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Rewiew

Determination of DNA damage caused by food additives using comet assay method

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Abstract. Additives have been used to improve specific characteristics of food products. As a result of the rapid increase in the use of chemical substances such as food additives in every area, it has become very im-portant to determine whether these chemicals have negative effects on the genetic structure of living things. Food additives are substances and used to preserve flavor or enhance its taste, appearance, or other qualities of foods. Especially; food preservatives within food additives are in use to protect the food against to micro-organisms negative effect. The risk assessing from food additives to human health carried out by WHO, in cooperation with FAO. However, the increased consumption of food additives may result to toxic reactions. It was indicated by different studies that some food additives have genotoxic and carcinogenic effects in differ-ent test organisms including plants, bacteria, human lymphocytes and in different organs, mice and rats. The effects of genotoxic and cytotoxic agents on living cells can be determined bu using comet assay anaysis. In recent years, the comet assay method has been widely preferred because of its advantageous, precise and fast results. Becasue of being carcinogenics possibility it should be more concious and be more carefull in taking food additives to our life.

Key Words: DNA Damage, Comet, Food Additives and Genotoxic

Introduction

During the food production, preparation, processing, packaging, transportation and storage, which are not consumed as food with or without nutritive value, used in accordance to selected technology that are allowed to be used to prevent adverse effects are defined as food additives (1).

As a result of the rapid increase in the use of chemical substances such as food additives in every area, it has become very important to determine whether these chemicals have negative effects on the genetic structure of living things. Additives have been used to improve specific characteristics of food products (2). Food additives (FA) are agents and used to change the properties like its taste, appearance, or other qualities of food in the desired direction. Food additives also include substances that may be introduced to food indirectly (called "indirect additives") in the manufacturing process, through packaging, storage or transport. Salts, vinegar, spices and smoking products have been used for a long time. Food additives can be divided into

several groups, such as: Acidulents; provides acid taste like vinegar and citric acid. Acidity regulators; used for controlling the pH of foods. Anticaking agents; keep powders from caking or sticking. Foaming agents provide foaming; antifoaming; reduce or prevent foaming in foods. Bulking agents; like starch effects the bulk of a food increasingly. Antioxidants; such as vitamin C inhibits the degradation of food via oxygen. Fortifying agents; like vitamins, minerals enhances the nutritional value of the foods. Colorings; are added colors to food providing more attractive. Color retention agents; preserve food's existing color. Emulsifiers; keep water and oils mixed together in an emulsion, like in mayonnaise. Flavors; are used to give a particular taste or smell to foods. Flavor enhancers; enhance a food's existing flavors. Flour treatment agents; are used for improvent of flour in baking. Humectants; keep away foods from drying out. Glazing agents; provide protective coating to foods. Tracer gas; prevent foods from being exposed to atmosphere, resulting with shelf life enhancement. Stabilizers, thickeners and gelling agents; such as agar or pectin (used in jam for example) provide foods a firmer texture. Sweeteners; are added more flavoring to foods. Thickening agents; are added to the mixture, for increase in its viscosity. Packaging Bisphenols, phthalates, and perfluoroalkyl chemicals (PFCs); are indirect additives used in packaging. Preservatives; prevent or inhibit food from the effect of fungi, bacteria and other microorganisms. They are used for prevention of microbial growth or by undesirable chemical changes (3,4).

There are more than 8000 food additives today. The Food and Drug Administration of the United States (FDA) has approved the use of 2800 food additives. The number of FAs approved by the European Union is approximately 297. FAs permitted to be used in our country, products that can be used and their usage limits are in line with EU directives (5).

To regulate these additives, and inform consumers, each additive is assigned a unique number, termed as "E numbers", which is used in Europe for all approved additives. This numbering plan has now been adopted by the Codex Alimentarius Commission to identify all additives and whether they are approved for use. WHO, in cooperation with the Food and Agriculture Organization of the United Nations (FAO), is responsible for assessing the risks to human health from food additives (6).

Some food additives (FA) have genotoxic and carcinogenic effects, some of them have been found to play a role in the formation of neurodegenerative diseases, hyperactivity, allergy, diabetes, obesity, reproductive and gastrointestinal system disorders (5).

