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GENOTOXIC EFFECTS OF SOME FOOD ADDITIVES ON MAMMALS

ABSTRACT

Food additives are substances that are added to foods for many purposes such as prevention of microbiological deterioration and increase of durability, nutritive value preservation, assisting technological operations, correction of sensory characteristics such as color, appearance, taste, smell. In this review, it is aimed to summarize the data on the genotoxic effects of the various food additives on the mammals and to provide a source for further work to be done thereafter. It has been reported that food additives cause genotoxicity compared to the results obtained from studies using various genotoxicity tests. In vivo and in vitro studies, it has been determined that additives increase genotoxicity and cytotoxicity depending on the applied dose. As a result; in this review, various in vivo and in vitro studies have shown that exposure to food additives is both genotoxic and cytotoxic on mammals, where findings are generally parallel to each other as a result of assessed studies.

Keywords: Food Preservatives, Mammals, Genotoxic Effect, Microbiological Deterioration, Cytotoxicity

BAZI GIDA KATKI MADDELERİNİN MEMELİLER ÜZERİNDEKİ GENOTOKSİK ETKİLERİ

ÖZ

Gıda katkı maddeleri, gıdalarda mikrobiyolojik bozulmayı önleme ve dayanıklılığı arttırma, besleyici değeri koruma, teknolojik işlemlere yardımcı olma, renk, görünüş, lezzet, koku gibi duyusal özellikleri düzeltme gibi pek çok amaçla katılan maddelerdir. Bu derlemede, çeşitli gıda katkı maddelerinin memeliler üzerindeki genotoksik etkilerine dair yapılan çalışmalar değerlendirilerek verilerin özetlenmesi ve bundan sonra yapılacak olan diğer çalışmalara kaynak teşkil etmesi amaçlanmıştır. Çeşitli genotoksisite testleri kullanılarak yapılmış çalışmalardan elde edilen verilere göre, gıda katkı maddelerinin genotoksisiteye neden olduğu rapor edilmiştir. Yapılan invivo ve invitro çalışmalarda katkı maddelerinin uygulanan doza bağlı olarak genotoksisite ve sitotoksisiteyi arttırdığı tespit edilmiştir. Sonuç olarak; bu derleme ile, değerlendirilen çalışmalar neticesinde bulguların genel olarak birbiriyle paralel olduğu, gıda katkı maddelerine maruziyetin memeliler üzerinde hem genotoksik hem de sitotoksik olduğu çeşitli invivo ve invitro çalışmalarla gösterilmiştir.

Anahtar Kelimeler: Gıda Koruyucu Maddeler, Memeliler, Genotoksik Etki, Mikrobiyolojik Bozulma, Sitotoksisite

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1. INTRODUCTION

The use of chemical food additives has increased rapidly with the increase in the production of various processed foods in the modern food technology. These preservative food additives are added to food in order prevent nutritional losses that occur due to microbiological and chemical changes as well as extending the shelf life of various foods (Saad et al., 2005). Food additives are also used for many purposes such as improving sensory attributes like color, appearance, texture and taste. The use of these substances dates back as the history of the humankind. When humankind discovered fire and began to consume food by cooking raw materials, spices and salt have begun to be used as the first natural additives. Rapid industrialization, rapid growth of world population and urbanization have increased the demand for convenience foods. Thus, processed and marketed food products have been extremely diversified and the number of food additives used in the processing of these products has been increased. The most important factor to be considered when using chemical substances used as food additives is the protection of human health. People can be exposed to such additives for many years. It is known that continuous intake of such chemical substances cause mutations and toxicity. It has been shown in various studies that food additives, which are allowed to be used consistently, show genotoxic effects in living organisms. Additives used randomly are harmful for public health. Even if these substances are used at doses that won't harm human health, they accumulate in the body after a while. It has been reported that accumulation of these additives can be genotoxic for humans, through damage in tissues (Briggs, 1997; Sarıkaya and Solak, 2003).

In the United States, 2800 food additives are allowed to be used, whereas this figure is approximately 300 in Turkey (Özkaya, 2004). As the consumption of these substances increases, some symptoms associated with some disorders have also begun to emerge. The most common of these are eczema, asthma, headache, allergic rashes, gastric disorders, diarrhea especially in children, hyperactivity and hypersensitivity (Güneşli, 2000; Hill and Belsito, 2003; Breiteneder, 2004; Hedge and Venkatesh, 2004). However, research on this subject has been increasing gradually and the use of these additives has been started to be restricted since studies have shown that food additives can cause genotoxic effects in many living organisms (Yurttagül and Ayaz, 2008; Altuğ, 2009).

