determinations were conducted with regard to LD50 dose of pymetrozine, the dose killing half of the (50 %) treated cells. In the preliminary study, LD50 dose of pymetrozine was determined as 40 µg/ml. This is the highest dose of pymetrozine to be used in the study. Other doses were determined as 20, 10, and 5 µg/ml which are the 50 % decreasing folds of the LD50 dose. So, 5, 10, 20 and 40 µg/ml of pymetrozine used in the experiments. Stock solutions were prepared just before each experiment and stored at room temperature.

Blood samples

Human peripheral blood samples were taken from four (n= 4) healthy, volunteer donors (two males and two females, all nonsmokers) aged from 22 to 25 years and heparinized to 1/10 ratio.

Cell cultures

SCE and CA Assay: For SCE and CA tests, 6 drops of heparinized blood samples were added to the tubes containing 2.5 mL chromosome medium supplemented with 5-bromodeoxyuridine (10 µg/mL) and the cultures incubated for 72 hours at 37 °C. Lymphocytes were treated with 5, 10, 20 and 40 µg/mL pymetrozine for 24 and 48 hours in the cell culture. At the 70 th hour of incubation, 0,06 µg/mL colchine was added to the tubes. SCE and CA analyses were conducted using the methods developed by Evans [14] and Perry and Thompson [15], with minor modifications. The cells were treated with a

hypotonic solution (0.4% KCl) for 15 min at 37 °C and then fixed three times in a cold solution consisting of methanol:glacial acetic acid (3:1 v/v) at room temperature. Finally, the centrifuged cells were dropped onto clean slides.
The air-dried slides were stained with 5% Giemsa (pH 6.8) for 10 min for CA analysis and with a modified Fluorescence Plus Giemsa method (FPG) for SCE analysis [16]. For FPG staining, slides were covered with Sorensen Buffer (pH 6.8) and were irradiated with UV light (254 nm) for 30 min. After irradiation, the slides were incubated in 1X SSC at 58-60 ˚C for 60 min and then stained with 5% Giemsa for 30 min.
For scoring of SCE, fifty well-seperated second- division metaphase cells were analyzed per donor (a total of 200 second-division metaphase for each concentration). Two hundred well-spread cells per donor were examined to determine the number of CAs (a total of 800 metaphase per concentration). Thus, structural and numerical chromosome aberrations were obtained. Percentages of cells with CAs were calculated for each donor separately. In addition, a total of 800 cells (100 cells per donor) were scored to determine the PI, which was calculated using the formula: $PI=1 X M_1+ 2 X M_2+ 3 XM_3 /100$, where M1, M2, and M3 represent the number of cells at the first, second, and third metaphases, respectively. In addition, the number of dividing cells (i.e., cells in metaphase) was counted in a total of 12,000 cells (3,000 cells per donor) per concentration to calculate MI. MI was calculated according to the formula MI = 100 X cells in metaphase/3.000.

Micronucleus Assay: For the MN test, whole blood cultures were started as described for the SCE and CA assay except for 5-bromodeoxyuridine. Peripheral lymphocytes were incubated at 37 ˚C for 68 h. The cells were exposed to pymetrozine at concentrations of 5, 10, 20 and 40 µg/ml for 24 and 48 h. Cytochalasin B (final concentration of 6 µg/ml) was added after 44 h of incubation to block cytokinesis and obtain binucleated cells. After an additional 24 h incubation at 37 ˚C, cells were harvested by centrifugation and slides were prepared for MN test [17]. Finally, slides were stained with 5% Giemsa in Sorensen Buffer (pH 6.8) for 8 min.
In this study, the criteria used for binuclear cell and MN evaluation were those suggested by Titenko-Holland et al. [18] and Fenech [11]. To determine MN formation, 2.000 binuclear cells were analyzed for each donor (8.000 binucleated cells were scored per concentration). Furthermore, cytotoxicity was estimated by using the nuclear division index (NDI) using the following formula: $NDI = 1 X M_1+ 2 X M_2+ 3 X M_3+ 4 X M_4 /N$; where M_1 to M_4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored.

