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EVALUATION OF THE GENOTOXIC POTENTIALS OF SUNFLOWER LECITHIN, XANTHAN GUM, AND MALIC ACID ON HUMAN PERIPHERAL LYMPHOCYTES IN VITRO

Doğukan EROĞLU¹ , Selçuk ÇEKER² 

¹Bolu Abant İzzet Baysal University, Graduate Institute, Department of Medical Biology and Genetics, Bolu, Türkiye

²Bolu Abant İzzet Baysal University, Faculty of Medicine, Department of Medical Biology, Bolu, Türkiye

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ABSTRACT

Food additives are substances used to preserve or improve the safety, freshness, flavor, texture, or appearance of food. As the consumption of processed foods has increased, so has the consumption of food additives. However, whether food additives have a negative impact on human health is a matter of debate. In this study, the potential genotoxicity of sunflower lecithin, malic acid, and xanthan gum, used as food additives, on human peripheral lymphocytes was determined using the micronucleus test. Preliminary tests conducted in this study investigated the potential genotoxic effects of different concentrations of sunflower lecithin (v/v - 1.2‰, 1.8‰, 2.7‰, 4.05‰, 5.4‰, 7.2‰), xanthan gum (500, 1000, 1500, 2000, 3000, and 4500 µg/ml), and malic acid (31.25, 62.50, 125, 250, 375, and 500 µg/ml). Based on the findings, the studied food additives exhibited statistically significant genotoxic effects ($P<0.05$) at elevated concentrations. Specifically, significant increases in micronucleus frequency were initiated at 4.05‰ (v/v) for sunflower lecithin, 2000 µg/ml for xanthan gum, and 250 µg/ml for malic acid compared to the control groups, reaching the highest genotoxic responses at the maximum tested concentrations.

Key words: Genotoxicity, sunflower lecithin, xanthan gum, malic acid

AYÇİÇEĞİ LESİTİNİ, KSANTAN SAKIZI VE MALİK ASİDİN İNSAN PERİFERİK LENFOSİTLERİNDE İN VİTRO GENOTOKSİK POTANSİYELLERİNİN BELİRLENMESİ

ÖZ

Gıda katkı maddeleri, gıdaların güvenliğini, tazeliğini, lezzetini, dokusunu veya görünümünü korumak ya da geliştirmek amacıyla kullanılan maddelerdir. İşlenmiş gıda tüketiminin artmasıyla birlikte gıda katkı maddelerinin tüketimi de artış göstermiştir. Ancak gıda katkı maddelerinin insan sağlığı üzerinde olumsuz bir etkisinin olup olmadığı tartışma konusudur. Bu çalışmada, gıda katkı maddesi olarak kullanılan ayçiçeği lesitini, malik asit ve ksantan sakızının insan periferik lenfositleri üzerindeki potansiyel genotoksitesi mikronükleus testi kullanılarak belirlenmiştir. Bu kapsamda, ayçiçeği lesitini (v/v - ‰1.2, ‰1.8, ‰2.7, ‰4.05, ‰5.4, ‰7.2), ksantan sakızı (500, 1000, 1500, 2000, 3000 ve 4500 µg/ml) ve malik asidin (31.25, 62.50, 125, 250, 375 ve 500 µg/ml) farklı konsantrasyonlarının potansiyel genotoksik etkileri araştırılmıştır. Elde edilen bulgulara göre, incelenen gıda katkı maddeleri yüksek konsantrasyonlarda istatistiksel olarak anlamlı ($P<0.05$) genotoksik etkiler sergilemiştir. Kontrol grupları ile kıyaslandığında, mikronükleus frekansındaki anlamlı artışların ayçiçeği lesitini için ‰4.05, ksantan sakızı için 2000 µg/ml ve malik asit için 250 µg/ml konsantrasyonlarında başladığı ve test edilen en yüksek konsantrasyonlarda en üst genotoksik yanıtı ulaştığı belirlenmiştir.