Food additives and preservatives lead to many negative effects such as inhibition of in vivo enzymes, denaturation of proteins, alteration or destruction of the cytoplasmic membrane of DNA or cell (Altuğ, 2009). Regulation of food additives has become an international issue besides being a national concept. The International Codex Alimentarius Commission (CAC) has been established as a result of joint efforts of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). The Joint FAO/WHO Expert Committee on Food Additives (JECFA), a subsidiary of the CAC, sets the recommended standards for all countries at meetings held annually on food additives. By utilizing the allowable quantities determined by the authorized committees of WHO and FAO, the health authorities in each country determine the foods, in which the food additives will be used, and the amount of use according to the conditions of their own country. Regulations have been made on the use of additives by the Turkish Food Codex Regulation (TGKY) prepared by the Ministry of Agriculture and Rural Affairs in Turkey. The food additives have been defined in the regulated codex and the E codes of

these additives, their names, the food groups that can be used and the maximum allowable quantities have been listed. The E-code refers to codes issued by the SCF (EU Scientific Committee on Food) for food additives. Classification according to the basic functions of food additives with the "E" numbering system (Bağcı, 1997; Altuğ, 2001; Barlow et al., 1999.) is as follows:

- Colorants: E100-180,
- Preservers: E200-297,
- Antioxidants: E300-321,
- Emulsifiers and Stabilizers: E322-500,
- Acid-base suppliers: E500- 578,
- Sweeteners, fragrances: E620-637,
- Food additives for general purposes: E900-927.

In order to be able to use food additives, it is necessary to carry out various analyses, to measure the results correctly, and to provide safety with various tests on animals under strictly controlled conditions (Yaman, 1996). Even if an additive's use is legally allowed, it can only be used in accordance with certain criteria. The additive permitted to be used must be used only in the food for which it is permitted, without exceeding the maximum specified dose. They should not be used to cover any fault in the food or to mislead the consumer, and the amount of use should be clearly written on the label of the food (Yılmaz, 1999).

Genotoxicity of many food additives in Turkey and in other countries is not known and these substances are still being used carelessly. For this reason, scientists have tried to determine the possible clastogenic, mutagenic and genotoxic effects of various food additives through in vivo and in vitro test methods. As a result of the studies, it has been determined that many food additives have mutagenic effect (Abe and Sasaki, 1977; Luca et al., 1987; Meng and Zhang, 1994; Sasaki et al., 2002; Arslan, 2004; Kaya and Topaktaş, 2007; Yılmaz, 2008; Mpountoukas et al., 2008).

In recent years, the short-term genotoxicity tests such as chromosomal abnormality (CA), sister chromatid exchange (SCE) and micronucleus (MN) tests have been extensively used in human lymphocyte cultures for the detection of mutations in human genomes caused by various chemical substances. Whether the chemicals induce primary DNA damage is determined using the comet test. The use of genotoxicity testing methods for detection of mutagenic/carcinogenic potentials or antimutagenic/anticarcinogenic characteristics of chemicals used as food additives are of great importance in terms of human health and food safety. This review aims to evaluate studies on the genotoxic effects of some preservative food additives on mammals, and to summarize the data in order to provide a resource for further studies to be carried out in this regard.

2. RESEARH SIGNIFICANCE

With the increasing technology in the food sector, different production techniques, rapid diversification of the products, awareness of the consumer, consumption of the foods in every period of the year, etc. have mandated the use of food additives. While recent innovations and developments in food services have eliminated many problems, they have caused new problems to arise. Although many of the additives used in food do not pose any problems, some are dangerous when taken continuously. The studies on the genotoxic effects of some food additives used as preservatives on mammals were evaluated and the data were summarized.

3. MATERIALS USED IN THE EXPERIMENTS

Current literature information were compiled and reviewed in the light of studies in the Department of Biology, Molecular Biology, Genetics and Zoology laboratories of the Faculty of Arts and Sciences at the Kafkas University.

