



GENOTOXIC AND ANTIGENOTOXIC EFFECT OF BORIC ACID

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Fahima Hamoud Moussa FAHIMA HAMOUD MOUSSA

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Fahima Hamoud Moussa

FAHIMA HAMOUD MOUSSA

07/08/2023

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BORIC ACID

(M. Sc. Thesis)

Fahima Hamoud Moussa FAHIMA HAMOUD MOUSSA

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ABSTRACT

Mitomycin-C (MMC) is an alkylating chemotherapeutic drug used to treat several cancers. It prevents DNA synthesis (even RNA and protein synthesis at higher concentrations) by causing DNA crosslinks, generally in guanine and cytosine pairs, and then inhibits cell mitosis and causes cell cycle arrest. Boron is one of the most abundant and naturally occurring metalloid elements with unique characteristics. In recent years, boron-containing compounds such as borax, colemanite, ulexite, sodium perborate, and boric acid, have been utilized as food preservatives and anti-cancer medications. Especially boric acid (BA- H_3BO_3) is extensively applied in agriculture, industry, cosmetics, and medicine. BA is recognized as a critical micronutrient because of its intriguing biological functions. It can boost the body's natural antioxidant defense. The main goal of this research was to investigate the genotoxic potential of BA and its antigenotoxic effect against MMC-induced DNA damage *in vitro* in human peripheral lymphocytes (HPLs) using four types of genotoxicity tests; chromosomal aberration-CA, sister chromatid exchange-SCE, cytokinesis block micronucleus cytome-CBMN-cyt, and comet. HPLs were treated with four concentrations of BA (0.25, 0.5, 1, and 2.5 $\mu\text{g/mL}$) either alone or concurrently with MMC (0.20 $\mu\text{g/mL}$) or hydrogen peroxide (H_2O_2 ; 100 μM in comet assay). Single treatment of BA did not significantly increase the frequency of abnormal cells, CA/Cell, SCE/Cell (except 2.5 $\mu\text{g/mL}$ at 48 h), and MN compared with negative control. In the comet assay, BA significantly increased the comet parameters (except 0.25 and 0.5 $\mu\text{g/mL}$ in tail intensity). Cotreatment of BA+MMC significantly diminished the frequency of CA, SCE, and MN in all the applied concentrations compared with positive control. BA also significantly decreased DNA damage triggered by H_2O_2 . BA+MMC applications did not significantly affect the mitotic index (MI), replication index (RI), the frequency of nuclear bud (NBUD), nucleoplasmic bridge (NPB), nuclear division index (NDI), and cytokinesis-block proliferation index (CBPI). In conclusion, we determined for the first time that BA alone does not induce significant damage but rather generates antigenotoxic potential against genetic damage induced by MMC and H_2O_2 in HPLs *in vitro*. Therefore, these results will contribute remarkable information on the pharmacological assessment of BA, which has been started to use for nutritional, pharmaceutical, and medical applications.

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BORİK ASITIN GENOTOKSİK VE ANTİGENOTOKSİK ETKİSİ
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Fahima Hamoud Moussa FAHIMA HAMOUD MOUSSA

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ÖZET

Mitomisin-C (MMC), bir çok kanserin tedavisinde kullanılan alkilleyici bir kemoterapi ilacıdır. DNA’da, genellikle guanin ve sitozin çiftlerinde çapraz bağlar oluşturarak DNA (yüksek konsantrasyonlarda RNA ve protein) sentezini engeller ve böylece hücre mitozunu ve hücre siklusunu durdurur. Bor, doğal olarak oluşan ve kendine has özellikleri ile en fazla bulunan metalloid bir elementtir. Son yıllarda, boraks, kolemanit, üleksit, sodyum perborat ve borik asit gibi bor ihtiva eden bileşikler gıda katkı maddesi ve anti-kanser ilacı olarak kullanılmaktadır. Özellikle borik asit (BA- H_3BO_3), tarımda, endüstride, kozmetikte ve tıpta yaygın olarak kullanılmaktadır. İlginç biyolojik işlevleri nedeniyle BA, önemli bir mikro besin olarak kabul edilmektedir. Vücudun antioksidan savunmasını artırmaktadır. Bu çalışmanın ana amacı, BA’in olası genotoksik potansiyelini ve MMC ile oluşturulan genotoksositeye karşı olası antigenotoksik potansiyelini *in vitro* insan periferik lenfositlerinde (İPL) kromozom anormallikleri-KA, kardeş kromatid değişimi-KKD, sitokinezi bloklanmış mikronükleus-sitom-CBMN-cyt ve komet testleri ile incelemektir. İPL’leri BA’in dört farklı konsantrasyonu ile (0,25; 0,5; 1 ve 2,5 $\mu g/mL$) ya tek başına veya eş zamanlı olarak MMC (0,20 $\mu g/mL$) (KA, KKD ve MN testinde) veya hidrojen peroksit (H_2O_2 ; 100 μM komet testinde) ile muamele edilmiştir. BA’in tek başına uygulaması, negatif kontrole kıyasla, anormal hücre frekansı, KA/Hücre, KKD/Hücre düzeyinde (48 saat, 2,5 $\mu g/mL$ hariç) ve MN frekansında anlamlı bir artış oluşturmamıştır. BA, komet testinde, bütün parametrelerde anlamlı artış oluşturmamıştır (kuyruk yoğunluğunda, 0,25 ve 0,5 $\mu g/mL$ hariç). Diğer yandan, BA+MMC muamelesi, pozitif kontrole (MMC) kıyasla, tüm konsantrasyonlarda, KA, KKD ve MN frekanslarını anlamlı seviyede düşürmüştür. BA ayrıca komet testinde H_2O_2 tarafından tetiklenen DNA hasarını anlamlı ölçüde azaltmıştır. BA+MMC uygulaması, mitotik indeks (MI), replikasyon indeksi (RI), nükleer tomurcuk frekansı (NBUD), nükleoplazmik köprü frekansı (NPB), nükleer bölünme indeksi (NBI) ve sitokinez-blok proliferasyon indeksi (CBPI) üzerinde anlamlı değişime neden olmamıştır. Sonuç olarak, bu çalışmada, BA’in *in vitro* insan periferik lenfositlerinde anlamlı hasar oluşturmadağı, daha ziyade, MMC ve H_2O_2 tarafından tetiklenen genetik hasarlara karşı antigenotoksik etkili olduğı ilk kez tespit edilmiştir. Bu nedenle, elde edilen sonuçlar, tıbbi, farmakolojik ve beslenme amacıyla kullanılmaya başlanan BA’in farmakolojik değerlendirmeleri üzerindeki araştırmalarına önemli katkılar sağlayacaktır.

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SYMBOLS AND ABBREVIATIONS

The symbols and abbreviations used in this research are given below along with their explanations.

Symbols	Explanation
%	Percent
~	Tilde
°C	Celsius degree
μg/g	Microgram/gram
μg/kg	Microgram/kilogram
μg/L	Microgram/liter
μg/mL	Microgram/milliliter
μM	Micromolar
μm	Micrometer
g/kg	Gram/kilogram
g/mol	Gram/molar
g/cm³	Gram/cubic centimeter
mg	Milligram
mg/kg	Milligram/kilogram
mg/L	Milligram/liter
mL	Milliliter
mM	Millimolar
nm	Nanometer
ppm	Part per million
α	Alpha
β	Beta

Abbreviations	Explanation
AFB1	Aflatoxin b1
B	Boron
B(OH)₃	Boric acid
B(OH)₄	Tetrahydroxyborate
BNCT	Boron neutron capture therapy
BrdU	Bromodeoxyuridine
CA	Chromosomal aberration
Ca	Calcium
CAT	Catalase
CBMN	Cytokinesis-block micronucleus
CCL₄	Carbon Tetrachloride
CPM	Cyclophosphamide
Cyt-B	Cytochalasin-B
DNA	Deoxyribonucleic acid
DSB	Double strand break
GIN	Transgenerational genomic instability
GSH	Glutathione
GSH-PX	Glutathione peroxidase
H₂O₂	Hydrogen peroxide
HepG2	Hepatitis G2
HPV	Human Papillomavirus
HR	Homologue recombination
IC₅₀	Half maximal inhibitory concentration
IGF-1	Insulin growth factor-1
IWGT	International Workshop on Genotoxicity Testing
K	Potassium
KCL	Potassium chloride
LD₅₀	Median lethal dose

Abbreviations	Explanation
MDA	Malondialdehyde
MI	Mitotic index
MMC	Mitomycin-C
MN	Micronucleus
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBUD	Nuclear bud
NDI	Nuclear division index
NPB	Nucleoplasmic bud
NPBA	3-nitrophenyl boric acid
NRU	Neutral red uptake
OECD	Organization for Economic Cooperation and Development
PAC	Paclitaxel
pH	Potential hydrogen
PSA	Prostate-specific antigen
PVC	Polyvinyl chloride
RBC	Red blood cell
RI	Replication index
RNA	Ribonucleic acid
ROP	Reactive oxygen particles
ROS	Reactive oxygen species
rpm	Rotation per minute
SCE	Sister chromatid exchange
SMART	Somatic mutation and recombination test
SOD	Superoxide dismutase
SSB	Single strand break
SSC	Saline sodium citrate
USDA	United States Department of Agriculture

Abbreviations**Explanation****UV**

Ultraviolet

WHO

World Health Organization

1. INTRODUCTION

Cancer may result from internal (immune system dysregulation, hereditary mutations, hormones) or external (infections, lifestyle, professional) factors. These factors may work in collaboration to gradually initiate or accelerate the development of cancer. It can be cured either by one type or a combination of several treatments (such as chemotherapy, surgery, radiotherapy, hormone therapy, immune therapy, and target therapy). Mitomycin-C is one of the widely used chemotherapeutic agents to cure cancer.

Mitomycin-C (MMC) is a natural substance produced by the bacterium *Streptomyces caespitosus*. MMC is an antineoplastic and antibiotic isolated from bacteria. Acting as an alkylating agent, MMC inhibits DNA and RNA synthesis by causing DNA crosslinks and cleaves DNA by nuclear lysis. It is more effective in cells in the late G1 and early S stages. Because of these effects, it is widely utilized to combat some human malignancies, including cancer of the pancreas, bladder, lung, stomach, breast, rectum, uterus, cervix, and colon.

Since malignant and normal cells are not distinguished during chemotherapy, MMC may induce some adverse effects on healthy cells. Transgenerational genomic instability (GIN) is a side effect of prolonged exposure to chemotherapeutic agents that can result in pro-oncogenic mutations and ultimately raise the risk of subsequent malignancies.

Oxidative DNA damage generated by reactive oxygen species (ROS) and free radicals is crucial in the development of various diseases, including cancer and aging. Hydrogen peroxide (H_2O_2) can induce oxidative stress and damage DNA by generating reactive oxygen species and free radicals, which lead to single-strand breaks. They also change purines and pyrimidines to their hydroxyl derivatives, such as 8-hydroxyguanine. Failures in the repair of such damages may have carcinogenic effects.

Boron (B) is the fifth element of the periodic table and the only nonmetal in group 3A. B is one of the most abundant elements in nature after silicon. Due to its physical and chemical properties, it is located next to carbon in the periodic table. B has remarkable properties and is one of the most commonly used elements in the world. B minerals and products have numerous applications that are expanding every day. It is a trace mineral with various vital

metabolic roles, making it necessary for animal, plant, and human health. Türkiye has the world's largest boron reserves (73%). It is used in targeted drug therapies for cancer and other diseases. It is also used in pesticides, antimicrobial agents, detergents, textile, nuclear, fiberglass, and aerospace industries for various purposes.

There are various derivatives of boron, such as borax, boric acid, ulexite, colemanite, and sodium perborate. Of these, boric acid (BA- H_3BO_3) is colorless, odorless, and easily soluble transparent crystalline or white granular powder, light organic Lewis acid with structural properties similar to carbon, and its specific gravity is 1.43 gr/cm^3 . BA is used in many areas, such as agriculture, wood protection chemicals, hydrogen energy storage chemicals, glass, iron, steel, and ceramic industries, and construction of lightweight building materials. It is applied in health as a cleaning product and to improve bone fractures. It is used in many cosmetics and personal care products and foods. It is also used in military technologies and nuclear energy.

Several studies have demonstrated that boric acid is not genotoxic and mutagenic. The results of genotoxicity studies in mammalian test systems were all negative. In contrast, it may have hypolipidemic, anti-inflammatory, and anti-carcinogenic effects and minimize adverse effects of anticancer drugs and oxidative stress induced by chemicals. BA was found to be effective in reducing the accelerated amount of chromosome aberrations, micronucleus, and sister chromatid exchange formation generated by paclitaxel (PAC-an anticancer drug). Due to all these reasons, BA and its compounds remain an exciting research area and attract scientists for its unknown valuable effects.

The purpose of this investigation is to examine whether boric acid is genotoxic alone and also to examine its possible antigenotoxic potential against genotoxic damages induced by MMC (an alkylating agent used as an anticancer drug) and H_2O_2 (a compound inducing genotoxic effect due to ROS and free radical formation). Therefore, in this research, the genotoxic and antigenotoxic potentials of boric acid were evaluated in human lymphocytes *in vitro* using Chromosomal Aberrations (CA), Sister Chromatid Exchange (SCE), Cytokinesis Block Micronucleus Cytome (CBMN-cyt), and Comet tests for the first time.

This research will determine whether boric acid, frequently preferred and widely used in industrial and medical areas today and to which people are increasingly exposed, carries any

genetic risk. Moreover, the possible antigenotoxic potential of boric acid against MMC and H_2O_2 , known to be genotoxic, will also be evaluated for the first time.

2. GENERAL INFORMATION

2.1. Cancer

The word tumor, synonymously with the concept of cancer, is used from the word 'oncos' and includes the concepts of benign and malignant. It is a genetic disease identified when the cells in the body proliferate without control and propagate throughout the body. Tumors are formed by cancerous cells, which are clumps of tissue. As cancer cells develop, there will be additional changes. Different genetic alterations can occur in distinct cells within the same tumor (National Cancer Institute, 2021b). The risk of cancer increases in people whose immune system is suppressed due to factors such as chronic disease, old age, chronic stress, chemotherapy use, analgesics, and corticosteroid-like drugs (Roy and Saikia, 2016).

Malignant tumors invade the tissues by spreading to nearby tissues. They can also spread to distant tissues in the body and cause new tumors. This spreading process is known as metastasis. Benign tumors do not spread or invade nearby tissues and often do not regrow when removed from the site of their origin. They can endanger life by causing severe symptoms in many organs, especially the brain (National Cancer Institute 2021b).

The second important reason for death is cancer, a significant barrier to increasing life expectancy. More than 200 different cancer types and more than 500 cancer-related genes are known to affect the human body (Pavlopoulou et al., 2015; WHO, 2021).

Cancerous cells are generally characterized by continuous division ability, getting rid of growth inhibitory factors, resistance to cell death, activating invasion and metastasis, genomic instability and mutation, escaping from the immune system, and inducing angiogenesis (Hanahan and Weinberg, 2011; Koh et al., 2019).

2.1.1 Causes of cancer

The causes or etiology of cancer is one of the most interesting topics today. There are many factors affecting the formation of tumors. The most thoroughly studied or recognized risk factors are genetic factors, mutation in genes, immune conditions, infectious organisms,

environmental and occupational exposures, chemicals, air pollution, waste factories, ionizing radiation, tobacco use, dietary factors, chronic infection, age and family history (Saini, Kumar et al., 2020).

Genetic factors

Cancer is produced by changes in genes, genetic disorders, and physical units of inheritance carrying information to produce a protein or several proteins. Cancer is a genetic disease induced by gene changes that help cancer cells form, divide, grow, and spread. For example, the p53 tumor suppressor gene mutation is a crucial gene linked to DNA synthesis, gene transcription, DNA repair, and apoptosis (Abbas and Rehman, 2018).

Environmental and occupational exposures

Environmental risks that cause cancer include exhaust from cars, sewage treatment plants, smelters, toxic waste sites, cigarette smoke, and ultraviolet rays from the sun. Exposure to ionizing radiation at high levels, such as cancer radiation therapy, nuclear accidents, or excessive medical imaging (such as X-rays and CT scans), can increase the risk of developing cancer (Saini, Kumar, et al., 2020).

Dietary factors

A poor diet and specific dietary habits can contribute to cancer development. A diet heavy in processed and red meats, poor in fiber, and low in vegetables and fruits can elevate the chance of colorectal cancer. Excessive alcohol intake is also related to a raised risk of various tumors, including colon, liver, breast, and oral cancer. The two main dietary risk factors for cancer are commonly recognized in research as obesity and alcohol consumption (Mariosa et al., 2019).

Chronic infections

Certain persistent infections can raise the risk of certain malignancies. The studies also reveal that specific co-factors, including smoking, high parity, and prior exposure to other

sexually transmitted illnesses, might alter the risk of cervical cancer among human papillomavirus (HPV) DNA-positive women (Abbas and Rehman, 2018).

Age and family history

Cancer risk rises with age as genetic mutations accumulate over time. Once their DNA-repair capability is depleted, the consequent genetic instability in stem cells can be linked to cancer growth in later ages. The types of cancer that occur with age are breast, uterus, prostate, colon, kidney, liver, pancreas, stomach, bone marrow, head, and neck (Ugai, Sasamoto et al., 2022).

2.1.2. Cancer treatment

It is known that many cancer treatments have developed over the centuries and are still developing, and determining the type of treatment after a cancer diagnosis varies depending on the form of cancer stage. While some people recover with only one type of treatment, in some types of cancer, more than one treatment is preferred depending on the stage. Most people diagnosed with cancer have a combined treatment protocol consisting of different types of therapy such as surgery, radiation therapy, immunotherapy, hormone therapy, targeted therapy, and chemotherapy (Saini, Kumar et al., 2020; National Cancer Institute, 2022).

Surgery

It is a procedure that can be used to treat cancer, where the tumor is completely removed from the body. Surgery may be preferred to prevent, diagnose, stage, and treat cancer. The types of cancer treated with surgery are breast, esophageal, colorectal, and brain tumors (Wyld et al., 2015).

Radiation therapy

Radiotherapy is beneficial at high radiation doses and the most active cytotoxic cure available for healing localized cancers (Schau and McBride, 2015). Radiation stimulates a

damage repair period in normal tissues. It is used as part of cancer treatment to help shrink and kill tumor cells, and in some cases, it can be used alone. Radiotherapy causes early side effects during treatment, immediately after treatment, or within a few days of radiotherapy cure (Barazzuol et al., 2020).

Immunotherapy

This treatment method uses substances from living organisms (biological treatment agents). Immunotherapy utilizes the immune system to damage cancer cells and activate white blood cells that attack cancer cells. In this way, the immune system attacks cancer cells and can destroy them. It is used to cure many types of cancer (bladder, cervical, colorectal, sarcoma, and ovarian) and often in combination with other cancer treatments. However, it may also cause some side effects such as fatigue, dizziness, muscle and joint aches, nausea, and vomiting, but most are temporary (Riley et al., 2019).

Hormonal therapy

Hormones are substances or proteins the body produces to coordinate different behaviors among organs, skin, muscles, and other tissues. Some cancers (uterine, pituitary gland tumor, neuroendocrine tumor, thyroid cancer) are known to rely on hormones to grow. Therefore, cures that prevent or change hormones may rarely help decrease or stop these cancers' growth (American Cancer Society, 2022). The goal of hormone therapy is to reduce or stop the growth of malignancies that use hormones as a growth factor (Saini, Kumar et al., 2020).

Targeted therapy

This type of cancer therapy is designed to target proteins that control how tumor cells divide, spread, and grow. As researchers learn about deoxyribonucleic acid (DNA) alterations and proteins that generate cancer, they can better design treatments targeting cancer-causing DNA proteins (National Cancer Institute, 2022). The most essential feature distinguishing them from standard chemotherapy drugs is that some drugs are designed to "target" cancer cells without harming normal cells (American Cancer Society, 2022). Cancers like lymphoma, melanoma, and chronic myeloid leukemia are cured with targeted therapy.

Chemotherapy

Chemotherapy treats tumor cells with anticancer drugs and prevents them from proliferating, invading, metastasizing, and finally reduces the chance of recurrence. This treatment can be applied sequentially or simultaneously with other treatments (radiotherapy and surgery), depending on the stage of the disease (National Cancer Institute, 2022).

Chemotherapy has many side effects, including anemia, loss of appetite, changes in taste, changes in sensitivity to food temperature, constipation, diarrhea, dysphagia, dry mouth, fatigue, and early satiety, which can reduce food intake and lead to excessive weight loss (Schirmacher, 2019).

In the treatment of cancer by chemotherapy, various drugs such as cisplatin, cyclophosphamide, docetaxel, doxorubicin, adriamycin, epirubicin, fluorouracil, methotrexate, paclitaxel (Prahalthan et al. 2006), and mitomycin-C (MMC) are used. Among them, MMC is the most used chemotherapeutic drug.

Mitomycin-C, a medication administered to treat some cancers, is isolated from *Streptomyces caespitosus*. Its molecular weight is 334 daltons. It is effective against various gastrointestinal system cancers, lung, bladder, uterine, neck, and head cancers, and chronic leukemia (Kersey and Vivian, 2008; Zhou et al., 2022). MMC forms DNA crosslinking on the opposite strands of the double helix and subsequently blocks transcription and replication by stopping DNA strand separation (Guo et al., 2017). As an alkylating agent, MMC forms free radicals and causes apoptosis (Abraham et al. 2006). Harm to biological molecules like nucleic acids, proteins, and lipids occurs when reactive oxygen species (ROS) interact with cells and surpass endogenous antioxidant protection systems (Ortega-Gutierrez et al., 2009). If this impairment is not restored correctly, genetic instability may result, raising the possibility of evolving cancer, DNA alterations such as alkaline labile sites, single-strand breaks (SSBs) and double-strand breaks (DSBs), and several types of oxidized purines and pyrimidines (Shay et al. 2009). During the chemotherapy, the normal cells and tumor cells are not differentiated, so side effects also occur in normal cells. Long-term exposure to chemotherapeutic drugs causes transgenerational genomic instability (GIN), leading to pro-oncogenic mutations and increasing the risk for secondary cancers (Zhang, 2020).

So, to minimize cancer risk (Williams, 2005), scientists suggest a dietary pattern emphasizing regular physical exercise, low-fat dairy products, legumes, whole grains, fruits, vegetables, nuts, and seeds. Applying antioxidants to the cells together with DNA-injuring agents can eliminate free radicals before they harm the macromolecules like proteins, lipids, and DNA. Additionally, there is proof that antioxidants enhance the antitumor effects of chemotherapy and cause apoptosis in cancerous cells but not in healthy cells (Drisko et al., 2003).

2.2. Oxidative Stress

The mechanism of removing or neutralizing the antioxidants that prevent the free radicals created in the organism from accumulating in the environment requires a certain equilibrium. An increase in the formation of free radicals and reactive oxygen species and a delay in the response of antioxidants means that the balance in this mechanism is disturbed. This condition is known as oxidative stress. The imbalance in the oxidative stress mechanism means that protein, lipids, carbohydrates, nucleic acids, and beneficial enzymes, which are the building blocks of the organism, deteriorate and cause tissue damage (Ray et al., 2012; Pizzino et al., 2017).

2.3. Free Radicals and Reactive Oxygen Species

Free radicals are chemical species formed by ROS such as hydroxyl, superoxide, peroxy, alkoxy, hydrogen peroxide, and nitrogen species like nitric oxide, nitrogen dioxide, and peroxy nitrile that contain one or more electrons in their outer orbitals that are unpaired. They are generally unstable and show remarkable reactivity, prone to "thieving" electrons from other molecules around them (Di Meo and Venditti, 2020).

In biological systems, oxygen-derived radicals are the most important free radicals. These radicals are not called radicals because they do not have unpaired electrons. However, they can be effective on cells by quickly passing through cell membranes. Therefore, the term "reactive oxygen species (ROS)" is used for reactive and non-radical species that can produce free radicals (Sadiq, 2023). ROS are exceptionally reactive molecules and can harm cell buildings such as nucleic acids, carbohydrates, lipids, and proteins and change their behaviors.

There are many ROS formed in the organism. The most common of these occur in lipid structures. When the lipid radical reacts with molecular oxygen, the process ends with forming the lipid peroxy radical. The initiation of a chain reaction of the lipid peroxy radical with other lipids causes the formation of lipid hydroperoxides. Lipid radicals can also turn into highly toxic products. The most well-known product among these is the formation of malondialdehyde (MDA) from the aldehyde group. Aldehydes like MDA may be damaging due to their mutagenic, protein crosslinking, and carcinogenic features (Sezer and Keskin, 2014).

2.4. Antioxidant Defense Systems of the Cell

In the cell, ROS levels can be determined as an equilibrium between the intensities of ROS production and the level of detoxification by antioxidants. Sustaining homeostatic levels of ROS as an end of this equilibrium is significant both for inhibiting oxidative damage and keeping a suitable redox situation for normal signaling pathways within the cell. Therefore, a vital defense mechanism called the "antioxidant defense system" has developed in the human body to prevent the production of free oxygen radicals. This mechanism neutralizes the endogenous and exogenous damage to the cell and cell structures. The defense mechanism must reduce the local oxygen density to prevent the initiation of lipid peroxidation by scavenging hydroxyl radicals and binding and neutralizing metal ions; it performs its defense in the form of preventing inflammatory cells from attacking the lesion site and accumulating there (Snezhkina et al., 2019).

The defense mechanism can be generated by endogenous and exogenous molecules, either by enzymatic or non-enzymatic molecules. Both of them act to diminish the ROS injury in the cells. Endogenous enzymatic cellular antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). Other enzymatic antioxidants include glutathione-S-transferase and glucose-6-phosphate dehydrogenase. As for the endogenous non-enzymatic antioxidant molecules, they are found extracellularly (in the plasma and blood) and include albumin and uric acid in the plasma and bilirubin, and β -carotene and α -tocopherol (vitamin E) in the blood (Liguori et al., 2018).

Exogenous antioxidants comprise vitamins like (vitamin C or ascorbic acid and vitamin E or α -tocopherol) which clear $O_2\bullet^-$ and $\bullet OH$ radicals and prevent lipid peroxidation in cellular

membranes, respectively. Other exogenous molecules are drugs (acetylcysteine) and phenolic antioxidants such as resveratrol, flavonoids, phenolic acids, zinc, and selenium. Some antioxidants can be found in some vegetables, fruits, dietary boron intakes, and endogenous and exogenous melatonin, which aids in clearing free radicals and upregulating other antioxidant enzymes (Pisoschi et al., 2021).

2.5. Boric Acid [H_3BO_3]

Boron, in group 3A (IIIA) of the periodic table, is shown with the symbol B and has semi-metal properties. Its atomic number is 5, and its weight is 10.81 g/mol (Ediz and Özdağ, 2001). Türkiye is first place by hosting the world's boron reserves (73%) and has reached a certain level in production (Özkul et al., 2017; Selimoğlu, 2018). Boron is found in low concentrations in both human and animal tissues. It is essential for some plants and animals. Because boron is not present in nature in its purest form, it is found in boric acid (H_3BO_3) at 98.4% and borate at 1.6% by binding to oxygen (Henderson et al., 2009). The most prevalent soluble form is boric acid (BA) among boron compounds. BA; a Lewis acid with a density of 1.43 gr/cm³, is a colorless and transparent crystal-shaped white-looking granule that dissolves easily in water (Bhami and Das, 2015; Ghanizadeh et al., 2020).