Some food additives, however, have been prohibited from use because of their toxicity. Different studies finding indicate that these additives induce DNA damage in bacteria, fungi, insects and mammalian cells in vivo and in vitro. They also cause chromosomal anomalies in mammalian cells, including human cells. The individual response varies on the basis of used dose, age, gender, nutritional status and genetic factors (7). Different content of these substances, such as nitrous compounds, have been found as carcinogenic.

With the increasing use of these substance take into consideration of their harmfull effect. Among these harmful effects caused by regular use of food additives are hypersensitivity, various allergic reactions, lesions and tumors in body, genotoxicity, mutagenicity etc. It has been reported that certain food additives have an genotoxic effect according to different test systems. Azo dyes, allura red, amaranth and new coccine, mainly used as food color additives in Japan, were reported to cause DNA damage in colon in mice (8).

Many different physical and chemical agents and physiological metabolic reactions can cause molecular changes in the living cell. DNA is an important target for ultraviolet, X rays and chemical agents. DNA damage either occurs spontaneously or under the influence of environmental factors (9). Damage to DNA or inadequate DNA repair systems may result with cell death.

Toxicity is defined as the damage caused by chemical substances in the organism. In toxicity studies, the experimental animals like rats, mice, guinea pigs are given different doses of chemicals to be tested and all possible toxic effects are searched.

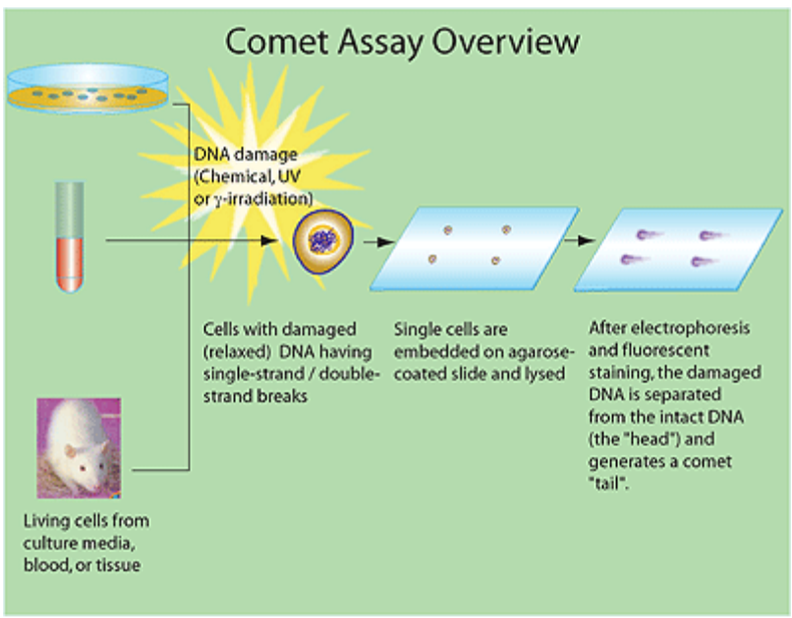
In these days, different tests, are widely used in the detection of genotoxic and carcinogenic potentials of chemical agents including FA. The most common of these tests are: AMES (Salmonella microsome mutagenicity test), Abnormalities, sister chromatite exchange, micronucleus and Comet tests (5).

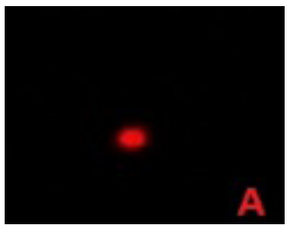
In the determination of these DNA damage; several different micro-bioassay methods can be used (10).

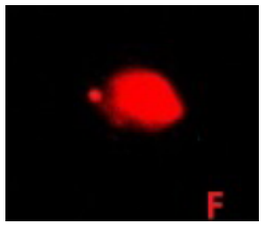
The single cell gel electrophoresis (SCGE) or Comet technique, which is based on the determination of DNA fractures, has been widely used in the determination of DNA damage and repair mechanism level and genetic toxicology. Comet assay is used to measure and analyze DNA damage in various organisms and especially in mammalian cells (11,12).