Anahtar kelimeler: Genotoksite, ayçiçeği lesitini, ksantan sakızı, malik asit

Corresponding author/Yazışmalardan sorumlu yazar: Selçuk ÇEKER

ORCID No: 0000-0002-2795-8863 **E-mail:** selcuk.ceker@ibu.edu.tr



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INTRODUCTION

In the rapidly evolving pace of modern life, consumer preferences increasingly turn toward faster and more convenient food products. To meet this growing need for quick and easy meals, and to ensure that products reach consumers without spoilage or alteration, the production and use of food additives have continuously increased (Karatepe & Ekerbiçer, 2017). Food additives are substances used to provide the flavors people seek in their preferred products and to prevent food from spoiling throughout its shelf life. Food additives can be classified into two main categories, artificial and natural, depending on their production methods. Natural food additives are primarily produced by purifying components obtained from plant or animal sources, while artificial food additives are chemically synthesized (Wu et al., 2022). They are also categorized into broad categories such as preservatives, colorants, antioxidants, sweeteners, thickeners, emulsifiers, and flavor enhancers, depending on their intended use (Wu et al., 2022).

The usage rates of food additives in food are determined by relevant organizations because of long-term studies. However, despite a cautious approach to the quality and quantity of food additives within the framework of food safety, it is observed that in some countries that do not pay enough attention to these issues, the incidence of certain diseases increases in direct proportion to the increase in ready-to-eat food consumption habits (Eroglu & Ayaz, 2018). Studies on human health have shown that food additives such as emulsifiers, sweeteners, and preservatives in ultra-processed foods reduce the diversity of the gut microbiota, disrupt bacterial balance, and cause irritable bowel syndrome, low-grade inflammation, and metabolic disorders. They reduce beneficial bacterial populations while promoting the growth of harmful ones (Rinninella et al., 2020). Crucially, even within permissible daily intake limits, certain additives can accumulate in the human body if not effectively eliminated. This toxic accumulation in the body can cause tissue damage, mutations, and cancer formation (Pressman et al., 2017). A comprehensive study conducted in France, involving more than 100,000 adults, shows that a person consumes an average of 4 kg to 10 kg of food additives per year (Chazelas et al., 2021).

Sunflower lecithin and xanthan gum, used as emulsifiers and thickeners in foods, and malic acid, used as a preservative, are among the commonly used food additives. Sunflower lecithin is a valuable byproduct obtained during the refining of sunflower oil, containing a high percentage of phosphatidylcholine and essential oils. Sunflower lecithin functions as an emulsifier, dispersing agent, viscosity reducer, separating agent, dietary supplement, and more. It is widely used in various industries such as pharmaceuticals, food, and cosmetics (Guiotto et al., 2015). Xanthan gum is used especially as a thickening agent because its network structure prevents the separation of insoluble solid particles. Xanthan gum is used in salad dressings, baked goods, desserts, dairy products, processed cheese, mayonnaise, and ready-to-eat meals (Sworn, 2021). Malic acid is a substance that can be used in hard candies, processed fruits and fruit juices, soft drinks, soft candies, chewing gum, jams and jellies, gelatins, and puddings. Malic acid is particularly used to prevent taste deterioration caused by the rancidity of fats in food (Marques et al., 2020).

Although the health impacts of food additives remain a subject of intense debate, the potential genotoxicity of sunflower lecithin, xanthan gum, and malic acid on peripheral lymphocytes-critical components of the human immune system-remains unexplored in the current literature. To address this gap, the present study investigates the potential genotoxic effects of these three widely used additives on human peripheral lymphocytes in vitro.

Material and Methods

Ethical approval for this research was obtained from the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University Faculty of Medicine (decision no. 2023/307 dated 10.10.2023). Blood samples were collected from 4 volunteers aged 22-25 who met the criteria of being non-smokers, non-consumers of alcohol, and free of medication use in the last six months. Sunflower lecithin, xanthan gum, and malic acid, whose genotoxic potential was investigated, were commercially available. Aflatoxin B₁ (AFB₁, 5 µM) was utilized as the positive control group, while untreated cells served as the negative control group.

Determination of Food Additive Concentrations

The concentrations of sunflower lecithin, xanthan gum, and malic acid investigated in this study were determined through preliminary trials informed by literature reviews, as well as LD50, NOAEL, and ADI values. Accordingly, the concentrations selected for the study were set as follows: 1.2‰, 1.8‰, 2.7‰, 4.05‰, 5.4‰, and 7.2‰ for sunflower lecithin; 500, 1000, 1500, 2000, 3000, and 4500 µg/ml for xanthan gum; and 31.25, 62.50, 125, 250, 375, and 500 µg/ml for malic acid.