Statistical Analysis

All of the subjects (i.e., the 4 donors; N = 4) were used as the experimental unit (N) for all statistical analyses. Results are expressed as the mean ±S.D. (Standard Deviation). Data obtained from microscopic examinations were statistically compared with control group using Student's t-test in SPSS (17.0) package program.

RESULTS

In this study; four different concentrations (5, 10, 15, and 20 µg/mL) and six different parameters (SCE, CA, MN, PI, MI, NDI) were evaluated in two exposure periods (24 and

48 h) to determine the possible genotoxic and cytotoxic effects of pymetrozine in human peripheral blood lymphocytes *in vitro*. Obtained results are presented in Table 1, 2, and 3. The observed frequencies of SCE and PI in the peripheral lymphocytes are shown in Table 1. When 24 and 48 h treatment of human peripheral lymphocytes with pymetrozine was compared in terms of SCE frequency per cell, there was no significant difference in all doses except for 20 µg/mL compared to the control group ($p>0.05$). Smilarly, there were no marked differences betweenthe PI values of treated cells and that of control group in all doses for both 24 and 48 h treatments ($p>0.05$), as shown in Table 1.

Table 1. Effects of Pymetrozine on Sister Chromatid Exchanges (SCE) and Proliferation Index (PI) in Human Peripheral Lymphocytes for 24 and 48 h Treatment Periods.

Test substance	Treatment		SCE/Cell ± SD	PI ± SD
	Time (h)	Concentration (µg/mL)		
C (-)[a]	24	0.00	3.03±0.85	2.69±0.69
C (+)[b]		0.10	34.42±3.21	1.76±0.12
Pymetrozine		5	3.90±1.54	2.18±0.21
		10	4.48±1.92	1.92±0.12
		20	4.50±0.74*	1.94±0.19
		40	5.00±1.59	1.82±0.47
C (-)	48	0.00	3.03±0.85	2.69±0.69
C (+)		0.10	34.42±3.21	1.76±0.12
Pymetrozine		5	3.39±0.59	2.46±0.48
		10	4.11±0.90	2.88±1.34
		20	3.50±0.88	2.50±1.04
		40	3.68±0.81	2.35±1.02

[a]: Control
[b]: Positive control, Mitomycin C
* $p<0.05$

In cells treated with pymetrozine for 24 and 48 hours, the frequency of chromosomal abnormalities per cell and the percentage of abnormal cells was found to be significantly increased in all doses, as compared to the control ($p<0.05$) (Table 2). Cell cultures treated with 20 µg/mL ($p<0.05$) and 40 µg/mL ($p<0.01$) of pymetrozine for 24 h and also in the 48 h treatment period, at the dose of 40 µg/mL, statistically significant decrease in MI was observed when compared with the control ($p<0.01$). As shown in Table 2, pymetrozin caused chromosomal abnormalities such as chromatid break Figure 2), chromosomal break (Figure 3), fragment (Figure 4), sister union, dicentric chromosome, and polyploidy. The most observed anomalies in this study are the chromatid breaks, as well as sister union, chromosomal breaks, and fragments are the most frequently observed chromosomal abnormalities.

Table 2. Percentage of chromosomal abnormalities per cell (CA/Cell), types of chromosomal abnormalities, frequency of abnormal cells (AC), and mitotic index (MI) in human peripheral lymphocytes treated with pymetrozine.

