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RESEARCH ARTICLE

Investigations About Genotoxicity of Deltamethrin [1] [2]

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Summary

Deltamethrin is a member of pyretroids with a potent effect on parasites. It is a very effective compound having the general characteristics of this group in a synthetic dibromo pyrethroid structure. Deltamethrin has a wide range of usage particularly in agriculture, preventive medicine programs, farm and pet animals. In the studies on toxicity, results were characterized by cholinergic effects, and no evident chronic toxic effect was observed in 2 years of feeding studies on rats. Although it is reported by EPA and FDA that it has no particular toxic effect, these findings are not in accordance with the results of genotoxicity studies. The result of the genotoxicity studies changes according to the live material used, the application route and dose of the compound. In the present study, genotoxicity of delthamethrin at the doses of 50, 100, 200 mg/kg/bw was investigated by *in vivo* micronucleus test. Deltamethrin was given to the mice intraperitoneally in 10% tween 80 with the doses presented above. To the positive control group, Mitomycin C, which is a mutagen agent, prepared in saline was given in 2 mg/kg/bw doses. At the 48th h after the application, by collecting bone marrow, blood and spleen samples, the formation of micronuclei in erythrocytes and splenocytes was investigated microscopically. In deltamethrin treatment groups, micronuclei frequency was determined and the results were found statistically significant when compared with negative control group ($P<0.001$). The micronuclei numbers obtained from different tissue samples of the same animal were also statistically significant ($P<0.001$). Particularly in the highest dose group, results were similar to the positive control group. According to these results, it was determined that in acute toxic dose deltamethrin showed genetic toxicity on somatic cells of mice.

Keywords: *Genotoxicity, Deltamethrin, Mice, Bone marrow, Mutagenicity, Micronuclei*

Deltametrin'in Genototoksisitesi Üzerine Araştırmalar

Özet

Parazitlere karşı yüksek düzeyde etkinliğe sahip piretroidlerin bir üyesi olan Deltametrin bu grubun genel özelliklerini taşıyan sentetik dibromo - piretroid yapısında çok etkili bir bileşik olarak tanımlanmaktadır. Deltametrin'in tarım, halk sağlığı koruma programları, çiftlik ve ev hayvanları başta olmak üzere geniş bir kullanım alanı vardır. Deltametrin ile ilgili toksisite çalışmalarında akut toksisite bulgularının kolinerjik etkiler ile karakterize olduğu tespit edilmiş olup 2 yıl süreyle ratlarda yapılan yedirme çalışmaları sonucunda belirgin bir kronik toksik etkisine rastlanmamıştır. EPA ve FDA tarafından özel toksik etkisinin olmadığı bildirilmekle beraber yapılan genotoksisite taramalarında elde edilen sonuçlar arasında bir uyum bulunmamaktadır. Genotoksisite taramalarının sonuçları, kullanılan canlı materyale, bileşiğin verilme yolu ve dozuna göre değişiklik göstermektedir. Çalışmamızda *in vivo* mikronukleus testi yöntemiyle, 50, 100, 200 mg/kg/canlı ağırlık dozda fareler üzerindeki genotoksik deltametrinin etkisi araştırılmıştır. Deltametrin yukarda belirtilen dozlarda %10'luk tween 80 içerisinde farelere intraperitonel olarak verilmiştir. Pozitif kontrol grubuna serum fizyolojik içerisinde hazırlanmış, mutajen bir ajan olan Mitomycin C, 2 mg/kg/canlı ağırlık dozda verilmiştir. Uygulamayı izleyen 48. saatte hayvanlardan kemik iliği, kan ve dalak numuneleri alınarak eritrosit ve splenositlerde mikronuklei oluşumu yönünden mikroskobik olarak incelenmiştir. Her üç doz grubunda da mikronuklei oluşumu görülmüştür ve sonuçlar istatiksel olarak da önemli bulunmuştur ($P<0.001$). Aynı hayvana ait farklı doku numunelerinden elde edilen mikronuklei sayıları da istatiksel olarak önemli çıkmıştır ($P<0.001$). Özellikle en yüksek doz grubunda pozitif kontrol grubuna yakın sonuçlar elde edilmiştir. Bu sonuçlardan yola çıkarak, Deltametrin'in akut toksik dozlarda fare somatik hücrelerinde genetik toksisite gösterdiği kanısına varılmıştır.