Micronucleus Test

The protocol used by Stich et al. for the micronucleus (MN) test was adopted and modified (Ceker, 2021). A 7 mL cell culture tube, prepared with a peripheral blood sample and the respective chemicals, was incubated at 37°C for 72 hours. At the 44th hour, 6 µg/ml cytochalasin B was added to each tube. At the 72nd hour, the cultured tubes were centrifuged at 1000 rpm for 10 minutes, and the supernatant was reserved for biochemical analysis. A hypotonic solution was added to the pellet. The tubes were incubated at 37°C for 25 minutes. The supernatant was discarded after a second centrifugation at 1000 rpm for 10 minutes. Fixation was performed by adding a 3:1 methanol-acetic acid fixation solution to the pellet. The fixation process was repeated three times. At the end of the fixation process, all but the bottom portion of the supernatant were discarded. The remaining pellet was then used to prepare slides, each containing 5-6 drops of the pellet, which were maintained in cold fixation. The prepared slides were left to dry at room temperature for three days. After drying, the slides were stained with Giemsa stain for 10 minutes. After staining, the slides were rinsed with distilled water to remove excess stain. To determine the number of micronuclei, 1000 binucleated cells with well-defined cytoplasm and intact boundaries were examined in preparations from each donor group and control group, and those containing micronuclei were recorded. The MN frequency (MN/1000 cells) was then calculated (Ceker, 2021).

RESULTS AND DISCUSSION

Food additives have become a focus of attention due to their increasing use in daily diets and their effects on human health. Numerous studies exist in literature on this topic. Research

on the effects of food additives on human health is of great importance in terms of protecting consumer health. These health impacts can vary depending on the amount consumed, the duration of exposure, as well as the age, gender, health status, and genetic factors of the consumer (Atalay & Celep, 2024).

Long-term exposure to food additives has been shown to cause toxic, genotoxic, mutagenic, and immunotoxic effects, reproductive and developmental disorders, and hypersensitivity/allergic reactions in living organisms (Abo-El-Sooud et al., 2018). Various studies have demonstrated that long-term and high-quantity consumption of certain food additives can trigger oxidative stress (Bischoff et al., 2022; Ko et al., 2022; Atalay & Celep, 2024). The relationship between oxidative stress and genotoxicity is particularly relevant to the development of many health problems, including cancer (Veltman et al., 2023).

A literature review revealed no studies on the genotoxicity of sunflower lecithin, malic acid, and xanthan gum in human peripheral lymphocytes, which are critical components of the human immune system. Therefore, this study investigated the potential genotoxic effects of sunflower lecithin, malic acid, and xanthan gum, which are present in many packaged ready-to-eat products, on human peripheral lymphocytes. When aflatoxin B₁ (AFB₁), used as the positive control, was introduced to the culture medium, it significantly increased the micronucleus frequency across all experimental groups, exhibiting a robust genotoxic effect (Table 1). On the other hand, it was observed that sunflower lecithin, xanthan gum, and malic acid did not show genotoxic effects on human peripheral lymphocytes at low concentrations compared to the negative control group. However, they increased the micronucleus frequency in a concentration-dependent manner, and these increases were found to be statistically significant at higher doses (Table 1).

In this study, malic acid concentrations of 31.25, 62.50, 125, 250, 375, and 500 µg/ml were used. The findings showed that when comparing the negative control (2.52 ± 0.52^a) and positive control groups (3.74 ± 0.32^c), concentrations starting from 250 µg/ml up to the maximum tested dose of 500 µg/ml exhibited statistically significant ($P < 0.05$) genotoxic trends on human peripheral lymphocytes, with the highest chromosomal damage observed at 500 µg/ml (3.19 ± 0.38^d) (Table 1).