Comet technique; based on determination of the effects of genotoxic and cytotoxic agents or biological, chemical and physical reasons on living cells and lies the migration of DNA molecules in alkaline environment (13). Swedish scientist Östling & Johansson developed this technique in 1984 (14) Singh et al., later modified this technique, in 1988, as the Alkaline Comet Assay. On the examination of the results by fluorescence microscopy, DNA chains form a comet image, resulted naming as Comet Assay, meaning comet (15). It has been powerfull method of detecting damage in DNA by obtaining similar images of comets. In recent years, the comet assay method has preferred for its advantageous, precise and fast results (16). The advantages of the in vivo comet assay include its applicability to various tissues and/or special cell types, its sensitivity for detecting low levels of DNA damage, its requirement for small numbers of cells per sample, general ease of test performance, the short time needed to complete a study and its relatively low cost (17). The Comet test is now widely used to determine the damage caused by mutagenic, UV and ionized radiations, genotoxic, alkylating agents, intercalating agents and oxidative damage, resulted from stress. (18).

In the Comet assay method, the extent of the damage is determined by the length of the tail that the cells generate when they migrate. The results are examined in 5 categories according to the degree of damage. Accuracy of damage can be evaluated with comet parameters such as comet length, comet height, comet area, comet density, head diameter, head area, head density, DNA head, tail length, tail area, tail density, average tail density, DNA tail moment in tail, olive moment with using software programs (19).







Visual evaluation of Comet Assay cells; A: "0" Nondamage Grup; F: Apoptotic Cells (20).

In vivo comet assay (single cell gel electrophoresis) is highly used in genotoxicity testing. Genotoxicity testing in vivo is formed for reveal of possible hazardous effect of subjected additives (i.e. what is the possible genotoxic/mutagenic effect of substance to humans) and to dose–response assessment (i.e. the relationship between the dose of a substance and a possible adverse effect of it) and understanding of a substance's mode of action (17). It is a reality that food additives especially preservatives play an important role in the safety of food transportation, several different studies indicate the possible genotoxic and mutagenic effects of the additives.

This article attempts to summarize the possible genotoxic effects of food additives and the use of the comet assay method to determine this.

In the light of these studies, more attention should be paid to the use of food additives.

Literatüre Review

One of the study related to food additives to genetoxic effect determantion included some antioxidant additives, such as citric acid (CA) and phosphoric acid (PA) and their combination, as well as antimicrobial additives, such as benzoic acid (BA) and calcium propionate (CP), and their effect on human lymphocytes with using alkaline single-cell gel electrophoresis.

They found a significant increase in the DNA damage in human lymphocytes after 1 h of in vitro exposure to CA, PA, BA and CP (200, 25–200, 50–500, 50–1000 mg/mL, respectively). The combination of CA and PA significantly increased the mean tail intensity at all the concentrations used (25–200 mg/mL) and significantly increased the mean tail length mainly after higher concentrations (100 and 200 mg/mL). Data in this study showed that the concentrations of food additives used induce DNA damage and PA was the most genotoxic and CA was less genotoxic additives among them (21).

In another study food color additives genetoxic effect into different species was searched. Azo dyes, allura red, amaranth and new coccine, used as food color additives in Japan, have been indicated to cause specific DNA damage in mice. To search effect differencess according to species, and to see its effect into rats as well, each of dyes was administered to male mice (1 and 10 mg/kg) and male rats (10, 100 and 1,000 mg/kg) by gavage. Brain, kidney, lung, liver, colon, glandular stomach, urinary bladder and bone marrow were sampled 3 hr (for mice) and 3, 6, 12 and 24 hr (for rats) after the treatment. The DNA damage in the mouse colon was seen 3 hr after the administration of all of the dyes at 10 mg/kg according to comet assay results, however, none of the dyes showed DNA damage in rats indicating there is a possibility that rats are not effected or tolerated these dyes (22).

Calcium propionate (CP; E-282) is an approved preservative in bread and inhibits the growth of mold and other microorganisms. Genotoxicity data of the CP are very rare; it was found to be mutagenic in fibroblast cells of Chinese hamster (23).

Citric acid (CA; E-330) is a weak organic acid and used widely as an acidulant, pH regulator, flavor enhancer, and antioxidant effect in many foods, such as soft drinks, jelly sweet, baked nutrients, marmalade, jam, and candy (24) .

