https://dergipark.org.tr/tr/pub/ataunivbd
2020; 15(2): 130-137

Research Article/Araştırma Makalesi
DOI: 10.17094/ataunivbd.699247

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
Atatürk University Journal of Veterinary Sciences

Investigation of The Genotoxic Effect of Acetamiprid in Mouse Bone Marrow Cells by CA (Chromosomal Aberration) and MN (Micronucleus) Test Methods*

Safak SANDAYUK[1a], Pınar AKSU KILICLE[1b]✉

1. Kafkas University, Faculty of Arts and Sciences, Department of Biology, Kars, TURKEY.
ORCID: 0000-0002-0247-6462[a], 0000-0002-3567-5775[b]

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
05.03.2020	24.04.2020	27.10.2020

Bu makaleye atıfta bulunmak için/To cite this article:
Sandayuk S, Aksu Kilicle P: Investigation of The Genotoxic Effect of Acetamiprid in Mouse Bone Marrow Cells by KA (chromosomal aberration) and MN (micronucleus) Test Methods. Atatürk University J. Vet. Sci., 15(2): 130-137, 2020. DOI: 10.17094/ataunivbd.699247

Abstract: In this study, we aimed to determine acetamipridine's (ACE) genotoxic effect in *mus* musculus var. *albinos* bone marrow cells by chromosomal aberration (CA) and micronucleus (MN) analysis. Mice were divided into four groups. Group 1 determined as negative control (NC), group 2, 3 and 4 were determined as 5 mg/kg ACE, 10 mg/kg ACE, and 15 mg/kg ACE groups respectively. ACE was given orally for 14 days. CA, mitotic index (MI) and MN frequencies were determined in femoral cells. As a result, no genotoxic effects were observed in groups treated with 5-10 mg/kg ACE compared to negative control. CA rates of 15 mg/kg ACE group was determined to be high (P<0.001). In addition, MI ratio in the 15 mg/kg ACE group was lower than the control group. When the ratios of the polychromatic erythrocyte with micronucleus were examined, no significant difference was found between the 5 mg/kg and 10 mg/kg ACE groups and control group. However, a statistically significant increase in MNPCE rates was observed in the 15 mg/kg ACE group compared to control ones (P<0.001). It was concluded that ACE at 15 mg/kg dose was genotoxic-cytotoxic.

Keywords: Genotoxic, Micronucleus, Mitotic index, Mus musculus albino.

Asetamipridin Fare Kemik İliği Hücrelerinde Genotoksik etkisinin KA (Kromozomal Aberasyon) ve MN (Mikronükleus) Test Yöntemleri ile Araştırılması

Öz: Bu çalışma ile asetamipridin (ACE) *mus musculus* var. albino türü farelerin kemik iliği hücrelerinde kromozomal aberasyon (KA) ve mikronükleus (MN) analizi aracılığı ile genotoksik etkisinin belirlenmesini amaçladık. Fareler dört gruba ayrıldı. 1. grup negatif kontrol grubu olarak belirlendi. 2. grup 5 mg/kg ACE grubu, 3. grup 10 mg/kg ACE grubu ve 4. grup, 15 mg/kg ACE grubu olarak belirlendi ve ACE 14 gün boyunca oral olarak verildi. Femur hücrelerinde kromozomal aberasyon, mitotik indeks (MI) ve MN frekansları belirlendi. Sonuç olarak negatif kontrol grubuyla karşılaştırıldığında 5 mg/kg ve 10 mg/kg ACE uygulanan gruplarda genotoksik etki gözlenmedi. 15 mg/kg ACE uygulanan grupta kromozomal aberasyon oranının yüksek olduğu (P<0.001) belirlendi. Ayrıca 15 mg/kg ACE uygulanan grupta mitotik indeks oranın kontrol grubuna göre düşük olduğu saptandı. MN frekansları incelendiğinde, 5 mg/kg ve 10 mg/kg ACE uygulanan gruplar ile kontrol grubu arasında anlamlı bir fark bulunamadı. Ancak kontrol grubuna göre 15 mg/kg asetamiprid uygulanan grupta, Mikronükleuslu polikromatik eritrosit oranlarında istatistiksel olarak anlamlı bir artış gözlendi (P<0.001). ACE 15 mg/kg dozlarının genotoksik-sitotoksik etkili olduğu sonucuna varıldı.