2.5.1. Boric acid uses

BA is the most frequent soluble form of boron. It is applied in the health, agricultural, industrial, and cosmetic industries (Ince et al., 2012). The first report indicating that B may be beneficial for bone health appeared in 1981. It has an essential place in bone formation (osteogenesis), and its deficiency has been reported to affect bone regeneration and development negatively. In a study, its deprivation caused significant bone anomalies in chicks fed high vitamin D levels (Pizzorno, 2015; Nielsen, 2017). Studies have shown that boron is effective in bone development and preservation. It also increases the osteogenic specialization of human dental germ stem cells (Taşlı et al., 2013).

In a project, 100 osteoporosis patients were fed for six months by adding 6 mg of boron-based nutritional supplement calcium fructoborate to the vegetables, fruits, and margarine they consumed. It was stated that bone density improved in sixty-six of the patients (Scorei and Rotaru, 2011).

B considerably stimulates the rate and quality of vegetable growth, according to research on the nutrition and physiology of plants. Regarding flowering, fruit production, and fruit quality, boron is a crucial element for fruit trees. Recent research has shown that some fruit trees produce more fruit when boron is sprayed on the leaves (Sokmen et al., 2018).

Boron an essential element that is typically found as a non-ionized molecule in soil solution form, making it unusual among the other essential elements (H_3BO_3). It is neither an enzyme component nor is there convincing evidence that it directly affects enzyme activities, but it plays a significant part functions in lignification and RNA metabolism (Krishna Chaitanya et al., 2014). Boron is necessary for plant growth and development because its accessibility to soil and water is crucial in agronomic production (Tanaka and Fujiwara, 2008). Some scientists have noted that roughly 90% of boron exists in the cell walls of plants (Goldbach and Wimmer, 2007). Plants need a continuous boron supply to maintain development and growth (Waqar Ahmad et al., 2009; Çetinkaya et al., 2014). Boron cooperates in cell wall formation and stabilization, pollen germination, tube growth, lignification, and xylem differentiation. It gives drought tolerance to plants. Boron regulates tuber plants' potassium/calcium (K/Ca) ratio and sugar translocation. Promoting protein synthesis and cell division is necessary, which facilitates potassium transport in guard cells. Its deficiency causes brown heads and holly stems in cauliflower, potatoes, sugar beet, etc. (Krishna Chaitanya, 2014).

It was reported that boron increases angiogenesis (the formation of blood vessels), which is critical for wound healing and tissue engineering (Haro Durand et al., 2014). Boron affects the regulation of the inflammatory response (Pizzorno, 2015). A study investigating the relationship between boron and DNA damage showed that boron applications to the human primary epithelial cell line (HS-2) reduced the production of DNA DSBs and accelerated the wound-curing procedure. For this reason, it has been revealed that boric acid has significant therapeutic activity and shows an essential role in oxidative stress and wound-curing progression. It is also thought that it can be used in the dealing of inflammatory diseases and may have a protective effect against DNA damage (Tepedelen et al., 2016).

The most common use (52%) of boron minerals is the glass industry. Borosilicate glass is a significant element of textile glass fiber (Chauhan, Hosmane, and Mozafari, 2019). Detergents and soaps contain boron minerals because of their water-softening, antiseptic,

and bleaching properties. Borates have been applied as flame-retardants in numerous materials in wood, polyvinyl chloride (PVC), cellulosic shields, textiles, etc. Boron represses the burning by shielding the material's surface and disrupting its connection with oxygen (Sokmen and Buyukakinci, 2018).

Boric acid has also been utilized as a pesticide to kill fungi, insects, algae, mites, fleas, termites, and cockroaches (Cox, 2004). BA keeps fresh meats, caviar, and dairy goods (Ministry of Agriculture of Turkey 2004). It can be used as an antimicrobial, antibacterial additive in food at an extreme concentration of 4 g/L and an antiseptic against skin infections (Ommaty, 2000).

2.5.2. Boric acid effects

A nonmetal, an essential plant element, boron has many metabolic and biochemical effects on human and animal health (Başkan et al., 2022; Aydın et al., 2018; Kuru and Yarat, 2017). Each of the oceans and the crust of the earth contains boron (Lemarchand et al. 2000). It can be found in two distinct ways, including trigonal boric acid $B(OH)_3$ and tetrahedral borate anion $B(OH)_4$. In acidic solutions, the trigonal $B(OH)_3$ form is prevalent (Aggarwal, 2017).

Boric acid is the most critical boron compound used for several purposes and in many industrial areas. However, depending on the concentration and duration, it can be harmful (genotoxic) or protective (antigenotoxic) against certain toxic chemicals.

2.5.3. Genotoxic effect

BA and borax both exhibit similar hazardous consequences on living things, as demonstrated by WHO research released in 1998. Borax and BA were investigated in a study, and it was discovered that at physiological pH, borax can be completely transformed into boric acid (USDA Forest Service, 2006). As a result, both borax and boric acid have the same impact on organisms.

Excess boron exposure in humans can result in toxic effects. When exposed to excessive levels of B, symptoms such as vomiting, diarrhea, nausea, and tiredness may occur (Korkmaz et al., 2011). According to animal tests, dietary boron intakes above 100 $\mu\text{g/g}$ can

cause toxicity in mice. Administration of BA (17.5 and 9.6 mg/kg per day) in rats produced aberrant animal development (Scialli et al., 2010). According to the available information, it is improbable for human exposure to boric acid or borax daily to result in signs of reproductive toxicology. However, it was claimed that rats given 400 mg of boric acid per kilogram per day under the skin experienced histological alterations in their kidney tissues (Sabuncuoglu et al., 2006). Additionally, certain damages, such as weight loss and suppression of DNA synthesis at the molecular level, were determined in rats after receiving 1 g/kg borax and BA for three weeks (Dani et al., 1971).

A study conducted in Texas, USA, 2007 showed that boron concentrations in groundwater were inversely correlated with prostate cancer incidence and death risk (Barranco et al., 2007). As a result of BA treatment to the hepatoma cell line (HepG2), DNA damage, increased apoptosis activity, and cell cycle arrest were observed (El-Hefny et al., 2020; Tombuloglu et al., 2020).

Aydın et al., in 2021, investigated the cytotoxic effects of BA on glioblastoma cells. BA was applied in three doses for 48 hours. The cell viability test was established colorimetrically utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. Based on the statistical evaluation conducted after the treatment, the scored percentage of cell viability was 23% for 50 mM, 46% for 25 mM, and 90% for 2.5 mM boron application (Aydın et al., 2021). In addition, it has been observed that high-dose boron administration causes cytotoxic effects on glioblastoma cells.

Arslan et al., (2008) look into the genotoxic impact of BA on the formation of chromosome aberration and sister chromatid exchange. The results obtained stated that BA caused an increase in chromosome aberration, especially in structural abnormality. BA revealed a cytotoxic effect in human lymphocytes because it reduced the mitotic and replication indexes. According to Donbak et al., (2002) and Turkoglu (2006), BA lowered the MI and increased aberrant mitosis in the root tip cells of *Allium cepa*.

Ku and Chapin evaluated the mechanism underlying BA's testicular toxicity and found that in adult rats, BA led to testicular lesions (Ku and Chapin, 1994). It was concluded that BA had a teratogenic impact, resulting in atrophy and germinal cell loss (Parry, 2000).

In a separate study, BA revealed a mitogenic effect when it was employed on HeLa cells in a concentration of 100-500 mg/L. When the dose increased up to 1,000 mg/L or above, the study revealed that BA suppressed the cell's ability to proliferate. The inhibition of cell division and reproduction caused by boron maybe because it affects the signaling pathways involved in cell division (Geyikoglu and Turkez, 2008).

2.5.4. Antigenotoxic effect

It is known that boron is essential for human and animal health. Various cancers, for example, prostate, head, breast, liver, lung, and neck cancer, hepatocellular carcinoma, recurrent cancers, and pediatric cancers, can be treated with substances that are found in natural resources (Ram and Kumari, 2001; Ayhancı et al., 2022; Turkez et al., 2022). Investigations have revealed that boron and its products are potentially valuable substances for treating various cancers. Current *in vitro* and *in vivo* investigations have demonstrated the impact of B and its derivatives on several tumor cells.

According to recent research, boron can act as a protective agent against hazardous chemicals, including chemotherapeutic drugs, heavy metals, nanoparticles, pesticides, etc. In research, BA was used as a protector against the side consequences of a chemotherapeutic agent, paclitaxel (PAC). The outcomes of this investigation showed that BA had no effects on human blood and that it assisted in reducing the genotoxic and cytotoxic effects generated by PAC (Turkez et al., 2010). In another research, boric acid was shown to reduce tumor size as well as prostate-specific antigen (PSA) and Insulin-like growth factor- 1 (IGF-1) levels (Gallardo-Williams et al., 2004).

Üstündag et al., (2020) examined the ameliorating impact of BA against heavy metals (lead and cadmium) genotoxicity using comet assay and micronucleus test in V79 cells. The results revealed that BA strongly decreased MN frequency and, in the comet assay, the DNA damage produced by hydrogen peroxide. Therefore, it was concluded that BA efficiently protected V79 hamster lung fibroblast cells from the damaging effects of Pb and Cd.

Bakir and Agar also investigated the antigenotoxic effect of BA in opposition to vanadium (V), a heavy metal, in *Triticum aestivum L.* The administration of V changed and decreased

the antioxidant activity of enzymes such as SOD, POD, and MDA. These enzymes' activity was elevated by the cotreatment of V and oric acid (Bakir and Agar, 2020).

Recent epidemiological, *in vitro*, and *in vivo* research revealed the effectiveness of boric acid as a chemotherapeutic drug. Because of its unique characteristics, BA has a pharmaceutical effect for cancer treatments. Boric acid, a weak organic Lewis acid, shares identical structural properties to carbon. For this reason, most enzymes containing carbon can act as substrate inhibitors. With this feature, boric acid can suppress many enzymes, such as proteasome, peptidase, protease, arginase, transpeptidase, and nitric oxide synthase. The pharmacological characteristics of boric acid, such as low toxicity, ease of use, and excretion within 24 hours without being metabolized in the urine, make it an attractive candidate for cancer treatment (Das, Shareef, et al., 2022).

The findings of epidemiological research disclosed that BA might have anti-carcinogenic effects. In a study conducted in 2004, dietary boron was found to be effective on prostate cancer (Cui et al., 2004). 3-nitrophenyl boric acid (NPBA) and boric acid were found to inhibit the enzymatic action of PSA. Boric acid inhibits tumor progression and metastasis. The results showed that boron compounds can reduce PSA activity (Gallardo-Williams et al., 2003).

In human skin melanoma cell lines, high concentrations of BA have been reported to have an anti-proliferative effect and induce apoptosis (Acerbo and Miller, 2009). Korkmaz et al., made an investigation and compared women who live in boron rich and poor regions. The finding of this study showed that cytopathological signs of cervical cancer were not detected in 472 women who live in boron-rich areas. In contrast, cervical cancer findings were found in 15 out of 587 women living in boron-poor areas (Korkmaz et al., 2007).

Boron is widely used as a drug carrier in anticancer therapy due to its high biocompatibility, its similarity to the carbon atom in terms of its physical and chemical properties, and its ability to easily pass through the cell pores and enter the cell owing to its small atomic diameter (Mahabir et al., 2008; Henderson et al., 2015).

The best-known example of this therapy is boron neutron capture therapy (BNCT), which has been applied for over 50 years (İdiz et al., 2018). With the BNCT method, which has

been used recently in cancer treatment, a non-radioactive and ^{10}B -bearing boron compound is injected intravenously and selectively taken into the cell by the target tumor cells. The boron compound taken into the cell breaks down after capturing the neutrons from the neutron source, releasing high-energy charged particles. This method is primarily used in dealing with brain cancer since the target is tumor cells directly, and it can be preferred because the damage to healthy cells is minimal (Coderre et al., 2003). Consequently, it was discovered that boron molecules can be used as natural anticancer agents in daily diet, and boron can be presented as a source for potential drugs for cancer treatment. However, additional *in vitro* and *in vivo* research are needed to conceive the mechanisms of action and risk of toxicity for boron to be used appropriately.

Due to all these results obtained from the investigations carried out on the genotoxic and protective effects of boron and boric acid, this study aimed to research whether boric acid has any genotoxic effect and/or if it can provide antigenotoxic protection in human lymphocytes against the chemotherapeutic agent and oxidant chemical using chromosomes aberrations, sister chromatid exchanges, cytokinesis-block micronucleus cytome, and comet tests.

2.6. Tests Used in the Evaluation of Genotoxicity and Antigenotoxicity

Genetic toxicology is a multidisciplinary study investigating how numerous physical and chemical agents induce damage in a cell's genetic material (DNA and RNA) and/or chromosomes and how these damages cause mutations when they are not appropriately repaired on time. Damage in DNA refers to gene mutations, alteration in the chemical structure, breaks in single and double strands, a nucleobase missing from the backbone, or a chemically modified base such as 8-OHdG. DNA damage may change the sequence of base pairs, induce alterations in the structure of genetic materials, and inhibit the replication and transcription of genetic material. As a result, it can not function properly. Damages may also occur in chromosomes. Chromosomal abnormality, or chromosomal aberration, refers to a condition categorized by a structural or numerical change in one or more chromosomes, affecting the sex chromosomes, the autosomes, or both. Most structural aberrations, such as deletions, inversions, translocations, duplications, and ring chromosomes, are produced from the unequal exchange between chromosomes or enzymatic misrepair of two chromosome breakages (Zeiger, 2004; Canedo ve Rocha, 2021). Various toxic chemicals may induce

DNA rearrangements, such as point mutations, chromosomal aberration, genomic instability, and epigenetic modifications (Mamur, 2022: 31,60; Barabadi et al., 2019).

Various chemicals are used in every industrial area, such as agriculture, chemistry, health, and material and military technologies. It is essential to determine whether these chemicals cause mutations in the living genome (Zimmermann et al., 2020). In the 1970s, chemical mutagenesis became a significant concern in studying chromosome and gene activities (Heddle et al., 1983; Zeiger, 2004).

If the damages in the hereditary material remain unrepaired, variations can be considered deviations from the normal condition. Consequently, mutagen-target interaction, diverse types of DNA damage (like DNA adduct, alkaline sensitive regions, chain breaks) may occur, and mutations can be at different levels, from a nucleotide change (gene mutation) to a structural (chromosome mutation) or numerical chromosome change (genome mutation). As a result, the damages in the genetic material can be very diverse. These range from point mutations to breaks in chromosomes. It can be assessed within an extensive range of changes (Aminetzach et al., 2005; Sawyer et al., 2007; Aksu-Kılıç and Doğan, 2020). DNA damage that occurs is repaired by various repair mechanisms in which many proteins work in coordination. Therefore, many molecular repairing mechanisms against DNA damage have developed. The vast majority of DNA damage is repaired by repair systems (Saks et al. 2017; Lord et al. 2006). DNA damage can be removed by repairing, but if the damage is quite extensive and can not be repaired, these cells can be eradicated by apoptosis from the organism (Decordier et al., 2002). Damages that cannot be eliminated either way may cause mutations and can source permanent hereditary changes and undesirable conditions such as tissue damage, aging, infertility, and cancer (Figure 2.1) (Corvi and Madia, 2017; Saks et al., 2017; Mamur, 2022: 31,60).

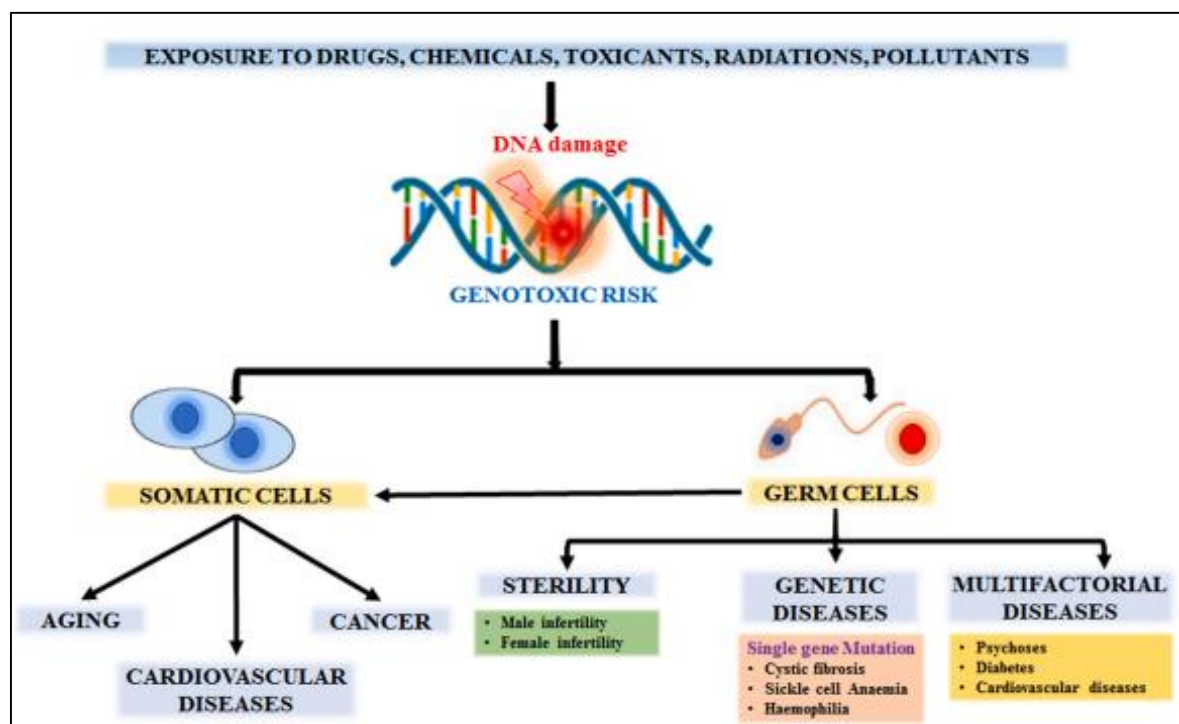


Figure 2.1. Major effects in human health on exposure to genotoxic agents (Choudhuri et al., 2021)

Currently, numerous techniques are employed to evaluate the damage level of various agents on DNA, chromosome, and cellular events. The main ones are chromosomal aberration, sister chromatid exchange, cytokinesis-block micronucleus cytome, and comet tests that are recognized as standard tests by the World Health Organization (WHO) for the evaluation of genotoxicity in humans. The guidelines for these tests have been published by the Organization for Economic Cooperation and Development (OECD). The International Workshops on Genotoxicity Testing (IWGT) bring together researchers in the discipline of genetic toxicology nearly every four years to advance recommendations on genetic toxicology testing procedures and testing strategies (Eastmond et al., 2009; Słoczyńska et al., 2014; Mamur, 2022: 31,60). Since no test can determine all the types of DNA and chromosome damage, a battery of tests is used to determine the genotoxic potential of a chemical or pro-drug. Genetic toxicity assays are used in drug development technologies because they are fast, reasonably inexpensive, and highly predictive for rodent carcinogens (Alnabbat et al., 2022; Mamur, 2022: 31,60).

2.6.1. Chromosomal aberration (CA) test

Numerical and structural changes in chromosomes that occur spontaneously or with the effect of various mutagenic agents are called chromosome aberrations. It is one of the frequently applied techniques to detect chromosomal abnormalities. It has been approved for use in genotoxicity studies and has been included in OECD guidelines as "Test Guideline 473" (Corvi and Madia, 2017). It is applied in two ways: *in vitro* and *in vivo*. While chromosomal abnormalities are identified using mammalian cell cultures in the *in vitro* CA test, bone marrow cells are used in the *in vivo* CA test. In both methods, chromosomes are examined in the metaphase stage of mitosis. A substance called colchicine is used to stop mitosis at metaphase. Colchicine administration is usually applied two hours before the end of the culture in the *in vitro* and two hours before the animals are sacrificed in the *in vivo* CA test (Preston et al., 1981; Russo et al., 2015; Basri Ila and Tahir Hüsün, 2022: 153,184).

Chromosomal abnormalities are generated as the result of damage to DNA. They are seen in two ways: structural and numerical anomalies. Structural chromosome aberrations comprise the dicentric chromosome, chromatid, chromosome break, fragment, sister chromatid union, ring chromosome, translocation, inversion, and isochromosome. Numerical chromosomes are euploidy and aneuploidy. Abnormalities occurring in the chromosomes are one of the critical biological outcomes of exposing living things to ionizing radiation and genotoxic chemicals (Natarajan, 2002; Hollingsworth et al., 2020).

In epidemiological studies, the risk of developing cancer was found to be significantly higher in people with an increased frequency of CAs. Dose-response curves for CAs differ according to the inducer exposed and the cell type analyzed (Aksu-Kılıç and Doğan, 2020).

2.6.2. Sister chromatid exchange (SCE) test

One of the *in vitro* genotoxicity tests is sister chromatid exchange (SCE), which is used to determine the reciprocal interchange of fragments occurring at homologous loci of chromatids of a chromosome (Lee et al., 2016). This technique may easily demonstrate repairs occurring in DNA double-strand breaks by recombination. Mutagenic and carcinogenic substances, viral infections, radiation, various hereditary diseases such as bloom syndrome, and other genotoxic environmental variables increase the frequency of

SCE. An increase in SCE frequency and tumor formation was found to be associated (Çelik, 2022: 185, 217; Atlı Şekeroğlu and Şekeroğlu, 2011). Due to an incomplete understanding of the mechanisms of SCE testing, with the OECD ruling in 2014, it was removed from the genetic toxicology test guides (Test no: 479) (OECD, 2015).

Since the 1970s, the SCE test has been employed to assess the "chromosomal mutagenicity" level of chemical agents (Perry and Evans, 1975). Interstrand crosslinking chemicals, such as MMC, are substantial causes of SCE because homolog recombination (H.R.) is needed to repair the damage caused during duplication (Thompson, 2005). 5-bromo-2'-deoxyuridine (BrdU), which will participate in two replication processes, is added to the culture medium to display DNA breaks. As a result of the first DNA replication in the S phase, one strand of newly formed DNA is normal, while the other strand contains BrdU, which replaces thymine. Then, as a result of the second replication in the medium, sister chromatids differ from each other in terms of BrdU amount. During the second mitosis's metaphase, cell division is stopped by the addition of colchicine. Chromosomes of cells that are kept under fluorescent light and stained with Giemsa are examined under a light microscope. Chromosome regions containing BrdU are seen in light color, while areas without BrdU are seen in dark color. These different staining areas indicate a reciprocal exchange of fragments between sister chromatids (Figure 2.2) (Nazlıgül, 2009; Atlı Şekeroğlu and Şekeroğlu, 2011; Stults et al., 2020; Çelik, 2022: 185,217; Ünal, Yüzbaşıoğlu, and Erikel 2023).

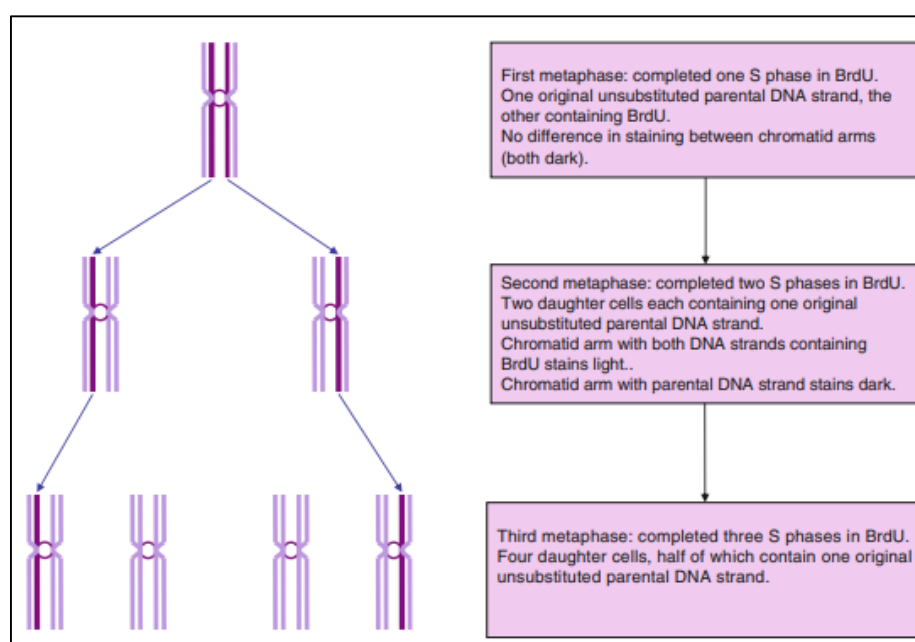


Figure 2.2. Sister chromatid exchange (Clare, 2012)

2.6.3. Cytokinesis-block micronucleus cytome (CBMN-cyt) test

This method is frequently used as one of the preferred tests in genetic toxicology and cytogenetic studies. The test is published as "Test Guideline 487" in OECD guidelines and describes its appropriate use in genotoxicity testing (Cao et al., 2013). Today, the CBMN-cyt method is used to evaluate chromosome loss, chromosome breakage, inhibition of cell division, gene amplification, necrosis, apoptosis, and nucleoplasmic bridges in genotoxicity research (Yüzbaşıoğlu and Avuloğlu-Yılmaz, 2022: 219, 249; Fenech, 2018; Fenech, 2006). CBMN-cyt test is extensively utilized in the detection of the genotoxic and malignant risk of all kinds of biological, chemicals, and physical factors (such as UV and irradiation) that may affect the chromosomes because of its short-term, economical, and easy application test, in researching the toxic effects and safety of drugs before they are put on the market (Ocampo et al., 2013; Yüzbaşıoğlu and Avuloğlu-Yılmaz, 2022: 219, 249).

Micronuclei are small, extranuclear fragments that do not join the newly formed daughter cell nucleus in telophase after anaphase. They may consist of chromatids in the dividing cell or chromatid fragments, acentric chromosomes, or whole chromosomes (Fenech and Morley, 1985; Mateuca et al., 2006; Bonassi and Fenech, 2019). Deficiencies in genes, kinetochore, errors in the mitotic spindle, and chromosomal damage are the leading causes of micronucleus.

In the CBMN test, an actin inhibitor cytochalasin-B (Cyt B) is added to the lymphocytes at the 44th hour of the cell culture. This substance inhibits cytokinesis and forms binucleate (double-nucleated) cells that complete a cell cycle. Various mutagenic agents lead to a rise in micronuclei quantity, which is considered a secondary indicator of structural and numerical chromosome aberrations. In addition, micronucleus increase is considered as genomic instability in somatic cells. The CBMN-cytome assay is a genotoxicity technique that is applied both *in vitro* and *in vivo* to evaluate the aneugenic and clastogenic levels of various physical and chemical substances (Figure 2.3) (Ünal, Yübaşıoğlu, and Avuloğlu-Yılmaz, 2023).