Test Substance	Treatment		CA/Cell±SD	ChromosomalAbnormalites[a]						AH±SD (%)	MI±SD
	Time (h)	Concentration (µg/mL)		B'	B"	F	SU	DS	P		
C (-)[b]	48	0.0	0.05±0.01	5	-	1	1	-	-	5.25±2.06	3.67±0.31
C (+)[c]		0.1	0.34±0.22	8	4	11	3	6	8	29.32±2.32	1.25±0.27
Pymetrozine		5	0.08±0.01*	7	2	1	2	-	-	8.25±1.50*	3.40±1.20
		10	0.11±0.01**	10	2	-	-	-	1	11.00±1.15*	3.60±1.40
		20	0.14±0.02**	9	1	-	2	-	-	13.50±2.64*	3.17±0.20*
		40	0.12±0.01**	12	-	1	3	2	-	12.25±1.89**	2.00±0.53**
C (-)	48	0.0	0.05±0.01	5	-	1	1	-	-	5.25±2.06	3.67±0.31
C (+)		0.1	0.52±0.40	6	6	20	12	9	10	44.15±3.70	1.22±0.23
Pymetrozine		5	0.09±0.02*	7	2	-	1	2	-	8.25±2.50	3.44±1.19
		10	0.11±0.02**	12	3	1	-	-	-	10.75±1.70**	2.84±1.02
		20	0.08±0.01*	11	1	1	1	2	-	8.75±1.89*	2.71±0.84
		40	0.12±0.01**	15	2	1	6	2	-	12.50±1.91**	1.45±0.44**

[a]: B', Chromatid breaks; B", Chromosomal breaks; F, Fragment; SU, Sister union, DS, Dicentric chromosome; P, Polyploidi;
[b]: Control
[c]: Positive control, Mitomycin C
* p<0.05; **p<0.01

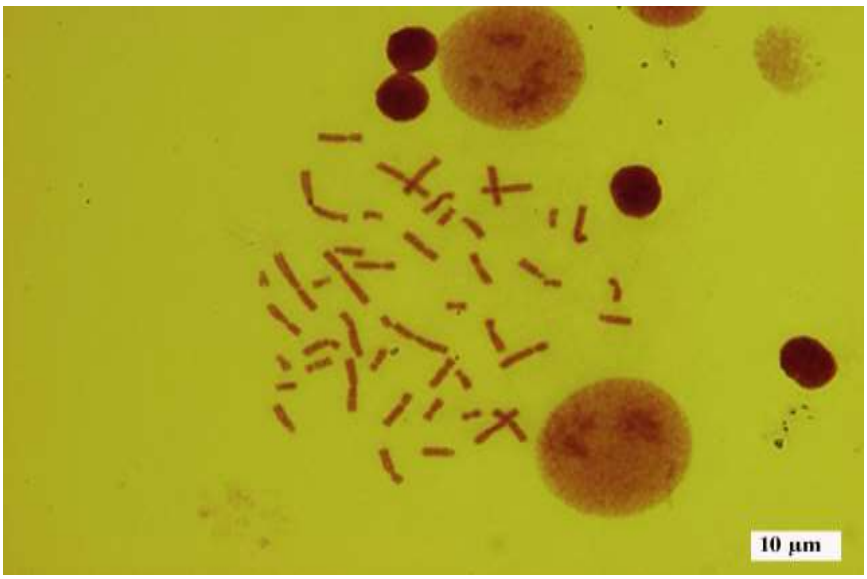

Figure 2. Chromatid break (X1000) (20 µg/mL pymetrozine, 48 h treatment, ♂).