Anahtar sözcükler: *Deltametrin, Mutajenite, Mikronuklei, Genototoksisite, Kemik iliği, Fare*

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INTRODUCTION

Pyrethroids are semi-synthetic derivatives of natural chrysanthemumic acid which have a high anti-parasitic effect on mature and larval forms of many parasites. These kinds of compounds are anti-parasitic drugs preferred for their widespread use, low-toxicity for the mammals and absence of residues [1-7].

Deltamethrin; [(S)-a-cyano-3-phenoxybenzyl-(1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate] is a member of pyrethroids, and possesses the general properties of this group. It is a synthetic dibromo-pyrethroide and is defined as the compound that has the greatest effect. Deltamethrin is widely used in agriculture, public health preservation programs for farm animals and pets. Once taken by insects orally or from the cuticula, they cause paralysis and death in a short time with a "knock down" effect. The most important advantages of the compound are that they have a low acute toxicity for the non-target mammals and they do not cause phyto-toxicity despite their strong insecticide effect. Although deltamethrin is reported to have a fast metabolism in living organisms, and a low level of residues in the environment; these may vary depending on the environmental conditions. Though ether bonds are broken down via UV and sun lights, it is quite tolerant to storage and can preserve its activity for 6 months at 40ºC. Deltamethrin is also found to have a more stabile formulation with acidic carriers, compared to alkalis [5-8].

As pyrethroids are widely used for their high activity on mature and larval forms of insects, they have a high risk to be taken by mammals [1,5,6,9]. Scanning of xenobiotics for mutagenic effect is a vital stage for toxicologic studies [10-16]. There are many studies assesing the mutagenic potential of deltamethrin. Baeza-Squiban *et al*. reported that deltamethrin was not carcinogenic in rats and mice [17].

Dass *et al*. found that while Deltamethrin did not cause a prominent increase in Micronuclei (MN) and Sister Chromatid Exchange (SCE) incidence, it caused DNA damage in Single Cell Gel Electrophoresis Assay (COMET) test [18]. Grisolia used the commercial formulation of Deltamethrin (Decis 25) both in rats and mice and assessed genotoxic effect of the compound through MN test. After a single dose application of 90 mg/kg in mice and 5 mg/kg in fish *(Tilapia rendalli)*, Grisolia detected an increase MN frequency [19]. Clearly, the foregoing information indicates there is controversy regarding the genotoxic effects of deltamethrin.

Mutational changes covering the most part of geno toxic effects are among the ones assessed in genotoxicity studies. Thus; the mutagenic effect of a compound must be determined firstly by the identification of its genotoxic effect [5,15-17]. Therefore, genotoxic activity of deltamethrin was investigated to determine the mutagenic changes in somatic bone marrow, blood and spleen cells.

MATERIAL and METHODS

Experiment Design

Four-six month-old CD 1 mice were used as the subjects. To enable environmental adaptation, the animals, which weighed around 23±5 g were kept and fed in the experimental room for 15 days prior to injection. Water and food were given ad libitum, pelleted food was used. All the animals were kept in light for 12 h and in dark for the other 12 h. 120 mice used were divided into 6 groups randomly. The 1st group was the control group (K); the 2nd was the negative control group (NK), which was given 10% tween 80 in 10 mg/kg body weight dose; the 3rd group was the Positive control group which was given Mitomycin c (PK); group 4 was given deltamethrin at a dose of 50 mg/kg body weight (D1); group 5 was given deltamethrin at a dose of 100 mg/kg body weight (D2); group 6 was given deltamethrin at a dose of 200 mg/kg body weight (D3). This study was approved by the Experimental Ethic Committee of Istanbul University, Faculty of Veterinary Medicine.

Application of Deltamethrin

Deltamethrin solution was applied in a single dose based on (10 mg/ml in 10% Tween 80) body weight and was given to the groups intraperitoneally as 50, 100, and 200 mg/kg body weight doses. As the positive control, the mutagenic compound Mitomycin C, 2 mg/kg dose was also applied intraperitoneally to the group [11,16-18]. Tween 80, which was used as the vehicle of deltamethrin, was applied intraperitoneally to the negative control group. No injection was applied to the control group.

Preparation of Blood and Tissue Samples

Blood, bone marrow and spleen samples were taken in the 48th h after the injection. As peripheral blood sample, around 100 µl blood was taken from the tail veins of the animals anesthetized with ether, into heparinized-tubes using pasteur pipette.