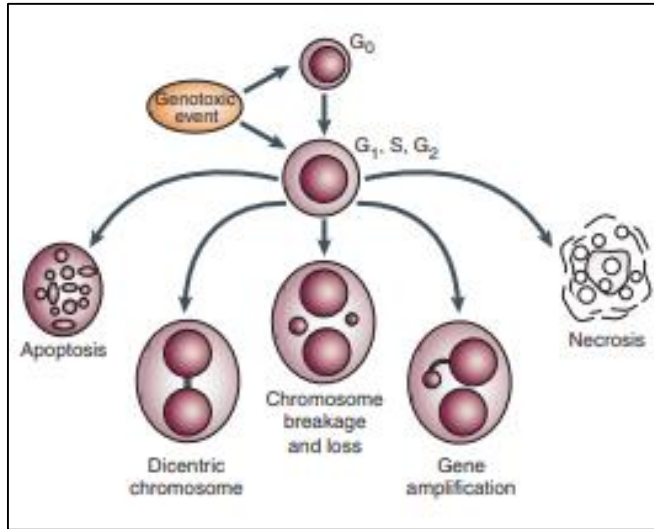


Figure 2.3. Cytokinesis-block micronucleus cytome test (Fenech, 2007)

2.6.4. Comet (Single Cell Gel Electrophoresis-SCGE) test

Comet is a method to determine DNA damage and its repair disorder caused by various agents in many mammalian cells. This test, which relies on the idea of identifying DNA breaks, is a fast, sensitive, simple, and inexpensive method, but also a biomonitoring test used in the determination of damages in DNA caused by many mutagenic substances, especially in humans (Östling and Johanson, 1984; Choy, 2001; Şekeroğlu et al., 2011). Comet assay is one of the genotoxicity tests applied as a valuable and reliable method. Therefore, it was approved by the OECD in 2016 (Test Guideline: 489) (Ünal, Saygılı and Akbaş, 2022: 313, 340).

The advantages of this test are that it is possible to perform using a small number of cells, has a very short working time, is low in cost, can easily applied to any eukaryotic cell that can reproduce or not, and allows visual detection of DNA breaks (Sasaki et al., 2002; Dinçer and Kankaya, 2010; Ünal, Saygılı and Akbaş, 2022: 313, 340; Ünal, Yüzbaşıoğlu and Avuluoğlu-Yılmaz, 2023). This method is used to evaluate the level of damage and degrees in cancer cells and prenatal diagnosis of some hereditary diseases (Şekeroğlu et al., 2011).

This method is based on the idea that DNA molecules with varying molecular weight and electrical charge will move in the electrical field at an alkaline pH differently. According to this method, cells are first placed on agarose in a slightly alkaline pH environment; relaxation and denaturation are performed to open the DNA double helix and then stained with

fluorescent dye ethidium bromide (Møller et al., 2020; Ünal, Saygılı and Akbaş, 2022: 313, 340).

When evaluating DNA damage, three different parameters are taken as a basis: tail length (tail migration) (μm), tail intensity (DNA%), and tail moment. Tail length represents the distance between the last point in the tail and the comet head. The proportion of DNA fragments in the tail refers to tail intensity. The tail moment represents the product of the tail length and the DNA percentage in the tail (Boeck and Geys, 2013; Azqueta, Ladeira, et al., 2020).

This thesis examines boric acid's genotoxic and antigenotoxic potential against MMC and H_2O_2 by using chromosome aberration, sister chromatid exchange, cytokinesis-block micronucleus cytome, and comet tests in human lymphocytes for the first time.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. material for chromosome examination

This study examined BA's genotoxic potential and its antigenotoxic effect against Mitomycin-C (MMC) *in vitro* in human lymphocytes. Peripheral blood was obtained from 3 donors (women) aged 22-30 who had no health problems, did not smoke or consume alcohol, did not use any medication in the last three months, and signed the voluntary consent form. This research was approved by the Gazi University Faculty of Medicine Clinical Research Ethics Committee (Date: 06.03.2023, No: 10).

3.1.2. Test materials and other chemicals used

Boric acid

Boric acid (H_3BO_3) from Eti Maden was obtained from Prof. Dr. H. İbrahim Ünal, Department of Chemistry, Gazi University, Ankara. Cas No is 10043-35-3, its chemical formula is H_3BO_3 , and its molecular weight is 61,83 g/mol. The structural formula of BA is given in Figure 3.1.

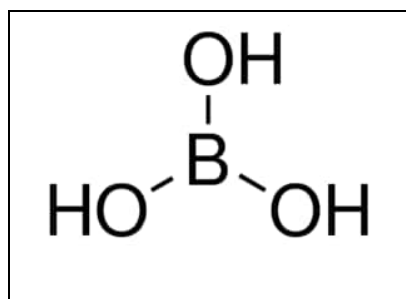


Figure 3.1. The structural formula of Boric acid (Eti Maden)

Mitomycin-C (MMC)

MMC isolated from *Streptomyces caespitosus* is an alkylating agent with chemotherapeutic action. It was commercially obtained from Sigma. Mitomycin-C: 6-amino-4,7-dioxo-8-

methoxy-5-methyl-1,1,2,8,8,8-hexahydroazirin [2,3] pyrrole [1,2] indole-8-methylcarbamate. The chemical formula is $C_{15}H_{18}N_4O_5$, Cas No is 50-07-7, and the molecular weight is 334.33 g/mol. The structural formula is shown in Figure 3.2.

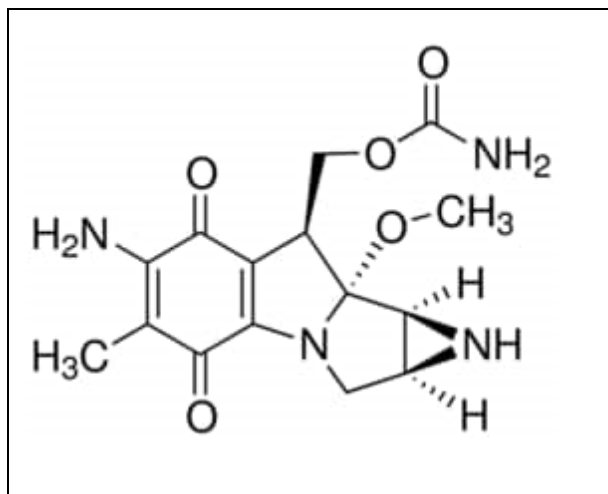


Figure 3.2. The structural formula of Mitomycin-C (Sigma-Aldrich)

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (35%), used in this study, is a compound capable of oxidizing all organic and inorganic compounds and causing severe damage, especially in DNA. Its structural formula is given in Figure 3.3. Its molecular formula is H_2O_2 , and its molecular weight is 34.01 g/mol. Cas No: 7722-84-1. It was commercially obtained from Applichem.

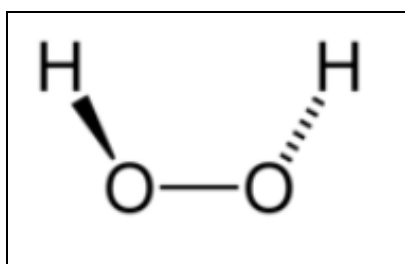


Figure 3.3. The structural formula of hydrogen peroxide (Sigma-Aldrich)

Other chemicals used in the study

Chromosome medium LymphoPlus (CAS No: CY100-100) was obtained from Cegrogen Biotech, Methyl alcohol (Cas No: 67-56-1), Glacial acetic acid (CAS No: 64-19-7), Giemsa (CAS No: 1092040500), Entellan (CAS No: 1079610100), KCl (CAS No: 7447-40-7),

Formaldehyde (CAS No: 50-00-0), and Buffer tablets pH 6.8 (CAS No: 111374) were purchased from Merck, Bromodeoxyuridine (CAS No: 59-14-3), Colchicine (CAS No: 64-86-8), and Cytochalasin-B (CAS No: 14930-96-2) were commercially obtained from Sigma. All the other chemicals were analytical grade.

3.2. Methods

In the present research, Chromosomal Aberration, Sister Chromatid Exchange, and Cytokinesis-Block Micronucleus Cytome (CBMN-Cyt) tests were carried out in human lymphocytes *in vitro* and comet test in isolated lymphocytes *in vitro*.

3.2.1. Determination of the concentrations used in the study

The lethal doses of BA obtained from the literature searches to determine the concentrations to be used are given in Table 3.1.

Table 3.1. LD₅₀ doses of boric acid from the literature searches

Chemicals	Concentrations	Cell types	References
Boric acid (H ₃ BO ₃)	2.5, 5, and 10 µM	Human blood (lymphocytes)	Turkez (2008)
	0.25, 0.5, 1, 2, 5, 10 and 20 ppm	Human blood (lymphocytes)	Turkez and Geyikoğlu (2010)

A preliminary test was applied using various concentrations (2.5, 5, 10, and 20 µg/mL) to determine the effect of BA on the mitotic index. Depending on the results obtained, the concentrations to be used were determined as 0.25, 0.5, 1, and 2.5 µg/mL.

3.2.2. Application of test materials to human lymphocytes

Chromosomal aberration and sister chromatid exchange tests

The chromosome aberrations test was applied following Evans et al.'s (1984) technique with a few changes (Yüzbaşıoğlu, Çelik, Yılmaz, Ünal and Aksoy, 2006, Akbaş et al., 2022). Perry and Wolf's (1974) method with some modifications (Speit and Haupter, 1985; Yüzbaşıoğlu, Çelik, Yılmaz, Ünal and Aksoy, 2006; Akbaş et al., 2022) was implemented for sister chromatid exchange test. 2.5 mL of LymphoPlus medium was added to culture tubes along with 0.2 mL of peripheral blood collected from volunteers using a heparin syringe. The same culture was given 50 µg/mL of 5-Bromo-2-deoxyuridine=Bromodeoxyuridine (BrdU) and kept at 37°C for 72 h.

Human cells were treated with various doses of boric acid (BA) (0.25, 0.5, 1, and 2.5 µg/mL) both alone and concurrently with 0.20 µg/mL MMC (BA+MMC), a mutagen. They were added to each tube separately during the last 24 and/or 48 hours of culture. Besides, a negative (distilled water) and a positive (0.20 µg/mL MMC) control was also run. Colchicine was added at a concentration of 0.06 µg/mL at the 70th hour of incubation. At the 72nd hour of incubation, the cultures were terminated. Cell cultures were centrifugated at 1,100 rpm for 10 min, then 5 mL of 0.075 M KCl solution was added to the bottom. Cultures were centrifuged at 1,100 rpm for 10 minutes and then incubated at 37°C for 20 min. The lymphocytes were subsequently fixed three times in 3:1 methanol: acetic acid. The culture cells were homogenized, and the supernatant was discarded and then spread on slides that were left to dry at room temperature for one day (Basri İla and Tahir Hüsün, 2022: 153,184; Çelik, 2022: 185,217).

Cytokinesis-block micronucleus cytome assay

Micronucleus test of Fenech (2000) was performed with few modifications (Kirsch-Volders et al., 2011). Peripheral blood specimens (0.2 mL) obtained from donors were added to the culture tubes containing 2.5 mL LymphoPlus. Cultures were incubated for 72 h at 37°C. At 24 hours of culture, lymphocytes were treated with various doses of boric acid (BA) (0.25, 0.5, 1, and 2.5 µg/mL) both alone and simultaneously with 0.20 µg/mL MMC (BA+MMC). Treatments were applied for the last 48 hours. To stop cytokinesis, cytochalasin-B (5.2

$\mu\text{g/mL}$) was supplemented at the 44th hour of incubation. A negative and positive (MMC-0.20 $\mu\text{g/mL}$) control was also run in each Application. The tubes were centrifuged (1,000 rpm), and the supernatant was discarded. Then, a hypotonic solution (0.075 M KCl) was added. Following centrifugation, the cells underwent three rounds of fixation in a cold fixative. Formaldehyde was added to the final fixation process. The cell suspension remaining in the tube was spread on the slide and left to dry at ambient temperature (Yüzbaşıoğlu and Avuloğlu-Yılmaz, 2022: 219,249).

Staining of slides

The slides were stained for (5-10 minutes) in 5% Giemsa (pH 6.8) in Sorensen buffer to determine CA, SCE, and MI. To evaluate micronucleus, slides were stained for 1-3 minutes.

For observing sister chromatid exchanges, the test developed by Speit and Haupter in 1985 was applied with some modifications. In this Application, covered slides in Sorensen buffer (pH 6.8) were irradiated for 13 minutes at 254 nm and kept in 1xSSC (saline sodium citrate buffer) solution at 58-60°C for 1 h. Subsequently, the slides were stained for five to ten minutes with 5% Giemsa (pH 6.8). All the slides were made permanent with entellan and then examined microscopically.

Detection of chromosome aberrations and mitotic index

Cells with well-distributed chromosomes were evaluated for each application and control group to detect chromosomal abnormalities. In total, 300 metaphases (100 from each donor) were evaluated. All treatment and control groups were identified based on the number of chromosome aberrations per cell and the percentage of abnormal cells. Three thousand cells (1,000 cells per donor) from each application and control group were analyzed to determine the mitotic index. For the mitotic index, the percentage of dividing cells by the total number of cells was used (Basri İla and Tahir Hüsün, 2022: 153,184; Çelik, 2022: 185,217).

Detection of sister chromatid exchange and replication index

Seventy-five cells from three donors (25 cells from each) were examined to assess the number of sister chromatid exchanges per cell. In this evaluation, cells undergoing the

second mitosis were scanned. To observe SCE, BrdU was added previously to the culture medium. SCEs were determined by counting the dark-stained segments in the light-stained chromatid of a chromosome or vice versa.

Replication index (RI) was determined from 300 cells/3 donors (100 cells/donor) from each slide. Among these, the first (M₁), second (M₂), and third (M₃) mitotic stages were determined, and the RI was obtained using the following formula (Çelik, 2022: 185,217).

$$RI = [1 \times (M_1) + 2 \times (M_2) + 3 \times (M_3)] / N$$

M₁: Number of cells in the first metaphase

M₂: Number of cells in the second metaphase

M₃: Number of cells in the third metaphase

N: Total number of cells examined

Evaluation of micronucleus frequency, nuclear division index, and cytokinesis block proliferation index

Binucleate cells that completed the nuclear division but could not complete the cytoplasmic division were assessed to determine the micronucleus frequency. One thousand binucleate cells per donor (3,000 binucleate/3 donors) were scored for this. The number of MN per cell (MN/cell) was also determined for each Application. The following formulas were used to calculate the nuclear division index (NDI) and CPBI from a total of 1,500 cells (500 cells/donor) for each treatment (Yüzbaşıoğlu and Avuloğlu-Yılmaz, 2022: 219, 249).

$$NDI = [(1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4)] / N$$

N₁-N₄ reveals the number of cells with 1-4 nuclei, respectively.

$$CBPI = [1 \times (1MN) + 2 \times (2MN) + 3 \times (3MN + 4MN)] / N$$

N: Total number of cells examined

Under an oil immersion lens, x1,000 magnifications were performed for microscopic examination of CAs and SCEs. 40x magnification was used to score 1,000 binucleated cells for each subject. An Olympus light microscope (CX23) was used to score the slides.

To obtain the images of chromosome aberrations and sister chromatid exchange, a Leica-DM-LB2 microscope attached with a CCD camera was used at x1000 magnification. For MN analyses, x400 magnification was used.

3.2.3. Studies in isolated human lymphocytes

Comet assay

To evaluate the DNA damaging effect of a single treatment of BA and the possible protective effect of BA against H_2O_2 (BA+ H_2O_2), an oxidant was also investigated using comet assay.

Some modifications of Singh et al.'s (1988) procedure were applied to the comet technique. The lymphocytes were separated from blood samples obtained from healthy subjects using lymphocyte separation media. The cell viability of isolated lymphocytes was defined as $\geq 97\%$ using the trypan blue exclusion test. They were treated with either four single doses of boric acid (0.25, 0.5, 1, and 2.5 $\mu\text{g/mL}$) or concurrently with BA and 100 μM H_2O_2 (BA+ H_2O_2) at 37°C for one hour. Control groups were also run in both treatments, which were negative (distilled water) and positive (100 μM H_2O_2). The supernatant was collected, followed by centrifugation, and then re-suspended with PBS. Lymphocytes were combined with low-melting-temperature agar and applied to slides previously covered with high-melting-temperature agar. Slides were dipped in the lysing solution for 1 hour in the refrigerator. After 20 min in the electrophoresis buffer, electrophoresis was performed on the slides for 20 min at 25 V, 300 mA. Slides immersed in neutralization buffer following electrophoresis were stained with EtBr and examined (Ünal, Saygılı and Akbaş, 2022: 313, 340).

For scoring comets at x400 magnification, the fluorescent microscope (Olympus BX51) (with a filter of 546 nm and a barrier filter of 590 nm) was employed. Two slides were prepared for each treatment and each donor. To determine the DNA damage, a total of 300 nucleoids per treatment (100 comets for each donor; 50 comets from each slide) were evaluated in terms of tail length (μm), tail intensity (%), and tail moment by a specialized Image Analysis System (Comet Assay IV, Perceptive Instruments Ltd., UK). The same microscope attached to a CCD camera obtained comet images at x400 magnification.

3.2.4. Statistical analyses

In this thesis, IBM SPSS 23.0 Statistics One-Way ANOVA (post-Tukey) was utilized to reveal the dose-effect relationship for abnormal cell frequency, CA/Cell, mitotic index,

SCE/Cell, MN/Cell, nuclear bud, nucleoplasmic bridge (NPB), tail length, tail intensity, and tail moment. The replication index, cytokinesis block proliferation index, and nuclear division index results were evaluated by z-test.

4. RESEARCH FINDINGS

4.1. Determination of the Genotoxic and Antigenotoxic Effects of Boric Acid Against Mitomycin-C-Induced Genetic Damage

4.1.1. Chromosome aberration test results

The types of chromosome aberrations, abnormal cell frequencies, and chromosome aberrations per cell determined as a result of the treatment with Boric acid alone and simultaneous treatment with BA + Mitomycin-C (0.20 $\mu\text{g/mL}$) in human lymphocytes for 24 and 48 hours are given in Table 4.1.

24-hours treatment

The abnormal cell and CA/Cell frequency remarkably increased in MMC-treated lymphocytes over negative control (Table 4.1). However, a non-significant increase in the frequency of abnormal cells and CA/cell occurred in applying boric acid alone. In contrast, simultaneous treatment of boric acid with MMC markedly reduced the frequency of aberrant cells and CA/Cell (except 2.5 BA+MMC) against positive control. These reductions were slightly concentration-dependent, $r=-0.46$ and $r=-0.49$, respectively (Table 4.1, Figure 4.1 and Figure 4.2).

MMC caused five different structural aberrations. These are chromatid and chromosome break, fragment, dicentric chromosome, and sister chromatid union. It also formed one type of numerical aberration (polyploidy). Chromatid break was the most prevalent type of structural aberration. A single application of boric acid induced seven types of structural abnormalities. Chromatid break was the most observed type of structural aberration, followed by fragment, chromosome break, sister chromatid union, dicentric chromosome, chromatid exchange (only at 1 $\mu\text{g/mL}$), and ring chromosome (only at 0.25 $\mu\text{g/mL}$) (Table 4.1). Polyploidy, as a numerical aberration, was also found in all the concentrations of BA (Table 4.1). In the co-treatment of BA+MMC, six different types of structural abnormalities were observed. These abnormality types are chromatid and chromosome break, dicentric chromosome, fragment, chromatid exchange, and sister chromatid union (only in 0.25 BA+MMC) (Figure 4.1). While polyploidy was found in all the concentrations applied,

endoreduplication was only present in 0.5 BA+MMC. In the negative control, chromatid and chromosome break, fragment, and sister chromatid union were observed in a pretty small number (Tables 4.1, Figure 4.1, and Figure 4.2).

Table 4.1. Effect of Boric acid (BA) and BA+MMC applications on the frequency of chromosome aberrations.

Test substance	Treatment		Aberrations									Abnormal cell $\pm$ SE (%)	CA/Cell $\pm$ SE
	Time (h)	Conc. (μ g/mL)	ctb	csb	f	dc	scu	cte	r	p	e		
NC	24	0.00	6	1	1	-	1	-	-	-	-	3.00 $\pm$ 0.58	3.00 $\pm$ 0.58
PC	24	0.20	28	6	5	2	5	-	-	3	-	15.00 $\pm$ 1.00 ^a	16.33 $\pm$ 1.76 ^a
BA	24	0.25	11	1	5	1	2	-	1	2	-	6.33 $\pm$ 1.45	7.67 $\pm$ 2.33
		0.5	7	1	3	-	5	-	-	2	-	5.33 $\pm$ 0.88	6.00 $\pm$ 1.15
		1	11	2	-	-	1	1	-	7	-	7.33 $\pm$ 1.20	7.33 $\pm$ 1.20
		2.5	12	2	2	-	2	-	-	3	-	6.67 $\pm$ 0.67	7.00 $\pm$ 0.58
BA + MMC	24	0.25	12	3	1	1	-	1	-	3	-	6.67 $\pm$ 1.86*	7.00 $\pm$ 2.00*
		0.5	3	1	4	1	3	-	-	5	1	6.00 $\pm$ 0.58*	6.00 $\pm$ 0.58*
		1	16	3	2	1	1	-	-	1	-	8.33 $\pm$ 0.88*	7.33 $\pm$ 1.45*
		2.5	18	3	1	-	2	-	-	1	-	8.00 $\pm$ 2.08*	8.33 $\pm$ 2.40
NC	48	0.00	5	2	1	-	-	-	-	3	-	3.67 $\pm$ 0.33	3.67 $\pm$ 0.33
PC	48	0.20	28	8	7	2	2	2	1	2	-	16.33 $\pm$ 1.45 ^a	17.33 $\pm$ 2.03 ^a
BA	48	0.25	11	1	1	-	5	-	-	1	-	5.33 $\pm$ 0.33	6.33 $\pm$ 0.88
		0.5	9	1	1	1	1	-	-	3	-	5.33 $\pm$ 0.88	5.33 $\pm$ 0.88
		1	7	5	2	1	3	-	-	1	-	5.67 $\pm$ 0.33	6.33 $\pm$ 0.88
		2.5	13	2	1	-	3	-	-	-	-	6.33 $\pm$ 1.20	6.33 $\pm$ 1.20
BA + MMC	48	0.25	8	2	1	-	1	-	-	1	-	4.33 $\pm$ 0.88*	4.33 $\pm$ 0.88*
		0.5	8	4	1	-	2	-	-	1	-	5.33 $\pm$ 0.33*	5.33 $\pm$ 0.33*
		1	12	3	4	-	3	-	1	-	-	7.00 $\pm$ 1.15*	7.67 $\pm$ 0.67*
		2.5	14	5	3	-	2	-	-	1	-	7.67 $\pm$ 0.33*	8.33 $\pm$ 0.88*

NC: Negative Control, PC: Positive Control-Mitomycin-C, Conc.: Concentration, SE: Standard Error, ctb: chromatid break, csb: chromosome break, f: fragment, dc: dicentric chromosome, scu: sister chromatid union, cte: chromatid exchange, r: ring chromosome, p: polyploidy, e: endoreduplication

^aSignificant compared to negative control $p < 0.05$ (One-Way ANOVA test)

*Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

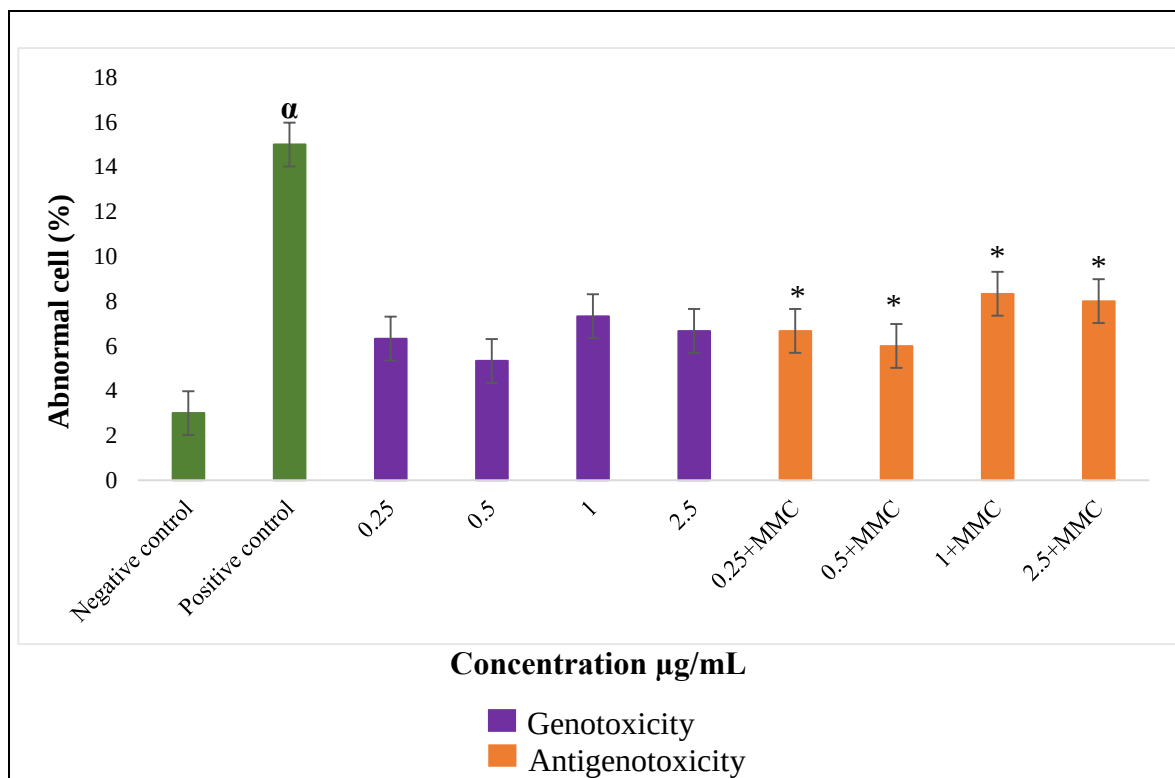


Figure 4.1. Abnormal cell frequency in lymphocytes after 24 h treatments of Boric acid and Boric acid+MMC. ^aSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

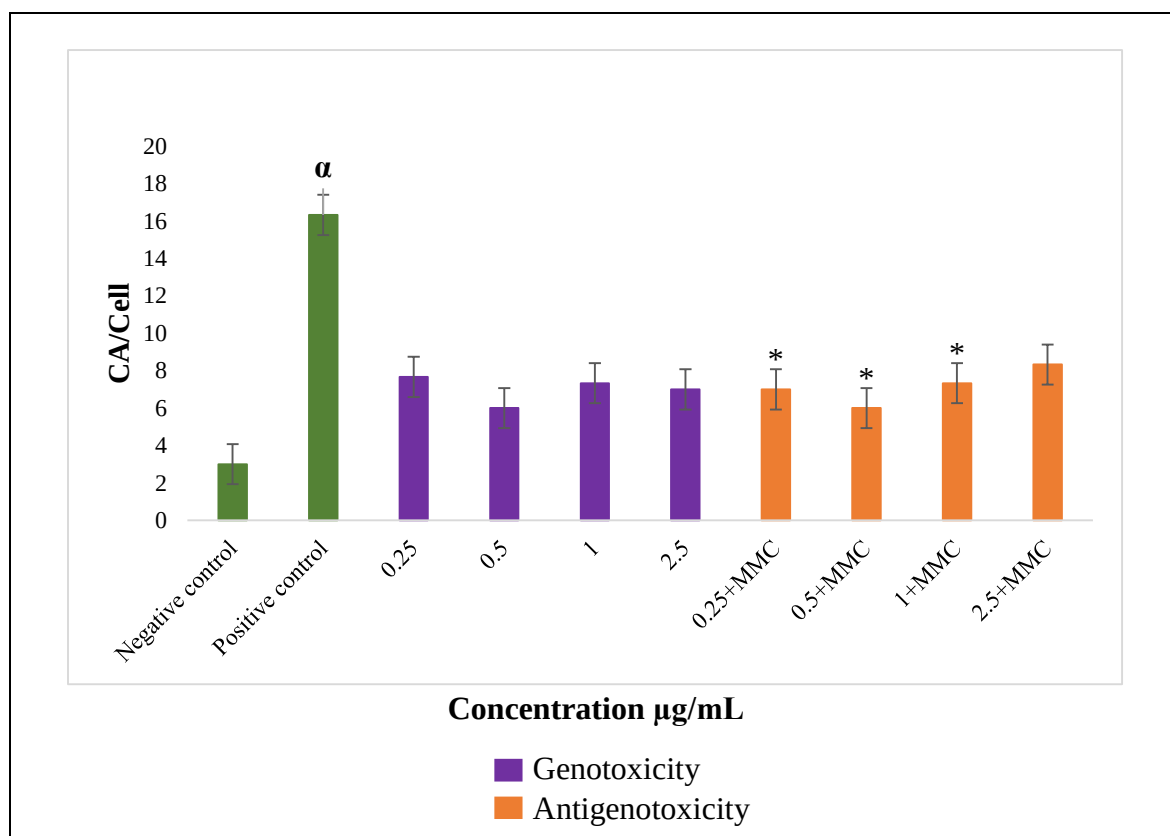


Figure 4.3. Number of abnormalities per cell in lymphocytes after 24 h treatments of Boric acid and Boric acid+MMC. ^αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

48-hours treatment

MMC significantly elevated the frequency of abnormal cells and the number of abnormalities per cell over the negative control. On the other hand, boric acid alone slightly increased abnormal cell frequency and the CA/Cell in all the applications, but none were significant. Interestingly, all the concentrations of BA in the simultaneous treatment with MMC significantly reduced aberrant cell frequency and CA/Cell compared to positive control. Decreasing was marginally concentration-dependent, $r = -0.46$ and $r = -0.43$, respectively (Tables 4.1, Figure 4.3 and Figure 4.4).