Table 1. Effects of sunflower lecithin, xanthan gum, and malic acid on micronucleus frequencies in human peripheral lymphocytes

Sunflower lecithin		Xanthan gum		Malic acid	
Conc.	MN Freq. $\pm$ S.E.	Conc.	MN Freq. $\pm$ S.E.	Conc.	MN Freq. $\pm$ S.E.
Control	2.48 $\pm$ 0.37 ^a	Control	2.43 $\pm$ 0.10 ^a	Control	2.52 $\pm$ 0.52 ^a
AFB ₁ 5 μ M	3.63 $\pm$ 0.46 ^c	AFB ₁ 5 μ M	3.86 $\pm$ 0.60 ^d	AFB ₁ 5 μ M	3.74 $\pm$ 0.32 ^c
1.2‰	2.51 $\pm$ 0.72 ^a	500 μ g/ml	2.48 $\pm$ 0.68 ^a	31.25 μ g/ml	2.52 $\pm$ 0.62 ^a
1.8‰	2.55 $\pm$ 0.62 ^a	1000 μ g/ml	2.53 $\pm$ 0.35 ^a	62.50 μ g/ml	2.55 $\pm$ 0.38 ^a
2.7‰	2.57 $\pm$ 0.34 ^a	1500 μ g/ml	2.54 $\pm$ 0.43 ^a	125 μ g/ml	2.56 $\pm$ 0.40 ^a
4.05‰	2.63 $\pm$ 0.30 ^{ab}	2000 μ g/ml	2.60 $\pm$ 0.80 ^b	250 μ g/ml	2.78 $\pm$ 0.62 ^b
5.4‰	2.80 $\pm$ 0.46 ^c	3000 μ g/ml	2.76 $\pm$ 0.34 ^{bc}	375 μ g/ml	2.94 $\pm$ 0.80 ^{cd}
7.2‰	2.92 $\pm$ 0.52 ^{cd}	4500 μ g/ml	2.80 $\pm$ 0.42 ^c	500 μ g/ml	3.19 $\pm$ 0.38 ^d

Note : a,b,c,d,e, Differences between values represented by different exponents within the same column are significant at the $P<0.05$ level. S.E.: Standard error, Conc.: Concentrations, Freq.: Frequencies

In research conducted by Arslan (2021), it was determined that malic acid has an anticarcinogenic effect on glioblastoma cells via the necrotic pathway at high concentrations and exhibits apoptotic properties in fibroblast cell lines. Studies on substances with similar properties to malic acid (sodium benzoate, sodium nitrite, sodium metabisulfite, sodium propionate, calcium propionate, potassium propionate, benzoic acid, and potassium benzoate) are also available in the literature. Various studies on benzoates show that benzoic acid increases genotoxicity at high concentrations and causes a decrease in survival time (Abo-El-Sooud et al., 2018; Chatterjee et al., 2021). Some studies have shown that potassium sorbate, sodium benzoate, and potassium nitrate exhibit genotoxicity at low concentrations. Sodium benzoate, in particular, has been associated with mutagenic and cytotoxic effects such as micronucleus formation and chromosome breaks in lymphocytes (Pongsavee, 2015; Oladele et al., 2020). In rat bone marrow cells, exposure to sodium metabisulfite for 12 and 24 hours was found to produce a high number of abnormal cells (Kayraldiz & Topaktas, 2007). Sodium propionate, calcium propionate, and potassium propionate have been found to significantly reduce the mitotic index and greatly increase the frequency of chromosomal abnormalities (Türkoğlu, 2008). The results obtained in this study are consistent with and support similar studies found in literature (Table 1). On the other hand, some studies in the literature regarding malic acid report that the substance is not genotoxic (Al-Qayim & Mashi, 2014; Guo et al., 2017). Furthermore, various studies have

reported the protective effect of malic acid against aluminum-induced toxicity both *in vitro* and *in vivo* (Al-Qayim & Saadoon, 2013; Al-Qayim & Mashi, 2014).

Emulsifiers are used to ensure a homogeneous distribution of substances in food and to preserve the structural form of the food (Gültekin, 2021). Sunflower lecithin, used in this study, is one of the substances used as an emulsifier. In the study conducted with sunflower lecithin, concentrations of 1.2‰, 1.8‰, 2.7‰, 4.05‰, 5.4‰, and 7.2‰ were used. The study results showed that statistically significant genotoxic effects were initiated at the 4.05‰ concentration (2.63 $\pm$ 0.30^{ab}) and became distinctly pronounced at higher concentrations up to 7.2‰ (2.92 $\pm$ 0.52^{cd}) compared to the negative control group (Table 1). Studies in the literature regarding lecithin support this research finding (Taner et al., 2014; Mortensen et al., 2017a; Johnson Jr et al., 2020). In allergic reaction tests, immunological and respiratory changes were detected in individuals exposed to lecithin (Mortensen et al., 2017a). However, there are also studies in the literature reporting that lecithin does not have a toxic effect (Marangoni et al., 2017; Younes et al., 2020).