After collecting the blood samples, the animals were killed via over dose ether inhalation. Both femurs were taken out and cut from both ends, the bone marrow was rinsed by 1 ml FBS (Fetal Bovine Serum) and taken into the test tube [20].

Immediately after this process, the whole spleen was taken into a petri dish containing 9 ml FBS (40%) and RPMI 1640 (60%) mixture.

The 5 µl blood taken from the blood sample in the heparinized-tube was smeared on 1 ml 65% percoll prepared with PBS (Phosphate buffer solution). It was centrifuged at 600 g for 20 min. Platelets and supernatants rich in big nucleated cells were taken and removed carefully. Pellet involving the erythrocytes remaining at the bottom of the tube and a limited number of nucleated

cells was diluted with 40 µl PBS to make smear preparate, and was allowed to dry on air [21].

Spleen samples within the RPMI 1640 (60%) and FBS (40%) mixture were filtered through a 100 µm steel filtration apparatus. Spleen capsule left on the apparatus was discarded. The suspension obtained in the petri dish was transferred into the 15 ml test tube and 5 min passed. At the end of this time, the suspension was divided equally into 2 conic test tubes. The tubes were centrifuged at 600 g for 5 min. The supernatant was carefully removed after the centrifuge. The remaining pellet in the tube was diluted with 40 µl PBS, and then was transferred into the tube involving 1 ml 67% percoll. These tubes were also centrifuged at 600 gr for 20 min; the supernatant was carefully removed and discarded after the centrifuge. The residue in the tube was diluted with 40 µl PBS to make smear preparate, and allowed to dry on air [21].

Once all the preparates got dried, they were fixed with methanol for 10 min. Following the drying of the preparates, they were stained with giemsa solution for 15 min. After this process, the preparates were kept in phosphate buffer solution for 5 min and enriched. The preparates were left to dry, and then they, were closed with lamella and examined through a microscope.

Microscopic Examination

All preparates were examined under light microscope at 100 multiples using immersion oil.

2.000 polychromatic erythrocytes were counted in blood and bone marrow for each sample. In these erythrocytes, number of cells involving micronuclei were determined. As for the preparates of spleen samples, 2.000 splenocytes were counted to identify the number of splenocytes with micronuclei.

Statistical Analyses

The data were analyzed by ANOVA which was followed by the post-hoc test *Duncan* [22].

RESULTS

Bone Marrow

Average number of Micronuclei *(Fig. 1A)* obtained from control and negative control groups were similar (5.9±0.223 and 5.4±0.210 respectively) *(Table 1)*. The 10% Tween 80 was found to have no effect on micronuclei formation. On the other hand; 50 and 100 mg/kg body weight doses of deltamethrin were found to cause significantly micronuclei formation (17.6±0.222 and 18.8±0.421 respectively, *Fig. 2)* ($P<0.001$). The change in MN formation was found dose-dependent between the two dose groups however, the difference between them was also found to be statistically insignificant. The number of micronuclei in the highest dose group was found to be significantly high compared to the control and negative control groups ($P<0.001$), and this was reported to cause the formation of micronuclei that is close to the amount in the positive control group. While this group showed no significant difference from the other two dose groups, no positive relationship was found among them, either.

Blood

Although no difference *(Fig. 3)* was found between

Table1. *Control, negative control, positive control and experimental groups, the bone marrow, blood, and spleen, the number of samples micronuclei*
Tablo1. *Kontrol, negatif kontrol, pozitif kontrol ve deney gruplarının kemik iliği, kan ve dalak numunelerindeki mikronuklei sayıları*

Tissue	n	P<	D1		D2		D3		PK		K		NK	
			x	Sx	x	Sx	x	Sx	x	Sx	x	Sx	x	Sx
Spleen	20	***	6.5^{d}	0.224	7.5^{c}	0.154	11.7^{b}	0.309	11.65^{a}	0.473	2.9^{e}	0.161	2.9^{e}	0.124
Blood	20	***	9.25^{c}	0.676	9.55^{c}	0.432	13.4^{a}	0.184	20.5^{b}	0.221	4.93^{d}	0.24	4.4^{d}	0.134
Bone marrow	20	***	17.6^{b}	0.222	18.8^{b}	0.421	27.7^{a}	0.711	26.55^{a}	0.763	5.9^{c}	0.223	5.4^{c}	0.210

