Effects of Bendiocarb Insecticide on Lipid Peroxidation, Antioxidant Enzymes and DNA Damage in Human Leukocytes

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Abstract: In this study, we aimed to determine the toxic effect of increasing doses of Bendiocarb, an insecticide from the carbamate group, on human blood cells *in vitro* via by Comet test, determination of total antioxidant capacity, antioxidant enzyme activity and malondialdehyde (MDA) level. Leukocyte isolation was performed from blood samples taken from six healthy male individuals who did not smoke and use alcohol and were not exposed to any chemicals in the working environment, and then their leukocytes exposed to increasing doses of Bendiocarb (20, 40, 80, 160 µg / mL). Comet test, iron reduction antioxidant capacity (FRAP), 2-2'azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS/TEAC) analysis with superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and MDA were evaluated. At the end of the study, it was determined that antioxidant enzyme levels decreased significantly due to dose increase, whereas MDA levels increased significantly in all doses ($p<0.05$). According to FRAP and TEAC methods, the antioxidant capacity of blood cells decreased significantly in increasing doses of Bendiocarb compared to the control group ($p<0.05$). In the Comet test, DNA tail percentage, length and moment were increased significantly in increasing doses of Bendiocarb and DNA damage was detected ($p<0.05$). This study is a preliminary study on this subject, and it has been shown in our study that the uncontrolled use of Bendiocarb pesticide in nature will have harmful effects on the environment and living things. We believe that the research will contribute to the literature and shed light on future comprehensive studies.

Bendiokarb İnsektisitinin İnsan Lökositlerinde Lipid Peroksidasyonu, Antioksidan Enzimler ve DNA Hasarı Üzerindeki Etkileri

Anahtar Kelimeler
Bendiocarb,
Genotoksisite,
Komet Testi,
Pestisit,
Antioksidan Enzimler

Özet: Bu çalışma da karbamat grubundan bir insektisit olan Bendiokarb'ın artan dozlarının insan kan hücreleri üzerine *in vitro* koşullarda toksik etkisini komet testi, total antioksidan kapasite tayini, antioksidan enzim aktivitesi ve malondialdehit (MDA) seviyesini değerlendirmek suretiyle belirlemeyi amaçladık. Sigara ve alkol kullanmayan, çalıştığı ortamda herhangi bir kimyasal maddeye maruz kalmayan sağlıklı altı erkek bireyden heparinli tüplere alınan kan örneklerinden lökosit izolasyonu yapılmış ve lökositleri artan dozlarda Bendiokarb'a (20, 40, 80, 160 µg/mL) maruz bırakılmıştır. Komet testi, demir indirgeme antioksidan kapasitesi (FRAP), 2-2'azinobis 3-ethilbenzotiazolin-6-sülfonik asit (ABTS/TEAC) analizleri ile antioksidan enzimler olan süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx) ve lipid peroksidasyon göstergesi olan MDA düzeyleri üzerine etkileri değerlendirilmiştir. Çalışma sonunda antioksidan enzim düzeylerinin doz artışına bağlı olarak anlamlı azaldığı, tüm dozlarda ise MDA düzeylerinin anlamlı olarak arttığı belirlenmiştir ($p<0.05$). FRAP ve TEAC yöntemlerine göre, kan hücrelerinin antioksidan kapasitesi, kontrol grubuna göre artan Bendiokarb dozlarında anlamlı olarak azalmıştır ($p<0.05$). Comet testinde artan Bendiokarb dozlarında DNA kuyruk yüzdesi, uzunluğu ve momenti anlamlı olarak artmış ve DNA hasarı tespit edilmiştir ($p <0.05$).
Bu çalışma bu konuda yapılmış bir ön çalışma niteliği taşımakta olup, çalışmamızda Bendiokarb pestisitinin doğada kontrolsüz kullanımının çevreye ve canlılara zararlı etkileri olacağı gösterilmiştir. Araştırmanın literatüre katkı sağlayacağı gibi bundan sonraki kapsamlı çalışmalara da ışık tutacağı düşünülmektedir.