Thickening agents are used in foods to achieve the desired consistency. One of these agents is xanthan gum. In this study, xanthan gum was used at concentrations of 500, 1000, 1500, 2000, 3000, and 4500 μ g/ml. The data obtained from the study showed that a statistically significant increase in micronucleus frequency was triggered starting at the 2000 μ g/ml application

(2.60 ± 0.80^b) and systematically advanced throughout the 3000 $\mu\text{g}/\text{ml}$ (2.76 ± 0.34^{bc}) and 4500 $\mu\text{g}/\text{ml}$ (2.80 ± 0.42^c) experimental groups (Table 1). In one study, albino rats were given 3% or 6% xanthan gum, and a decrease in body weight was observed. When the xanthan content in the diet was increased to 7.5% and 10%, a decrease in growth rate, abnormal stools, and reduced feed intake were observed in the rats (Mortensen et al., 2017b). On the other hand, there are also studies reporting that xanthan gum has no toxic effects (Hobbs et al., 2012).

In toxicological risk assessment, projecting *in vitro* findings into real-world human exposure scenarios is essential to clarify the practical relevance of the data. In the current study, statistically significant increases in MN frequencies were initiated at relatively high concentrations: 4.05‰ (v/v) for sunflower lecithin, 2000 $\mu\text{g}/\text{mL}$ for xanthan gum, and 250 $\mu\text{g}/\text{mL}$ for malic acid. From a regulatory standpoint, authorities like the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have allocated an Acceptable Daily Intake (ADI) of "not specified" for xanthan gum (E 415), lecithins (E 322), and malic acid (E 296), reflecting their exceptionally low systemic toxicity under typical conditions of use. In commercial processed foods, these compounds are typically utilized at much lower functional levels (ranging from 0.1% to 1% by weight). Therefore, the threshold concentrations at which genotoxic trends were observed in our *in vitro* system substantially exceed the standard physiological amounts encountered through ordinary daily dietary intake. These elevated experimental doses simulate maximum, worst-case cellular exposure or cumulative overconsumption scenarios. While our data indicate that these additives possess the biological capacity to induce chromosomal damage when direct cellular contact occurs at extreme thresholds, these concentrations are highly unlikely to be achieved within the human circulatory system or peripheral lymphocytes through normal, balanced nutritional habits.

CONCLUSION

In conclusion, sunflower lecithin, malic acid, and xanthan gum demonstrated concentration-dependent genotoxic potential only at elevated experimental thresholds, while remaining safe at low concentrations. However, toxicological studies on these specific additives remain limited. Consequently, further research is warranted to elucidate the long-term safety profiles and individual physiological effects of their chronic consumption.

LIMITATIONS OF THE STUDY AND FUTURE PERSPECTIVES

Although this study provides valuable insights into the cellular risks of cumulative food additive exposure, certain limitations should be acknowledged. First, the *in vitro* cytotoxicity and genotoxicity assessments were conducted using peripheral blood samples from a limited cohort ($n=4$) within a narrow age range (22-25 years). This constraint restricts the representation of wider population-based genetic variations, age-dependent metabolic differences, and diverse physiological responses. Second, direct cellular exposure in an *in vitro* system does not fully replicate complex *in vivo* physiological processes, including gastrointestinal digestion, enzymatic degradation, and hepatic first-pass metabolism. Finally, the statistically significant micronucleus increases observed at high concentrations ranging from 250 to 500 $\mu\text{g}/\text{mL}$ for malic acid and 2000 to 4500 $\mu\text{g}/\text{mL}$ for xanthan gum represent exposure levels that substantially exceed typical daily dietary intake and established ADI thresholds (EFSA, 2020). Therefore, while these findings highlight potential biological mechanisms under maximum exposure scenarios, future *in vivo* and epidemiological studies are required to clarify the clinical relevance of these additive concentrations under real-world dietary conditions.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this article.

AUTHOR CONTRIBUTIONS

Doğukan Eroglu: Investigation, Methodology, Formal analysis, Data curation, Writing - original draft. Selcuk Ceker: Project administration, Conceptualization, Supervision, Writing - review & editing.

DISCLOSURE ON ARTIFICIAL INTELLIGENCE USAGE

No artificial intelligence program was used in the preparation of this article.

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