^{a,b,c} *P<0.001*

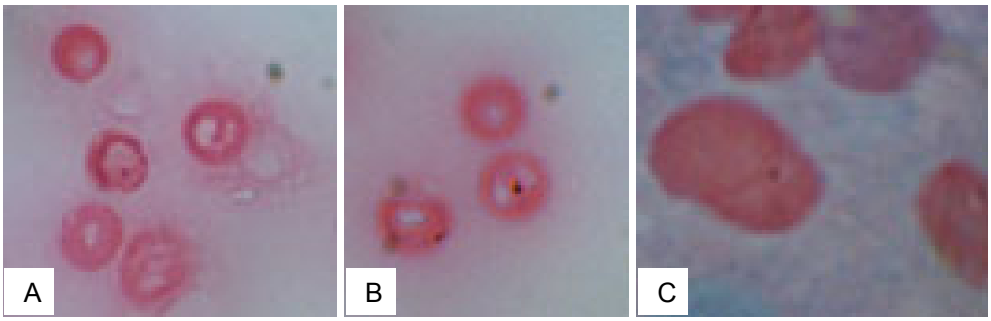

Fig 1. Bone marrow sample, polychromatic erythrocytes containing micronuclei **(A)**, Blood sample, red blood cells containing micronuclei **(B)**, Splenocytes in the spleen with micronuclei **(C)**

Şekil 1. (A) Kemik iliği numunesi, mikronukleili polikromatik eritrosit, **(B)** Kan numunesi, Mikronukleili kırmızı kan hücresi, **(C)** Dalakta mikronukleili splenosit

the control and negative groups in the microscopic examination of the blood samples, all the three dose groups (50, 100, 200 mg/kg body weight) caused to induce micronuclei formation *(Fig. 1B)*. The groups to which 50 mg/kg and 100 mg/kg doses of deltamethrin were given, had a similar number of micronuclei (9.25±0.676 and 9.55±0.432 respectively) and the difference between them were statistically insignificant *(Table 1)*. Whereas; the difference between the control groups and the first two dose group concerning the number micronuclei was significant (P<0.001) *(Table 1)*. The group which was given 200 mg/kg dose deltamethrin, had the greatest number of micronuclei formation among all experimental groups (13.4±0.184). Compared to other groups, the greatest dose group showed statistically significant results (P<0.001) concerning the number of micronuclei.

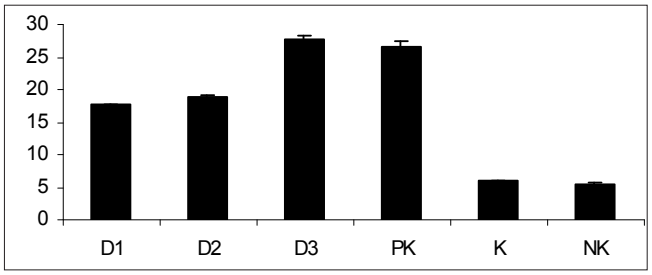

Fig 2. Number of Bone Marrow Micronuclei. D1: 50 mg/kg, D2:100 mg/kg, D3: 200 mg/kg, PK: MMC 2 mg/kg, K: control, NK: negative control

Şekil 2. Kemik İliği Mikronüklei Sayıları. D1:50 mg/kg, D2:100 mg/kg, D3: 200 mg/kg, PK: MMC 2 mg/kg, K: kontrol, NK: negatif kontrol

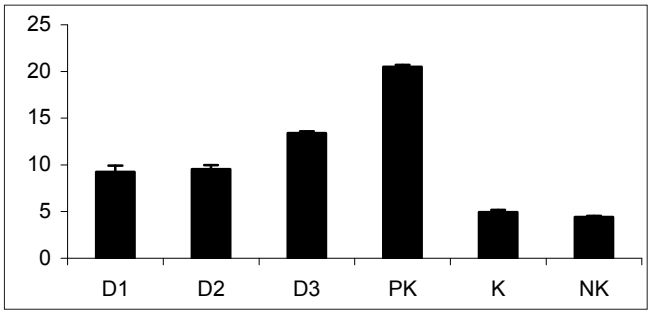

Fig 3. Number of red blood cells containing micronuclei. D1: 50 mg/kg, D2: 100 mg/kg, D3:200 mg/kg, PK: MMC 2 mg/kg, K: control, NK: negative control