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1. Introduction

Pesticides, which are widely used in agriculture, especially in the world, the residues left in the environment and foods become a serious public health problem. If the residual levels of pesticides in food exceed the daily acceptable limits, this can cause acute and chronic poisoning, as well as teratogenic effects, as well as very serious health problems such as mutagenic and carcinogenic effects [1].
Pesticides are chemicals used to reduce the destructive effects of living forms such as insects, rodents, weeds, fungi that reduce or damage nutritional values during production, storage and consumption of food sources living on or around humans, animals and plants, and because of their toxic effects on all organisms. They cause serious harmful effects in the ecosystem [2, 3].
Bendiocarb carbamate group is an insecticide [4]. Bendiocarb, whose commercial name is Ficam W and synonyms such as Garvo^R, Seedox^R, Turcam^R, Multamat^R, Niomil^R, Tattoo^R, Ent-27695, OMS 1394, NC 6897, was first prepared in 1967 at Chesterford Park Research Station, Fisons Agrochemical Division (UK) (5). It is in solid white crystalline form and its chemical formula is $C_{11}H_{13}NO_4$ and its molecular weight is 223.25 kDa [6].
Carbamate insecticides enter the bodies of both insects and mammals through contact, respiration and stomach. The LD_{50} dose of bendiocarb has been reported as 40 mg/kg orally in rats [7]. Symptoms of bendiocarb poisoning are weakness, blurred vision, headache, drop in blood pressure, irregular heartbeat, loss of reflexes and dizziness [4,8]. In many studies, it is revealed that Bendiocarb increases lymph-related tumors such as lymphoid and lymphosarcoma [9]. Although it is widely used worldwide, there are a limited number of studies on Bendiokarb use. In the literature review, studies related to DNA damage and oxidative stress caused by Bendiocarb in blood tissue are also extremely limited.
In this study, we aimed to determine the toxic effect of increasing doses of Bendiocarb on human blood cells *in vitro* conditions by evaluating comet test, total antioxidant capacity determination, antioxidant enzyme activity (SOD, CAT and GPx) and malondialdehyde (MDA) level.

2. Material and Methods

2.1. Chemicals

In the study, Bendiocarb was used in increasing doses (20, 40, 80, 160 µg/mL). Bendiocarb, chemicals used in biochemical analysis and comet test were obtained from Sigma-Aldrich (Germany).

2.2. Leukocyte isolation and treatment of Bendiocarb

Venous blood samples of approximately 20 ml were obtained in heparinized dry tubes from each of six male volunteers (range 25–30 years). All volunteers were healthy, had no drugs, no smoking, and none were exposed to any chemicals. Lymphocytes were isolated using the Biocoll (Source BioScience, Nottingham, U.K.) separating solution [10].
The samples were divided into two groups as control group (n = 6) and application group (n = 6). The application group is also divided into four groups and exposed to Bendiokarb for 1 hour at the determined doses. These;
Group 1: 20 µg / mL Bendiocarb applied group (n = 6),
Group 2: 40 µg / mL Bendiocarb applied group (n = 6),
Group 3: 80 µg / mL Bendiocarb applied group (n = 6),
Group 4: 160 µg / mL Bendiocarb applied group (n = 6),

2.3. Biochemical Analysis

MDA content, antioxidant enzyme activity and total antioxidant capacity analysis were determined by measuring the absorbance of the samples in a spectrophotometer. Protein concentration was determined according the method described by Lowry [11] using bovine serum albümin (BSA) as a standard.

Malondialdehyde (MDA) is one of the last products of polyunsaturated fatty acids peroxidation in the cells and is a very toxic molecule. A rise in free radicals causes overproduction of MDA. Malondialdehyde level is mostly known as a marker of oxidative stress. For the determination of MDA levels cells were incubated with thiobarbituric acid (TBA) at 95°C (pH 3.4). MDA reacts with TBA to form a colored complex. MDA levels were determined as a result of the measurements made in the spectrophotometer at a wavelength of 532 nm [12].

The activity of SOD was determination assaying the autooxidation and elucidation of pyrogallol by the method of Marklund and Marklund [13].This analysis was observed at 440 nm. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation prevention.

CAT activity was measured according to the method described by Aebi [14] via assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25°C. For CAT activity samples were diluted with TritonX-100. Changes in absorbance per unit time using the constant number, ε240: 0.0394 mM / cm, were taken as the measurement of catalase activity.

GPx activity was determined with H_2O_2 as a substrate. Analysis mixtures included reduced glutathione, glutathione reductase, NADPH and Tris–HCl. GPx activity was measured as the alter in absorbance at 340 nm [15]. GPx activity (ε340: 6220 M/cm) was calculated as the amount of NADPH spent by 1 mg of protein in 1 minute and the specific activity of its enzyme was determined.

The FRAP analysis was done by measuring changes in absorbance at 593 nm due to the production of FeII-tripridiyltriazine compound from the oxidized form of FeIII. The FRAP reaction was prepared by stir 300 mmol/L acetate buffer with 10 mmol/L 2,4,6 tripyridyl-s-triazine in 40 mmol/L HCl and with 20 mmol/L ferric chloride [16].