Anahtar Kelimeler: Genotoksik, Mikronükleus, Mitotik indeks, Mus musculus albino.

✉ Pınar AKSU KILICLE
Kafkas University, Faculty of Arts and Sciences, Department of Biology, Kars, TURKEY.
e-mail: pinar-aksu@hotmail.com
*This study was supported by the Scientific Research Projects Coordination Unit at Kafkas University within the scope of project number 2017-FM-30. This article is derived from the master's thesis.

INTRODUCTION

Despite the limited agricultural areas, the world population is increasing day by day. This situation increases the need for food. According to the Food and Agriculture Organization (FAO), people need 15-20 million tons of food each year (1). However, the limitation in the agricultural field and the limitation of animal production have become unable to meet the increased nutrient requirements. The inability to protect agricultural products against diseases and pests often has a negative effect on obtaining healthy food. This has led people throughout history to pursue various quests to meet food needs, increase agricultural productivity, and preserve limited food resources. This quest started a struggle that which can be called agricultural war (2).

Pesticides are chemical substances produced to kill insects and unwanted animals (3). Pesticides are very useful if administered with appropriate dosage and suitability. Intense and unconscious use raises the problem of pesticide residues (4). Pesticides have different levels of toxic effects on animals and humans other than agricultural pests as well. Therefore, it is necessary to evaluate the effects of pesticides not only with the organism which they affect, but also with the plants, human and other organisms in the area where they are applied (5-7).

Neonicotinoids are new generation insecticides developed based on the mechanism of action of nicotine (8,9). Neonicotinoids, nicotine-derived insecticides, which are used to control sucking insects, scabbard pests and some butterflies, are considered to be new generation insecticides (10,11). ACE, thiacloprid, imidacloprid and thiamethoxam are the drugs of this group. Neonicotinoids have high specific effects and are widely used in plant protection. Neonicotinoids, which accumulate in surface waters, are named as group 2 and 3 toxins in the classification made by the World Health Organization (WHO) and EPA (12,13).

Intense and unconscious use of ACE causes toxicity in humans and animals either directly or by entering the food chain. ACE is cytotoxic and genotoxic in mammals (14). It has been found to cause sibling chromatid changes in human peripheral lymphocyte cultures, micro-nucleus formation in blood lymphocytes and chromosomal anomalies (15). The effects of ACE on the living organisms and the environment need to be determined. Some studies have shown that ACE causes acute toxicity and also damages the nervous, reproductive and immune systems (5). It has been found to have genotoxic effects alone or in combination with other pesticides (15-18). In this study, we aimed to determine whether ACE in the neonicotinoid group shows genotoxicity in mouse bone marrow cells by using CA and MN analysis system.

MATERIALS and METHODS

Animal Material

Approval of the study was obtained with the permission of 17.03.2017/036 by Animal Experiments Local Ethics Committee at Kafkas University.

A total of 40 male mice of *Mus musculus* albino, weighing 20-30 g, were used in the study. The dose of the substances to be administered was determined according to the daily weights of the animals, and dissolved in distilled water and then administered through oral gavage.

Method

Mice were divided into 4 groups (10 mice). Group 1, NC group and were given distilled water through orally for 14 days. Group 2., 3. and 4. received (5,10 and 15 mg/kg) ACE through orally for 14 days (19).

Chromosomal Examination and Detection of Mitotic Index

The preparation of metaphase preparats for the detection of chromosomal aberration and mitotic index was performed according to Preston, taking into consideration our laboratory and working

conditions (20,21). For mitotic index and micronucleus test, mice in all groups were injected i.p at 4 mg/kg dose of colchicine prepared by being dissolved in distilled water 2 hours before euthanasia at the beginning of the 15th day. The animals were dislocated and femoral bones were removed. The bone marrow was removed from the femur by an injector and transferred to a centrifuge tube containing 3 mL of calf serum. Tubes of one of the femoral bones were centrifuged. Cells were incubated for 20-30 minutes in a heated hypotonic solution. After incubation, the cells were centrifuged. Cells were fixed in freshly prepared 5 mL of Carnoy's, then centrifuged to discard the supernatant. The fixation was repeated 3 times by discarding more than 0.5 mL supernatant in the tube after each centrifugation. The remaining cells were suspended by a pastor pipette, were dropped from 3-4 cm above and spread to moist clean slides. Preparations which were stained with 10% Giemsa solution prepared for 10 minutes. The number of cells in the metaphase stage was determined by counting and their percentages were determined. In this way, five preparations were prepared for one animal from each dose group. From these preparations, 100 metaphase cells were counted for the CA and 1000 cells for the MA.