Mitomycin-C induced seven different kinds of structural aberrations: chromatid and chromosome break, sister chromatid union, dicentric chromosome, fragment, chromatid exchange and ring chromosome, and one type of numerical aberration (polyploidy) (Table 4.1). Single treatment of boric acid caused five kinds of structural abnormalities: chromatid and chromosome break, fragment, sister chromatid union, and dicentric chromosome, while

polyploidy, which is a numerical abnormality, was found in every concentration except for 2.5 $\mu\text{g/mL}$ (Tables 4.1, Figure 4.3 and Figure 4.4). While five kinds of structural aberrations were observed in the simultaneous administration of boric acid with MMC, chromatid break was the most frequent aberration, followed by chromosome break, fragment, sister chromatid union, and the ring chromosome (only in 1 BA+MMC). The numerical abnormality, polyploidy, was found in all the concentrations except 1 BA+MMC (Table 4.1, Figure 4.3, and Figure 4.4). In the negative control, chromatid and chromosome break, fragment, and polyploidy were observed in quite small numbers (Tables 4.1, Figure 4.3, and Figure 4.4).

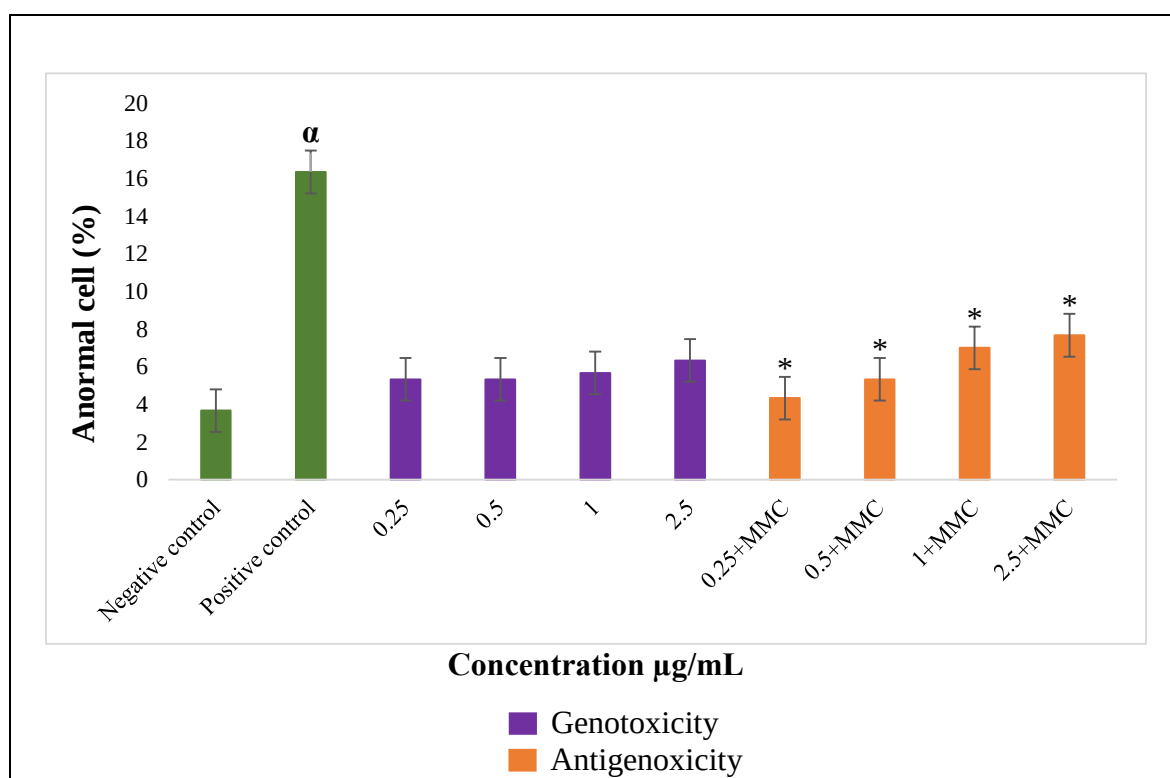


Figure 4.3. Abnormal cell frequency in lymphocytes after 48 h treatments of Boric acid and Boric acid+MMC. ^aSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

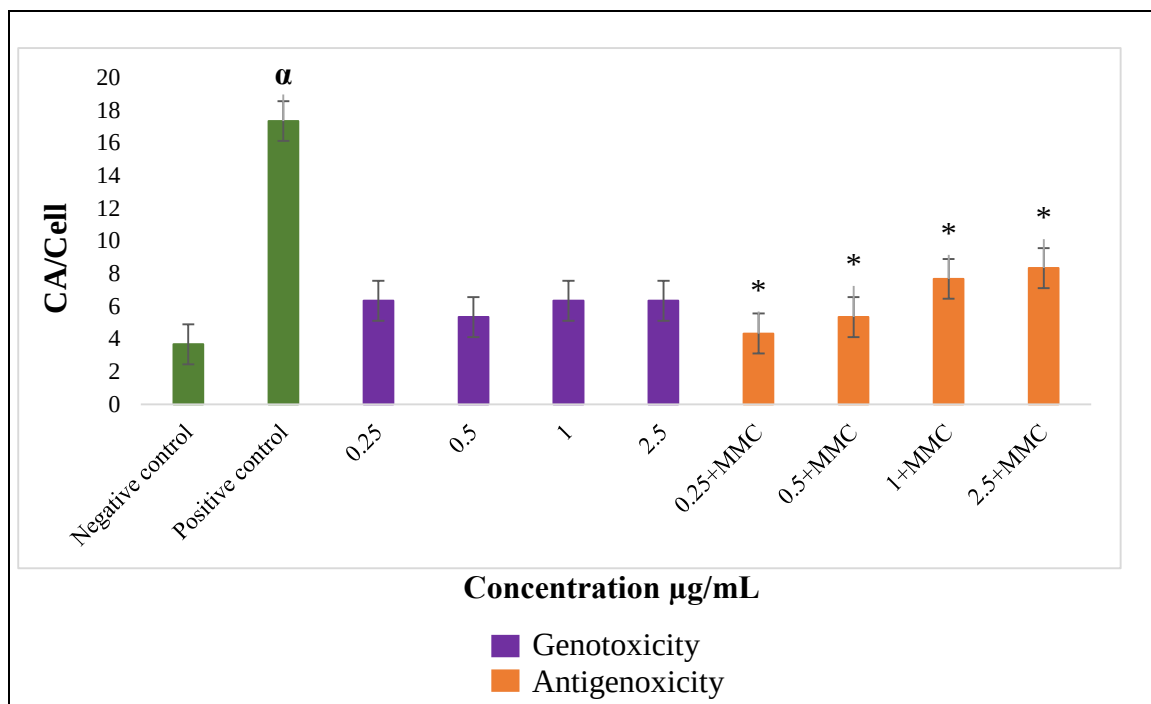
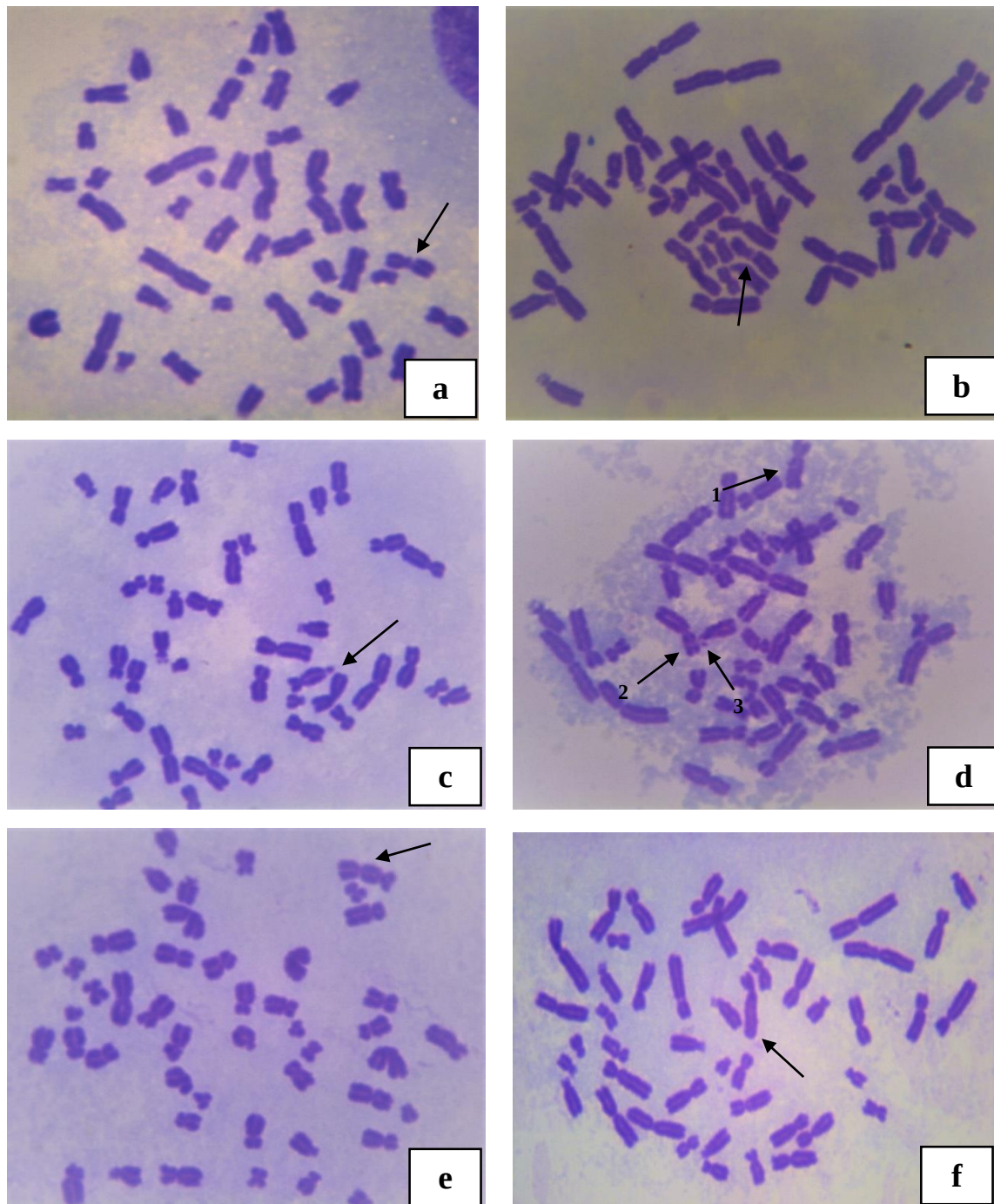
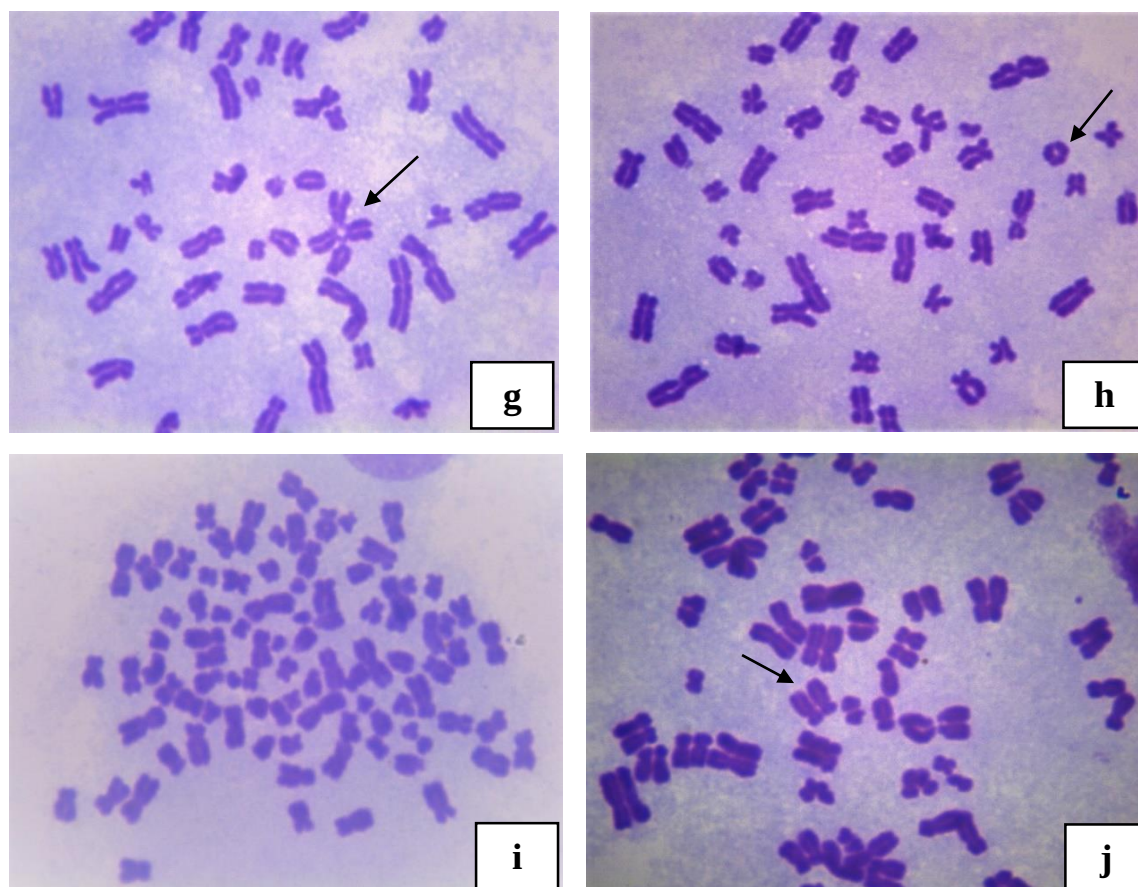


Figure 4.4. Number of abnormalities per cell in lymphocytes after 48 h treatments of Boric acid and Boric acid+MMC. α Significant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)



Picture 4.1. Micrographs from the chromosome aberration test following boric acid and BA+MMC administration. a) chromatid break, b) chromosome break, c) fragment, d) 1-chromatid break, 2-chromosome break and 3-fragment, e) dicentric chromosome, f) sister chromatid union, g) chromatid exchange, h) ring chromosome, i) polyploidy, j) endoreduplication



Picture 4.1. (Continue) Micrographs from the chromosome aberration test following boric acid and BA+MMC administrations. a) chromatid break, b) chromosome break, c) fragment, d) 1-chromatid break, 2-chromosome break and 3-fragment, e) dicentric chromosome, f) sister chromatid union, g) chromatid exchange, h) ring chromosome, i) polyploidy, j) endoreduplication

4.1.2. Sister chromatid exchange test results

The impact of boric acid on SCE, MI, and RI was investigated following the application of boric acid alone and simultaneously with MMC for 24 h and 48 h in human lymphocytes. The data obtained are shown in Table 4.2.

24-hours treatment

Following 24 hours of applications, MMC significantly raised the frequency of SCEs per cell over the negative control (Table 4.2). MMC induced the formation of 2-18 sister chromatid exchanges per cell. However, while a single application of boric acid produced 1-11 SCEs/Cell in human lymphocytes and slightly augmented SCE/Cell, none of the increase was statistically remarkable compared to the negative control (Tables 4.2 and Figure 4.5). In

contrast, in the co-treatment of boric acid and MMC, a statistically significant diminution occurred in SCE/Cell in every concentration applied compared to positive control. The most effective concentration in reducing SCE/Cell was 0.25 µg/mL BA against MMC (Table 4.2 and Figure 4.5). However, the reductions observed were not concentration-dependent ($r=-0.32$). Up to 14 SCEs/Cell were determined in some lymphocytes following BA+MMC treatments. Neither negative nor positive control induced significant variation in human lymphocytes in the replication index. While boric acid alone slightly increased the replication index compared to the negative control, simultaneous treatment of boric acid with MMC caused minimal changes compared to both the negative and positive controls. However, these variations were not statistically significant (Table 4.2).

In terms of mitotic index (MI), while MMC induced a significant decrease compared to negative control, single treatment of different doses of boric acid did not make a significant change, although there was a slight increase. On the other hand, in BA+MMC applications, the mitotic index augmented slightly but insignificantly at all the concentrations applied compared to the positive control (Table 4.2 and Figure 4.6).

Table 4.2. Effect of Boric acid (BA) and BA+MMC applications on the frequency of SCE/cell, RI, and MI in lymphocytes.

Test substance	Treatment		Min- maks SCE	SCE/Cell ± SE	M ₁	M ₂	M ₃	RI ± SE	MI ± SE
	Time (h)	Conc. (µg/mL)							
NC	24	0.00	0-9	3.53±0.19	56	75	169	2.38±0.88	10.27±0.12
PC	24	0.20	2-18	10.24±0.42 ^a	47	86	167	2.40±0.88	6.60±1.06 ^a
BA	24	0.25	1-10	3.69±0.24	45	79	176	2.44±0.89	9.5±0.23
		0.5	1-7	4.24±0.22	50	76	174	2.41±0.88	9.37±0.91
		1	1-11	4.30±0.29	41	79	180	2.46±0.89	9.27±0.03
		2.5	2-11	4.36±0.24	53	78	169	2.39±0.88	7.23±1.17
BA + MMC	24	0.25	0-11	5.53±0.29*	37	81	182	2.48±0.90	8.03±0.69
		0.5	1-13	5.76±0.31*	46	80	174	2.43±0.88	8.33±0.24
		1	1-13	5.88±0.32*	54	75	171	2.39±0.88	8.57±0.47
		2.5	1-14	6.27±0.33*	49	86	165	2.39±0.88	6.97±1.03
NC	48	0.00	1-10	3.47±0.21	57	78	165	2.36±0.88	13.27±1.52
PC	48	0.20	4-31	13.03±0.72 ^a	59	83	158	2.33±0.87	8.00±0.45 ^a
BA	48	0.25	0-17	3.88±0.33	54	75	171	2.39±0.88	10.20±1.17
		0.5	1-7	4.15±0.20	67	78	155	2.29±0.86	9.27±0.57
		1	0-12	4.23±0.31	45	80	175	2.43±0.89	9.17±0.62
		2.5	1-12	5.01±0.28 ^a	47	83	170	2.46±0.89	9.43±0.92
BA + MMC	48	0.25	0-17	6.49±0.43*	44	79	177	2.44±0.89	8.53±0.38
		0.5	2-16	7.11±0.42*	39	88	173	2.45±0.89	8.57±0.12
		1	1-16	7.64±0.44*	48	83	169	2.40±0.88	8.27±0.89
		2.5	1-20	8.27±0.35*	44	90	166	2.41±0.88	8.07±0.33

NC: Negative Control, PC: Positive Control-Mitomycin-C, Conc.: Concentration, SE: standard Error, SCE: sister chromatid exchange, M1: mitosis 1, M2: mitosis 2, M3: mitosis 3, RI: replication index, MI: mitotic index

^aSignificant compared to negative control $p < 0.05$ (One-Way ANOVA test)

*Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

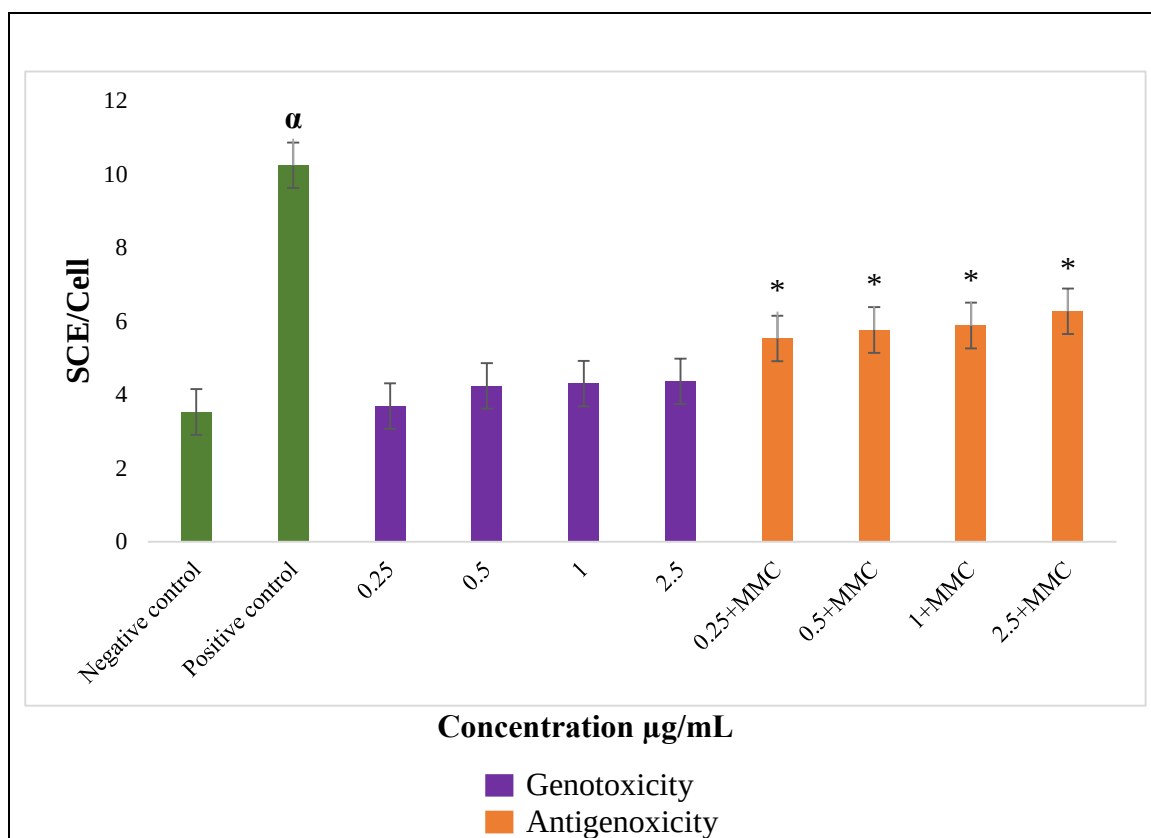


Figure 4.5. Sister chromatid exchange/Cell in lymphocytes after 24 h treatments of Boric acid and BA+MMC. ^αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

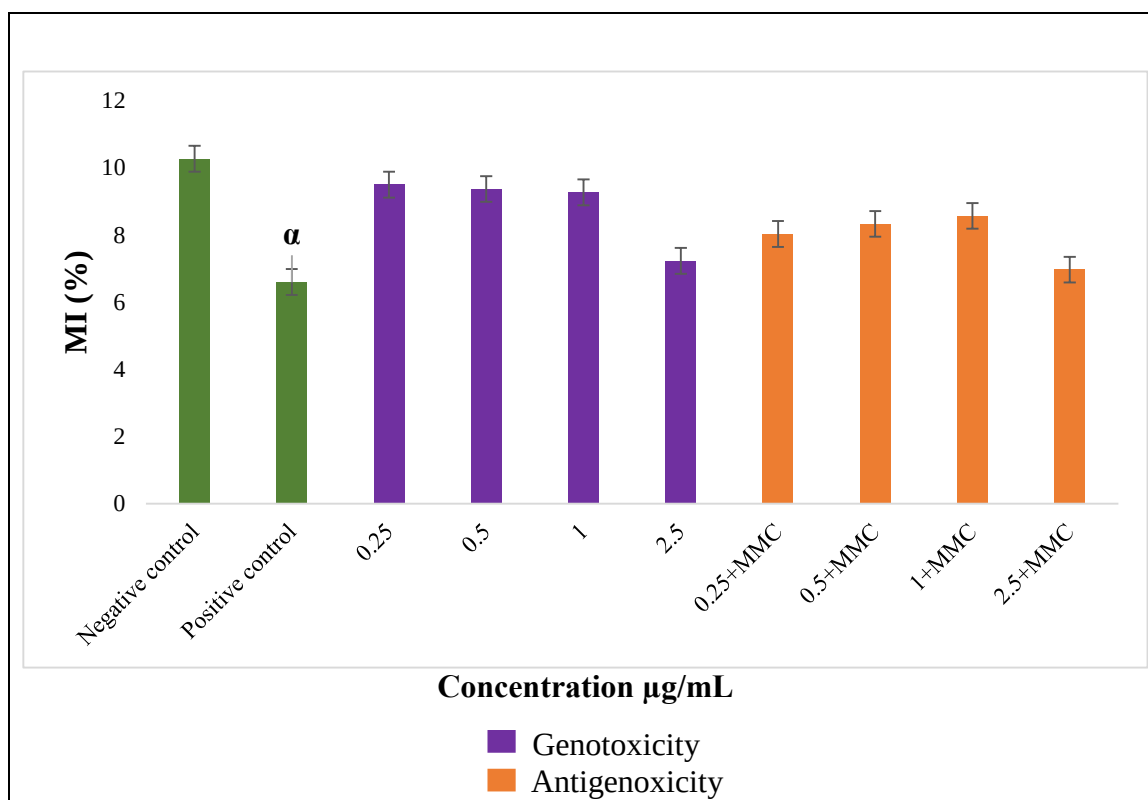


Figure 4.6. Mitotic index in lymphocytes after 24 h of treatments of Boric acid and BA+MMC. ^αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

48-hours treatment

MMC significantly elevated the frequency of SCEs per cell in lymphocytes compared to the negative control (Table 4.2). MMC caused the formation of 4-31 SCEs per cell. On the other hand, between 0-17 SCE formation was observed following the single boric acid treatment. While the frequency of SCE per cell generated by boric acid slightly increased at 0.25, 0.5, and 1 $\mu\text{g/mL}$, a significant rise was determined at 2.5 $\mu\text{g/mL}$ compared to the negative control. However, this increase was not concentration-dependent ($r=0.20$) (Table 4.2 and Figure 4.7). In the concurrent treatment of BA+MMC, while up to 20 SCE formation was observed (Table 4.2), SCE/Cell was significantly reduced by all the BA+MMC applications compared to positive control. However, these decreases were not concentration-dependent ($r=-0.25$). The most effective dose of boric acid in reducing SCE/Cell was 0.25 $\mu\text{g/mL}$ (Table 4.2 and Figure 4.7).