The TEAC analysis is based on the prevention of the absorbance of the ABTS by tested antioxidant [17]. Solution of ABTS was diluted with phosphate buffer saline (PBS) to an absorbance of 0.70 (± 0.02) at 734 nm. ABTS was mixed with biological samples and kept for 6 minutes. Then at 734 nm, the decrease in absorbance was measured.

2.4. Determination of DNA Damage by Comet Method

10 µL of blood tissue that was exposed to Bendiocarb for 1 hour in increasing doses in the tubes was taken and centrifuged by adding RPMI. Supernatant was thrown and agarose was added to the pellet and smeared on the slide. Slides are covered with coverslips and incubated at 4 C for 1 hour. Then, it was kept in electrophoresis diluted from stock for 1 hour [18]. Preparations were placed in an electrophoresis tank and carried out at 200 volts. The resulting preparations were stained after cleaning with pure water and examined under a fluorescent microscope. Data were analyzed using BS 200 ProP with software image analysis. As a result of imaging, tail DNA percentage, tail length and tail moment were determined for 50 comet cells and the differences between the groups were calculated statistically.

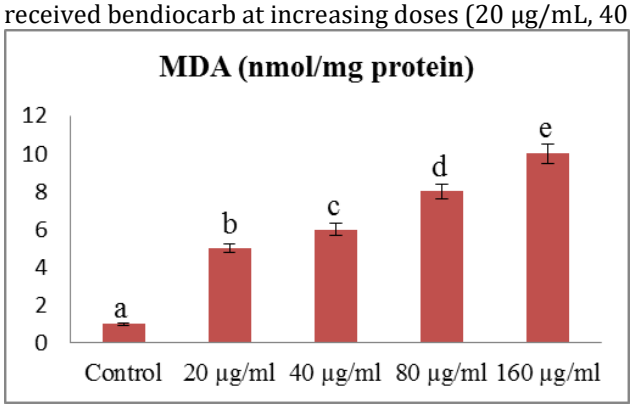
2.6. Statistical analysis

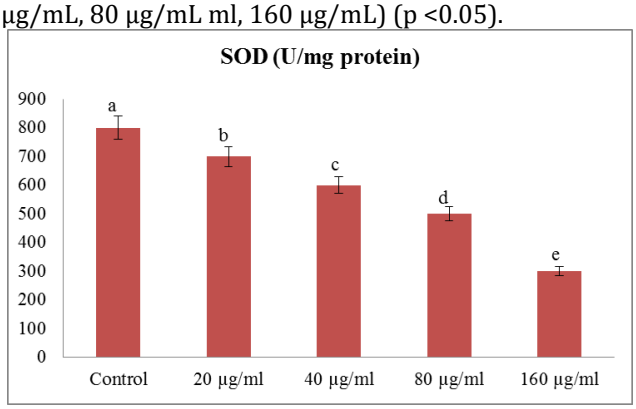
All of the statistical analyzes were done with one-way variance analysis (ANOVA) and Tukey test in Windows SPSS 11.5 computer program. P <0.05 value was considered statistically significant.

3. Results

3.1. Biochemical Results

MDA levels in blood cells were measured and results are given as nmol/Hb and nmol/protein for blood cells. The results obtained in determining the level of MDA in erythrocytes are shown in Figure 1. When the groups with increased doses of Bendiocarb and the control group were compared, it was observed that there was a statistically significant increase in MDA level due to dose increase ($p < 0.05$).
When compared to the control group, there was a statistically significant decrease in SOD enzyme activity due to dose increase in the groups that received bendiocarb at increasing doses (20 µg /mL, 40 µg/mL, 80 µg/mL, 160 µg/mL) ($p < 0.05$). (Figure 1). Compared to the control group, a statistically significant decrease in CAT enzyme activity was found in groups that received bendiocarb at increasing doses (20 µg/mL, 40 µg/mL, 80 µg/mL, 160 µg/mL) ($p < 0.05$). (Figure 1). GPx enzyme activity results in blood tissue erythrocytes are shown in Figure 1. Compared to the control group, a statistically significant decrease in GPx enzyme activity was found in groups that received bendiocarb at increasing doses (20 µg/mL, 40 µg/mL, 80 µg/mL ml, 160 µg/mL) ($p < 0.05$).