Micronucleus Test

Micronucleus detection in bone marrow preparations was performed by modifying the method firstly developed by Schmid (22,23). The femoral bones that were cleaved from their muscles were cut at both ends and removed by a bone marrow syringe and placed in a centrifuge tube containing 3 mL calf serum. Cells were suspended by adding a drop of calf serum to the remainder of the tube. A drop of this was taken and spread on clean slides. The slides were dried in air and fixed in methyl alcohol for 10 minutes. After fixation, the preparations were stained with May Grunwald (5 minutes) and Giemsa (30 minutes). 1000 cells were counted from each preparation. The numbers of NCE, PCE and MNPCE in these cells which are used for the micronucleus detection and percentages of these cells were calculated.

Statistical Analysis

SPSS 22 package program was used for statistical analysis of the data in our study. One-way ANOVA was used to determine the difference between the negative control and test groups. Tukey test was used to identify differences between groups. $P<0.05$ was statistically considered significant.

RESULTS

Chromosomal Aberration Rates

When the parameters of the mice in the NC group and other groups treated with ACE were examined, it was found that CA rate of animals in the group receiving ACE at 15 mg/kg dose was highest compared to the other groups and this difference was statistically significant compared to the NC group ($P<0.001$). Differences in CA rate in the groups receiving ACE at a dose of 5-10 mg/kg compared to the NC group were not found to be statistically significant (Figure 1). Chromatide fusion, chromosomal fracture, chromatide fracture and fragment images obtained from group 4 are given below (Figure 2), respectively.

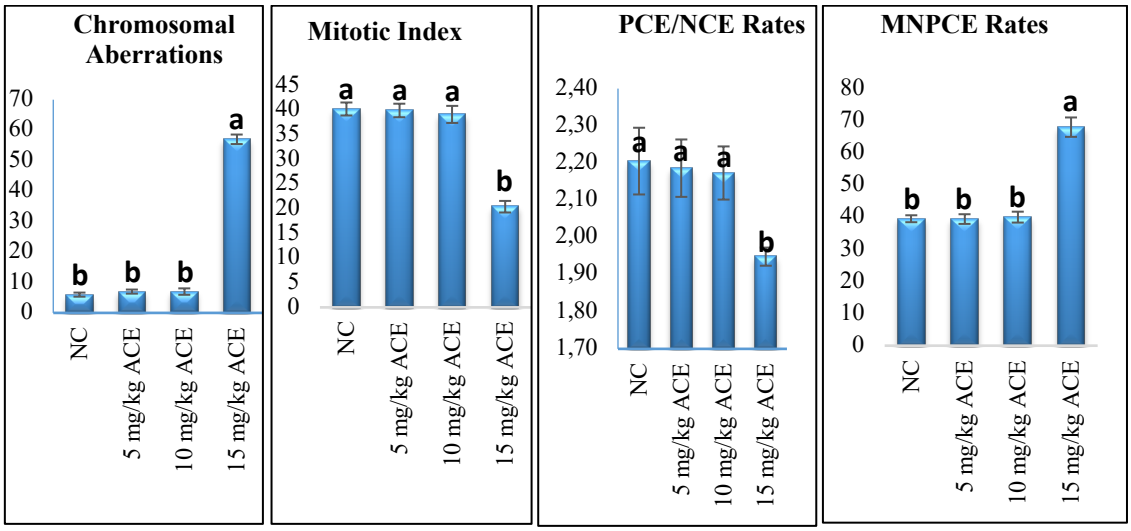

Figure 1. Chromosomal aberration (P<0.001), Mitotic index (P<0.001), PCE/NCE (P<0.001), and MNPCE rates (P < 0.001) of negative control and test groups.
*ACE: Acetamiprid, NC: Negative control, PCE: Polychromatic erythrocyte, NCE: Normochromatic erythrocyte, MNPCE: Micronucleated polychromatic erythrocyte.
Şekil 1. Negatif kontrol ve test gruplarında kromozomal aberasyon, mitotik aktivite, PCE/NCE ve MNPCE oranları.
*ACE: Asetamiprid, NC: Negatif kontrol, PCE: Polikromatik eritrosit, NCE: Normokromatik eritrosit, MNPCE: Mikronükleuslu polikromatik eritrosit.