Regarding the replication index, neither positive control nor any concentrations of a single boric acid treatment produce a significant difference at both treatment periods, compared to

negative control. Similarly, co-treatment of BA+MMC did not make a substantial difference at 24 and 48 h compared with positive control in RI (Table 4.2).

MMC significantly ($p < 0.05$) decreased mitotic index in lymphocytes over negative control at both 24 h and 48 h applications (Table 4.2). While all the doses of boric acid at a single treatment reduced MI compared to negative control, none was significant. The most lowering effect was observed at 2.5 $\mu\text{g/mL}$. Nevertheless, all the concentrations of BA+MMC slightly and insignificantly increased mitotic index compared to positive control. Increasing was most prominent at 0.25, 0.5, and 1 $\mu\text{g/mL}$ doses compared to 2.5 $\mu\text{g/mL}$ BA (Figure 4.8). Similar results were observed at 48 h treatments; while a single treatment of BA did not significantly reduce MI compared to negative control, concurrent treatment of BA+MMC unremarkably increased MI (Table 4.2 and Figure 4.8).

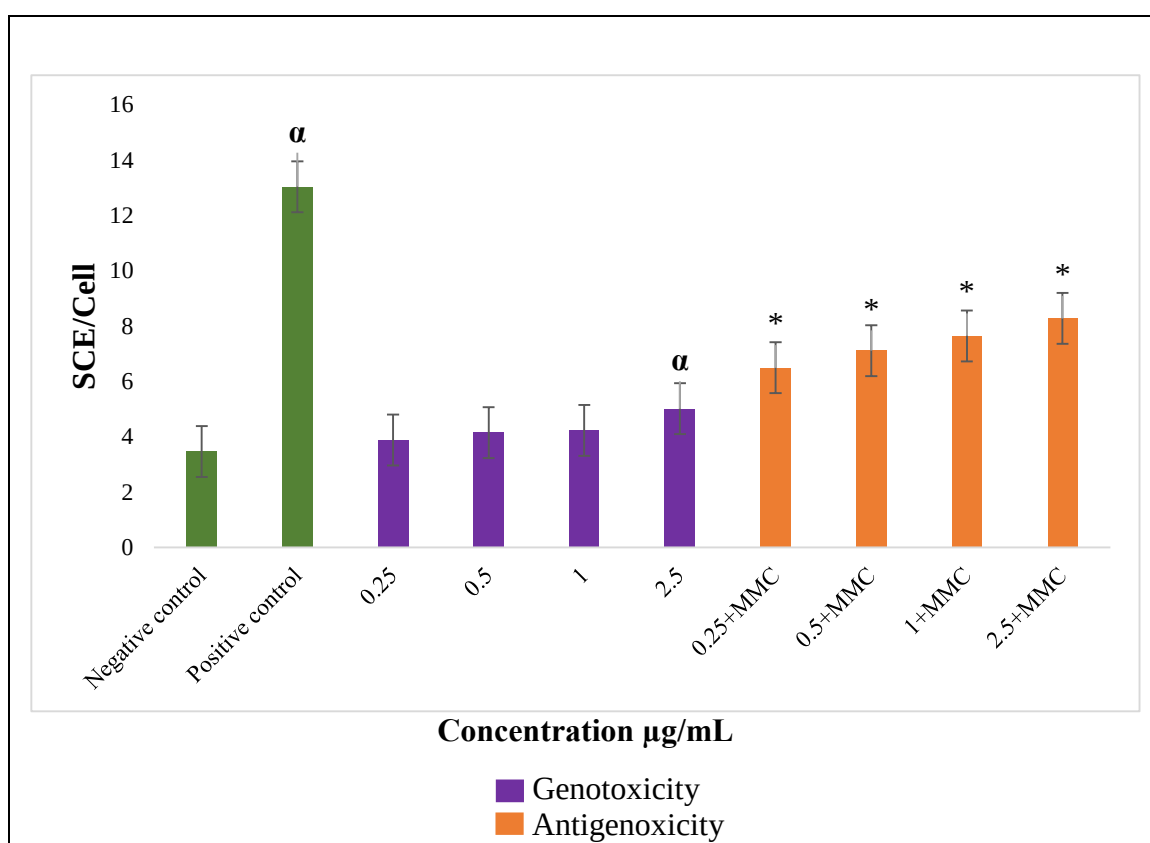


Figure 4.7. Sister chromatid exchange/Cell in lymphocytes after 48 h of treatments of Boric acid and BA+MMC. ^αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

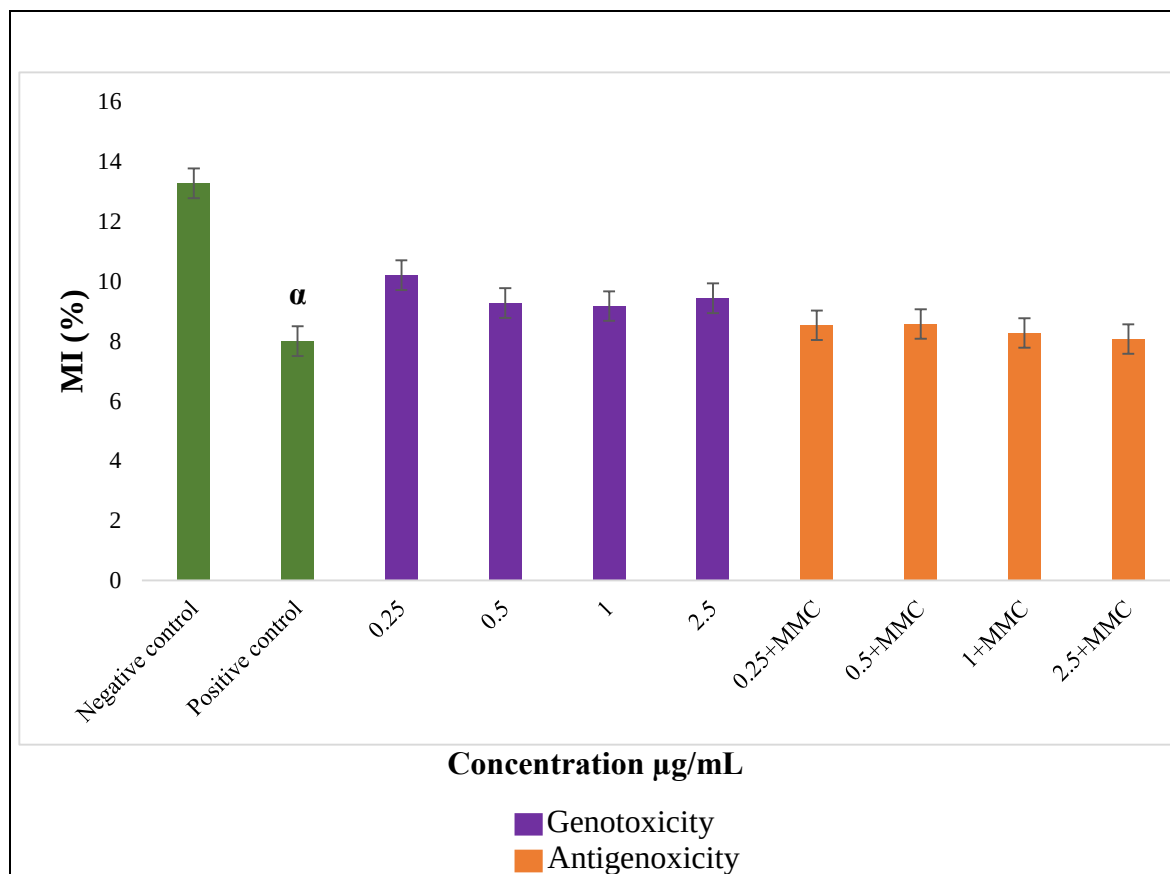
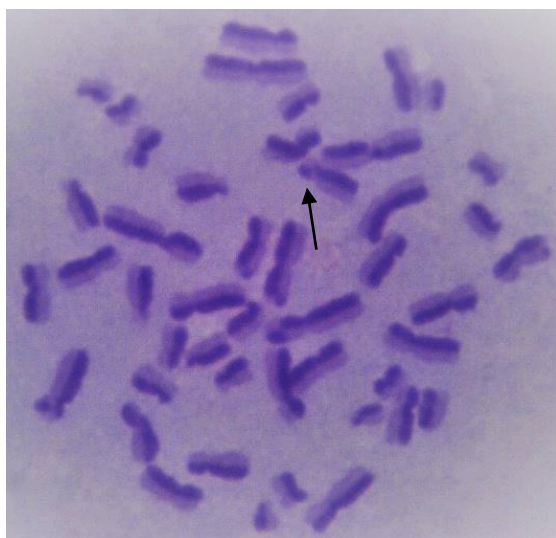


Figure 4.8. Mitotic index in lymphocytes after 48 h of treatments of Boric acid and BA+MMC. ^aSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)



Picture 4.2. Micrographs from the SCE test following Boric acid and BA+MMC administrations.

4.1.3. Cytokinesis-block micronucleus cytome test results

The effects of a single application of boric acid and simultaneous treatment with MMC on the formation of micronucleus, nucleoplasmic bridge (NPB), nuclear bud (NBUD), cytokinesis-block proliferation index and nuclear division index were also determined in human lymphocytes following 48 h treatment. The results observed are given in Table 4.3.

MMC significantly increased ($p < 0.05$) the frequency of MN over the negative control. While there is a slight augmentation in the frequency of micronucleated cells due to a single boric acid treatment, none of them was statistically significant over the negative control. Moreover, a statistically significant reduction in MN frequency was determined in the simultaneous treatment of all BA+MMC concentrations compared to the positive control. This decrease was concentration-dependent, $r = -0.54$ (Figure 4.9).

MMC generated a significant rise in the frequency of NBUDs in lymphocytes compared to negative control. Single treatment with boric acid did not markedly increase the frequency of NBUDs compared to negative control. In contrast, BA+MMC applications in all the concentrations decreased the frequency of NBUDs against MMC, but none of them was significant. However, neither MMC nor single and simultaneous treatment of BA together with MMC affects the frequency of the nucleoplasmic bridge, nuclear division index, and cytokinesis-block proliferation index (Table 4.3).

Table 4.3. Effect of Boric acid and BA+MMC applications on the frequency of MN, NBUD, NPB, NBI, and CBPI in lymphocytes.

Test substance	Treatment		Num . BN cells scored	Micronucleus frequencies in BN cells			MN ± SE	NBUD ± SE	NPB ± SE	NDI ± SE	CBPI ± SE
	Time (h)	Conc. (µg/mL)		1	2	3					
NC	48	0.00	3000	7	-	-	2.33±1.20	1.67±0.33	0.67±0.33	2.01±0.36	1.92±0.36
PC	48	0.20	3000	27	2	-	9.67±0.88 ^a	6.67±1.8 ^a	2.67±1.76	1.94±0.36	1.86±0.34
BA	48	0.25	3000	9	-	-	3.00±1.00	2.00±1.15	1.00±1.00	1.85±0.35	1.93±0.36
		0.5	3000	8	2	-	3.33±0.33	1.67±0.33	0.67±0.33	2.09±0.37	2.00±0.36
		1	3000	10	-	-	3.33±0.33	2.00±1.00	1.00±0.58	1.90±0.35	1.83±0.34
		2.5	3000	11	-	-	3.67±0.33	2.33±0.33	0.67±0.33	1.82±0.35	1.75±0.33
BA +MMC	48	0.25	3000	11	-	1	4.00±1.00*	3.00±1.53	1.00±0.58	1.76±0.35	1.70±0.33
		0.5	3000	12	-	-	4.00±1.15*	2.67±0.88	0.00±0.00	1.90±0.35	1.83±0.34
		1	3000	13	-	-	4.33±0.88*	2.67±0.33	1.00±0.58	1.86±0.35	1.79±0.34
		2.5	3000	14	-	-	4.67±0.33*	2.67±0.33	1.00±0.00	1.67±0.33	1.62±0.33

NC: Negative Control, PC: Positive Control-Mitomycin-C, Conc.: Concentration, Num. BN cells: Number of binucleate cells, SE: standard error, NBUD: nuclear bud, NPB: nucleoplasmic bridge, NBI: nuclear division index, CBPI: cytokinesis-block proliferation index

^aSignificant compared to negative control $p < 0.05$ (One-Way ANOVA test)

*Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

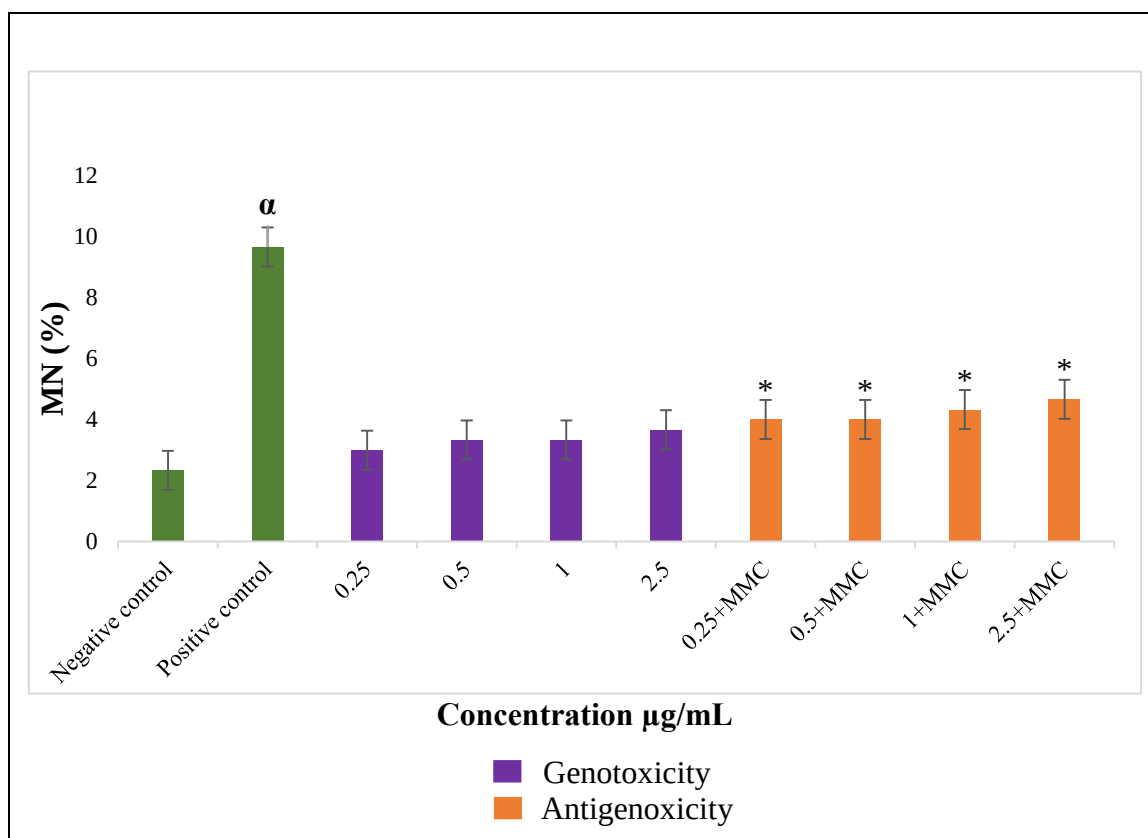
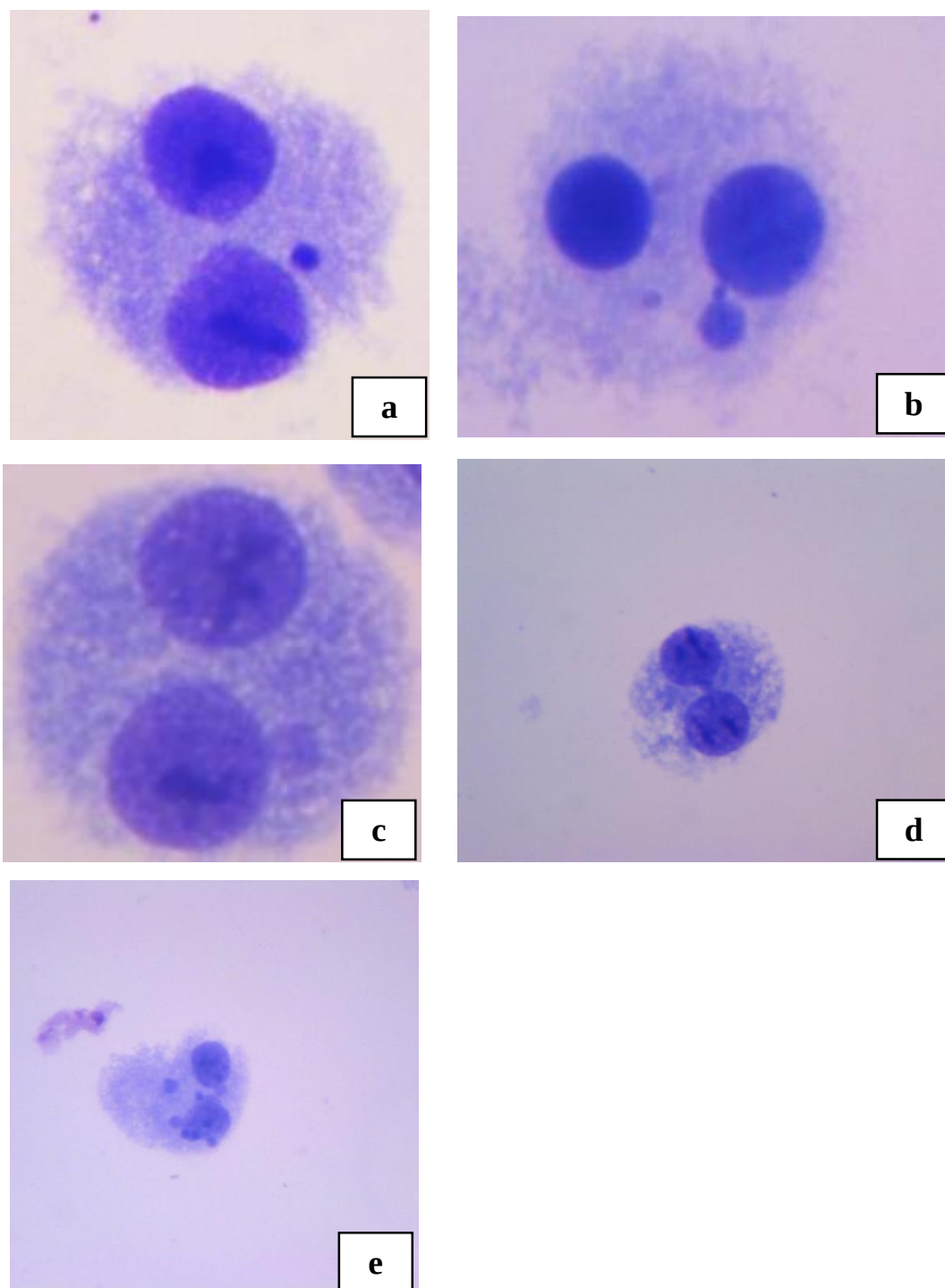


Figure 4.9. Micronucleus frequency in lymphocytes after treatments with Boric acid and BA+MMC. αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)



Picture 4.3. Micrographs from the MN test following boric acid and BA+MMC administrations. a) binucleate cell with one MN, b) binucleate cell with two MN and one nuclear bud, c) nuclear bud, d) nucleoplasmic bridge, e) binucleate cell with three nuclear buds and one MN

4.1.4. Comet assay results

In isolated lymphocytes, the effects of a single application of boric acid and co-treatment of boric acid with H_2O_2 (100 μM) on DNA damage were evaluated using three different

parameters by comet test. These parameters are comet tail intensity (DNA%), tail length (μm), and tail moment. The results obtained are shown in Table 4.4.

Hydrogen peroxide (H_2O_2) significantly increased DNA damage and elevated all the comet parameters compared to the negative group. Single treatment of boric acid in isolated lymphocytes for one hour considerably increased tail intensity (except 0.25 and 0.5 $\mu\text{g/mL}$) and tail length compared to negative control. The tail moment was also slightly but significantly increased compared to negative control. Simultaneous administration of boric acid and H_2O_2 was also examined, and observed that all concentrations of boric acid revealed a remarkable decrease in DNA damage induced by H_2O_2 in comparison with the positive control. Decreasing was significant in all three parameters (tail intensity, tail length, and tail moment) in lymphocytes (Table 4.4, Figure 4.10, Figure 4.11, and Figure 4.12). This decrease occurred regardless of the concentration for tail intensity, tail length, and tail moment ($r=-0.22$, $r=-0.25$, and $r=-0.11$), respectively.

Table 4.4. Effect of Boric acid (BA) and BA+ H_2O_2 applications on tail intensity, tail length, and tail moment in lymphocytes.

Test substance	Treatment		Mean tail intensity (%)	Mean tail length (μM)	Tail moment
	Time (h)	Conc.			
NC (H_2O)	1	0.00	3.26 $\pm$ 0.24	35.12 $\pm$ 0.24	0.62 $\pm$ 0.05
PC (H_2O_2)	1	100 μM	16.46 $\pm$ 0.99 ^a	70.29 $\pm$ 1.59 ^a	5.27 $\pm$ 0.42 ^a
BA	1	0.25 $\mu\text{g/ml}$	4.23 $\pm$ 0.28	39.64 $\pm$ 0.41 ^a	0.83 $\pm$ 0.53 ^a
		0.5 $\mu\text{g/ml}$	4.22 $\pm$ 0.32	40.70 $\pm$ 0.58 ^a	0.95 $\pm$ 0.97 ^a
		1 $\mu\text{g/ml}$	4.92 $\pm$ 0.35 ^a	42.62 $\pm$ 0.60 ^a	1.31 $\pm$ 0.11 ^a
		2.5 $\mu\text{g/ml}$	5.11 $\pm$ 0.37 ^a	45.97 $\pm$ 0.68 ^a	1.48 $\pm$ 0.13 ^a
BA + H_2O_2	1	0.25 $\mu\text{g/ml}$	6.25 $\pm$ 0.40*	49.77 $\pm$ 0.66*	1.45 $\pm$ 0.09*
		0.5 $\mu\text{g/ml}$	7.95 $\pm$ 0.48*	49.90 $\pm$ 1.08*	2.09 $\pm$ 0.25*
		1 $\mu\text{g/ml}$	6.65 $\pm$ 0.46*	50.27 $\pm$ 0.86*	2.61 $\pm$ 0.28*
		2.5 $\mu\text{g/ml}$	6.84 $\pm$ 0.73*	52.44 $\pm$ 0.97*	2.67 $\pm$ 0.37*

Totally 300 comets were scored

H_2O : distilled water

H_2O_2 : hydrogen peroxide

Conc.: Concentration

^aSignificant compared to negative control $p<0.05$ (One-Way ANOVA test)

*Significant compared to positive control $p<0.05$ (One-Way ANOVA test)

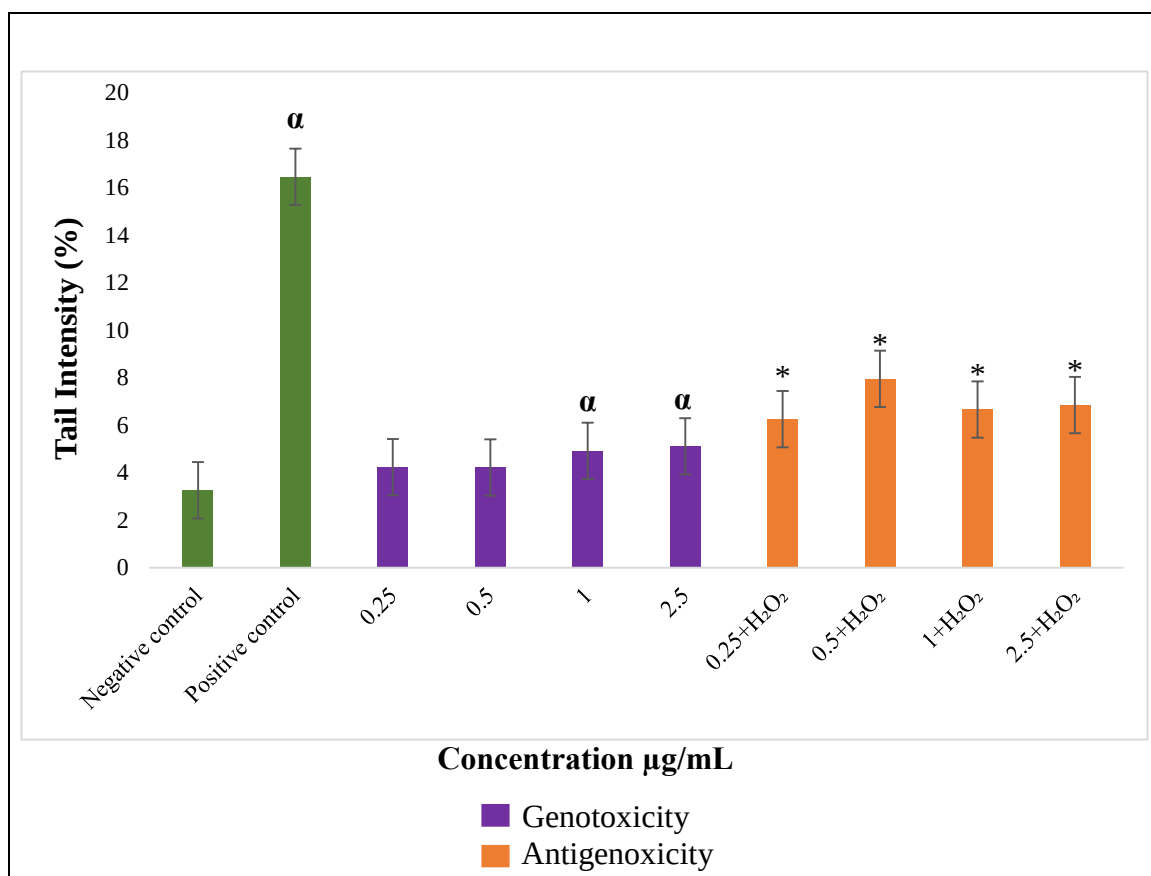


Figure 4.10. Tail intensity in lymphocytes after 1 h treatments with Boric acid and Boric acid+H₂O₂. αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