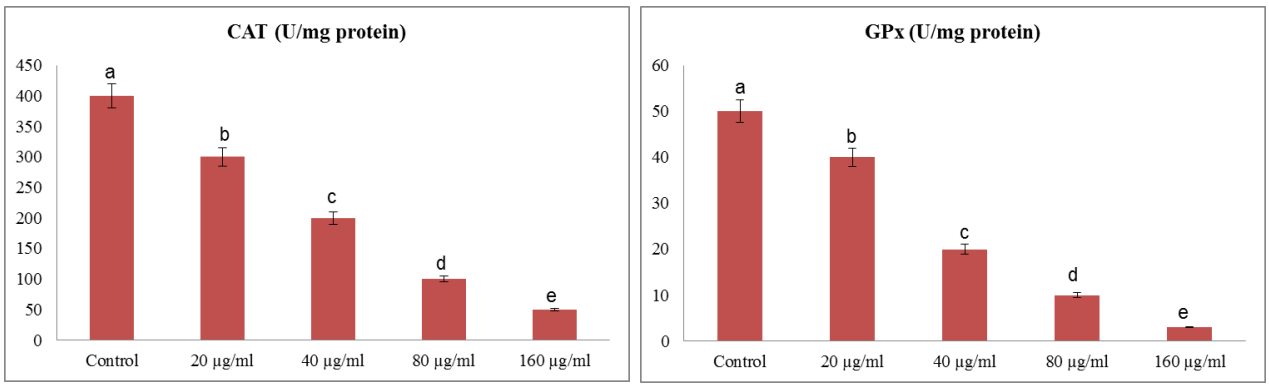


Figure 1. Biochemical analyses results (MDA, SOD, CAT, and GPx) ($P < 0.05$).

FRAP and TEAC values of the control and Bendiocarb application groups were calculated to determine antioxidant capacity changes in blood tissue and the results are shown in Figure 2. According to the FRAP and TEAC methods, the antioxidant capacity of blood cells decreased statistically significantly in increasing doses of Bendiocarb compared to the control group ($p < 0.05$) (Figure 2).

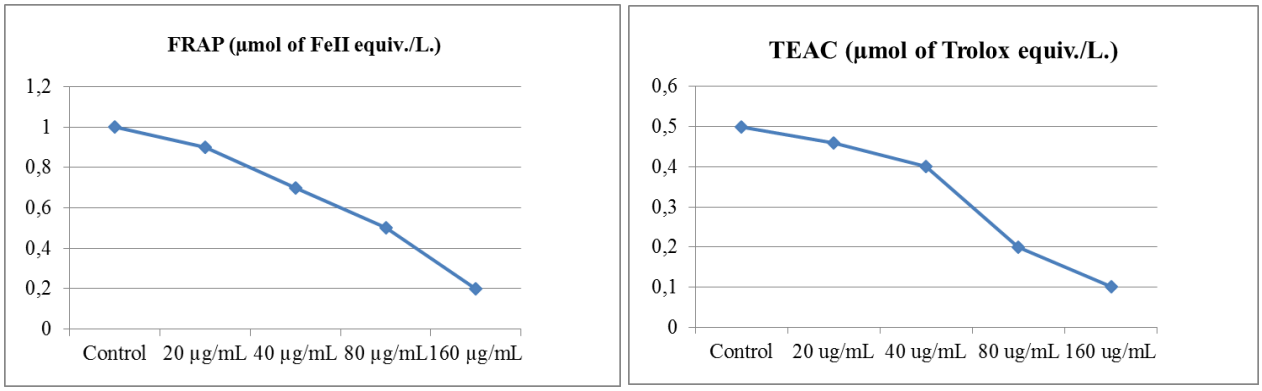


Figure 2. FRAP and TEAC results of human leukocyte cells exposed to increasing doses of bendiocarb.

3.2. Comet Assay Results

DNA tail percentage, tail length and tail moment values of the control group and application groups that we use to determine DNA damage in blood tissue are given in Table 1. When the control group and increasing doses of Bendiocarb application groups were compared in the Comet test, DNA tail percentage, tail length and tail moment were significantly increased in the increasing doses of Bendiocarb and DNA damage was detected (Figure 3).

Table 1. Average values of% DNA, tail length and tail moment of DNA damage (± SD) of human leukocyte cells

Doses	Tail% DNA ± SD	Tail Length ± SD	Tail Moment ± SD
Control	45.09±1.68^{a}	12.18±3.44^{a}	5.49±0.05^{a}
20 µg/mL	48.45±7.35^{a}	14.25±8.12^{a}	6.94±0.59^{a}
40 µg/mL	74.12±5.22^{b}	35.62±3.12^{b}	26.40±0.16^{b}
80 µg/mL	92.56±15.45^{c}	52.12±5.45^{c}	48.24±0.84^{c}
160 µg/mL	109.20±22.15^{d}	75.22±3.42^{d}	82.14±0,75^{d}