In another study; the possible toxic effect of two types of food additives, Sunset Yellow and Allura Red,

were investigated via assessing the physiological, histopathological and ultrastructural changes in the liver and kidney. Thirty adult male albino rats were divided into three groups of 10 animals each: control (received water), Sunset Yellow-treated (2.5 mg/kg body weight) and Allura Red-treated (seven mg/kg body weight). The doses were given orally for 4 weeks. The results showed an increase in the biochemical markers of hepatic and renal function in animals with azo dyes applied. Also a noticeable increase in MDA and a marked decrease in total antioxidant levels in azo dye-treated animals compared to controls were seen. It was seen also negative effect in the liver and kidney of albino rats and changings in their histological and fine structure, with downregulation of Bcl2 and upregulation of COX2 expression. According to comet assay results both of them caused histopathological and physiological aberrations in the liver and kidney of male Wistar albino rats. Also, it was seen a potential genotoxic effect caused by Sunset Yellow but not Allura Red (25).

In another study; investigation of the DNA damage caused by some food additives such as citric acid (CA), benzoic acid (BA), brilliant blue (BB) and sunset yellow (SY) were aimed in human male germ cells using comet assay. The sperm cells were incubated with different concentrations of these food additives (50, 100, 200 and 500 μg/mL) for 1 h at 32 °C. The results showed for CA, BA, BB and SY a dose dependent increase in tail DNA%, tail length and tail moment in human sperm when compared to control group. This studies' results indicate that SY and BB are more harmful than BA and CA to human sperm in vitro (26).

Different study were carried out to determine the genotoxic effect of colorings. For this aim tartrazine and chocolate brown as synthetic food coloring agents were used on rats. The rats were divided into five equal groups, each composed of 4 rats, as follows: The 1st group (G1) as control. The 2nd (G2) and 3rd (G3) groups were orally treated with a daily dose of tartrazine. The 4th (G4) and 5th (G5) groups were orally treated with a daily dose of chocolate brown for 7 weeks. Two rats from each of the experimental groups were sacrificed under anesthesia. The results revealed that tartrazine and chocolate brown caused DNA liver and kidney damage detected by comet assay. Chromosome ring were the most common abnormalities seen on bone marrow cells of treated rats. The results indicated that some of the colorants have an destructive effect on some vital organ functions. Because of this, large quantities and/or long periods of colorants administration should be restricted from diets of man's and generally from children' menu (27).

This study was carried out to assess the long-term daily administration of benzoic acid (BA), potassium sorbate (PS), chlorophyll (CPL), tartrazine (TAZ), and butylated hydroxyanisole (BHA) on hepato-renal changes and DNA damage in rats. Animals were orally applied with the 10 times of the acceptable daily intake (ADI) from each tested substance daily for 60 days. Blood, liver, and kidney samples were collected to evaluate hematological, histopathological, biochemical, and genotoxic alterations. The liver and kidney damage was evaluated by comet assay and as a result; DNA damage was obtained in liver and kidney at different degrees. Moreover, the histopathological findings of liver and kidneys support destructive and degenerative changes. The study indicates that most of food additives may induce genotoxicity and hepatonephropathy, which can be serious for human health. Because of this, it is necessary to be informed about the hazardous effects of food additives (28).

Four food preservatives (sodium nitrate, sodium nitrite, potassium nitrate and potassium nitrite) have been evaluated for genotoxicity in Drosophila melanogaster by Sarıkaya et al. It was found that the genotoxic and toxic effects produced by the combined treatments were considerably increased, especially when the four chemicals were mixed. In a similar study carried out by Demir et al. benzyl derivatives (benzaldehyde, benzylacetate, benzylalchol and benzoic acid) were evaluated for their genotoxic effects and benzaldehyde was found to have highest genotoxic effect (29,30).

In another study; it was aimed to determine the genotoxic potential of oregano essential oil using both the micronucleus (MN) test and comet (standard and

enzyme-modified) assays in Wistar rats treated with 50, 100, or 200 mg/kg body weight administered daily for 90 days. Essential oils from Origanum spp. is suitable for use as food additives via antioxidant and antimicrobial activities of them. According to genotoxicity assays results; no apparent oxidative damage was seen in the comet assay in any of all examined tissues of rats. As a result, oregano essential oil appears to be safe in Wistar rats and can be used safely in food packaging industry (31).