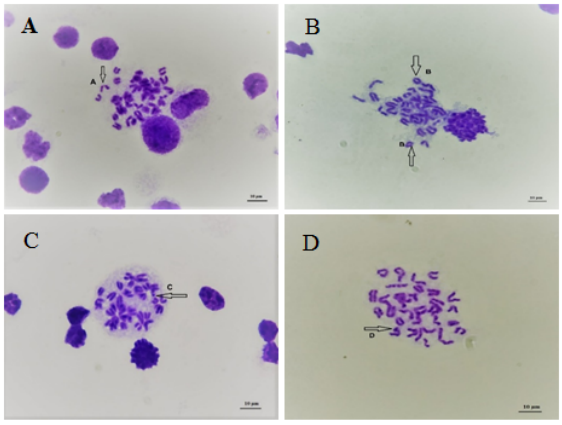

Figure 2. Chromosome fracture image (A), sister chromatid fusion image (B), fragment image (C) and chromid fracture image (D) (x 1000).
Şekil 2. Kromozom kırığı (A), kardeş kromatid birleşmesi (B), fragment (C) ve kromatid kırığı (D).

Mitotic Index Rates

It was observed that the MA decreased in the group with 15 mg/kg ACE and this was found to be statistically significant compared to the NC group (P<0.001). The difference between the NC group and the groups with 5-10 mg/kg doses of ACE was not statistically significant (Figure 1).

Micronucleus Frequency

It was observed that the implementation of ACE at a dose of 15 mg/kg to mice reduced the PCE/NCE ratio (Figure 1). While the difference in the increase in MNPCE rates was statistically insignificant between the NC group and the groups with 5-10 mg/kg ACE, it was found to be significant in the groups with ACE at 15 mg/kg dose (P<0.001, Figure 1). Polychromatic erythrocyte figures of single MN in the bone marrows are shown below (Figure 3).

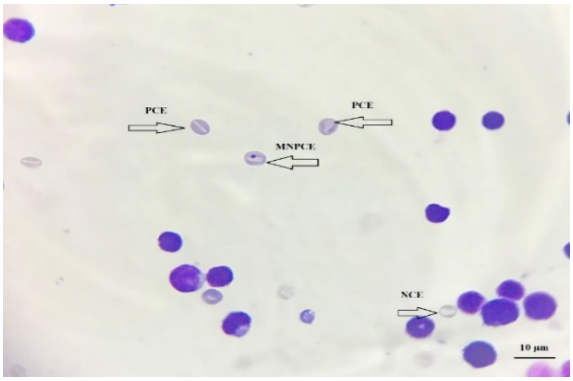

Figure 3. Single-micronucleated polychromatic erythrocyte (MNPCE) (x 1000).
Şekil 3. Tek mikronükleuslu polikromatik eritrosit (MNPCE).

DISCUSSION and CONCLUSION

Vertebrates exposed to insecticides, there is an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) (24). The resulting ROS and RNR cause damage by reacting with biological systems such as DNA, protein and cell membranes in the body (25). The antioxidant enzymes normally found in the body eliminate the harmful effects of these radicals by detoxifying them. Superoxide dismutase (SOD) and catalase (CAT) enzymes protect the cell against insecticide by destroying ROS (26,27).

Yao et al. (28) in their study reported that the ACE increases the SOD and CAT enzyme levels in three bacteria species for a short time. The presence of SOD and CAT enzyme activities is important to indicate the presence of superoxide radicals (29). In physiological conditions, superoxide anions (O_2^-) are reduced by SOD to hydrogen peroxide (H_2O_2). CAT enzymes prevent the formation of hydroxyl radicals by converting hydrogen peroxide into H_2O and O_2 (29). However, when the production of ROS and RNR is too high, an imbalance occurs between the antioxidant system and free radicals, which is called oxidative stress (30). This leads to the formation of hydroxyl free radicals which can cause DNA strand breakage by increasing superoxide and hydrogen peroxide anions (29). ACE-induced cytotoxicity has been reported to be caused by superoxide anions (31).