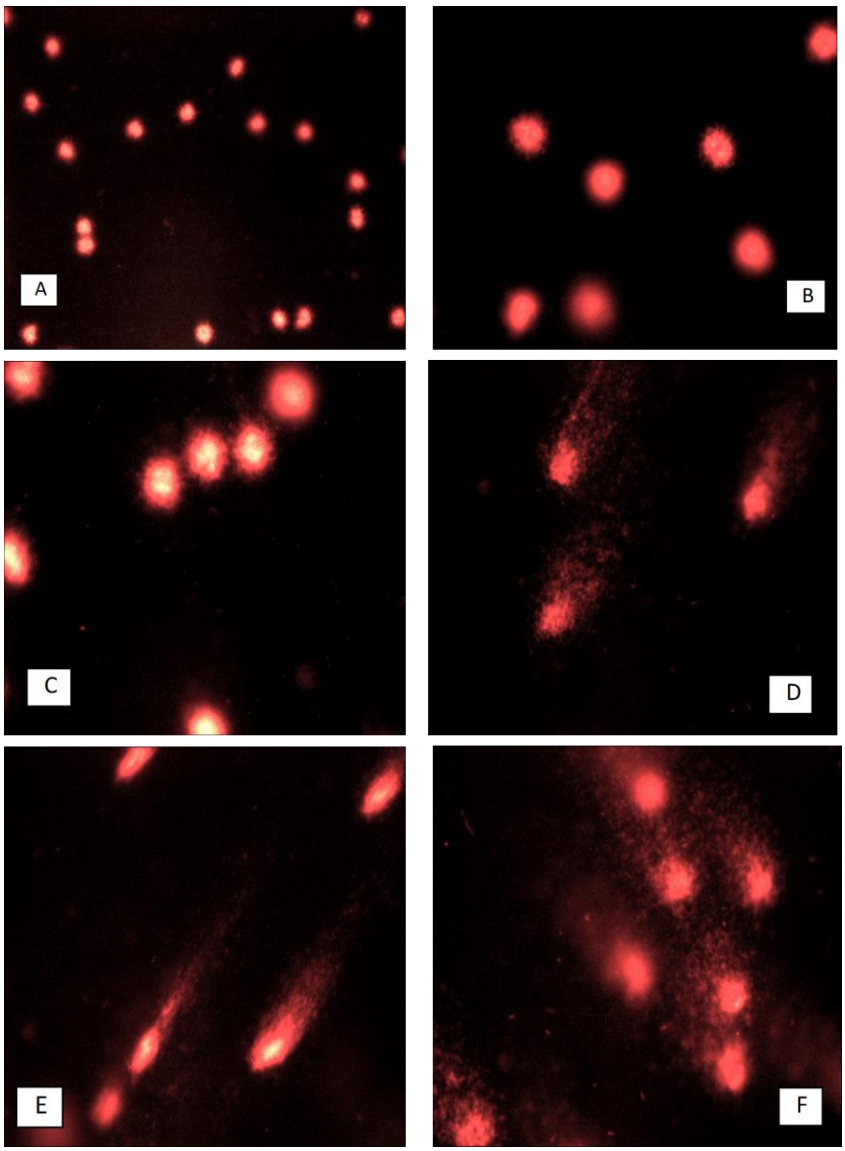


Figure 3. Comet analysis of the effect of increasing doses of Bendiocarb on the DNA of human leukocyte cells. (A-B) control, (C) 20 µg/mL, (D) 40 µg/ml, (E) 80 µg/mL, (F) 160 µg/mL.

As a result of incubation of bendiocarb with leukocytes, DNA damage at a concentration of 20 µg/mL was determined to be low and close to the control group ($p < 0.05$). DNA damage at 40, 80 and 160 µg/mL concentrations was statistically significant ($p < 0.05$) and higher than the control group. In addition, the highest percentage of Tail DNA was determined as 100% at a concentration of 160 µg/mL.

4. Discussion and Conclusion

Pesticides are frequently used in agriculture and applied to the environment to control pests. Since they are permanent in the environment, their toxicity must be known and risk assessments must be made.
Carbamate insecticides are carbamic acid derivatives used in agriculture in previous years due to their wide biological activity and low mammalian toxicity. Bendiocarb belongs to the class of carbamate insecticides and is effective against a wide range of agricultural pests. Bendiocarb is toxic for a lot of organisms, fish, birds and bees [19]. Determining pesticides left on air, soil and surface are essential to provide the grade and security of the products and reduce the risk to the health of people or other living things [20].