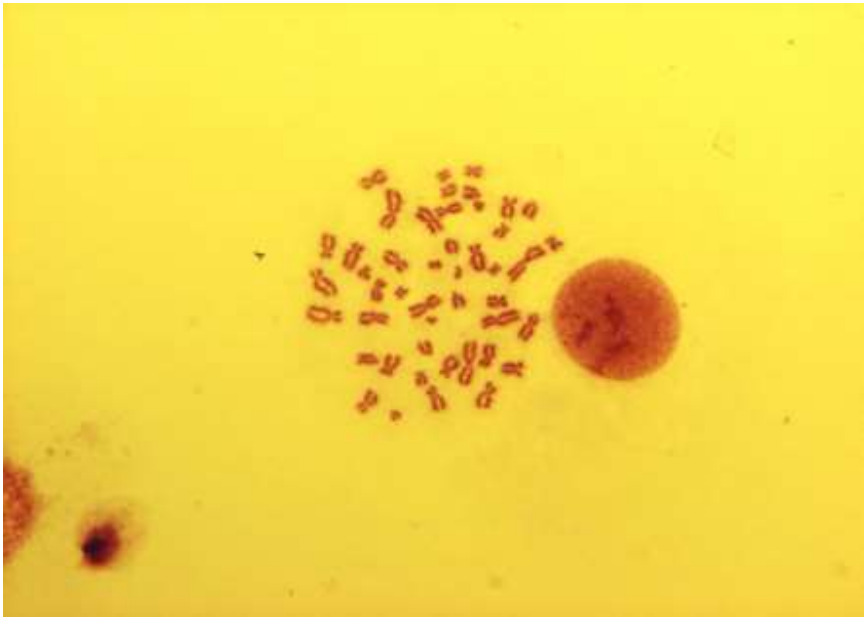

Figure 3. Sister union (X1000) (40 µg/mL pymetrozine, 48 h treatment, ♂).

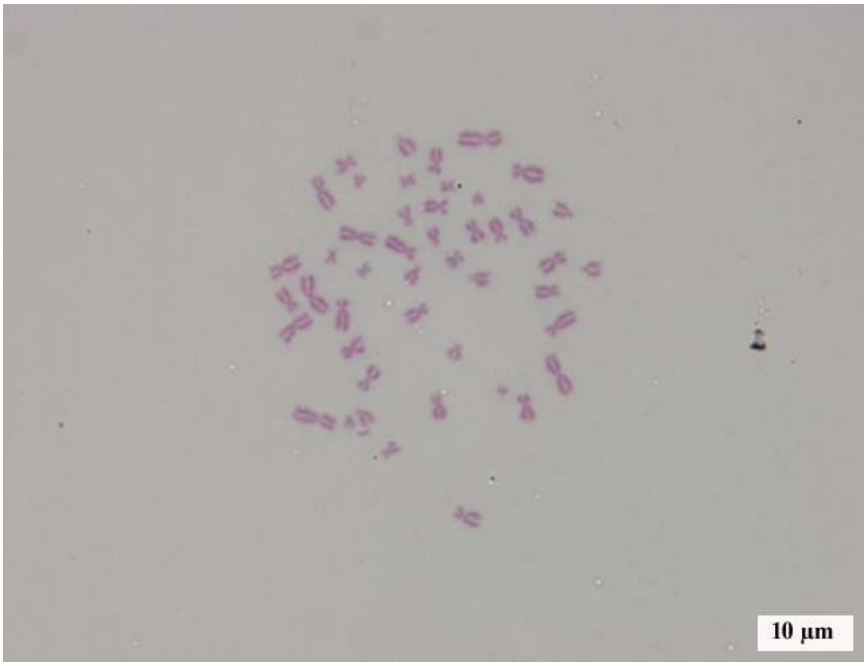

Figure 4. Fragment (X1000) (20 µg/mL pymetrozine, 48 h treatment, ♂).

As shown in Table 3, where the results of MN test are given, treatment with 20 μg/mL pymetrozine for 24 h significantly increased frequencies of MN and BNMN. Also, there was a marked elevation in the MN and BNMN frequencies of the treated cells for 48 h in all doses except for the highest dose (40 μg/mL) ($P<0.05$; $P<0.01$). Concerning the NDI values, pymetrozine did not cause significant differences in the NDI values of treated cells ($p>0.05$) as compared to control for all doses and treatment periods.

Table 3. Frequencies of micronucleus (MN), micronucleated binuclear cells (BNMN), and nuclear division index (NDI) in Pymetrozine treated human peripheral lymphocytes.