Sasaki et al. studied with currently used 39 additive including food coloring, color fixing, preservatives, antioxidants, fungicides and sweeteners, aiming to investigate whether these substances were genotoxic. After oral administration of these substancess to animals their stomach, colon, liver, kidney, bladder, bone marrow, lung and brain tissues were collected and used for comet test. They indicated that among all additives the food dyes have the highest genotoxicity effect. Amaranth, allura red, new coccin, erythrocyte, tartrazine, floxin and rose bengal food dyes in the stomach, colon and bladder cells cause to dose-dependent DNA damage. They also remarked that these dyes situmulated DNA damage in the gastrointestinal organs, even at low doses (7).

The occuring of DNA damage is considered to be an important in progress of carcinogenesis. The single cell gel electrophoresis (comet) assay is technically simple, fast, cheap and DNA damage can be investigated with highly reliable in animal experimental systems for all mammalian cell types. Especially, the comet assay applications are valuable for detection of genotoxic exposure in humans. The comet assay results indicate that DNA damage can be seen mostly in mammalian cells and affected from lifestyle and several different environmental exposures like diet, hypoxia, exercise and sunlight (32).

Another study based on determination of the impacts of sodium acetate (SA), sodium acid pyrophosphate (SAPP), and citric acid (CA) on the proliferation, viability and DNA damage of isolated lymphocytes in vitro. The comet assay results showed SA, SAPP and CA increased DNA damage percentage, tail DNA percentage, tail length and tail moment on the basis of their concentration. They summarized that SA, SAPP and CA are cytotoxic and genotoxic to isolated lymphocytes in vitro (33).

Perillaldehyde, a natural monocyclic terpenoid present mostly in the herb perilla, is being used as an flavouring compound to give spiciness and citrus taste to foods. For checking its using safely as a flavouring agent; perillaldehyde was chosen by the European Food Safety Authority as a representative of a subgroup of alicyclic aldehyde flavouring substances, it is tested for genotoxic potential via this study. Perillaldehyde was tested for several different assays including comet assay as well. According to findings; most of the genotoxicity assays results were negative. This study findings do not provide an indication of any genotoxic potential for perillaldehyde (34).

In another study; DNA damage were determined from liver, stomach, and bone marrow of rats fed with 2000 mg/kg of benzene, di(2-ethylhexyl) phthalate, and trisodium ethylenediamine tetraacetic acid monohydrate given orally with three times. All three compounds analysis gave negative results for liver and stomach. On the other hand a bone marrow comet and micronucleus analysis revealed that benzene, but not di(2-ethylhexyl) phthalate or trisodium ethylenediamine tetraacetic acid monohydrate caused to a significant increase in the median % tail DNA and micronucleated polychromatic erythrocytes indicating genotoxic effect of these substance according to comet assy (35).

This study was carried out to determine the toxicity of the food additives including sunset yellow (SY) and brilliant blue (BB) on Allium cepa root meristematic cells. It is known that food additives are used for aiming preservation, sweetening and coloring. In this study the control and treatment groups were formed from germinated roots. Group 1 (control group) with no application. Group 2 (SY or BB-treatment group), received increasing doses of SY (25, 50, 100 and 500 ppm) and BB (100, 200, 400 and 500 ppm) for tree times. DNA damage was measured via comet assay and RAPD-PCR technique. The tail DNA% and tail length were obtained as significantly increased in all application period compare to the control. Increasing doses of SY and BB caused to enhancement in toxicity level according to all parameters of A. cepa. As a conclusion, the SY and BB were obtained as cytotoxic and have an mutagenic potential. In comparion within; the SY was obtained as more harmful than BB in the A. cepa root meristematic cells (36).

In these days, agro-food by-products stand for a possible low-cost source of biologically active ingredients. In this study, a sugar and mineral enriched fraction (SMEF) from olive mill wastewater (OMWW) Cerasuola OMWW recovered. The in vitro cytotoxicity was investigated with comet assays on human fibroblasts for this fraction. Also, intracellular reactive oxygen species (ROS) production, apoptosis and cell morphological changes were determined as well. The results indicated that the SMEF have an toxic effect at higher concentrations (such as cell viability reduction, DNA fragmentation and morphological alterations) related to high ROS levels (37).