Agrochemical manufacturers, practitioners and people who consume pesticide agricultural products in the last link of the chain are exposed to pesticides at different rates, either acute or chronic. Pesticides entering the organism in various ways affect the nervous system, endocrine system, immune system, liver, heart and muscle tissues negatively. One of the most important systems among these negatively affected systems is the defense system, that is, the antioxidant system. There are various defense mechanisms in the body to prevent the formation

of ROS and the damage caused by them. These classes are known as 'antioxidant defense systems' and 'antioxidants'; They neutralize free radicals and help repair damage caused by free radicals and minimize the body's exposure to free radicals or restore the body's renewal [21].

In our study, changes in CAT, SOD and GPx activity, which are antioxidant defense enzymes, were investigated in human leukocytes exposed to increasing concentrations of Bendiocarb, the carbamate group insecticide. In line with the results obtained from the study, it was determined that the activity of CAT, SOD and GPx enzymes decreased with increasing concentration of bendiocarb.

In addition to antioxidant enzyme activity, FRAP and TEAC values of the control and Bendiocarb application groups were calculated to determine antioxidant capacity changes in blood tissue. According to the results obtained, the antioxidant capacity of blood cells decreased significantly with increasing doses of Bendiocarb compared to the control group ($P < 0.05$).

In addition, in our study, MDA levels in blood cells were measured and the results were given as nmol/protein for blood cells. The results obtained in determining the MDA level of leukocytes from blood tissue elements are shown in Figure 1. When increasing doses of Bendiocarb and control group were compared, it was observed that there was a statistically significant increase in MDA level due to dose increase ($P < 0.05$).

Sobekov et al. [22] investigated SOD, CAT, GSHPx, glutathione reductase (GR), glutathione-S-transferase (GST) and thiobarbituric acid (TBARs) activities in rabbit liver and kidneys after exposure to bendiocarb. They found that SOD, CAT and GR activity in liver tissue was not affected by bendiocarb, SOD activity in the kidney increased significantly, new MnSOD isoenzymes were formed and CAT and GSHPx-H_2O_2 activities decreased significantly in experimental groups. In addition, the TBARs content in the kidney was not affected by bendiocarb. They showed that the response of organs to bendiocarb is different and may depend on specific organ damage and protective abilities. Changes in the anti-cocci defense system have shown that the toxic effect of bendiocarb may be related to the production of ROS in addition to acetylcholine esterase inhibition.

Sakr and Al-Amoudi [23] studied histopathological changes in the kidney tissues of mammals exposed to deltamethrin to investigate the protective effect of basil against deltamethrin. As a result of these examinations, they observed a significant increase in the concentration of malondialdehyde (MDA) in kidney tissues of mammals exposed to deltamethrin, a decrease in superoxide dismutase (SOD) and catalase activity.

Although there are restrictions on the use of pesticides, it should be remembered that they can harm human health due to their chemical structure, biological activity and misuse. For this reason, most studies have been investigating their mutagenic effects in recent years, and for this purpose studies have been conducted with *Bacillus subtilis* and *Salmonella typhimurium* test strains [24], and fewer studies on their direct effects on DNA. DNA damage determination studies have been investigated in recent years in vivo and in vitro and especially in human lymphocyte cells by the Comet technique [25-27].

Comet technique is preferred in the evaluation of genotoxic effect because it is sensitive, economical, easy and in a short time, and it is possible to detect DNA damage in a single cell. The genotoxic effects of chemicals in the lymphocytes of human and experimental animals can be monitored with this method [28].

In our study, Comet test was used to investigate the genotoxic effects of bendiocarb on leukocytes, and it was determined that increased DNA breaks occurred at increasing levels depending on the dose. As a result of incubation of bendiocarb with leukocytes, DNA damage at a concentration of 20 μg/mL was determined low and close to the control group. DNA damage at 40, 80 and 160 μg/mL concentrations was statistically significant ($P<0.05$) and higher than the control group. Also, the highest tail DNA at 160 mg/mL concentration was determined to be 100 %.

DNA damage was investigated by adding 3, 10, 30 and 100 μg/mL to lymphocytes and MDCK (Mine-Darby Canine Kidney) cells of aminocarb, carbaryl, methiocarb, promecarb and propoxur, in a similar project study with our study. It has been observed that pesticides at a concentration of 100 μg / mL are highly toxic in MDCK cells, in particular, carbaryl and methiocarb cause the death of almost all cells at this concentration. DNA damage in lymphocyte and MDCK cells of pesticides at concentrations of 10 and 30 μg/mL has been investigated, and it has been observed that methiocarb and carbaryl cause DNA damage in the 4th degree at a concentration of 30 μg/mL for 16 hours. Carbamate pesticides caused significant DNA damage to lymphocytes, while causing lower damage to MDCK cells (30 μg / mL concentration and 1st degree damage to carbaryl, methiocarb and promecarb in 48 hours). Low genotoxicity in MDCK cells suggests that DNA damage repair mechanisms may be effective as a result of active cleavage [29].