Test substance	Treatment		MN ± SD (‰)	BNMN ± SD(‰)	NDI±SD
	Time (h)	Concentration (μg/mL)			
C (-)[a]	24	0.0	3.75±0.95	3.75±0.95	1.71±0.12
C (+)[b]		0.1	45.78±3.85	39.23±3.21	1.03±0.01
Pymetrozine		5	5.50±2.88	5.25±2.62	1.70±0.15
		10	7.25±1.25	6.25±0.95	1.67±0.25
		20	7.50±1.29*	7.50±1.29*	1.69±0.08
		40	4.00±0.81	4.00±0.81	1.64±0.31
C (-)	48	0.0	3.75±0.95	3.75±0.95	1.71±0.12
C (+)		0.1	62.25±4.82	67.12±2.62	1.00±0.01
Pymetrozine		5	10.25±1.70*	10.00±2.16*	1.44±0.28
		10	20.75±2.06**	18.75±2.06*	1.61±0.15
		20	10.25±3.77*	9.25±3.09*	1.60±0.09
		40	7.00±3.55	6.75±3.09	1.64±0.17

[a]: Control
[b]: Positive control, MMC
* $p<0.05$; **$p<0.01$

DISCUSSION

Pesticide application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and crop yields. On the other hand, several researches indicated the many adverse effects of pesticide usage such as leaving residues on crops, soil, water and air, causing persistence in pests and weeds, and harmful effects on human health. Hundreds of millions of people are exposed to insecticides through occupational settings, domestic use, consumption of contaminated food or drinks and living close to treated areas. Relationships between pesticide exposure and increased risk of cancers, neurodegenerative and neurodevelopmental disorders, and respiratory diseases have been demonstrated by numerous studies [6, 19 - 23]. Occupationally or incidentally, humans are exposed not only to single pesticides, but also to pesticide mixtures. Previous studies

have demonstrated that mixtures of some pesticides cause more genotoxic effects than the individual compounds themselves [24 - 25].

Assessment of potential genotoxicity of insecticides is critical for a better regulation and protection. However, there is no study concerning the genotoxic and cytotoxic effects of pymetrozine. So, in this study; possible genotoxic and cytotoxic potentials of pymetrozine have been investigated using human peripheral lymphocytes. Blood samples taken from healthy volunteer donor (two men and two females aged 20-24) were treated with four different concentrations of pymetrozine (5, 10, 20, and 40 μg/mL) for 24 and 48 h. In this treated cells, the frequencies of SCE/Cell, CA/Cell, AC, MN, and BNMN and also cell kinetic parameters of PI, MI, and NBI were determined.

In this study, pymetrozine did not significantly induce the SCE frequency per cell in all doses ($p>0.05$). Many chemical agents affect the formation of sister chromatid exchanges in different forms. It has been determined that some chemicals may cause cross-linkages in polynucleotides due to alkylation of DNA. These linkages cause to increase in the frequency of SCE because it can not be repaired by the DNA repair mechanism [26 - 27]. However, this study shows that pymetrozine does not cause DNA damage observed due to crosslinks resulting from alkylation of DNA.

In cells treated with pymetrozine for 24 and 48 hours, the frequency of chromosomal abnormalities and abnormal cells were significantly increased at all doses tested compared to the control ($p<0.05$). The most common type of chromosomal abnormalities observed was the chromatid breaks that occur in double chain of DNA of a chromatid of the chromosome. The reason of the chromatid breaks are the effects of the applied substances in the late S or G2 phase [28]. The second most common abnormality, chromosomal breaks, occur in double chain of DNA in two chromatids of a chromosome that are unrepaired [29]. This suggests that the insecticides are also effective at the G1 phase [30]. DNA double strand breaks in the chromatides have been identified as breaks in the phosphodiester bonds of DNA.