In another study, the genotoxic effects of antimicrobial food additive sodium sorbate (SS) was determined with using comet assay in isolated human lymphocytes and some other test. Four concentrations (100, 200, 400 and 800 μg/ml) of SS were tested with negative (sterile distilled water) and a positive control. The result indicated that this SS additive caused DNA damage at all concentrations and it is genotoxic to the human peripheral blood lymphocytes at highest concentrations (38).

Caramel color has been used in foods and beverages for years as a color additive. Several different safety testing including toxicokinetics, genotoxicity, subchronic toxicity, carcinogenicity, and reproductive/developmental toxicity has been carried out with different classes of caramel color. All obtained results indicated that caramel colors are not genotoxic or carcinogenic, and intake of caramel colors do not cause to any safety risks (39).

Tartrazine is approved as a food color additive in Europe with E number 102. The in vivo genotoxicity study was carried out according to OECD Guidelines. The findings of this study indicate the absence of genotoxic activity for Tartrazine, according to Comet assay as well as some other test carried out in liver, stomach and colon. In the conclusion, they revealed that there is no genotoxicity concern for Tartrazine. This similar negative genotoxity result was also obtained for Allura Red AC and Ponceau 4R, indicating same sign for all azo dyes used as food colors as well (40).

Aspartame is 200× sweeter than sucrose and is used in food products in more than 90 countries in the world. Aspartame has been tested for genotoxic effects. According to findings, obtained from in vivo bone marrow micronucleus, chromosomal aberration and Comet assays, aspartame is not genotoxic in somatic cells in vivo (41).

This study was carried out to determine the genotoxic possibility of Monosodium glutamate (MSG) different concentration via using alkaline comet assays in isolated human lymphocytes. Because MSG is used widely as flavor enhancers in the world. According to test result, MSG caused DNA damage at all concentrations and genotoxic to the human peripheral blood lymphocytes in vitro (42).

One of the important suggestion related to cancer induction is aspartame. It was found high level of relationship between the use of aspartame and the incidence of breast and prostate cancer (43).

There have been several studies indicating that saccharin, which has been used for a long time, is mutagenic and caused to cancer in experimental animals. Wolff et al. reported that sodium saccharin induces PPE formation in human lymphocytes and Chinese hamster cells (CHO), has an carcinogenic and mutagenic effects; causing to bladder cancer in mice (44).

Different kinds of additives are widely used in food industry and different studies supported that some additives have an cytotoxic effect but still in use. This study was carried out to determine DNA damage at which a group of selected food additives cause to via Comet assay. According to findings five substances (sodium nitrite and caffeine, the coloring agents fast green, erythrosine and indigo carmine) commonly added to foods and one pharmaceutical drugs (4-aminoantipyrine) caused to DNA damage at lower concentrations then normal using levels. On the basis of the comet assay results of all six substances have an certain genotoxic activity. Sodium nitrite genotoxic activity was found at the lowest concentration. Caffeine also showed strong genotoxic effect, while the coloring agents demonsrated mid level of genotoxicity (45).

Conclusion

Food additives are agents and used to change the foods' some specialities like its taste, appearance, or other qualities of food in desired direction. The increased consumption of food additives may result to toxic reactions. It was indicated by different studies that some food additives have genotoxic and carcinogenic effects in different test organisms. The effects of genotoxic and cytotoxic agents on living cells can be determined bu using comet assay anaysis. According to different summarized studies in our paper there is a huge possibility to be a genotoxic effect of using of food additives becasue of this possibility we should have more concious and be more carefull in taking food additives to our life.