4. FINDINGS AND DISCUSSIONS

As a result of the increased exposure of humans to food additives in today's conditions, assessing genotoxicity of food additives, which their genetic effects have not been fully under control yet, is of great importance for human health. Although all the research and inventions on the technical field are conducted in order to provide a healthier life and better nutrition, they are accompanied by countless problems. Despite being used in allowed quantities, food additives are taken almost daily through food and hence accumulated in the body over time due to the very long exposure. This accumulation leads to toxic effects in the body, resulting in various damages. These damages cause genotoxic effects when occurred on the genetic material. Short-term genotoxicity test systems are used to determine whether a chemical substance is genotoxic (Arslan, 2004; Yılmaz, 2008; Mpountoukasetal, 2008; Çelik, 2003; Parlak, 2007; Yüzbaşıoğlu et al., 2006). Many studies have been carried out to investigate the genotoxic effects of food additives to date. Ozdemir et al. (2012) investigated the genotoxic effects of Potassium Sorbate (200-500-1000µg/ml), Sodium Benzoate (100-300-800µg/ml) and Sodium Nitrite (1-10-100µg/ml) using micronucleus test technique in human lymphocyte cell culture. It has been put forth that potassium sorbate and sodium benzoate showed no genotoxic effect at concentrations used in food, but all concentrations of sodium nitrite were found to be genotoxic (Figure 1).

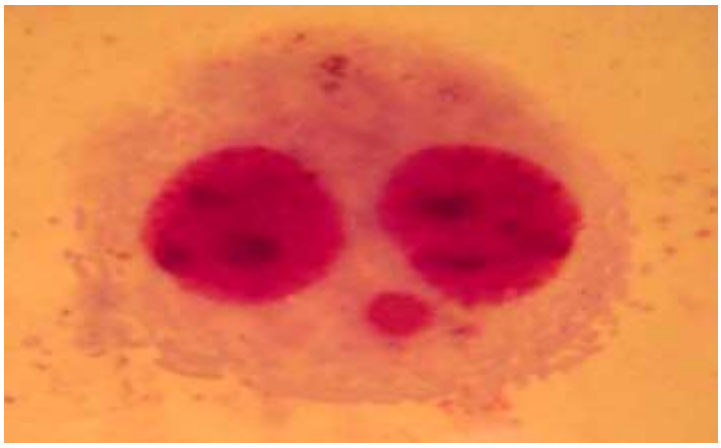

Figure 1. Cytokinesis-blocked two-nucleated lymphocyte cells and micronucleus (Özdemir et al., 2012)

Table 1. Control and MN concentrations at different preservative concentrations (%) (Özdemir et al., 2012)

Preservatives	Control (Ort±SS) (%)	Low Dose (Ort±SS) (%)	Medium Dose (Ort±SS) (%)	High dose (Ort±SS) (%)
Potassium Sorbate	1.20±0.41	1.20±0.86	1.33±1.29	1.73±1.71
Sodium benzoate	1.20±0.41	1.33±0.82	1.40±0.63	1.47±0.74
Sodium Nitrite	1.20±0.41	1.93±1.10a	2.07±1.33a	2.87±1.92b,c

a According to control $p<0.05$,
b According to control $p<0.01$,
c According to low dose $p<0.05$
Ort:Average, SS:Standard deviation

In order to investigate the genotoxic effect of potassium sorbate on lymphocyte cells, Mamur et al. (2010) used four different concentrations of potassium sorbate (125, 250, 500 and 1000µg/ml) in their studies using micronucleus, Comet and Sister Chromatid Exchange (SCE) techniques, and as a result they have observed the genotoxic effect of potassium sorbate. Ishdate&Odashima and Abe&Sasaki have found a positive effect of potassium sorbate the in vitro CA test at concentrations of 3-4mg/ml in studies conducted with Chinese Hamster cells (Abe and Sasaki, 1977; Ishidate and Odashima, 1977).

In another study conducted by Hasegawa et al. on the potential genotoxic risk of sorbic acid and potassium sorbate, CA, SCE and gene mutation test methods have been applied in cultured Chinese hamster V79 cells. Sorbic acid and potassium sorbate have been observed to cause chromosomal abnormalities at concentrations of 1000 to 2000 µg/mL (Hasegawa et al., 1984). Prival et al. found that potassium sorbate and sodium benzoate at low concentrations (2.0, 0.2 and 0.02 mM) were not genotoxic but showed a genotoxic effect as the amount of preservatives increased (4 and 8mM) (Prival et al., 1991). Another study by Zengin et al. showed that sodium benzoate doses of 6.25, 12.5, 25, 50 and 100µMg/ml caused chromosome anomalies, sister chromatid exchanges and micronucleus formation in vitro in human lymphocyte cells. In the same study, high concentrations of Sodium benzoate have been reported to be genotoxic in human peripheral lymphocytes (Zengin et al., 2011).

Mukherjee et al. investigated the possible genotoxic effects of sorbic acid and sodium nitrite alone and in mixture in bone marrow cells of mice. As a result of the study, it was reported that sorbic acid increased sister chromatid exchange (SCE) and micronucleus frequencies at high concentrations, whereas sodium nitrite found to increase SCE frequency at all applied concentrations. It has been found that the solution mixtures increase the sister chromatid-exchange frequency twice compared to single application of the additives (Mukherjee et al., 1988).