In this study, pymetrozine has also been found to cause the formation of dicentric chromosomes and sister unions. Association between the sister chromatids are generally originated from the deletions in the terminal regions of the chromosomes [31]. In the formation of discentric chromosomes, the associations between non-homologous chromosomes or between two long or two short arms of homologous chromosomes is effective [32]. According to this study, Pymetrozine also causes fragment formation. Fragments are thought to be subchromatid and chromatid fractures caused by pesticides [33]. The increase in chromosomal abnormalities is indicative for clastogenicity and increases in genetic diseases and cancer risk. It is also known that when certain chemical agents are administered in combination with another agent, the genotoxic effect on the cell may be further increased.

Several studies have demonstrated the efficiency of the MN assay to detect DNA damage induced by pesticides. So in this study, MN assay was performed by using peripheral blood lymphocytes. In the study 20 μg/mL pymetrozine significantly increased the MN and BNMN frequencies in the cells treated for 24 h ($p<0.05$). In the 48 h treatment, MN and BNMN level were also found to be markedly elevated in all doses except for the highest dose (40 μg/mL). Micronucleus are formed due to acentric fragment or a complete chromosome that can not be pulled into the poles, during mitosis. With the formation of cell membrane around the chromosomes and fragments separated at the

telophase stage, micronuclei are formed which are smaller than the main nucleus [11, 34 - 35]. The effect of pymetrozine causes the formation of micronuclei at high frequencies by destroying the structure of spindle fibers (tubulin and tubulin B proteins), causing to stay one or more than one chromosomes in the metaphase, as well as multipolar anaphase-telophase.

Although there was a decrease in PI values of the cells, this decrease was not statistically significant ($p>0.05$) as compared to control for all doses and treatment periods. On the other hand, pymetrozine was significantly reduced the MI values of cells treated with 20 µg/mL ($p<0.05$) and 40 µg/mL ($p<0.01$) for 24 h treatment and also at 40 µg/mL concentration ($p<0,01$) for 48 h treatment period. Pymetrozine also did not cause a marked decrease in the frequency of NBI ($p> 0.05$) when compared to control for all doses tested and treatment times.

In *in vitro* CA assays, MI is used to monitor induced cellular toxicity. MI gives the percentage of cells in the metaphase in the cell cycle. Decreased MI indicates inhibition of cell cycle progression and / or loss of proliferative capacity [36]. Determination of the degree of cytotoxicity is necessary to select the appropriate preparation time and test concentrations, and is particularly important when the results are used to assess risk for compounds to which humans may be exposed [37].

The decrease of the MI could be due to blocking of G2, which prevents the cell from entering mitosis or it may be caused by a decrease in the ATP level and the stress from the energy-production centre [38]. Inhibition of certain cell cyle-specific proteins/enzymes remains as a possible pesticide target that inhibits DNA synthesis or may inhibit spindle production, assembly or orientation. Results obtained in this and in the other studies mentioned above showed that pymetrozin is DNA damaging chemical.

As a result; pymetrozine has genotoxic effect in human peripheral lymphocytes by increasing frequencies of CA and MN, and also has a weak cytotoxic effect by decreasing the MI level. However, it is suggested that pymetrozine should be analyzed with additional test systems to make a definitive judgment on the genotoxic activity of this substance.

CONCLUSION

We would like to thank Scientific Research Projets of Kahramanmaraş Sütçü Imam University for providing financial support to this project (Project No: 2014/3-25).

COMPETING INTERESTS

The authors declare no competing interest.