Referances

1. Boğa A, Binokay S. Gıda Katkı Maddeleri ve Sağlığımıza Etkileri. Arşiv 2010; 19: 141.
2. Yüzbaşıoğlu D, Zengin N, Ünal F. Gıda koruyucuları ve Genotoksisite Testleri. Gıda 2014; 39 (3): 179–186.
3. Özel B, imşek Ö, Akçelik M, Saris PEJ. 2018. Innovative approaches to nisin production. Appl Microbiol Biotechnol 2018; 102(15): 6299–6307.
4. http://www.apelasyon.com/Yazi/321-gida-katki-maddeleri-3---koruyucular-antimikrobiyal-katki-maddeleri-Eylül 2015.
5. Şen S, Aksoy H,&Yılmaz S. Gıda katkı maddelerinin genotoksik, karsinojenik potansiyeli ve insan sağlığı üzerindeki diğer etkileri. J Hum Sci 2017; 14(4): 3093–3108.
6. Altuntaş GE, Ayhan K. Süt ve Süt Ürünlerinde Bakteriyosinlerin Kullanımı. Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi 2010; 16 (1): 113–120.
7. Sasaki YF, Kawaguchi S, Kamaya A, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mut Res 2002; 519: 103–119.
8. Atrı R, Sıngh A, Mathur N, Verma A. Genotoxıc Effect of Food Addıtıves and Food Products: A Revıew. Int J Chem 2013; 11(4): 1753–1768.
9. Dıncer Y, Kankaya S. DNA Hasarının Belirlenmesinde Comet Assay. Türkiye Klinikleri 2010; 30: 1365–73.
10. Erısmıs U, Cıgercı HI, Konuk M. 2012. Comet Assay in Amphibian Biomonitoring. 21. Ulusal Biyoloji Kongresi, Ege Üniversitesi, İzmir, Türkiye.
11. 11-Sıngh NP, Mccoy MT, Tıce RR, Schneıder EL. "A simple technique for quantitation of low levels of DNA damage in individual cells". Exp Cell Res 1988; 175: 184–191.
12. Tıce RR, Agurell E, Anderson D, Burlınson B, Hartmann A, Kobayashı H. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000; 35: 206–21.
13. Faırbaırn DW, Olıve PL, O'neıl KL. The comet assay: a comprehensive review. Mutat Res 1995; 339: 37–59.
14. Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun 1984; 123: 291–8.
15. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 2004; 26: 249–261.
16. Tıce RR, Andrews PW, Hıraı O, Sıngh NP. The single cell gel (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells. Adv Exp Med Biol 1991; 283: 157–164.
17. Brendler-Schwaab S, Hartmann A, Pfuhler S, Speit G. The in vivo comet assay: use and status in genotoxicity testing. Mutagenesis 2005; 20(4): 245–54.
18. https://www.sigmaaldrich.com/life-science/cell-biology/cancer-research/learning-center/cancer-research-protocols/comet-assay.html
19. Anderson D, Dhawan A, Weı T, Plewa MJ. An investigation of bone marrow and testcular cells invivo using the comet assay. Mutat Res 1996; 370: 159–174.
20. Güleç S. 2019. Hibernasyonda "pelophylax caralıtanus" (amphibia: anura)'da DNA Hasarının Araştırılması, Msc Thesis, Afyon Kocatepe Üniversitesi Sağlık Bilimleri Enstitüsü Afyon.
21. Yilmaz S, Ünal F, Yüzbaşıoğlu D, Çelik M. DNA damage in human lymphocytes exposed to four food additives in vitro. Toxicol Ind Health 2014; 30(10): 926–937.
22. Shimada C, Kano K, Sasaki Yu F, Sato I, Tsuda S. Differential colon DNA damage induced by azo food additives between rats and mice. J Toxicol Sci 2010; 35(4): 547–554.
23. Ishidate M, Sofuni T, Jr Yoshikawa K, et al. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 1984; 22: 623–636.
24. Gürsoy S. 2002. Besinlerde katkı maddelerinin kullanımı ve sitrik asit toksisitesi. MSc Thesis, Trakya Üniversitesi Fen Bilimleri Enstitüsü, Edirne.
25. Khayyat LI, Essawy AE, Sorour JM, Soffar A. Sunset Yellow and Allura Red modulate Bcl2 and COX2 expression levels and confer oxidative stress-mediated renal and hepatic toxicity in male rats. Peer J 2018; 6:e5689.
26. Pandir D. DNA damage in human germ cell exposed to the some food additives in vitro. Cytotechnolog 2016; 68(4): 725–733.
27. Hassan GM. Effects of some synthetic coloring additives on DNA damage and chromosomal aberrations of rats. Arab J Biotechnol 2010; 13 (1):13–24.
28. Abo-El-Sooud K, Hashem MM, Badr YA, et al. Assessment of hepato-renal damage and genotoxicity induced by long-term exposure to five permitted food additives in rats. Environ Sci Pollut Res Int 2018; 25(26): 26341–26350.
29. Sarikaya R, Cakir S. Genotoxicity Testing of Four Food Preservatives and their Combinations in the Drosophila Wing Spot Test. Environ Toxicol Pharmacol 2005; (3): 424–430.