EPA reported that the ACE was not mutagenic in the ames test in an in vivo study of Chinese hamster ovary cells and did not induce DNA synthesis in rat liver primary cells and mammalian liver cells as in vivo. In an in vitro CA study using CHO cells, the ACE was genotoxically positive when tested under metabolic activation at the cytotoxic dose level (32). This effect was not detected when there was no metabolic activation. In a CA study in mouse bone marrow, the ACE was reported to be non-clastogenic, nor did it produce MN in the in vivo mouse bone marrow test. However, studies It has shown that the ACE leads to DNA damage by increasing CA and MN formation (32).

Few studies have investigated the genotoxic effect of ACE. The first study investigating the relationship between ACE and genotoxic damage was conducted by Kocaman and Topaktaş. Scientists investigated the genotoxicity in human peripheral blood lymphocytes using 25, 30, 35 and 40 µg/mL doses of Mosetam 20 S (ACE 20%), a trademark by sister chromatid exchange (SCE), CA and MN test methods. They found significant increases in the frequencies of the SCE, the CA and the MN after 24 hours of exposure (15). Besides, Kocaman and Topaktaş studied the genotoxic effect of the ACE and a mixture of α-cypermethrin (α -CYP), which is also a pyrethroid insecticide, at 12.5 (ACE) + 2.5 (α-CYP), 15.1 (ACE)+5 (α-CYP), 17.5 (ACE) + 7.5 (α-CYP), and 20 (ACE)+10(α-CYP) µg/mL doses in the human peripheral blood lymphocytes by CA, MN and SCE techniques in their study. They found that exposure to ACE+α-cymethrin mixture for 24 and 48 hours increased CA and SCE depending on concentration. When the increase in MN rates is compared to the control, ACE+α-cymethrin mixture synergistically induces cytotoxicity and genotoxicity (33). Gökalp Muranlı et al. studied the genotoxic effects of single and combined uses of ACE and propineb (PP) insecticides in human peripheral blood lymphocytes using micronucleus test technique. In their study, lymphocytes were exposed to ACE (0.625, 1.25, 2.5 µg/mL), PP (12.5, 25, 50 µg mL) and ACE-PP mixture (0.625 + 12.5, 1.25 + 25, 2.5 + 50 µg/mL) for 1 and 2 days). They found that exposure to a 48-hour ACE-PP mixture produced a significant increase in MN rates, whereas their use alone did not produce a significant increase in MN rates (34). Çavaş et al.(14) reported that ACE has cytotoxic and genotoxic potential on small intestine cells using MN, comet and γH2AX test methods on CaCo-2 cells. In another study they also suggested that ACE has genotoxic effects on human lung cells and that fullerenol nanoparticles improve the effect of ACE by cleaning ROS (35). Bagri and Jain investigated the genotoxicity of ACE using CA and MN

test method in the cells of a mouse in a study. ACE was administered intraperitoneally to mice at a dose of 4.6 and 2.3 mg/kg for 60 and 90 days. As a positive control, cyclophosphamide was administered at a dose of 50 mg/kg. As a result, ACE was reported to be both genotoxic and cytotoxic after administration of 4.6 mg/kg for 60 and 90 days (36).

ACE and imidacloprid are new generation insecticides belong to the neonicotinoid group. Although they are of the same class of insecticides, ACE is a cyanoamide and imidacloprid is a nitroguanidine derivative. This chemical difference suggests that lower doses of ACE may cause more genotoxic effects (4). Bansal et al investigated the genotoxic effect of ACE and imidaclopridin on Culex quinquefasciatus reproductive systems using the dominant lethal test (DLT) in their study. According to the results of the study, the ACE at the lower doses shows a very high effect on ACE-induced genotoxic damage compared to imidacloprid (37). Rust and Saran showed that the genotoxic effect of ACE is higher than imidaclopride. Rust and Saran, in their work with thermal species compared ACE toxicity with imidacloprid and thiamethoxam and found that ACE has got toxicity higher than imidacloprid, but less than thiamethoxam (38).

In this study, according to CA, MA and MN test results, it has been observed that the ACE administered at doses of 5-10 mg/kg was not gentotoxic (the differences were statistically insignificant), but ACE at 15 mg/kg significantly increased CA and MN frequency and decreased the PCE/NCE ratios together with MA. These results suggest that high doses of ACE have a genotoxic effect.

Consequently, doses, the studied concentrations, exposure period, the application routes way (orally) and the method used for the investigation of ACE were different than the previous studies on ACE genotoxic effects. With this study, it can be concluded that required precautions should be taken during the application of ACE. Moreover, this study sheds light on the studies on the effect of ACE.