In another study, in vitro genotoxic effects of sodium sorbate and potassium sorbate, which are frequently used as antimicrobial additives, in human peripheral lymphocytes have been determined using chromosome abnormality (CA), sister chromatid exchange (CSE), micronucleus (MN) and comet tests. Both food additives were found to increase the chromosomal abnormality and sister chromatid change statistically significantly compared to the negative control. According to the results of Comet analysis, it was found that both additives increased the primary DNA damage significantly at all concentrations. Comet tail intensity and comet tail length were found to increase significantly in all applications compared to the control (Figure 2 and Figure 3). The findings obtained indicate that the additives used in the study were clastogenic and mutagenic in vitro cultured and isolated human lymphocytes and also induced primary DNA damage (Mamur, 2009).

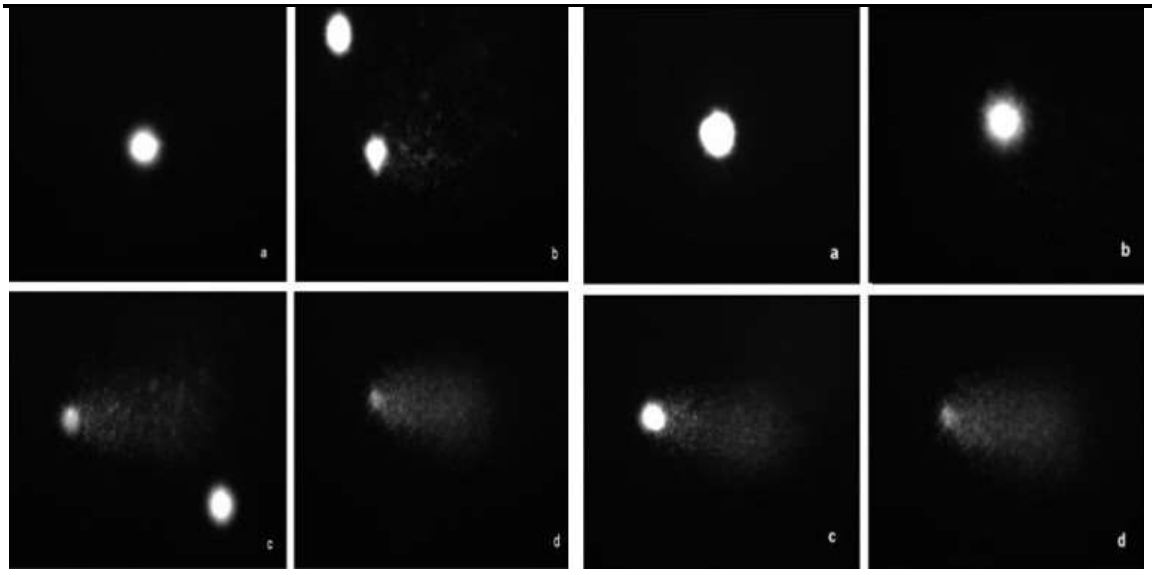

Figure 2. Comet test appearance of DNA damage in isolated human lymphocytes treated with potassium sorbate a) undamaged DNA b) little damaged DNA c) medium damaged DNA d) very damaged DNA (Mamur, 2009).

Figure 3. Comet test appearance of DNA damage in isolated human lymphocytes treated with sodium sorbate a) undamaged DNA b) little damaged DNA c) medium damaged DNA d) very damaged DNA damaged (Mamur, 2009).

5. CONCLUSIONS AND RECOMMENDATIONS

Food additives are added to food to increase and protect nutritional value, for this reason, there are some basic principles that must be followed regarding the use of food additives. Determining the risks posed by food additives as a result of voluntary or involuntary exposures to these substances due to many reasons is important for human health. Awareness of consumers should be increased about this as well as increasing the audits strictly. In order for these additives to be used safely in the food industry, they should be tested and evaluated thoroughly for a long time, allowed to be used only at the doses specified by the relevant institutions, and use of additives found to have adverse effects on human health should be prohibited. However, it should be noted that additives that are allowed to be used are taken continuously and accumulate in the body over time, affecting our health negatively. In order to minimize the effects of food additives, it is necessary to raise awareness of food producers and to prevent excessive use of additives used in production. Since consumer demand is a driving force behind the food industry, conscious consumers will force both producers and supervisory units to be more sensitive in making effective controls on the use of the right food additives.

NOTICE

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