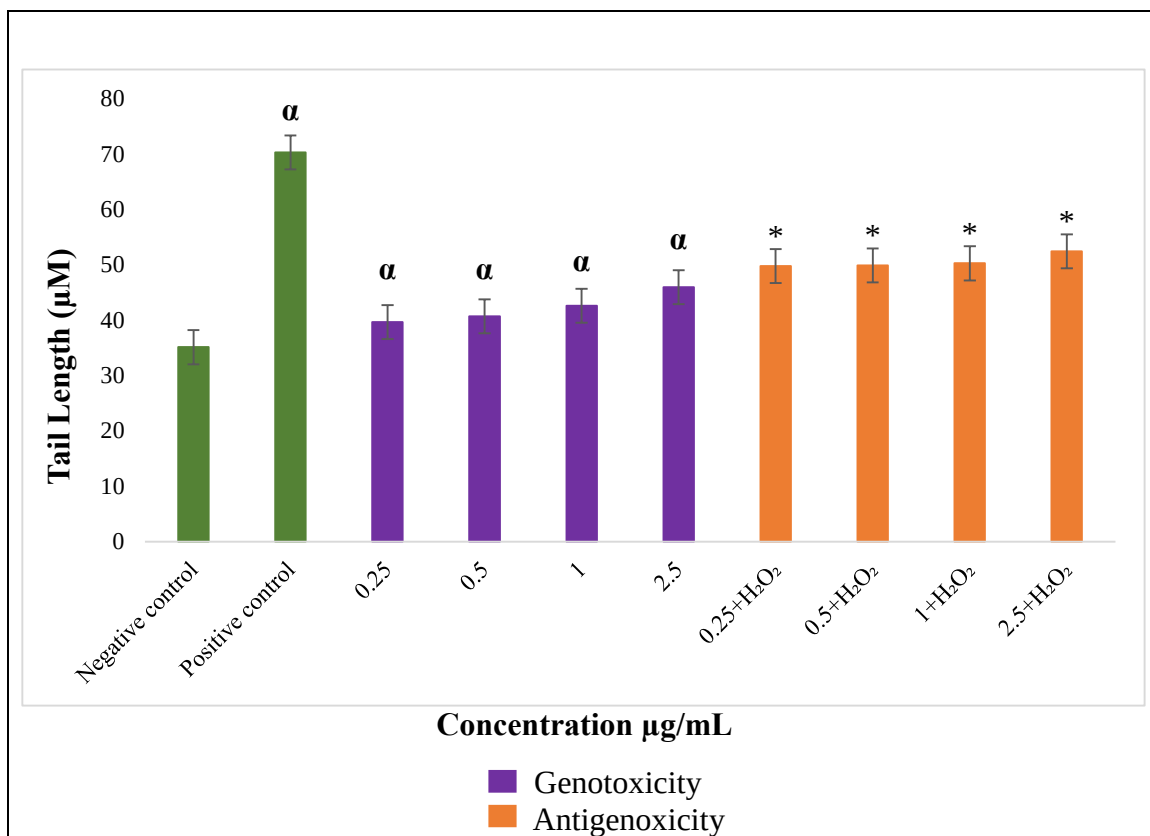


Figure 4.11. Tail length in lymphocytes after 1 h treatments with Boric acid and BA+H₂O₂.
 αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)

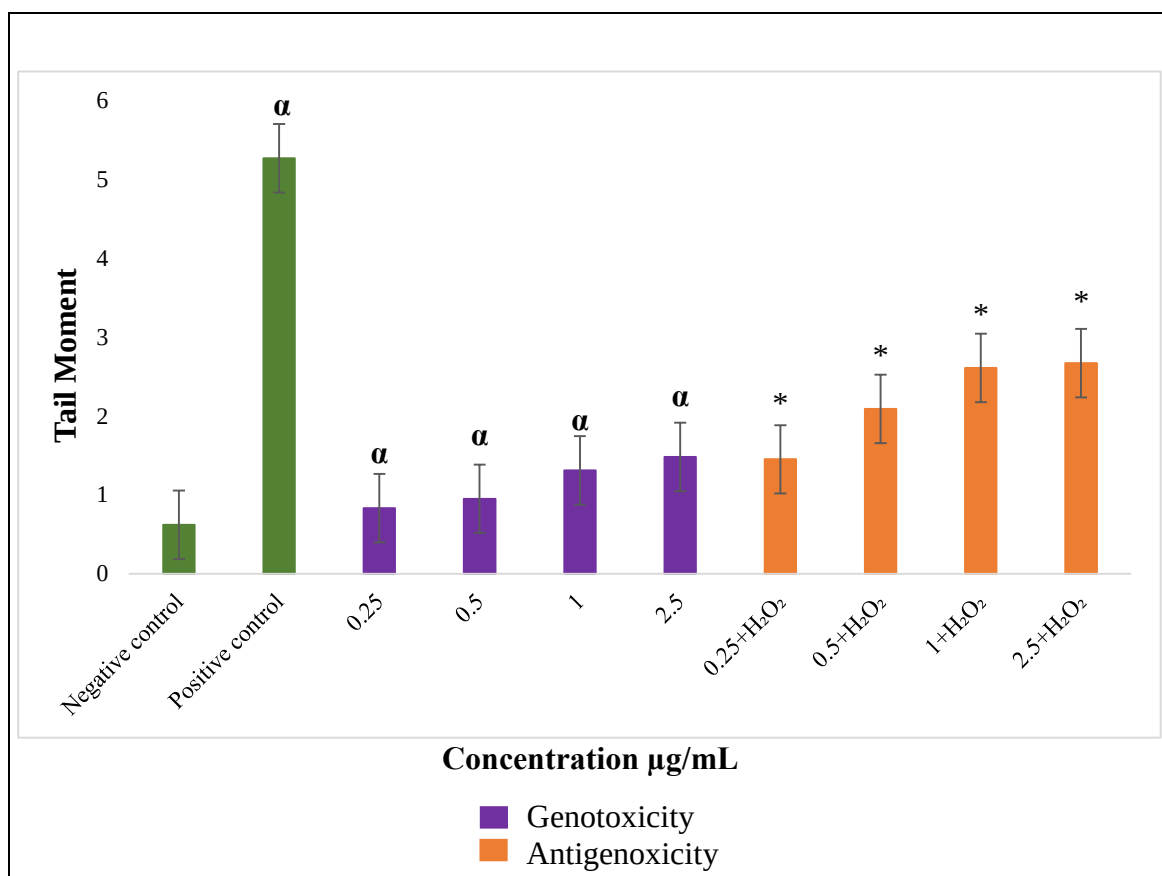
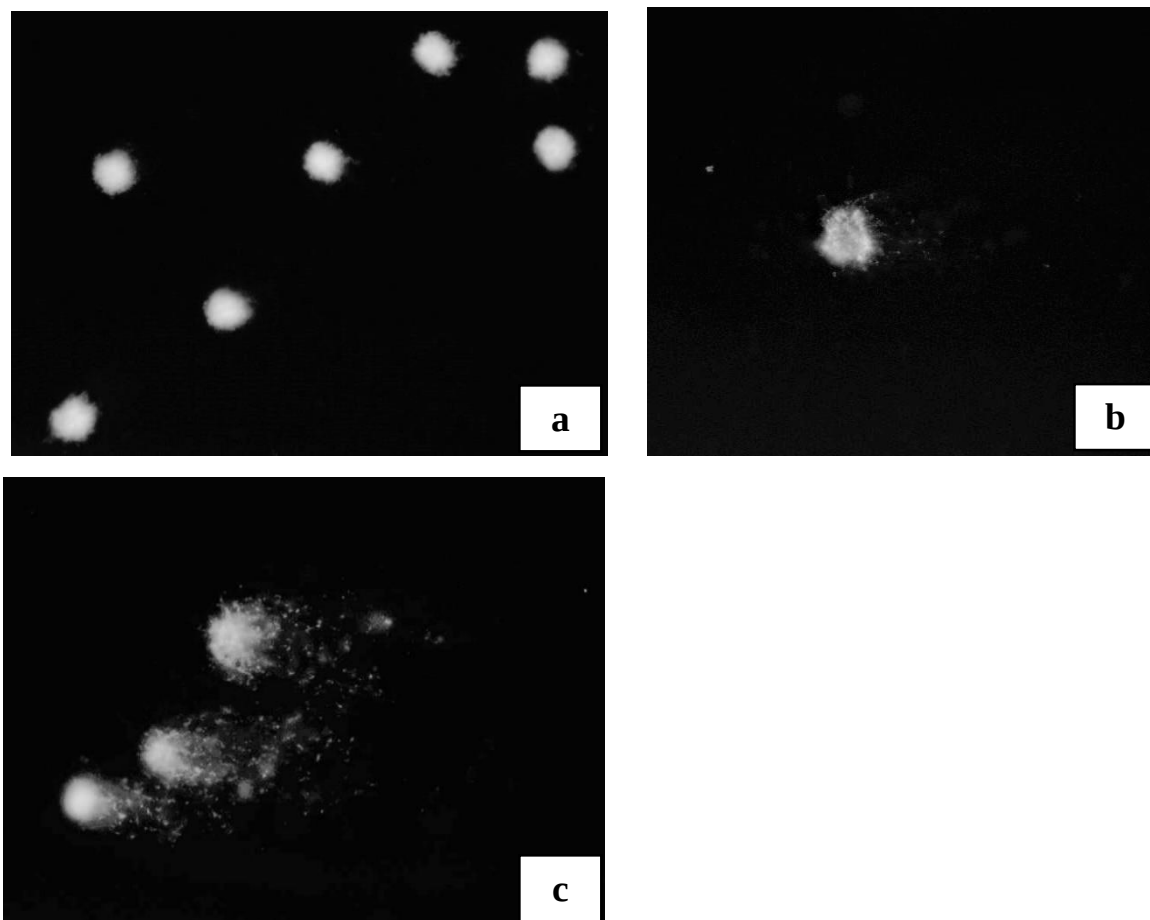


Figure 4.12. Tail moment in lymphocytes after 1 h treatments with Boric acid and Boric acid+H₂O₂. αSignificant compared to negative control $p < 0.05$, *Significant compared to positive control $p < 0.05$ (One-Way ANOVA test)



Picture 4.4. Micrographs from the comet assay following Boric acid and Boric acid+H₂O₂ administrations. a) undamaged DNA; b) slightly damaged DNA; c) severely damaged DNA

5. DISCUSSION

Cancer is a severe threat to human health caused by changes in the cellular genome, with a high global mortality rate; it is a complex and multifactorial disease. Cancer is a deadly disease in which cells multiply rapidly and uncontrollably. It ranks as the second most common cause of death in advanced countries. More than 200 different cancer types and more than 500 cancer-related genes are known to affect the human body (Pavlopoulou et al., 2015; Cao et al., 2021).

Many forms are still incurable and result in death, although there have been significant advances in the prevention and treatment of cancer. Surgery, chemotherapy, and radiotherapy are the most often used cancer treatment methods. However, these cancer therapies have numerous adverse effects, such as dizziness, nausea, vomiting, and hair loss, as well as the multidrug resistance (MDR) of tumor cells (Ministry of Health, 2013). It has been determined that, especially in radiotherapy and chemotherapy, normal cells are affected as well as cancerous cells, and they cause the formation of secondary tumors in cancer patients (National Cancer Institute, 2010; Nussbaumer et al., 2011; Saini et al., 2020). In chemotherapy, various types of drugs such as carmustine, altretamine, bendamustine, busulfan, carboplatin, cisplatin, chlorambucil, thiotepa, trabectedin, temozolomide, melphalan and mitomycin-C are widely used. MMC is an alkylating agent that causes DNA damage. Alkylating agents are chemicals that react with the alkyl group they contain to form covalent bonds with DNA, thereby, through a nucleophilic reaction (SN1 or SN2), exchanges a hydrogen group on a base with an alkyl group, which is generally linked with guanine (N2, N3, N7, O6), adenine (N3, N7), and cytosine (N3) binding (Khoury et al. 2020). As a result of this binding, not only DNA transcription but also cell proliferation is stopped (Del Castillo et al., 2021).

Boron (B) is a metalloid with properties midway between a metal and a nonmetal. It is essential for the health of humans, animals, and plants. It takes part in the synthesis and metabolism of multiple reactions. B is a trace mineral found in the environment in various forms, including boric acid, borates, ulexite, borax, and boric oxide. Boric acid (H_3BO_3) is one of the compounds of boron used for several purposes: as a food preservative, a disinfectant, a fertilizer, a flame retardant, and insulation material (Lopalco et al., 2020).

Boric acid functions as a regulatory ion by influencing membrane transition signals and motions in redox processes. It also controls the oxidative explosion process, which uses reactive oxygen particles to destroy bacteria on white blood cells (leukocytes). According to specific hypotheses, this arrangement protects some collaterals from destruction by activating reactive oxygen particles in the tissues nearby. On the other hand, boric acid lowers the risk of inflammatory illnesses. Depending on the concentration, it can cause hazardous effects or protect the organisms against various toxic agents. Therefore, determination of the exposure levels at which boric acid becomes harmful is crucial (Powell et al., 2019).

The objective of this research is to examine the potential genotoxic effect of BA and its potential protective effect against an anticancer drug (MMC) and H_2O_2 , an oxidant, by four different genotoxicity tests: chromosome aberration, sister chromatid exchange, cytokinesis block micronucleus cytome, and comet tests in human lymphocytes for the first time. Chromosomal aberrations, micronucleus, and comet tests are the tests whose standards are set in OECD guidelines and recommended for use in genotoxicity evaluations. On the other hand, although it has been removed from the OECD guideline, the SCE test is often used to support the findings of other genotoxicity tests (Yüzbaşıoğlu, Dilek, Erikel, and Ünal, 2023; Ünal, Akbaş, and Yüzbaşıoğlu, 2023).

Human peripheral blood lymphocyte cells are the example tissue and cells for monitoring the genotoxicity of any toxic agent. It is preferred firstly, because of its large quantity, and can be obtained easily. Secondly, lymphocytes are a significant cell type to investigate immunological responses and the potentially harmful effect of chemicals since they are essential to the immune system. Thirdly, human lymphocytes are potentially vulnerable cells since the most harmful environmental chemicals circulate throughout body fluids, passing back and forth between blood and cells (Rajakariar et al., 2008; Ünal et al., 2022).

Countless exogenous or endogenous substances constantly threaten our genetic material, DNA, and chromosomes. Exogenous agents refer to any chemical or physical factors we consciously or unknowingly are exposed to. Reactive oxygen species (ROS) produced as a result of metabolic activities and causing damage to a variety of macromolecules in the cell, particularly DNA can be named endogenous agents (Evans et al., 2004).

The discipline of genetic toxicology was developed in the late 1960s, and research was performed to find genotoxic and nongenotoxic carcinogens in the 1980s when it was seen that the association between genotoxicity and carcinogenicity posed a severe threat to human health. Short-term genotoxicity tests include studies investigating the mutagenic potential of chemicals in bacteria, whether they alter cells, their impact on DNA repair and synthesis, and their mutagenic effects in mammals (Hasselgren et al., 2019).

Genotoxicity tests are used to detect abnormal changes in chromosomal structure and number. Chromosome aberration, sister chromatid exchange, cytokinesis block micronucleus cytome, and comet tests are among the most frequently used and favored *in vitro* and *in vivo* assays for assessing the genotoxic effects of substances (Avuloğlu Yılmaz, Yüzbaşıoğlu, and Ünal, 2023; Yüzbaşıoğlu, Mahmoud, Mamur, and Ünal, 2022). For years, cytogenetic changes in human peripheral lymphocytes, such as CAs, SCEs, MNi, and comet formation, have been employed as early signs of genotoxic carcinogen exposure (Norppa, 2004; Roussel et al., 2019).

Antigenotoxicity can be defined as the ability of toxic chemicals or agents to decrease or stop genetic damage. It is known that genotoxic substances lead to mutations, damage in DNA, chromosomal abnormalities, and different genetic changes. By using several kinds of mechanisms, antigenotoxic substances can mitigate or minimize the negative effects of toxic compounds (Verschaeve, 2015; Figat et al., 2021). They may function as antioxidants, assisting in reducing the free radicals and ROS that could harm the DNA. Two types of antioxidants exist: endogenous or exogenous. They may be found in the extract of plants, food, and even boron intake. Antigenotoxic substances may enhance the DNA repair mechanism, allowing cells to repair any genetic damage successfully. Chromosome aberration, sister chromatid exchange, cytokinesis block micronucleus cytome, and comet tests are also widely used *in vitro* and *in vivo* for assessing antigenotoxic effects of many chemicals (Ünal, Yüzbaşıoğlu and Erikel, 2022).

For decades, chromosomal aberrations assay has been one of the most used methods to evaluate the carcinogenicity and mutagenicity of substances. It is applied to detect abnormalities due to exposure to physical, chemical, and biological agents. Environmental factors such as radiation, pesticides, nanoparticles, heavy metals, chemicals, and various

toxins during cell division can increase the formation of chromosome aberrations and genetic mutations (Andrey et al., 2019; Unal et al., 2020).

Chromosomal abnormalities are seen in two ways: structural and numerical. Structural abnormalities are the rearrangements or abnormal changes observed in the chromosome structure and are constituted of chromatid and chromosome break, dicentric chromosome, fragment, chromatid exchange, sister chromatid union, and ring chromosome. The abnormality in the number of chromosomes is referred to as numerical aberration and is mainly composed of polyploidy and endoreduplication (Basri Ila and Tahir Hüsünet, 2022: 153,184; Ünal, Yüzbaşıoğlu, and Erikel, 2022).

Single or double-chain breaks in the chromatid or chromosome cause structural abnormalities. Circulating lymphocytes are in the G₀ phase of the cell cycle. These cells can be stimulated to synthesize the DNA following stimulation by a polyclonal. Mitogen activation causes cells to transition from the cell cycle's G₀ (quiescent) phase to the G₁. Following stimulation and entry into G₁, the lymphocytes will go through the cell cycle phases of S (synthesis where DNA synthesis takes place), G₂ (gap 2), and M (mitosis, where cell division occurs) before returning to G₁. During the G₁ cycle, if lymphocytes are exposed to some genotoxic agents in the chromosome medium, the damage occurs in the whole chromosome since the chromosomes are not yet in two chromatids. However, if lymphocytes are exposed to these agents during the S or G₂ stages of the cell cycle, damage occurs in a single chromatid as two sister chromatids exist separately from each other (Ünal et al., 2013; Kirsch-Volders et al., 2002).

Fragments form as the result of chromatids or chromosome breaks, typically caused by terminal deletions (Anwar et al., 1994; Hasan Ila and Tahir Hüsünet, 2022: 153, 184). Dicentric chromosomes form when the centromeric regions of two broken chromosomes interchange. It arises as a result of conditions such as repairing DNA strand breaks, incorrectly repairing two chromosomes, or aberrant chromosomal replication (Agrawala et al., 2010; Ludovici et al., 2021). Sister chromatid union originates from the reciprocal fusing of these broken ends with terminal deletions at the ends of the chromosomes (Murli, 2003). Chromatid exchanges are quadriradial (four) or triradial (three) chromosomes and form from interchromosome or intrachromosome exchanges. In two adjacent regions of two separate chromosomes, symmetrical or chromatid breaks are known as quadriradial chromosomes. A

single chromatid and a single isochromatic break coinciding are critical components of triradial chromosomes (Sato et al., 2002). Ring chromosomes are circular DNA structures which are uncommon in eukaryotic genomes. Ring chromosomes typically occur from the fusion of broken ends of the same chromosome or by the fusion of two broken ends of two different chromosomes. Therefore, ring chromosomes reveal the loss of genetic material (Daber et al., 2012). In theory, removed, deactivated, or deleted telomeric sequences may cause it. Either double-strand DNA break or dysfunctional telomeres may result in ring formation. Polyploidy can arise following two cell divisions during mitosis or, more typically, from the inability of chromosomes to divide during meiosis. G1 and G2 phase-specific clastogens and substances that interact with the mitotic spindle are the causes of polyploidy (Van de Peer et al., 2017). Endoreduplication is the formation of two successive DNA syntheses due to replication without chromatid separation. While DNA damage and spindle fiber disruption are crucial factors in the development of endoreduplication, abnormalities in DNA topoisomerases, which are critical for chromatid separation during anaphase in mitosis, can also assist in the appearance of this aberration (Lima et al., 2007; Meredova et al., 2022).

Both structural abnormalities and numerical abnormalities have been associated with human health. In particular, they contribute significantly to the initiation, progression, and metastasis stage of carcinogenesis (Durante et al., 2013; Hasan Ila and Tahir Hüsnet, 2022: 153,184).

G2 is a crucial phase in the cell cycle because DNA damage is primarily repaired in this stage. Homologous recombination, which uses the sister chromatid that is still intact, is used to repair DNA double-strand breaks during the S and G2 phases of the cellular cycle. In the genetics process of homologous recombination, two DNA molecules exchange genetic information to create a new gene combination. It occurs in the midst of cell division and is essential for genetic diversity and DNA repair. A rise in the frequency of chromosomal abnormalities indicates that repair is either performed, completed improperly, or not at all (Toropov et al., 2019; El-Alfy et al., 2020).

In this study, the results obtained following chromosomal aberration assay revealed no increase in abnormal cell frequency and number of abnormalities per cell at 24 and 48 h administration of BA alone over negative control. Antigenotoxic potential of boric acid

against the genotoxic effect of mitomycin-C was also investigated. Interestingly, the co-treatment of BA+MMC for 24 and 48 h significantly diminished the number of abnormal cells and abnormalities per cell in each concentration (except 2.5 $\mu\text{g/mL}$) compared to positive control MMC. In the simultaneous application of BA+MMC, the most effective BA concentration was 0.25 $\mu\text{g/mL}$.

A single application of BA did not show any genotoxic effect in any concentration, but indeed, all the concentrations exhibited a mutagen-protective effect. The increased occurrence of CAs is considered a cancer biomarker, so BA's antigenotoxic potential *in vitro* raises the prospect of it playing an important role in chemoprevention. This is the first study to show that BA can protect against MMC. In the literature, there are some reports on the ameliorative effect of BA against some other genotoxic substances. For example, Geyikoglu and Turkez (2008) demonstrated that boron compounds (boric acid and borax) lowered vanadium-induced toxicity in human cells by employing micronucleus tests, sister chromatid exchange, and chromosome aberrations. The results obtained from this experiment stated that when BA and BX were applied alone no genotoxic effect was observed, which is by our findings. On the other hand, vanadium caused DNA damage and increased CA, SCE, and MN rates. Applying boron compounds and vanadium reduced CA, SCE, and MN frequency. These results were also coincident with our findings.

Celikezen et al., (2015) investigated ammonium tetra borate's genotoxic and antigenotoxic potential using chromosome aberration and micronucleus test in human lymphocytes. The result revealed that borate did not remarkably elevate CA and MN frequency. In contrast, when human lymphocytes were exposed to several boron compounds (such as borax, kernite, probitite, and boric acid), none of these induced a genotoxic effect (Geyikoglu et al., 2008; Türkez et al., 2010).

Çelikezen et al., (2021) also investigated the ameliorating effect of borax, one of the boron compounds, in opposition to doxorubicin (DXR), an anticancer drug, in rats. They demonstrated that DXR increased the MDA level in comparison to the control group. On the contrary, when DXR was treated with BX, a decrease has occurred. Reduced levels of antioxidant levels produce oxidative stress (OS); hence antioxidant enzymes levels in cells, including (SOD, G-6-PDH, CAT, GR, GSH, and GSH-Px), are essential for cellular defense mechanisms (Bukowska, and Kowalska, 2004; Tapiero et al., 2004). It has been highlighted

that SOD produced by these enzymes plays a significant key in the body's defense against OS and catalyzes the reaction that turns superoxidase radical into hydrogen peroxidase and oxygen (Kakarla et al., 2005). According to studies, antioxidant enzymes are crucial in limiting the damage caused by ROS and are either activated or inhibited in blood cells that have been exposed to many hazardous chemicals (Afaq et al., 1998; Prasad et al., 2006). In light of these results, it was noted that B and derivatives (such as borax and boric) were likely to affect mitochondrial oxidative phosphorylation metabolism by raising cAMP levels and reducing hydrolytic enzyme activity (Hall et al., 1980). On top of that, it was reported that elevated antioxidant enzymes resulted from a boost in cAMP levels. Boron may accumulate cAMP, which will subsequently improve SOD function (Sugino et al., 2002).

The working cell affects the interruption of NAD/NADP production. BA was found to alter Ca^{+2} release and NAD formation in cancer cells depending on the concentration. It was reported that the growth of cancer cells is reduced by 30-97% by borates (Barranco et al. 2009). In addition, tumor insulin growth factor-1 was significantly reduced by boron, which is essential for prostate cancer growth (Gallardo-Williams et al., 2004; Kawada et al., 2008). When BA was added to the diet of immunocompromised mice, human prostate cancer tumors transplanted into mice tended to decline (Kawada et al., 2008). Additionally, dietary boron reduced women's risk for lung and breast cancer (Mahabir et al., 2008).

In the literature, some studies opposed ours. For example, Arslan et al., (2008) postulated a study examining the toxic effect of BA in human lymphocytes treated with four concentrations (400, 600, 800, and 1000 $\mu\text{g/mL}$) using CA and SCE tests. The findings indicated that BA increased the frequency of CA and decreased MI and RI. The authors stated that BA increased the generation of CAs by disrupting DNA's phosphodiester backbone (Arslan et al., 2008). Our results were not coincident with these observations. The difference in results may be due to distinct concentrations, treatment periods, volunteer donors, and the conditions in which the test proceeded.

Another frequently used test in genotoxicity studies is the SCE, a rapid and effective test method for evaluating primary toxic effects caused by biological, physical, and chemical agents. At metaphase, SCE represents symmetrical exchanges of DNA segments between sister chromatids of chromosomes (Erikel, Yüzbaşıoğlu, and Ünal, 2023). The principle of the SCE test is to detect homologous chromosome changes that occur in DNA with the

addition of bromodeoxyuridine (BrdU). When the cell cycle is in the S phase, a thymidine analog called BrdU integrates DNA (Waisertreiger et al., 2020). Exposure of the cell to any physical and chemical genotoxic substance in the S phase during replication generates SCE. Repairing the broken region with identical loci of sister chromatids weakens the link between SCE formation and decreases cancer risk (Gajski et al., 2010; Sunada et al., 2019).

Free radicals formed from deprived oxygen have drawn attention in recent decades because of their significance in several serious diseases. Because they are highly reactive, they can exist in organic and inorganic compounds with one or more impaired electrons in their atomic structure. Free radicals put the viability of the cells at risk by potentially damaging lipids and nucleic acids (Stephens et al., 2009; Kara et al., 2012).

In this thesis, all concentrations of a single treatment of BA induced an increase in SCE frequency per cell at the 24 h application. However, none of them was remarkable in comparison to negative control. At the 48 h treatment, while SCE frequency increased at all the concentrations, only 2.5 µg/mL BA caused a significant elevation. In the simultaneous treatment of BA+MMC at both 24 h and 48 h administration, all the concentrations applied significantly reduced the SCE formation induced by MMC. There was no significant change in the replication index in any administration groups. These observations are by previous reports (Turkez et al., 2011). As a result, boric acid showed a protective effect against genetic damage formed by alkylating agents and oxidative stress by causing a decrease in the rate of SCE.

The mitotic index (MI) is a standard cytotoxicity parameter and a sensitive marker for assessing the mitotic changes brought on by a disruption in the cell's structure or function. The diminution in the MI may be inferred as the cell ceasing to grow during the interphase, the cell losing its ability to divide, or the activation of other mechanisms leading to cell death (Yüzbaşıoğlu, Erikel and Ünal, 2023). In the present study, we observed that the application of MMC significantly decreased MI. Studies conducted both *in vitro* and *in vivo* show that chemotherapy medications have harmful effects resulting in DNA single-chain breaks. As stated, the reduction of MI or the inhibition of DNA synthesis could be induced by a diminution in adenosine triphosphate (ATP) level and pressure from the site of cell energy production (Epel, 1963; Jain and Andsorbhoy, 1988; Chen et al., 2020). In this study, BA alone reduced the percentage of MI, but this decrease was not significant. On the other hand,

the co-treatment of BA and MMC insignificantly elevated the percentage of mitosis compared with the positive control.

Turkez et al., (2011) examined the ameliorating effect of BA against toxicity induced by a chemotherapeutic agent, paclitaxel (PAC), applied in human lymphocytes using SCE and micronucleus tests. Peripheral lymphocytes obtained from healthy donors were treated with four doses of BA alone and with PAC for 72 hours. BA diminished the number of PAC-induced SCE and CBMN formations and minimized the side effects of the chemotherapeutic agents PAC. The result of this study stated that BA improves tissue antioxidant capability and resistance to free oxygen radicals.