ACKNOWLEDGEMENT

This study was supported by Scientific Research Projects Coordination Unit at Kafkas University within the scope of project number 2017-FM-30. This article is derived from the master's thesis.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. FAO, 1993. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. Geneva, FAO Plant Production and Protection Paper, 122.
2. Akdoğan A., 2011. Bazı pestisitlerin kromatografik ayrılmaları ve tayinleri. Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü/ Kimya Anabilim Dalı, Türkiye.
3. Zahm HS., Ward HM., 1998. Pesticides and childhood cancer. Occupational epidemiology branch, division of cancer etiology, national cancer institute, Rockville, Maryland. Environ Health Perspect, 106, 893-908.
4. Kocaman AY., 2007. Acetamiprid ve alpha-cypermethrin pestisidlerinin tek başına ve karışım halinde kullanıldıkları zaman insan periferal lenfositlerindeki in vitro genotoksik etkileri. Çukurova Üniversitesi, Fen bilimleri Enstitüsü/Biyoloji Anabilimdalı, Türkiye.
5. Sheetsa LP., Lib AA., Minnemac DJ., Collierd RH., Creeke MR., Pefferc RC., 2016. A critical review of neonicotinoid insecticides for developmental neurotoxicity. Crit Rev Toxicol, 46, 153-190.
6. Qintana MM., Rivero Osimani V., Magnarelli G., Rovedatti MG., Guinazu N., 2018. The insecticides chlorpyrifos and acetamiprid induce redox imbalance in umbilical cord blood erythrocytes in vitro. Pestic Biochem Physiol,

148, 87-92.

7. Cepeda S., Forero Castro M., Cardenas-Nieto D., Martinez-Agüero M., Rondon-Lagos M., 2020. Chromosomal instability in farmers exposed to pesticides: high prevalence of clonal and non-clonal chromosomal alterations. Risk Manag Healthc Policy, 11, 97-110.
8. Şenyıldız M., Kılınç A., Ozden S., 2018. Investigation of the genotoxic and cytotoxic effects of widely used neonicotinoid insecticides in HepG2 and SH-SY5Y cells. Toxicol Ind Health, 34, 375-383.
9. Sanchez-Bayo F., Tennekes HA., 2020. Time-cumulative toxicity of neonicotinoids: experimental evidence and implications for enviromental risk assesmants. Int J Environ Res Public Health, 17, 1629-1649.
10. Casida J., Quistad GB., 2004. Why insecticides are more toxic to ınsect than people: the unique toxicology of insects. J Pestic Sci, 29, 81-86.
11. De Lima E Silva C., De Rooij V., Verweij RA., Van Gestel CAM., 2020. Toxicity in neonicotinoids to folsima candida and eisenia and andrei. Environ Toxicol Chem, 39, 548-555.
12. Dünya Sağlık Örgütü (World Health Organization, WHO), 2009. The WHP recommended classification of pesticides by Hazard and Guidelines to Classification, World Health Organization Programme on Chemical Safety, Geneva, Switzerland.
13. Environmental Protection Agency (EPA), 2003. Pesticides-fact sheet for clothianidin; pesticide tolerance, feredal register. Enviromentel Protection Agency (EPA), Pesticides–Fact Sheet for Thiacloprid, 68, 32390-32400.
14. Çavaş T., Çinkılıç N., Vatan Ö., Yılmaz D., Çoşkun M., 2012. In vitro genotoxicity evaluation of acetamiprid in Caco-2 Cells using the micronucleus, comet and γh2ax foci assays. Pestic Biochem Physiol, 104, 212-217.
15. Kocaman AY., Topaktaş M., 2007. Invitro evaluation of the genotoxicity of acetamiprid in human peripheral blood lymphocytes. Environ Mol Mutagen, 48, 483-490.
16. Göç Rastgele P., 2017. Assessment of the combined effects of acetamiprid and propineb in vivo. Igdir Univ. J Ins Sci Tech, 7, 79-86.
17. Kocaman AY., Topaktas M., 2010. Genotoxic effects of a particular mixture of acetamiprid and alpha-cypermethrin on chromosome aberration, sister chromatid exchange, and micronucleus formation in human peripheral blood lymphocytes. EnvironToxicol, 25, 157-168.
18. Bansal M., Chaudhry A., 2011. Evaluation of mutagenic potential of acetamiprid by dominant lethal test on Culex quinquefasciatus. J Appl Nat Sci, 3, 171-175.
19. Bagri P., Kumar V., Sikka AK., 2016. Assessment of imidacloprid-induced mutagenic effects in somatic cells of Swiss albino male mice. Drug Chem Toxicol, 39, 412-417.
20. Preston RJ., Dean BJ., Galloway S., Holden H., McFee AF., Shelby M., 1987. Mammalian in vivo cytogenetic assays anaylsis of chromosome aberrations in bone marrow cells. J Mutat Res, 189, 157-165.
21. Darwish IAEM., Mosallam SAER., 2019. Chromosome aberrations in bone marrow cells of rats treated with MTBE. Pak. J. Pharm. Sci, 32, 89-93.
22. Schmid W., 1975. The micronucleus test. J Mutat Res, 31, 9-15.
23. Nallani GC., Liu Z., Chandrasekaran A., 2020. Toxicokinetic testing strategies to demonstrate bone marrow exposure in in vivo micronucleus study for genotoxicity assessment of agrochemicals. Reg Toxicol Pharmacol, 110, 1045-1052.
24. Klauning JE., 1991. Alterations in intracellular communication during the stage of promotion. Exp Biol Med, 198, 688-692.
25. Scassellati SG., Moretti M., Villarini M., Angeli G, Pasquini R., Monarca S., Scarselli R., Crea M.G., Leonardis C., 1994. An evalution of toxic and genotoxic risk from work related exposur to chemical compounds. Prevenzione Oggi, 6, 125-