REFERENCES

1. Aktatr W, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol. 2009; 2:1 - 12.
2. Damalas CA, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health. 2011; 8:1402 - 19.
3. Deveci HA, Unal S, Karapehlivan M, Karasu Ayata M, Gaffaroğlu M, Kaya I, Yılmaz M. Effects of Glyphosate (Herbicide) on serum paraoxonase activity, high

density lipoprotein, total antioxidant and oxidant levels in Kars Creek Transcaucasian Barbs (Capoeta Capoeta [Guldenstaedt, 1973]. Fresenius Environ Bull. 2017; 26: 3514- 8.
4. Garaj-Vrhovac V, Zeljezic D. Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay: pesticide genotoxicity revealed by comet assay. Mutat Res. 2000; 469: 279 - 85.
5. Leiss JK, Savitz DA. Home pesticide use and childhood cancer: a case control study. Am J Public Health. 1995; 85:249 - 53.
6. Arbuckel TE, Server LE. Pesticides exposures and fetal death: a review of the epidemiologic literature. Crit Rev Toxicol. 1998; 28: 229 - 70.
7. Das SH. Mode of action of pesticides and the novel trends- a critical review. IRJAS. 2013; 3: 393 - 401.
8. Ishaaya I, Barazani A, Kontsedalov S, Horowitz AR. Insecticides with novel modes of action: Mechanism, selectivity and cross-resistance. Entomol Res. 2007; 37:148 -52.
9. Fuog D, Fergusson SC, Flückiger C. Pymetrozine: A novel insecticide affecting aphids and whiteflies. In: shaaya I, Degheele D, eds. Insecticides with Novel Modes of Action. Berlin Heidelberg:, Springer-Verlag, 1998.
10. Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG, Tice R, Waters MD, Aitio A. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. Mutat Res. 2000; 463:111 - 72.
11. Fenech M. The in vitro micronucleus technique. Mutat Res. 2000; 455: 81 - 95.
12. Bolognesi C. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat Res. 2003; 543:251 - 72.
13. Norppa, H, Bonassi S, Hansteen I-L, Hagmar L, Strömberg U, Rössner P, Boffetta P, Lindholm C, Gundy S, Lazutka J, Cebulska-Wasilewska A, Fabianova E, Sram R J, Kunudsen LE, Barale R, Fucic A. Chromosomal aberrations and SCE as biomarkers of cancer risk. Mutat.Res. 2006; 600: 37 - 45.
14. Evans HJ. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests, In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. Hahdbook of Mutagenicity Test Producers. 2nd ed. New York: Elsevier, 1984; 405 - 427.
15. Perry P, Thompson EJ. The methodology of sister chromatid exchanges. In: Kilbey BJ, Legator M, Nichols W, Ramel C, eds. Handbook of Mutagenicity Test Procedures. 2nd ed. New York, Elsevier1984; 495 - 529.
16. Speit G, Haupter S. On the mechanisms of differential giemsa staining of bromodeoxyuridine-substituted chromosomes. II. Differences between the demonstration of sister chromatid differentiation and replication patterns. Hum Genet. 1985; 70:126 - 9.
17. Rothfuss A, Schutz P, Bochum S, Volm T, Eberhardt E, Kreienberg R. Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carries of a BRCA1 mutation in breast cancer families. Cancer Res. 2000; 60: 390 - 4.
18. Titenko-Holland N, Windham G, Kolachana P, Reinisch F, Parvatham S, Osorio AM, Smith MT. Genotoxicity of malathion in human lymphocytes assessed using