SCE and MN tests in human lymphocytes examined the toxic effect of boron and its derivatives (boric acid, colemanite, ulexite, and borax). These findings demonstrated that boron had no mutagenic potential (Türkez et al., 2007), which aligns with our research. In another investigation, CAs and SCEs did not rise when treated with boric acid in Chinese hamster ovary cells. Similarly, no toxic effect was observed following BA treatment in the mouse lymphoma (Guo et al., 2020; Hadrup et al., 2021). Karatekeli et al., (2023) exposed rats to aflatoxin b1 (AFB1) and boron, both alone and simultaneously, and observed that boron exhibits hepatoprotective effects against aflatoxin b1 by antioxidant, anti-apoptotic, and anti-inflammatory pathways. The administration of AFB1 decreased the enzymes that participate in the antioxidant defense system mechanism (GSH, SOD, CAT) and damaged the liver and its functionality. In contrast, the application of boron enhanced the activity of transaminase enzymes. This proved that boron, which had been reported to have a preventive impact on tissues, also exhibited a protective effect against liver damage brought on by AFB1 (Karatekeli et al., 2023).

Alkylating agents (such as MMC, cisplatin, cyclophosphamide, etc.) cause crosslinks by preventing the DNA double-strands from being properly bonded. The homologous chromosome repair mechanism in the DNA repair system repairs these crosslinks. Therefore, agents that form DNA crosslinks are potent inducers of SCE (Wilson and Thompson, 2007; Michalková et al., 2018).

Mitomycin-C is a genotoxic substance that has a long history of research. ROS and mono or difunctional alkylating agents are produced due to MMC metabolism. Enzymes like the

cytochrome p450 reductase metabolize MMC, which promotes DNA molecules to crosslink between adenine and guanine residues, stopping DNA synthesis and eventually impairing mitosis in cells, resulting in cell cycle arrest. Even though the alkylation of the DNA can happen at any phase in the cell cycle, DNA synthesis, especially in the late G1 and S stages, has the most detrimental biological effects (Siegel et al., 1990).

Oxidative stress is characterized as a long-term imbalance within the body's ability to protect itself (antioxidant defense system) from free radicals and the production of ROS. Life-threatening diseases such as diabetes, cardiovascular disease, various cancers, neurological illnesses, and kidney disease are linked to oxidative stress. Researchers have conducted various host modulatory medicines to achieve a balance between oxidative and defense systems, including antioxidant therapy. Boron and its derivatives are preferred research topics for investigators due to their anti-inflammatory and antioxidant characteristics in preventing and treating many illnesses (Sezer et al., 2013).

The cytokinesis-block micronucleus cytome (CBMN-cyt) test is an important *in vitro* and *in vivo* test system that is mostly employed in the examination of genotoxic effects of chemicals and populations exposed to toxic agents, in molecular epidemiology, and in the determination of cytogenetic damage (Avuloğlu-Yilmaz, Yüzbaşıoğlu, and Ünal, 2020). CBMN is based on the suppression of cytokinesis by adding cytochalasin-B (cyt-B) at the 44th hour in cells undergoing the nuclear division following stimulation and identifying MNs in cells containing binucleate (BN). Actin polymerization is prevented by the *Helminthosporium dematiadeum* extract known as Cyt-B. During the cytokinesis phase, it binds to the terminals of actin filaments and stops actin from polymerizing (Fenech and Morley, 1985; Norppa et al., 2003).

A rise in MN frequency is regarded as an inexplicit marker of the structural and numerical abnormalities that distinct chemical substances cause in cells. Micronuclei may consist of non-centromere (acentric) chromosome particles or whole chromosomes that cannot be pulled to the poles due to some effects in the anaphase of the cell cycle. Micronuclei are primarily composed of deficiencies of genes involved in the cell cycle control during nuclear division in the cell cycle, errors in kinetochore, mitotic threads, and/or chromosomal degenerations (Fenech, 2007).

Single-strand breaks (SSBs) in DNA can turn into double-strand breaks (DSBs) due to problems in repair mechanisms, and the broken piece can form a micronucleus. Later, these fragments are covered with nucleoplasm during telophase and can be seen as a small membrane-enclosed nucleus in the resulting binucleate cell. In addition, agents such as radiation directly cause DSBs on DNA. Thus, the centromeres of the dicentric chromosome being dragged to different poles can result from the formation of the micronucleus (Bonassive and Fenech, 2019).

Factors such as defects in spindle fibers, changes in the structure of centromeres and centrosomes, problems in mitotic control points, and abnormal centrosome increase can cause a complete chromosome to form micronuclei by not going to any pole while the chromosomes are pulled towards the poles. Micronuclei have been reported to be a marker of impending tumorigenesis. The micronucleus frequency in cancerous cells was always found to be higher when compared to the micronucleus frequency in normal cells. Different studies have demonstrated that a rise in MN frequency is associated with cancer formation (Bochtler et al., 2019; Avuloğlu-Yılmaz, Yüzbaşıoğlu, and Ünal, 2020).

The occurrences of chromosomal instability are linked to nuclear budding (NBUD) by recombining amplified sequences' homologous regions to create mini circles of telomeric DNA and acentric; that way, amplified DNA can be removed. When centromeres of a dicentric chromosome are dragged opposing poles of the cell during anaphase, a nucleoplasmic bridge (NPB) occurs (Yüzbaşıoğlu and Avuloğlu-Yılmaz 2022: 219, 249).

One of the parameters used in assessing the cytotoxic effect is the nuclear division index (NDI), which provides information on the amount of nuclear division. Cells become mononucleated when cytokinesis is blocked or do not divide and binucleated if they divide once. Multiple divisions have occurred in cells with more than two nuclei (Fenech, 2006).

This study observed no change in micronucleus frequency in human lymphocytes administrated with BA alone. Therefore, BA did not exhibit a genotoxic effect. On the contrary, in the simultaneous application of BA+MMC, MN formation was significantly decreased by BA at all doses compared to the positive control, and all these reductions were shown to be statistically significant. No significant change was observed when all applications were evaluated in terms of cytokinesis block proliferation index and nuclear

division index. Boric acid is thought to provide protection, especially against MMC-induced MN formation.

Numerous studies have shown that boron has a low possibility of causing mutagenicity in cultured mammalian cells and microorganisms, although Turkez et al., (2012) point out that boron is a nongenotoxic agent in human lymphocytes. Although boron provides several advantages, excessive ingestion harms animals and humans. Our findings indicate that boron has no remarkable impact at the concentrations we applied (0.25, 0.5, 1, and 2.5 µg/mL).

The study of Turkez and Geyikoglu (2010) examined the protective effect of boric acid against aflatoxin B₁ in peripheral lymphocytes using biochemical tests (SOD, CAT, GSH-Px activity, MDA, GSH level) and cytogenetic methods (MN). After supplementation of BA (0.25, 0.5, 1, 2, 5, 10, and 20 ppm) and AFB₁ (1.56 and 3.12 ppm), both alone and in combination, the lymphocytes were incubated at 37°C for 1 h. The result revealed that BA alone did not induce any genotoxic effect using the MN test. In contrast, simultaneous treatment of BA + AFB₁ efficiently decreased the MN frequency against AFB₁-induced DNA damage in human lymphocytes. The authors reported that BA lowered the adverse effects of AFB₁ by increasing the antioxidant enzymes (SOD, CAT, and GSH-Px) but not affecting the MDA levels in the blood (Turkez and Geyikoglu, 2010).

BA decreases the side effects of anticancer drugs and other genotoxic agents such as heavy metals, nanoparticles, pesticides, etc. Türkez, Geyikoğlu, and Çolak investigated boric acid's protective effect against aluminum (Al) in the liver of rats using a liver micronucleus assay. Four concentrations of Al (3.2, 13, 36, and 58.5 mg/kg) were applied for ten days. The findings obtained indicate that Al elevated the micronucleated hepatocytes. However, BA administration diminished the genotoxic effect of Al (Türkez et al., 2011). According to Park et al., (2005), there is not enough information about how boron impacts animal cells after exposure. Despite the lack of knowledge regarding boron's physiological role in hepatocytes, one theory holds that the antioxidant properties of boron and its derivatives can protect against Al-induced damage. The reason may be that Al's genotoxic consequences are linked to forming free radicals (Abubaker et al., 2003). In this case, it is well recognized that DNA damage is frequently attributed to oxidative stress as a primary contributing factor.

In a study performed by Sun et al., (2013) and Oliveira et al., (2001), cytokinesis block micronucleus assay was used to investigate the genotoxic impacts of boron neutron capture therapy (BNCT) in melanoma cells. They discovered that BNCT has a significant therapeutic benefit for preventing tumor recurrence and may be an option for glioma treatment. BNCT is a type of cancer treatment that utilizes the unique properties of boron to target and eradicate specific tumor cells. The nuclear process occurs when high-energy particles are captured by the boron atoms in the tumor cells; then, the neutrons are released, which is localized to cancerous cells. This treatment offers the ability to cure several types of cancer in a targeted and efficient way (Dymova et al., 2020).

Nuclear bud (NBUD) and nucleoplasmic bridge (NPB) formation were also examined in this thesis. BA did not significantly increase NBUD and NPB. NBUD is employed as a biomarker of amplified DNA or the removal of DNA repair complexes. The majority of NBUD is sourced from terminal or interstitial acentric fragments. NBUD is probably induced when extra DNA is removed from the nucleus or when the nuclear membrane surrounding the DNA remains in the cytoplasm even after the nuclear division (Fenech, 2004 and 2006). NPB is used as an indicator of an improper repair of DNA strand breaks, or telomeres end fusions. During anaphase of mitosis, when the chromosomes are often pulled to the opposing poles of the cell, dicentric chromosomes are the primary source of nucleoplasmic bridges (Fenech, 2008).

In this research, comet assay, another practical, reliable, and sensitive test method frequently used to examine the genotoxic effects of any physical, chemical, and biological agents, is also applied. It is used to measure DNA damage triggered by applications such as chemotherapy and radiotherapy used to prevent cancer (Cordelli et al., 2021). The goal of this test is to detect DNA damage like SSBs, DSBs, and DNA-DNA crosslinks in single cells (Ünal, Saygılı and Akbaş, 2022: 313, 340; Collins, 2004). In this method, three parameters are evaluated: tail intensity (% tail DNA) is the percentage of DNA fragments in the tail; tail length (μm) is the distance from the comet head to the last visible point of the tail; and tail moment is the amount of DNA in the tail and the average migration interval (Karlsson, 2010; Burlinson, 2012; Ghebretinsae et al., 2013). In this test, hydrogen peroxide (H_2O_2), which is one of the oxidative DNA damaging agents in cells, is used as a positive control (Benhusein et al., 2010). Thus, the genotoxic potential of boric acid and its antigenotoxic potential against H_2O_2 -induced DNA damage was investigated by the comet test for the first time.

The single application of boric acid showed a significant increase in the comet parameters in all the concentrations applied, while the two highest concentrations (1 and 2.5 $\mu\text{g/mL}$) were significant in the tail intensity contrasted with the negative control. This might result from the 1 h of treatment. Because repairing of DNA is known to start after 1 h of DNA damage. On the contrary, the degree of DNA damage induced by H_2O_2 was remarkably reduced by BA in terms of all the parameters compared to the positive control itself (Figure 5.1). In the study carried out by El-Hefny et al., (2020), the genotoxic effect of three preservative foods (Sodium Sulphite 100 mM, Boric Acid 100 mM, and Benzoic Acid 100 mM) was investigated using comet assay and SMART in *Drosophila melanogaster*. All three preservatives induced DNA damage in comet assay parameters (tail DNA, tail length, and tail moment). Since the genes of humans and *Drosophila* share a remarkable similarity, and both species have a large number of extensively preserved genes and pathways that regulate the formation of stress responses (Misra et al., 2011; Sykiotis and Bohmann, 2010), the authors stated that the tested substances may have genotoxic impact that cannot be ignored for humans (El-Hefny et al., 2020). Excessive dose of boron affects negatively the aquatic environment. Gülsoy et al., (2015) examined the genotoxic effect of boric acid and borax on Zebrafish using comet assay. Four concentrations (1, 4, 16, and 64 mg/L) were employed for 24, 48, 72, and 96 h. The result indicated that BA and BX augmented the DNA damage, which may be toxic to the erythrocytes of the fish. However, the majority of the research found that boron has no genotoxic but instead antigenotoxic effect on different human cells and rat tissues (Türkez, 2008; Üstündag et al., 2014; Ince et al., 2014).

Cengiz (2018) found oxidative stress and renal injury formed by cyclophosphamide (CPM) on rats' kidneys and the protective effect of BA. CPM is a typical immunosuppressive and anticancer drug that has side effects and causes damage to specific organs as any other kind of cancer chemotherapeutic drug. The administration of CPM caused a decrease in GPx, CAT, GSH, and SOD activities and raised NO and MDA levels. On the other hand, BA administration was found to diminish lipid peroxidation by boosting antioxidant activities (Cengiz, 2018).

Üstündag et al., (2014) postulated research about the defensive effect of BA in opposition to lead (Pb) and cadmium (Cd) on V79 cells using comet and micronucleus tests for sixteen hours. Pb and Cd are significant environmental toxins that have major consequences on human health. Pb and Cd toxicity is mainly related to the oxidative stress induced by

attaching to protein sulfhydryl groups and depleting glutathione. It was reported that the cells were preadministrated with low concentrations of boron (2.5 and 10 mM), which diminished the effect of Pb and Cd in MN frequency and DNA damage in the comet assay. This reduction was due to minimizing the free oxygen and ROS. Not only does BA decrease the genotoxic effect of Pb and Cd, but also hydrogen peroxide. BA has an ameliorating impact on V79 cell cultures in opposition to the induced damage of cadmium chloride and lead chloride. Our research findings correlated with these results.

As a way, nucleic acids are protected by BA from the damage of free radicals and ROS, which can be produced in the context of regular metabolic activity as well as after exposure to chemicals and pollutants. The literature suggests that boron can control the tissue imbalance between oxidants and antioxidants in afflicted tissue. By raising blood glutathione levels and lowering lipid peroxidation damage brought on by ROS, boron can enhance antioxidant activity (Demirer et al., 2012). Furthermore, specific metabolic parameters and endocrine systems can also be affected, as well as the immunological responses (Demirer et al., 2012; Ince et al., 2014).

The results obtained in this investigation demonstrated that in all the tests we applied, 0.25 µg/mL was more effective than 2.5 µg/mL among the doses applied regarding BA's protective effect against MMC. This effect might result from the highest dose (2.5 µg/mL) administrated, which may have exceeded the physiological limits.

BA is a rare element found in nature and has antioxidant characteristics, although BA's mechanism remains not entirely understood (Pawa et al., 2006). Boric acid primarily lowers ROS and free radicals. Furthermore, experimental research demonstrated that BA has ameliorating effects against genotoxicity, hepatotoxicity, oxidative stress, and cardiotoxicity (Henderson et al., 2009). In addition, BA treatment decreased the proliferation of prostate cancer cells by diminishing intercellular Ca^{2+} reserves and signaling, which are crucial for the development of apoptosomes. BA can have an indirect effect on apoptosis or the death cell. It also inhibits the inflammatory process by reducing intracellular ROS, $\text{TNF-}\alpha$, and Ca^{2+} and raising antioxidant levels, presumably by limiting GSH degradation (Sogut et al., 2018).

It is well recognized that many chemotherapeutic drugs, including MMC, elicit multiple adverse impacts by inducing hazardous responses in both cancerous cells and normal cells, which generate ROS and free radicals. Therefore, to prevent its production and stimulate the antioxidant defense system, BA can change the genetic and cytotoxic effect caused by toxic substances like anticancer drugs. After chemotherapy, BA could have a similar and ameliorative impact on cancer patients. However, specific ameliorating agents with chemotherapeutic drugs like MMC or other drugs inducing side effects should not interfere with the drug's anticancer activity. Thus, the interactions between BA and MMC are yet unclear in the *in vivo* studies (Pisano et al., 2003).

Within this research, boric acid decreased the frequency of chromosome aberration, sister chromatid exchange, cytokinesis block micronucleus, and hydrogen peroxide-induced DNA damage *in vitro* human lymphocytes. The results of this study can be utilized to establish safe daily boron intake amounts in cancer patients. However, more examination using other *in vitro* and *in vivo* test systems is recommended to fully comprehend the antigenotoxic mechanism of boric acid against mitomycin-C (Figure 5.1; 5.2).

Figure 5.1. General view of the effects of boric acid and BA+MMC applications in human lymphocytes

	Substance (boric acid)	Abnormal cell %	CA/Cell	SCE/Cell	MI (%)	MN
24H	MMC	↑	↑	↑	↓	
	0.25 µg/mL					
	0.5 µg/mL					
	1 µg/mL					
	2.5 µg/mL					
24H	Boric acid+MMC					
	0.25 µg/mL	↓	↓	↓		
	0.5 µg/mL	↓	↓	↓		
	1 µg/mL	↓	↓	↓		
	2.5 µg/mL	↓		↓		
48H	Boric acid					
	MMC	↑	↑	↑	↓	↑
	0.25 µg/mL					
	0.5 µg/mL					
	1 µg/mL					
	2.5 µg/mL			↑		
	Boric acid+MMC					
48H	0.25 µg/mL	↓	↓	↓		↓
	0.5 µg/mL	↓	↓	↓		↓
	1 µg/mL	↓	↓	↓		↓
	2.5 µg/mL	↓	↓	↓		↓

Figure 5.2. General view of the effects of boric acid and BA+ H₂O₂ applications in isolated lymphocytes

		Tail intensity	Tail length	Tail moment
Boric acid				
1 Hour	H ₂ O ₂	↑	↑	↑
	0.25 µg/mL		↑	↑
	0.5 µg/mL		↑	↑
	1 µg/mL	↑	↑	↑
	2.5 µg/mL	↑	↑	↑
Boric acid+H ₂ O ₂				
1 Hour	0.25 µg/mL	↓	↓	↓
	0.5 µg/mL	↓	↓	↓
	1 µg/mL	↓	↓	↓
	2.5 µg/mL	↓	↓	↓



Significant increase/decrease compared to negative control



Significant increase/decrease compared to positive control

6. CONCLUSION AND RECOMMENDATION

Cancer is a significant universal health concern and is the main reason of death globally. The World Health Organization (WHO) ranks cancer as the second most common cause of mortality. Its development is generally attributed to genomic instabilities induced by environmental toxins and mutagens. There are numerous types of cancer, each with unique characteristics and treatments. However, these treatment methods have not been able to significantly lower the cancer's impact and stop its rising occurrence. As a result of chemotherapy and radiotherapy applications, some cancer patients struggle with the side effects, which can even lead to the risk of a second cancer. Therefore, to reduce the risk of the reappearance of the disease, it is suggested the use of natural substances (such as boron, an antioxidant found in extracts of plants, foods, and in the earth's crust) which can protect the normal cells from the adverse effects of the chemotherapeutic agents. Administering the suitable concentrations at the suitable period will minimize the adverse effects of chemotherapeutic drugs and block the effect of environmental mutagens that suppress this process by interfering with the affected cells or triggering the initiation of carcinogenesis.

Among the natural substances providing antioxidant protection against certain drugs is boron and its derivatives. This element, with metals and nonmetals properties, has many uses in various fields. Depending on the doses ingested, it can either cause damage or protect the organisms against the side effects of drugs and toxic agents. In this research, a significant reduction was observed in the frequency of chromosome aberration, sister chromatid exchange, micronucleus, and DNA damage that might have resulted from the ameliorating effect of boric acid against damages induced by MMC, the alkylating agent, and H_2O_2 , an oxidant agent. MMC affects DNA synthesis, especially during the cell cycle's late G1 and S phases, suppresses cellular RNA and protein synthesis at high concentrations, and induces apoptosis and necrosis.

The protective effect of BA induced a decrease in chromosomal aberration, sister chromatid exchange, MN frequency, and comet parameters (tail intensity, length, and moment) against MMC and H_2O_2 . Although BA's antioxidant mechanism is still not entirely understood, it is a well-known part of enzymatic and cell membrane processes. This might result from its

power to boost tissue antioxidant capacity and the body's natural defenses against free oxygen radicals.

To summarize, this is the first study investigating boric acid's genotoxic and antigenotoxic potentials alone and simultaneously with MMC using CA, SCE, CBMN-Cyt, and comet tests in human lymphocytes. The results indicated that BA is able to prevent genomic damage caused by MMC and H₂O₂ in human peripheral lymphocytes *in vitro*. However, additional *in vitro* and *in vivo* studies should be carried out to understand the antigenotoxic mechanism of boric acid fully.

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GENİŞLETİLMİŞ TÜKÇE ÖZET

1. GİRİŞ

Kanser, iç (bağışıklık sistemi düzensizliği, kalıtsal mutasyonlar, hormonlar) veya dış (enfeksiyonlar, yaşam tarzı, mesleki) faktörlerden kaynaklanabilir. Bu faktörler, kanser gelişimini kademeli olarak başlatmak veya hızlandırmak için işbirliği içinde çalışabilir. Kanser, tek bir tedavi türüyle veya birkaç tedavinin kombinasyonu ile (kemoterapi, cerrahi, radyoterapi, hormon tedavisi, bağışıklık tedavisi ve hedef tedavisi gibi) tedavi edilebilir. Mitomisin-C, kanseri tedavi etmek için yaygın olarak kullanılan kemoterapötik ajanlardan biridir.

Mitomisin-C (MMC), *Streptomyces caespitosus* bakterisi tarafından üretilen doğal bir maddedir. MMC, bakterilerden izole edilen bir antineoplastik ve antibiyotiktir. Alkilleyici bir ajan olarak hareket eden MMC, DNA çapraz bağlarına neden olarak DNA ve RNA sentezini inhibe eder ve nükleer lizis yoluyla DNA'yı parçalar. Geç G1 ve erken S evrelerindeki hücrelerde daha etkilidir. Bu etkileri nedeniyle, pankreas, mesane, akciğer, mide, meme, rektum, rahim, serviks ve kolon kanseri dâhil olmak üzere bazı insan kanserleri ile mücadelede yaygın olarak kullanılmaktadır.

Kemoterapi sırasında malign ve normal hücreler ayırt edilemediğinden, MMC sağlıklı hücreler üzerinde bazı olumsuz etkilere neden olabilir. Kemoterapötik ajanlara uzun süre maruz kalmak, pro-onkojenik mutasyonlara ve sonuçta bir nesilden diğerine aktarılabilen genomik kararsızlıklara (transgenerasyonel genomik instabilite (GIN) sebep olabilir.

Reaktif oksijen türleri (ROS) ve serbest radikaller tarafından oluşturulan oksidatif DNA hasarı, kanser ve yaşlanma dâhil olmak üzere çeşitli hastalıkların gelişiminde çok önemlidir. Hidrojen peroksit (H_2O_2) oksidatif stresi indükleyebilir ve tek iplikçik kırılmalarına yol açan reaktif oksijen türleri ve serbest radikaller üreterek DNA'ya zarar verebilir. Ayrıca pürin ve pirimidinleri 8-hidroksiguanin gibi hidroksil türevlerine dönüştürür. Bu tür hasarların onarımındaki başarısızlıklar kanserogen etkilere sebep olabilir.

Bor (B), periyodik tablonun 5. elementidir ve 3A grubundaki tek ametaldir. B, silisyumdan sonra doğada en bol bulunan elementlerden biridir. Fiziksel ve kimyasal özellikleri

nedeniyle periyodik tabloda karbonun yanında yer alır. B dikkat çekici özelliklere sahiptir ve dünyada en yaygın kullanılan elementlerden biridir. B mineralleri ve ürünleri her geçen gün artan, sayısız uygulamaya sahiptir. Hayati açıdan önemli metabolik rollere sahip bir eser mineral olduğundan, hayvan, bitki ve insan sağlığı için gerekli bir elementtir. Türkiye, dünyanın en büyük bor rezervlerine (%73) sahiptir. Bor, kanser ve diğer hastalıklar için hedefe yönelik ilaç tedavilerinde kullanılmaktadır. Ayrıca pestisitlerde, antimikrobiyal ajanlarda, deterjanlarda, tekstil, nükleer, fiberglas ve havacılık endüstrilerinde çeşitli amaçlarla kullanılmaktadır.

Borun boraks, borik asit, üleksit, kolemanit ve sodyum perborat gibi çeşitli türevleri bulunmaktadır. Bunlardan borik asit ($\text{BA-H}_3\text{BO}_3$) renksiz, kokusuz ve kolay çözünebilen şeffaf kristal veya beyaz granüler toz, karbona benzer yapısal özelliklere sahip hafif organik Lewis asidi olup ağırlığı $1,43 \text{ gr/cm}^3$ 'tür. BA, tarım, ahşap koruma kimyasalları, hidrojen enerjisi depolama kimyasalları, cam, demir, çelik ve seramik endüstrileri ve hafif yapı malzemelerinin yapımı gibi birçok alanda kullanılmaktadır. Sağlıkta temizlik ürünü olarak ve kemik kırıklarının iyileştirilmesinde uygulanır. Birçok kozmetik ve kişisel bakım ürünlerinde ve gıdalarda kullanılmaktadır. Askeri teknolojilerde ve nükleer enerjide de kullanılmaktadır.

Çeşitli çalışmalar borik asidin genotoksik ve mutajenik olmadığını göstermiştir. Memeli test sistemlerindeki yürütülen genotoksisite çalışmalarının sonuçlarının tümünün negatif olduğu belirtilmektedir. Buna karşılık, hipolipidemik, anti-enflamatuar ve anti-kanserojenik etkilere sahip olabileceği ve hatta anti-kanser ilaçların ve bazı kimyasalların neden olduğu oksidatif stresin olumsuz etkilerini azalttığı bildirilmiştir. BA'nın paklitaksel (PAC-bir antikanser ilacı) tarafından tetiklenen kromozom aberasyonları, mikronükleus ve kardeş kromatid değişimlerini azaltmada etkili olduğu bulunmuştur. Tüm bu nedenlerden dolayı, BA ve bileşikleri çok ilgi uyandıran bir araştırma alanı olmaya devam etmekte ve potansiyel yararlarının ortaya çıkarılması için bilim insanlarının ilgisini çekmektedir.

Bu araştırmanın amacı, borik asidin tek başına genotoksik olup olmadığını incelemek ve ayrıca MMC (anti-kanser ilacı olarak kullanılan bir alkilliyici ajan) ve H_2O_2 (ROS ve serbest radikal oluşumu nedeniyle genotoksik etkiye neden olan bir bileşik) tarafından tetiklenen genotoksik hasarlara karşı olası antigenotoksik potansiyelini incelemektir. Bu nedenle, bu araştırmada borik asidin genotoksik ve antigenotoksik potansiyelleri insan lenfositlerinde *in*

vitro olarak Kromozomal Anormallik (KA), Kardeş Kromatid Değişimi (KKD), Sitokinez Blok Mikronükleus Sitomu (CBMN-cyt) ve Komet testleri kullanılarak ilk kez değerlendirilmiştir.

Bu araştırma ile günümüzde endüstriyel ve tıbbi alanlarda sıklıkla tercih edilen ve yaygın olarak kullanılan, insanların giderek daha fazla maruz kaldığı borik asidin herhangi bir genetik risk taşıyıp taşımadığı ve ayrıca genotoksik olduğu bilinen MMC ve H₂O₂'ye karşı olası antigenotoksik potansiyeli ilk kez değerlendirilmiştir.