In another study, human peripheral lymphocytes of 10, 50, 100 and 200 µg/mL dimethoate, methylparathion, propoxur, pirimicarb were applied to human peripheral lymphocytes and DNA damage was investigated in vitro by Comet technique. As in our study, a dose-dependent examination was made and DNA damage was found to be high. [30].

Swiss albino mice were administered orally 0.28 and 8.96 mg/kg chlorpyriphos ethyl, 12.25 and 392.00 mg/kg acephate. DNA damage was examined with Comet technique at 24, 48, 72 and 96 hours after the application. It was reported that a significant increase in Comet tail length was observed at 24 and 48 hours after the application and that the DNA damage was dose dependent. It has been reported that DNA damage gradually decreases 48 hours after application and Comet tail length decreases to the levels of the control group 96 hours later [31].
In another study, genotoxic effects, oxidative stress parameters and AChE activities were investigated in the production of organophosphorus pesticides for 21 workers and 21 control groups working for an average of 97 months. In erythrocytes, lipid peroxidation levels, catalase, superoxide dismutase and glutathione peroxidase activities were measured as oxidative stress biomarkers, and AChE activity as toxicity biomarkers. To determine DNA damage, Comet tail length was measured in leukocytes. As a result of chronic exposure to organophosphorus pesticides, catalase, superoxide dismutase and glutathione levels were observed, while no statistically significant difference was observed in lipid peroxidation levels and AChE activity [32].

It has been determined that Bendiocarb insecticide has a toxic effect in all doses applied on blood tissue. Bendiocarb increased MDA levels in blood tissues at all doses administered, and decreased total antioxidant capacity and SOD, CAT, GPX levels statistically significantly in this study.

Our study demonstrates that Bendiocarb causes damage on DNA due to the increase in dose in all doses applied. We think Bendiocarb has all these negative effects by causing oxidative stress. Based on these data, uncontrolled use of Bendiocarb pesticide in nature has been shown to have harmful effects on the environment and living things. However this study is a preliminary study on this subject, we believe that it will contribute to the existing literature and shed light on further studies.

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References

[1] Çiftçioğlu, G., Issa, G. 2006. Çevre ve Gıdalardaki Pestisit Kalıntılarının Halk Sağlığına Etkisi. İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 32(3), 91-96.

[2] Alp, H., Aytekin, İ., Atakişi, O. 2011. Ratlardaki Akut Malathion Toksisitesinin Neden Olduğu Oksidatif Stres Üzerine Kafeik asit Fenil Ester ve Elarjik Asitin Etkileri. Atatürk Üniversitesi, Veteriner Bilimleri Dergisi, 6, 117-24.

[3] Öztürk, D. 2009. Cyprinus carpio'da Malathion Etkisinde Lipit Peroksidasyonu ve Serum Steroid Hormon Düzeylerindeki Değişimler. Çukurova Üniversitesi, Adana, Yüksek Lisans Tezi, 64s, Adana.

[4] Petrovova, E., Purzyc, H., Mazensky, D. Torma, N., Sopoliga, I., Sedmera, D. 2015. Morphometric Alterations, Steatosis, Fibrosisand Active Caspase-3 Detection in Carbamate Bendiocarb Treated Rabbit Liver. Environmental Toxicology, 30(2), 212-22.

[5] Bonsall, JL., Goose, J. 1986. The safety evaluation of Bendiocarb, a residual insecticide for vector control, Toxicology Letter, 33(1-3), 45-49.

[6] WHO-Pesticide residues in food-Evaluations, 1982, Report sponsored jointly by FAO and WHO, p: 57-75.

[7] Pazarbaşi, A., Kasap, M., Kasap, H. 1999. Çukurova'da Sivrisinek Mücadelesinde Kullanılan Bendiokarb'ın Memelilere (Sıçanlara) Dermal Etkisi. Turksh Journal of Zoology, 23, 303-308.

[8] Almasiova, V., Holovska, K., Tarabova, L., Cigankova, V., Lukacinova, V., Nistiar, F. 2012. Structural and ultrastructural study of the rabbit testes exposed to carbamate insecticide. Journal of Environmental Science Health, 47, 1319–1328.

[9] Dorko, F., Danko, J., Flesarova, S., Boros, E., Sobekova, A. 2011. Effect of pesticide Bendiocarbamate on distribution of acetylcholine- and butyrylcholine-positive nerves in rabbit'sthymus. European Journal of Histochemistry, 55, 206–209.