Şekil 3. Kan Mikronüklei Sayıları. D1: 50 mg/kg, D2:100 mg/kg, D3: 200 mg/kg, PK: MMC 2 mg/kg, K: kontrol, NK: negatif control

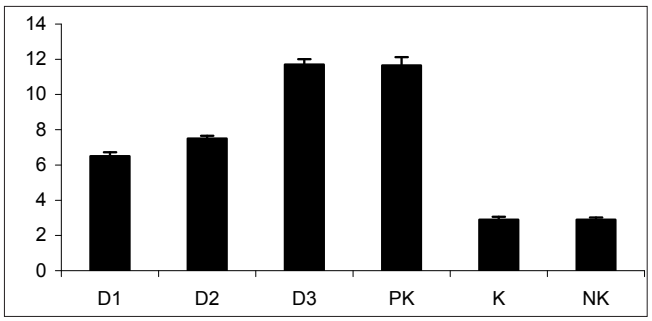

Fig 4. Number of Splenocytes with micronuclei. D1: 50 mg/kg, D2: 100 mg/kg, D3: 200 mg/kg, PK: MMC 2 mg/kg, K: control, NK: negative control

Şekil 4. Dalak mikronüklei Sayıları. D1: 50 mg/kg, D2: 100 mg/kg, D3: 200 mg/kg, PK: MMC 2 mg/kg, K: kontrol, NK: negatif kontrol

Spleen

In spleen samples *(Fig. 1C)*, control and negative control groups formed an equal number of micronuclei, and no statistically significant difference was found among them. However; micronuclei formation was found to be induced in each of the three dose groups (in groups which were given 50, 100 and 200 mg/kg dose deltamethrin; 6.5±0224, 7.5±0.154 and 11.7±0.309 respectively) *(Fig. 4)*. Although the number of micronuclei in the first two dose groups was quite close to each other, the difference between them was statistically significant (P<0.001) *(Table 1)*. The number of micronuclei in these two dose groups and the difference between them were found to be statistically significant (P<0.001). The greatest dose group (200 mg/kg) was identified to have the greatest number of micronuclei, and its difference from the control groups were found to be statistically significant (P<0.001).

Tissue samples of all groups were compared with each other and significant statistical differences were found. In study groups were presented in *Table 1* the distribution of micrunuclei numbers.

DISCUSSION

Although deltamethrin was reported to have no specific toxic effects such as teratogenic or carcinogenic, controversial results were reported regarding its genotoxicity. FDA (US Food and Drug Administration) and EPA (US Environmental Protection Agency) reported no specific toxic effects of deltamethrin, including genotoxicity. However; it is claimed that, it may cause DNA damage depending on the test methods, live material, compound delivery method and dose [1-4]. Many studies has been made to find out the genotoxicity of deltamethrin. Results obtained *from in vitro* bacterial, *in vitro* mammalian cell and *in vivo* genotoxicity tests do not show a complete agreement [1,5-7,23].

In vitro bacterial genotoxic activity of deltamethrin was assessed through different bacterial strains and different test methods. No genotoxic activity of deltamethrin was found in mutant strains of *S. typhimurium, E. coli* and *S. cerevisiae* in Ames test, DNA repair test, plate incorporation and fluctuation tests. In all these tests, the presence or absence of metabolic activation system was reported to have no effect on the results [6]. Villarini *et al.,* studied deltamethrin in human peripheral blood cells in tree different methods as *in vitro*. In Comet test, in the presence of metabolic activation, deltamethrin showed positive results at 100 µg/ml or higher concentrations and

a positive relationship was found between DNA damage and the doses applied. On the other hand, *in vitro* SCE and Micronuclei tests showed negative results. Authors attributed these controversial results to the fact that; Comet test was applied to resting cells while SCE and MN tests were applied to cells at proliferation stage, and that, strong DNA repair mechanisms may be involved in the process at this proliferation stage [24]. Pastor *et al*. performed a population study concerning mutagenicity and carcinogenicity on the farmers who had been exposed to pesticides among which deltamethrin was present. Exposure to deltamethrin or other pesticides were reported to cause no increase in MN and carcinogenicity frequencies [25].