30. Demir E, Kocaoglu S, Kaya B. Genotoxicity Testing of Four Benzyl Derivatives in the Drosophila Wing Spot Test. Food ChemToxicol 2008; 46(3): 1034–1041.
31. Llana-Ruiz-Cabello M, Puerto M, Maisanaba S, Guzmán-Guillén R, Pichardo S, Cameán AM. Use of micronucleus and comet assay to evaluate evaluate the genotoxicity of oregano essential oil (Origanum vulgare l. Virens) in rats orally exposed for 90 days. J Toxicol Environ Health A 2018; 81(12): 525–533.
32. Møller P1. Genotoxicity of environmental agents assessed by the alkaline comet assay. Basic Clin Pharmacol Toxicol 2005; 96 (1):1–42.
33. Abd-Elhakim YM, Anwar A, Hashem MM, Moustafa GG, Abo-El-Sooud K. Sodium Acetate, Sodium Acid Pyrophosphate, and Citric Acid Impacts on Isolated Peripheral Lymphocyte Viability, Proliferation, and DNA Damage. J Biochem Mol Toxicol 2018; 32(8):e22171. doi: 10.1002/jbt.22171.
34. Hobbs CA, Taylor SV, Beevers C, et al. Genotoxicity assessment of the flavouring agent, perillaldehyde. Food Chem Toxicol 2016; 97:232-242. doi: 10.1016/j.fct.2016.08.029.
35. Kitamoto S, Matsuyama R, Uematsu Y, et al. Genotoxicity evaluation of benzene, di(2-ethylhexyl) phthalate, and trisodium ethylenediamine tetraacetic acid monohydrate using a combined rat comet/micronucleus assays. Mutat Res Genet Toxicol Environ Mutagen 2015; 786-788:137–43. doi: 10.1016/j.mrgentox.2015.05.002.
36. Koç K, Pandir D. All aspect of toxic effect of brilliant blue and sunset yellow in Allium cepa roots. Cytotechnology 2018; 70(1): 449–463. doi: 10.1007/s10616-017-0161-9.
37. Di Mauro MD, Tomasello B, Giardina RC, et al. Sugar and mineral enriched fraction from olive mill wastewater for promising cosmeceutical application: characterization, in vitro and in vivo studies. Food Funct 2017; 8(12):4713-4722. doi: 10.1039/c7fo01363a.
38. Mamur S, Yüzbaşıoğlu D, Unal F, Aksoy H. Genotoxicity of food preservative sodium sorbate in human lymphocytes in vitro. Cytotechnology 2012; 64(5):553–62. doi: 10.1007/s10616-012-9434-5.
39. Vollmuth TA. Caramel color safety - An update. Food Chem Toxicol 2018; 111:578-596. doi: 10.1016/j.fct.2017.12.004.
40. Bastaki M, Farrell T, Bhusari S, Pant K, Kulkarni R. Lack of genotoxicity in vivo for food color additive Tartrazine. Food Chem Toxicol 2017; 105:278-284. doi: 10.1016/j.fct.2017.04.034.
41. Kirkland D, Gatehouse D. "Aspartame: A review of genotoxicity data". Food Chem Toxicol 2015; 84:161–8. doi: 10.1016/j.fct.2015.08.021.
42. Ataseven N, Yüzbaşıoğlu D, Keskin AÇ, Ünal F. Genotoxicity of monosodium glutamate. Food Chem Toxicol 2016; 91:8–18. doi: 10.1016/j.fct.2016.02.021.
43. Schwartz GR. Aspartame and breast and other cancers. West J Med 1999; 171(5-6): 300–1.
44. Wolff S, Rodin B. Saccharin-induced sister chromatid exchanges in Chinese hamster and human cells. Science 1978; 200: 543–545.
45. Peycheva E, Alexandrova R, Miloshev G. Application of the yeast comet assay in testing of food additives forgenotoxicity. LWT- Food Sci Technol 2014; 59: 510–517.

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