[10] Yılmaz, S., Unal, F., Yuzbasioglu, D., Celik, M. 2014. DNA damage in human lymphocytes exposed to four food additives in vitro. Toxicology and Industrial Health, 30, 926-37.

[11] Lowry, OH., Rosebrough, N. J., Farr, AL, 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 19, 265.

[12] Ohkawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Analytical Biochemistry, 95,351 - 358.

[13] Marklund, S., Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47,469-474.

[14] Aebi, H. 1984. Catalase in vitro. Methods Enzymology, 105,121-126.

[15] Paglia, DE., Valentine, WN. 1987. Studies on the quantative and qualitative characterization of glutathione peroxidase. Journal of Laboratory Medicne, 70,158-165.

[16] Benzie, I.F., Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) As a Measure of Antioxidant Power Q the FRAP Assay, Analytical Biochemistry, 239, 70–76.

[17] Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant Activity Applying An Improved ABTS Radical Cation Decolorization Assay, Free Radical Biology and Medicine, 26, 1231–1237.

[18] Özkan, D., Yüzbaşıoğlu, D., Ünal, F., Yılmaz, S., Aksoy, H. 2009. Evaluation of the cytogenetic damage induced by the organophosphorous insecticide acephate. Cytotechnology, 59, 73-80.

[19] Dikshith, T.S.S, 2011. Handbook of Chemicals and Safety, CRC Press, Boca Raton, FL, USA.

[20] Ghorabaa, Z., Aibaghia, B., Soleymanpoura, A., 2018. Ultrasound-assisted dispersive liquid-liquid microextraction followed by ion mobility spectrometry for the simultaneous determination of Bendiokarb and azinphos-ethyl in water, soil, food and beverage samples. Ecotoxicology and Environmental Safety, 165, 459–466.

[21] Ames, B.N., Shıgenaga, M.K., Hagen, T.M., 1993. Oxidants, Antioxidants and the Degenerative Diseases of Aging, Proceedings of the National Academy of Sciences, 90, 7915-7922.

[22] Sobekova, A., Holovska, K., Lenartova, V. 2009. The another toxic effect of carbamate insecticides. Acta Biologica Hungarica, 60(1), 45–54.

[23] Sakr, S.A., Al-Amoudi, W.M., 2012. Effect of leave extract of Ocimum basilicum on deltamethrin induced nephrotoxicity and oxidative stress in albino rats. Journal of Applied Pharmaceutical Science, 2(5), 22-27.

[24] Shiau, S.Y., Huff, R.A., Wells, B.C, 1980. Mutagenicity and DNA-damaging activity for several pesticides tested with Bacillus subtilis mutants. Mutation Research, 71,169-179.

[25] Piperakis, S.M., Kontogianni, K., Siffel, C. 2006. Measuring the effects of pesticides on occupationally exposed humans with the comet assay. Environmental Toxicology, 21, 355-359.

[26] Kisby, GE., Muniz, JF., Scherer, J., Lasarev, MR., Koshy, M., Kow, YW., McCauley, L., 2009. Oxidative stress and DNA damage in agricultural workers. Journal of Agromedicine, 14(2), 206-214.

[27] Castillo-Cadena, J., Tenorio-Vieyra, LE., Quintana-Carabia, AI., Garcia-Fabila, MM., Ramirez-San Juan, E., Madrigal-Bujaidar, E. 2006. Determination of DNA damage in floriculturists exposed to mixtures of pesticides. Journal of Biomedicine and Biotechnology,1-12.

[28] Dikilitaş, M., Koçyiğit, A, 2010. Canlılarda "Tek Hücre Jel Elektroforez" Yöntemi İle DNA Hasar Analizi, Comet Analiz Yöntemi, Harran Üniversitesi Ziraat Fakültesi Dergisi, 14(2), 77- 89.

[29] Yurdun, T., Turan, K., 2002. Pestisitlerin genotoksik etkilerinin araştırılması. M.Ü. Bilimsel Araştırma Projeleri Komisyon Başkanlığı. Proje No: SAĞ-061/131102.

[30] Ündeğer Ü, Başaran N. 2005. Effects of pesticides on human peripheral lymphocytes in vitro: induction of DNA damage. Archives of Toxicology, 79,169-176.

[31] Rahman, MF., Mahboob, M., Danadevi, K., Banu, BS., Grover, P., 2002. Assessment of genotoxic effects of chlorpyriphos and acephate by the comet assay in mice leucocytes. Mutation Research, 516,139-147.

[32] Shadnia, A., Azizi, E., Hosseini, R., Khoei, S., Fouladdel, S., Pajoumand, A., Jalali, N., Abdollahi, M., 2005. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulations. Human & Experimental Toxicology, 24(9), 439-445.