Deltamethrin was studied through *in vivo* tests for genotoxicity and different results were found. These results show differences depending on the animal type, test type, dose of deltamethrin applied, and the type of the studied organ [18,26]. Micronucleus formation was dose-dependent with respect to the treatment doses of deltamethrin. In a study, deltamethrin was orally given at as 5 and 10 mg/kg doses to the mice for two days. According to micronuclei frequency test results in immature erythrocytes and chromosome aberration test results in bone marrow cells, deltamethrin was reported to show a mutagenic activity [6]. Gandhi *et al.* applied 32.5, 162.5 and 300 mg/kg doses intraperitoneally to mice in order to study the genotoxicity of deltamethrin through *in vivo* micronucleus test of bone marrow samples at 6th and 30th h. While the results of 32.5 mg dose group test were found to be similar to those of the control group, 162.5 and 300 mg/kg dose groups were found to cause micronuclei [27]. Agarwal *et al.* applied deltamethrin to adult albino, female rats as a single dose through intraperitoneal, subcutan and oral route at 5.6, 8.4, 11.2 mg/kg doses and 2.24 mg/kg dose in IP route for 5 days. 24 h after the treatment, mitotic index was inhibited in accordance with the dose, and an increase in chromosome aberration frequency was observed in bone marrow. Authors reported a potential genotoxic effect of deltamethrin on mammals [28]. Cabral *et al.* applied 0.1,4 and 8 mg/kg dose deltamethrin in arachis oil to mice through oral gavage; and 0.3 and 6 mg/kg doses to the rats for 2 years. At the end of the study, an increase in lymphoma incidence in mice, and thyroid tumour incidence in rats was observed. However; this effect was reported to show no dose-response relationship and deltamethrin was reported to show no carcinogenic effects on both animal types [29]. Shukla and Taneja assessed the mutagenic potential of deltamethrin through dominant lethal test, and found that deltamethrin had a mutagenic potential [30]. Cesar Koppe Grisolia assessed two insecticides, two herbicides and three fungicides involving the commercial formulation of deltamethrin (Decis 25 CE, 25g/l deltamethrin) through mouse micronucleus and fish micronucleus tests for their genetotoxic potentials. Thus; a single 8, 33, 90 mg/kg doses of commercial preparations were applied to the mice and a single 1, 5, 15 mg/kg dose to the fish *(Tilapia rendalli)*. Twenty four h after these, MN tests were performed on bone marrow cells in mice, and peripheral blood in fish. While Decis 25 caused MN frequency only in 5 mg/kg dose in *T. rendalli*, it caused MN frequency only in 90 mg/kg dose in mice. According to these results, the researcher reported that, deltamethrin had a mutagenic effect [19]. On the other hand; Polokova *et al.* applied low doses of (1.36, 3.4 and 6.8 mg/kg per day) deltamethrin to mice every other day. Smear preparate was made from bone marrow at 6th, 24th and 48th h and micronuclei development was studied. According to these, deltamethrin was reported to be non-mutagenic [31]. The doses used in this study of Polokova *et al.* were much lower than dose of the currents study, and applications were made every other day. Authors reported the toxic effect may be dependent on differences in dosage and route of administrations [31].

These studies show that; while deltamethrin causes micronuclei formation in acute toxic doses. It provides a slight and statistically insignificant induction in lower doses.

Acute toxic doses are generally used to get a faster result from *in vivo* studies. In our study, acute toxic doses of deltamethrin were applied intraperitoneally. Application of the highest (200 mg/kg body weight) dose deltamethrin caused a high level of micronuclei formation which was also statistically significant compared to the control and negative control groups. Average numbers of micronuclei obtained in this dose group were found to be 27.7±0.711, 13.4±0.184 and 11.7±0.309 in bone marrow, blood and spleen respectively. The results of our study were in accordance with the results from other *in vivo* micronucleus studies in which deltamethrin were applied intraperitoneally. According to the results of our study, obtained from bone marrow, blood and spleen samples showed differences for groups to which the same doses were applied. In rodents, because the spleen are able to remove micro-nucleated polychromatic erythrocytes from the circulation [32]. The number of micronuclei obtained from spleen samples was lower. The ability of spleen to remove these cells from the circulation was observed similarly in blood samples. Although the numbers of micronuclei in blood samples were higher than in the spleen, they were less than the bone marrow. In conclusion, acute toxic doses of deltamethrin was found to have genotoxic activity on the test animals.

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