138.

26. Banerjee BD., Seth V., Bhattacharya A., Pahsa ST., Chakraborty AK., 1999. Effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett, 107, 33-47.
27. Kayis T., Altun M., Coskun M., 2019. Thiamethoxam-mediated alteration in multi-biomarkers of a model organism, Galleria mellonella L. (Lepidoptera: Pyralidae). Environ Sci Pollut Res Int, 26, 36623-36633.
28. Yao XH., MIN H., LV ZM., 2006. Response of superoxide dismutase, catalase, and ATPase activity in bacteria exposed to acetamiprid. Biomed Environ Sci, 19, 309-314.
29. Halliwel B., Gutteridge JMC., 1999. Free radicals in biology and medicine. 3rd ed., 968-974, Oxford University press, New York.
30. Aruoma OI., 1998. Free radicals, oxidative stress and antioxidants in human health and disease. J Am Oil Chem Soc, 75, 199-212.
31. Gokalp Muranli FD., Göç Rasgele P., Kekecoglu M., Kanev M., Ozdemir K., 2015. Potentıal genotoxıcıty of acetamiprid and propineb sıngly or ın combınatıon ın cultured human perıpheral blood lymphocytes by usıng mn assay. Fresen Environ Bull, 24, 3947-3955.
32. Environmental Protection Agency (EPA), 2004. Acetamiprid; notice of filing a pesticide petition to establish a tolerance for a certain pesticide chemical in or on food. In Notice, 47145-47149.
33. Kocaman AY., Topaktaş M., 2010. 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, 25, 157-168.
34. Gokalp Muranli FD., Goc Rasgele P., Kekecoglu M., Kanev M., Ozdemir K., 2005. Potential genotoxicity of acetamiprid and propineb singly or in combınation in cultured human peripheral blood lymphocytes by using mn assay. Fresen Environ Bull, 24, 3947-3955.
35. Çavaş T., Çinkılıç N., Vatan Ö., Yılmaz D., 2014. Effects of fullerenol nanoparticles on acetamiprid induced cytoxicity and genotoxicity in cultured human lung fibroblasts. Pestic Biochem Physiol, 114, 1-7.
36. Bagri P., Jain SK., 2019. Assessment of acetamiprid-ınduced genotoxic effects in bone marrow cells of swiss albino male mice. Drug Chem Toxicol, 42, 357-363.
37. Bansal M., Kaur G., Chaudhry A., 2012. Pestıcıdes effect on genetic components: a genotoxic study on Culex quınquefascıatus by applyıng domınant lethal test. Int. J. Adv. Biol. Biomed. Res, 2, 685-690.
38. Rust MK., Saran RK., 2008. Toxicity repellency and effects of acetamiprid on western subterranean termite (ısoptera: Rhinotermitidae). J Econ Entomol, 101, 1360-1366.