the micronucleus assay in vitro and in vivo: A study of malathion-exposed workers. Mutat Res. 1997; 388: 85 - 95.
19. Hilgert Jacobsen-Pereira C, Dos Santos CR, Troina Maraslis F, Pimentel L, Feijó AJL, Iomara Silva C, de Medeiros GDS, Costa Zeferino R, Curi Pedrosa R, Weidner Maluf S. Markers of genotoxicity and oxidative stress in farmers exposed to pesticides. Ecotoxicol Environ Saf. 2017; 148:177-83.
20. Ezzi L, Haouas Z, Salah IB, Sakly A, Grissa I, Chakroun S, Kerkeni E, Hassine M, Mehdi M, Cheikh HB. Toxicopathic changes and genotoxic effects in liver of rat following exposure to diazinon. Environ Sci Pollut Res Int. 2016; 23: 11163-70.
21. Yang M, Xiang G, Li D, Zhang Y, Xu W, Tao L. The insecticide spinosad induces DNA damage and apoptosis in HEK293 and HepG2 cells. Mutat Res. 2016; 812:12 -19.
22. Kataria SK1, Chhillar AK2, Kumar A1, Tomar M1, Malik V1. Cytogenetic and hematological alterations induced by acute oral exposure of imidacloprid in female mice. Drug Chem Toxicol. 2016; 39:59 - 65.
23. Valencia-Quintana R, Gómez-Arroyo S, Sánchez-Alarcón J, Milić M, Olivares JL, Waliszewski SM, Cortés-Eslava J, Villalobos-Pietrini R, Calderón-Segura ME. Genotoxic effects of the carbamate insecticide Pirimor-50® in Vicia faba root tip meristems and human lymphocyte culture after direct application and treatment with its metabolic extracts. Arh Hig Rada Toksikol. 2016; 67:266 - 76.
24. Sultana Shaik A, Shaik AP, Jamil K, Alsaeed AH. Evaluation of cytotoxicity and genotoxicity of pesticide mixtures on lymphocytes. Toxicol Mech Methods. 2016; 26:588 - 94.
25. Kocaman AY, Topaktas¸ M. Genotoxic effects of a particular mixture of acetamiprid and a-cypermethrin on chromosome aberration, sister chromatid exchange, and micronucleus formation in human peripheral blood lymphocytes. Environ Toxicol. 2010; 25:157 - 68.
26. Wilson DM, Thompson LH. Molecular mechanisms of sister-chromatid exchange. Mutat Res. 2007; 616:11 - 23.
27. Swenson DH, Harbach PR, Trzos RJ. The relationship between alkylation of specific DNA bases and induction of sister chromatid exchange. Carcinogenesis. 1980; 1: 931 - 6.
28. Natarajan AT. Chromosome aberrations: past, present and future. Mutat Res. 2002; 504: 3 -16.
29. Savage JRK. Update on target theory as applied to chromosomal aberrations. Environ Mol Mutagen. 1993; 22:198 - 207.
30. Natarajan T, Obe G. Mutagenicity testing with cultured mammalian cells: cytogenetic assays. New York, Academic Press, 1982; 171 - 213.
31. Palitti F. Mechanisms of the origin of chromosomal aberrations. Mutat Res. 1998; 404: 133- 7.
32. Obe G, Pfeiffer P, Savage JRK, Johannes C, Goedecke W, Jeppesen P, Natarajan AT, Martínez-López W, Folle GA, Drets ME. Chromosomal aberrations: formation, identification and distribution. Mutat Res. 2002; 504: 17 - 36.

33. Prakash NS, Laksmi N, Harini I. Cytological effects of agricultural chemicals: II. Effects of fungicides Bavistin and Deltan on chilli (Capsicum annuum L.). Cytologia. 1988; 53: 709 - 15.
34. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 2011; 26:125 - 32.
35. T Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Vande Loock K, Decordier I.he in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol. 2011; 85: 873 - 99.
36. Muehlbauer PA1, Schuler MJ. Measuring the mitotic index in chemically-treated human lymphocyte cultures by flow cytometry. Mutat Res. 2003; 537: 117 - 30.
37. Muehlbauer PA1, Spellman RA, Gunther WC, Sanok KE, Wiersch CJ, O'Lone SD, Dobo KL, Schuler MJ. Improving dose selection and identification of aneugens in the in vitro chromosome aberration test by integration of flow cytometry-based methods. Environ Mol Mutagen. 2008; 49: 318 - 27.
38. K. Šiviková, J. Dianovský. Mitotic Index and Cell Proliferation Kinetics as Additional Variables for Assessment of Genotoxic Effect of The Herbicide Modown. Acta Vet Brno. 2000; 69: 45 - 50.