2. GENEL BİLGİLER

2.1. Kanser

Kanser, vücuttaki hücrelerin kontrolsüz bir şekilde çoğalması ve vücutta yayılmasıyla tanımlanan genetik bir hastalıktır. Tümörler, doku kümeleri olan kanserli hücreler tarafından oluşturulur. Kanser hücreleri geliştikçe, ek değişiklikler de ortaya çıkmaktadır. Aynı tümör içindeki farklı hücrelerde farklı genetik değişiklikler de meydana gelebilir (National Cancer Institute, 2021b). Kronik hastalık, yaşlılık, kronik stres, kemoterapi kullanımı, analjezikler ve kortikosteroid benzeri ilaçların kullanımı gibi faktörler nedeniyle bağışıklık sistemi baskılanan kişilerde kanser riski artış göstermektedir (Roy ve Saikia, 2016).

Malign tümörler yakın dokulara yayılarak dokuları istila ederler. Ayrıca vücuttaki uzak dokulara da yayılabilir ve yeni tümörlerin oluşmasına neden olabilirler. Bu yayılma süreci metastaz olarak bilinir. Bu sebeplerden dolayı kanser, başta beyin olmak üzere birçok organda ciddi semptomlara neden olarak yaşamı tehlikeye atabilir. İyi huylu tümörler ise yakın dokulara yayılmaz veya istila etmez ve kaynaklandıkları bölgeden çıkarıldıklarında genellikle yeniden büyümmezler (National Cancer Institute 2021b).

Dünyada ikinci önemli ölüm nedeni olan kanser, ortalama yaşam süresinin artmasının önündeki en büyük engellerden biridir. İnsan vücudunu etkilediği bilinen 200'den fazla farklı kanser türü ve 500'den fazla kanserle ilişkili gen bulunmaktadır (Pavlopoulou ve diğerleri, 2015; WHO, 2021).

Kanserli hücreler genellikle sürekli bölünme yeteneği, büyümeyi engelleyici faktörlerden kurtulma, hücre ölümüne direnç, istila ve metastazı aktive etme, genomik kararsızlık ve mutasyon, bağışıklık sisteminden kaçma ve anjiyogenezi tetikleme gibi belli özelliklere sahiptir (Hanahan ve Weinberg, 2011; Koh ve diğerleri, 2019).

2.1.1 Kanserin nedenleri

Kanserin nedenleri veya etiyolojisi günümüzün en ilginç konularından biridir. Tümörlerin oluşumunu etkileyen birçok faktör vardır. En kapsamlı şekilde incelenen veya tanınan risk faktörleri genetik faktörler, genlerde mutasyon, bağışıklık koşulları, bulaşıcı organizmalar,

çevresel ve mesleki maruziyetler, kimyasallar, hava kirliliği, fabrikaları atıkları, iyonlaştırıcı radyasyon, tütün kullanımı, diyet faktörleri, kronik enfeksiyon, yaş ve aile öyküsüdür (Saini, Kumar ve diğerleri, 2020).

2.1.2. Kanser tedavisi

Yüzyıllar boyunca birçok kanser tedavisinin geliştiği ve halen gelişmekte olduğu bilinmektedir. Kanser tanısı konulduktan sonra tedavi şeklinin belirlenmesi, kanserin evresine bağlı olarak değişmektedir. Bazı kişiler tek tip tedavi ile iyileşirken, bazı kanser türlerinde evreye bağlı olarak birden fazla tedavi tercih edilmektedir. Kanser teşhisi konulan çoğu kişilere, cerrahi, radyasyon tedavisi, immünoterapi, hormon tedavisi, hedefe yönelik tedavi ve kemoterapi gibi farklı tedavi türlerinden oluşan kombine bir tedavi protokolü uygulanmaktadır (Saini, Kumar ve diğerleri, 2020; National Cancer Institute, 2022).

Cerrahi tedavi

Kanser tedavisinde kullanılabilen, tümörün vücuttan tamamen çıkarıldığı bir yöntemdir. Cerrahi, kanseri önlemek, teşhis etmek, evrelemek ve tedavi etmek için tercih edilebilir. Cerrahi ile tedavi edilen kanser türleri meme, özofagus, kolorektal ve beyin tümörleridir (Wyld ve diğerleri, 2015).

Radyasyon tedavisi

Radyoterapinin yüksek radyasyon dozlarında faydalı olduğu ve lokalize kanserlerin iyileşmesi için mevcut en aktif sitotoksik tedavi olduğu bilinmektedir (Schau and McBride, 2015). Radyasyon, normal dokularda bir hasar onarım sürecini uyarır. Tümör hücrelerini küçültmeye ve öldürmeye yardımcı olmak için kanser tedavisinin bir parçası olarak kullanılır ve bazı durumlarda tek başına da kullanılabilir. Radyoterapi, tedavi sırasında, tedaviden hemen sonra veya radyoterapi küründen sonraki birkaç gün içinde erken yan etkilere neden olabilir (Barazzuol ve diğerleri, 2020).

İmmünoterapi

Bu tedavi yönteminde canlı organizmalardan elde edilen maddeler (biyolojik tedavi ajanları) kullanılır. İmmünoterapi, kanser hücrelerine zarar vermek ve kanser hücrelerine saldıran beyaz kan hücrelerini aktive etmek için bağışıklık sistemini kullanır. Bu şekilde bağışıklık sistemi kanser hücrelerine saldırır ve onları yok edebilir. Birçok kanser türünü (mesane, rahim ağzı, kolorektal, sarkom ve yumurtalık) tedavi etmek için ve genellikle diğer kanser tedavileriyle birlikte kullanılır. Bununla birlikte, yorgunluk, baş dönmesi, kas ve eklem ağrıları, mide bulantısı ve kusma gibi bazı yan etkilere de neden olabilir, ancak bunların çoğu geçicidir (Riley ve diğerleri, 2019).

Hormonal tedavi

Hormonlar, organlar, deri, kaslar ve diğer dokular arasındaki farklı davranışları koordine etmek için vücut tarafından üretilen maddeler veya proteinlerdir. Bazı kanserlerin (rahim, hipofiz bezi tümörü, nöroendokrin tümör, tiroid kanseri) büyümek için hormonlara ihtiyaç gösterdiği bilinmektedir. Bu nedenle, hormonları önleyen veya değiştiren tedaviler nadiren bu kanserlerin büyümesini azaltmaya veya durdurmaya yardımcı olabilir (Amerikan Kanser Derneği, 2022). Hormon tedavisinin amacı, hormonları bir büyüme faktörü olarak kullanan malignitelerin büyümesini azaltmak veya durdurmaktır (Saini, Kumar ve diğerleri, 2020).

Hedefe yönelik tedavi

Bu tür kanser tedavisi, tümör hücrelerinin nasıl bölündüğünü, yayıldığını ve büyüdüğünü kontrol eden proteinleri hedef almak üzere tasarlanmıştır. Araştırmacılar kanseri oluşturan deoksiribonükleik asit (DNA) değişiklikleri ve proteinler hakkında bilgi sahibi oldukça, kansere neden olan DNA proteinlerini hedef alan tedavileri daha iyi tasarlayabilmektedir (Ulusal Kanser Enstitüsü, 2022). Bazı ilaçların normal hücrelere zarar vermeden kanser hücrelerini "hedef alacak" şekilde tasarlanmış olması, onları standart kemoterapi ilaçlarından ayıran en önemli özellik olarak bilinmektedir (American Cancer Society, 2022). Lenfoma, melanom ve kronik miyeloid lösemi gibi kanserler hedefe yönelik tedavi ile iyileştirilmektedir.

Kemoterapi

Kemoterapi, tümör hücrelerini anti-kanser ilaçlarıyla tedavi etmek ve çoğalmalarını, istila etmelerini, metastaz yapmalarını önlemek ve son olarak tekrarlama riskini azaltmak için kullanılmaktadır. Bu tedavi, hastalığın evresine bağlı olarak sırayla veya diğer tedavilerle (radyoterapi ve cerrahi) eş zamanlı olarak uygulanabilir (National Cancer Institute, 2022).

Kemoterapinin, gıda alımını azaltabilen ve aşırı kilo kaybına yol açabilen anemi, iştah kaybı, tat değişiklikleri, gıda sıcaklığına duyarlılıkta değişiklikler, kabızlık, ishal, disfaji, ağız kuruluğu, yorgunluk ve erken doyma gibi birçok yan etkisi vardır (Schirrmacher, 2019).

Kanserin kemoterapi ile tedavisinde sisplatin, siklofosfamid, dosetaksel, doksorubisin, adriamisin, epirubisin, flourourasil, metotreksat, paklitaksel ve mitomisin-C (MMC) gibi çeşitli ilaçlar kullanılmaktadır. Bunlar arasında MMC en çok kullanılan kemoterapötik ilaçtır (Siddique ve diğerleri, 2010; Yüzbaşıoğlu ve diğerleri, 2023; Mehmandoust ve diğerleri, 2022).

Bazı kanserlerin tedavisi için uygulanan bir ilaç olan Mitomisin-C, *Streptomyces caespitosus*'tan izole edilmektedir. Molekül ağırlığı 334 dalton'dur. Gastrointestinal sistem kanserleri, akciğer, mesane, rahim, boyun ve baş kanserleri ve kronik lösemi gibi çeşitli kanserlere karşı etkilidir (Kersey ve Vivian, 2008; Zhou ve diğerleri, 2022). Alkilleyici bir ajan olarak MMC, çift sarmalın zıt ipliklerinde DNA çapraz bağlanması oluşturur ve daha sonra DNA ipliği ayrılmasını durdurarak transkripsiyon ve replikasyonu bloke eder (Guo ve diğerleri, 2017). MMC, biyotransformasyon yollarına bağlı olarak, reaktif oksijen türleri (ROS) üretebilir. Bu ROS, hücre ile etkileşime girdiğinde ve endojen antioksidan koruma sistemlerinin düzeyini aştığında, hücredeki nükleik asitler, proteinler ve lipitler gibi biyolojik moleküllerde hasar oluşturur (Ortega-Gutierrez ve diğerleri, 2009; Yüzbaşıoğlu ve diğerleri, 2023). Bu hasarlar doğru şekilde onarılmazsa, genetik-genomik kararsızlık ortaya çıkabilir ve bu da alkali kararsız bölgeleri, tek iplik kırılmaları (SSB'ler) ve/veya çift iplik kırılmaları (DSB'ler) gibi DNA değişikliklerini ve çeşitli oksitlenmiş pürin ve pirimidin türlerine dönüşme olasılığını ve hatta kanser riskini artırır (Shay ve diğerleri, 2009). Kemoterapi sırasında tümör hücreleri ile normal hücreleri çok fazla ayırt edilemez, bu nedenle normal hücrelerde de yan etkiler ortaya çıkar. Kemoterapötik ilaçlara uzun süre maruz kalmak, pro-onkojenik mutasyonlara yol açabilen ve sonuçta ikincil kanser riskini

artıran ve hatta gelecek nesillere de aktarılabilen genomik kararsızlığa (transgenerasyonel genomik kararsızlık-GIN) neden olur (Zhang, 2020).

Kanser riskini en aza indirmek için (Williams, 2005), bilim insanları düzenli fiziksel egzersiz, az yağlı süt ürünleri, baklagilleri, tam tahılları, meyveleri, sebzeleri, kabuklu yemişleri ve tohumları vurgulayan bir beslenme düzeni önermektedir. Antioksidanların DNA'ya zarar veren maddelerle birlikte hücrelere uygulanması, serbest radikalleri, proteinlere, lipidlere ve DNA gibi makromoleküllere zarar vermeden önce ortadan kaldırılabılır. Ayrıca, antioksidanların, kemoterapinin antitümör etkilerini artırdığına ve kanserli hücrelerde apoptoza neden olduğuna, ancak sağlıklı hücrelerde böyle bir etki oluşturmadığına dair kanıtlar vardır (Drisko ve diğerleri, 2003).

2.5. Borik Asit [H_3BO_3]

Periyodik tabloda 3A (IIIA) grubunda yer alan ve B sembolü ile gösterilen bor, yarı metal özelliğine sahiptir. Atom numarası 5 ve atom ağırlığı 10,81 g/mol'dür (Ediz ve Özdağ, 2001). Türkiye, bor rezervleri açısından dünyada ilk sırada yer almakta (%73) ve üretimde belirli bir seviyeye ulaşmış durumdadır (Özkul ve diğerleri, 2017; Selimoğlu, 2018). Bor, hem insan hem de hayvan dokularında düşük konsantrasyonlarda bulunmaktadır. Bazı bitkiler ve hayvanlar için gerekli ve önemli bir mineraldir. Bor doğada en saf haliyle bulunmadığından, oksijene bağlanarak %98,4 oranında borik asit (H_3BO_3) ve %1,6 oranında borat formunda bulunur (Henderson ve diğerleri, 2009). Bor bileşikleri arasında en yaygın çözünebilir form borik asittir (BA). Yoğunluğu 1,43 gr/cm³ olan bir Lewis asididir. BA, suda kolayca çözünen renksiz veya şeffaf-beyaz kristal görünümlü bir granüldür (Bhami ve Das, 2015; Ghanizadeh ve diğerleri, 2020).

Borik asit, çeşitli amaçlarla ve birçok endüstriyel alanda kullanılan en önemli bor bileşiğidir, bor hem insan hem de hayvan sağlığı için gereklidir ve insanlarda aşırı maruziyet toksik etkilere neden olabilir, ancak konsantrasyona ve süreye bağlı olarak bazı toksik kimyasallara karşı zararlı (genotoksik) veya koruyucu (antigenotoksik) olabilir.

Bor ve borik asidin genotoksik ve koruyucu etkileri üzerine yapılan araştırmalardan elde edilen tüm bu sonuçlar nedeniyle, bu çalışmada borik asidin herhangi bir genotoksik etkisinin olup olmadığı ve/veya insan lenfositlerinde kemoterapötik ajan ve oksidan

kimyasala karşı antijenotoksik koruma sağlayıp sağlayamayacağının kromozom aberasyonları, kardeş kromatid değişimleri, sitokinez blok mikronükleus sitom ve komet testleri kullanılarak araştırılması amaçlanmıştır.

3. MATERİYAL VE METOT

3.1. Materyal

3.1.1. Kromozom incelemesi için materyal

Bu çalışmada, BA'nın genotoksik potansiyeli ve Mitomisin-C'ye (MMC) karşı antigenotoksik etkisi insan lenfositlerinde *in vitro* olarak incelenmiştir. Periferik kan, 22-30 yaşları arasında, herhangi bir sağlık sorunu olmayan, sigara ve alkol kullanmayan, son üç ay içinde herhangi bir ilaç kullanmayan ve gönüllü onam formunu imzalayan 3 donörden (kadın) elde edilmiştir. Bu araştırma Gazi Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu tarafından onaylanmıştır (Tarih: 06.03.2023, No: 10).

3.1.2. Test materyalleri ve kullanılan diğer kimyasallar

Borik asit

Borik asit (H_3BO_3) Eti Maden'den (Prof. Dr. H. İbrahim Ünal, Kimya Bölümü, Gazi Üniversitesi, Ankara) temin edilmiştir. Cas No 10043-35-3, kimyasal formülü H_3BO_3 ve molekül ağırlığı 61,83 g/mol'dür. BA'nın yapısal formülü Şekil 3.1'de verilmiştir (sayfa 27).

Mitomisin-C (MMC)

Streptomyces caespitosus'tan izole edilen MMC, kemoterapötik etkiye sahip alkilleyici bir ajandır. Ticari olarak Sigma'dan temin edilmiştir. Mitomisin-C: 6-amino-4,7-diokso-8-metoksi-5-metil-1,1,2,8,8-hekzahidroazirin [2,3] pirolo [1,2] indol-8-metilkarbamat. Kimyasal formülü $C_{15}H_{18}N_4O_5$, Cas No 50-07-7 ve molekül ağırlığı 334,33 g/mol'dür. Yapısal formül Şekil 3.2'de gösterilmiştir (sayfa 28).

Hidrojen peroksit (H_2O_2)

Bu çalışmada kullanılan hidrojen peroksit (%35), tüm organik ve inorganik bileşikleri oksitleyebilen ve özellikle DNA'da ciddi hasara yol açabilen bir bileşiktir. Yapısal formülü

Şekil 3.3'te verilmiştir. Molekül formülü H_2O_2 ve molekül ağırlığı 34,01 g/mol'dür. Cas No: 7722-84-1. Ticari olarak Applichem'den temin edilmiştir (sayfa 28).

Çalışmada kullanılan diğer kimyasallar

Kromozom besiyeri LymphoPlus (CAS No: CY100-100) Cegrogen Biotech'ten, Metil alkol (Cas No: 67-56-1), Glacial asetik asit (CAS No: 64-19-7), Giemsa (CAS No: 1092040500), Entellan (CAS No: 1079610100), KCl (CAS No: 7447-40-7), Formaldehit (CAS No: 50-00-0) ve Tampon tablet pH 6. 8 (CAS No: 111374) Merck'ten, Bromodeoxyuridine (CAS No: 59-14-3), Colchicine (CAS No: 64-86-8) ve Cytochalasin-B (CAS No: 14930-96-2) Sigma'dan ticari olarak temin edilmiştir. Diğer kimyasallar teknik formdadır.

3.2. Metot

Bu araştırmada, insan lenfositlerinde *in vitro* olarak Kromozomal Anormallik, Kardeş Kromatid Değişimi ve Sitokinez-Blok Mikronükleus Sitom (CBMN-Cyt) testleri ve izole lenfositlerde *in vitro* olarak komet testi gerçekleştirilmiştir.

3.2.1. Çalışmada kullanılan konsantrasyonların belirlenmesi

Kullanılacak konsantrasyonları belirlemek için yapılan literatür taramalarında, borik asit için elde edilen letal doz (LD_{50}) değerleri Çizelge 3.1'de verilmiştir (sayfa 29).

Çeşitli konsantrasyonlar (2,5, 5, 10 ve 20 $\mu\text{g/mL}$) kullanılarak BA'in mitotik indeks üzerindeki etkisini belirlemek için bir ön deneme uygulanmıştır. Elde edilen sonuçlara dayanarak, 0,25, 0,5, 1 ve 2,5 $\mu\text{g/mL}$ 'lik dozlar, kullanılacak en uygun konsantrasyonlar olarak belirlenmiştir.

3.2.2. Test materyallerinin insan lenfositlerine uygulanması

Kromozomal aberasyon ve kardeş kromatid değişimi testleri

Kromozom aberasyonları testi Evans ve arkadaşlarının (1984) tekniği birkaç değişiklikle takip edilerek uygulanmıştır (Yüzbaşıoğlu, Çelik, Yılmaz, Ünal ve Aksoy, 2006, Akbaş ve

diğerleri, 2022). Kardeş kromatid değişim testi için Perry ve Wolfun (1974) yöntemi bazı modifikasyonlarla (Speit ve Haupter, 1985; Yüzbaşıoğlu, Çelik, Yılmaz, Ünal ve Aksoy, 2006, Akbaş ve diğerleri, 2022) uygulanmıştır. Heparinli enjektör kullanılarak gönüllülerden alınan 0,2 mL periferik kan, 2,5 mL LymphoPlus besiyeri bulunan kültür tüplerine eklenmiştir. Aynı kültüre 50 µg/mL 5-Bromo-2-deoxyuridine=Bromodeoxyuridine (BrdU) eklenmiş ve 72 saat boyunca 37°C'de kültüre bırakılmıştır.

İnsan peripheral lenfositleri, hem borik asit (0,25, 0,5, 1 ve 2,5 µg/mL) ile tek başına hem de bir mutajen olan MMC (0,20 µg/mL) ile eş zamanlı olarak (BA+MMC) (BA-0,25, 0,5, 1 ve 2,5 µg/mL+MMC) muamele edilmiştir. Bunlar kültürün son 24 ve/veya 48 saatinde her tüpe ayrı ayrı eklenmiştir. Ayrıca, negatif (distile su) ve pozitif (0,20 µg/mL MMC) kontrol de eklenmiştir. Kolşisin, her bir tüpe, inkübasyonun 70. saatinde 0,06 µg/mL konsantrasyonda eklenmiştir. İnkübasyonun 72. saatinde kültürler sonlandırılmıştır. Hücre kültürleri 1.100 rpm'de 10 dakika santrifüj edilmiş, ardından dip kısma 5 mL 0,075 M KCl çözeltisi eklenmiştir. Kültürler 10 dakika boyunca 1.100 rpm'de santrifüj edilmiş ve sonra 37°C'de (20 dk) inkübe edilmiştir. Lenfositler daha sonra 3:1 metanol: asetikte üç kez fikse edilmiştir. Süspansiyondaki hücreler homojenize edilerek lam üzerine yayılmış ve oda sıcaklığında kurutulmuştur (Basri İla ve Tahir Hüsnet, 2022 : 153,184; Çelik, 2022 : 185, 217).

Sitokinez-blok mikronükleus sitom testi

Fenech'in (2000) mikronükleus testi birkaç modifikasyonla gerçekleştirilmiştir (Kirsch-Volders ve diğerleri, 2011). Donörlerden alınan periferik kan örnekleri (0,2 mL) 2,5 mL LymphoPlus içeren kültür tüplerine eklenmiştir. Kültürler 37°C'de 72 saat inkübe edilmiştir. Kültürün 24. saatinde lenfositler çeşitli dozlarda borik asit (BA) (0,25, 0,5, 1 ve 2,5 µg/mL) ile hem tek başına hem de MMC (0,20 µg/mL) ile eş zamanlı olarak (BA+MMC) (BA-0,25, 0,5, 1 ve 2,5 µg/mL+MMC) muamele edilmiştir. Muamele son 48 saat boyunca uygulanmıştır. Sitokinezisi durdurmak için inkübasyonun 44. saatinde kültüre sitokalsin-B (5,2 µg/mL) ilave edilmiştir. Her uygulamada ayrıca bir negatif ve pozitif (MMC-0,20 µg/mL) kontrol eklenmiştir. Kültürün sonunda tüpler santrifüj edilmiş (1.000 rpm) ve süpernatant atılmıştır. Ardından hipotonik çözelti (0,075 M KCl) eklenmiştir. Santrifüjün ardından hücreler soğuk fiksatif içinde üç kez fiksasyona tabi tutulmuştur. Son fiksasyon

işlemine formaldehit eklenmiştir. Tüpte kalan hücre süspansiyonu lam üzerine yayılarak oda sıcaklığında kurumaya bırakılmıştır (Yüzbaşıoğlu ve Avuloğlu-Yılmaz, 2022: 219, 249).

Preparatların boyanması

KA, KKD ve MI'yı belirlemek için lamlar Sorensen tamponunda %5 Giemsa (pH 6,8) ile (5-10 dakika) boyanmıştır. Mikronükleusu değerlendirmek için lamlar 1-3 dakika boyanmıştır.

Kardeş kromatid değişimlerinin gözlenmesi için 1985 yılında Speit ve Haupter tarafından geliştirilen test bazı modifikasyonlarla uygulanmıştır. Bu uygulamada, Sorensen tamponu (pH 6,8) içindeki lamlar 254 nm'de 13 dakika ışınlanmış ve 1xSSC (saline sodium citrate buffer) solüsyonunda 58-60°C'de 1 saat bekletildikten sonra %5 Giemsa (pH 6,8) ile 5-10 dakika boyanmıştır. Tüm lamlar entellan ile kalıcı hale getirildikten sonra mikroskopta taranmıştır.

Kromozom anormallikleri ve mitotik indeksin saptanması

Kromozomal anormalliklerini tespit etmek amacıyla, her bir uygulama ve kontrol grubu için iyi dağılmış kromozomlara sahip hücreler değerlendirilmiştir. Toplamda 300 (her donörden 100) metafaz analiz edilmiştir. Tüm uygulama ve kontrol gruplarında, anormal hücrelerin frekansı ve hücre başına düşen kromozom anormalliği sayısı (KA/Hücre) tespit edilmiştir. Mitotik indeksi belirlemek için her uygulama ve kontrol grubundan toplam 3.000 (donör başına 1.000) hücre analiz edilmiştir. Mitotik indeks, bölünen hücre sayısının toplam hücre sayısına yüzde cinsinden oranı ile belirlenmiştir (Basri İla ve Tahir Hüsnet, 2022: 153,184; Çelik, 2022: 185, 217).

Kardeş kromatid değişimi ve replikasyon indeksinin saptanması

Hücre başına düşen kardeş kromatid değişim sayısını değerlendirmek için üç donörden toplam 75 (her bir donörden 25) hücre incelenmiştir. Bu değerlendirmede ikinci mitozu geçiren hücreler kullanılmıştır. KKD'ni gözlemek için kültür ortamına önceden BrdU eklenmiştir. KKD'leri, bir kromozomun açık boyalı kromatidindeki koyu boyalı segmentler sayılarak veya bunun tersi uygulanarak hesaplanmıştır.

Replikasyon indeksini (Rİ) belirlemek için, her bir preparatta toplam 300 hücre (100 hücre/donör) incelenmiştir. Bunlar arasından birinci (M1), ikinci (M2) ve üçüncü (M3) mitoz evresindeki hücreler belirlenmiş ve Rİ aşağıdaki formül kullanılarak elde edilmiştir (Çelik, 2022: 185, 217).

$$Rİ = [1x(M1) + 2x(M2) + 3x(M3)]/N$$

M₁: Birinci metafazdaki hücre sayısı

M₂: İkinci metafazdaki hücre sayısı

M₃: Üçüncü metafazdaki hücre sayısı

N: İncelenen toplam hücre sayısı

Mikronükleus frekansı, nükleer bölünme indeksi ve sitokinez blok proliferasyon indeksinin değerlendirilmesi

Mikronükleus frekansını belirlemek için, çekirdek bölünmesini tamamlayan ancak sitoplazmik bölünmeyi tamamlayamayan binükleat hücreler değerlendirilmiştir. Bunun için donör başına 1.000 binükleat hücre (toplam 3.000 binükleat/3 donör) incelenmiştir. Hücre başına düşen MN sayısı (MN/hücre) da her uygulama için belirlenmiştir. Her bir uygulama için toplam 1.500 hücreden (500 hücre/donör) nükleer bölünme indeksi (NBI) ve sitokinez-blok proliferasyon indeksini (CBPI) hesaplamak için aşağıdaki formüller kullanılmıştır (Yüzbaşıoğlu ve Avuloğlu-Yılmaz, 2022: 219, 249).

$$NBI = [(1xN1) + (2xN2) + (3xN3) + (4xN4)]/N$$

N1-N4 sırasıyla 1-4 çekirdekli hücre sayısı

$$CBPI = [1x(1MN) + 2x(2MN) + 3x(3MN) + 4x(4MN)]/N$$

N: İncelenen toplam hücre sayısı

KA'ların ve KKD'lerin mikroskopik incelemesi için X1.000 büyütme kullanılmıştır. Her donör için toplam 1.000 binükleat hücre değerlendirmek için X400 büyütme kullanılmıştır. Hücrelerin incelenmesinde, Olympus ışık mikroskobu (CX23) kullanılmıştır.

Kromozom anormalliklerinin ve kardeş kromatid değişiminin görüntülerini elde etmek için CCD kamera takılı Leica-DM-LB2 mikroskobu (X1.000 büyütme) kullanılmıştır. MN analizleri için X400 büyütme kullanılmıştır.

3.2.3. İzole insan lenfositlerinde yapılan çalışmalar

Komet testi

Bu çalışmada, izole edilen insan peripheral lenfositlerinde, hem borik asitin tek başına (0,25, 0,5, 1 ve 2,5 µg/mL) uygulamasının ve hem de bir oksidan olan H₂O₂ ile birlikte eş zamanlı (BA+H₂O₂) (BA-0,25, 0,5, 1 ve 2,5 µg/mL+MMC) uygulamalarının, sırasıyla DNA hasarı oluşturu ve bu hasarı iyileştirici potansiyelini belirlemek için komet testi de uygulanmıştır.

Komet testi, Singh ve arkadaşlarının (1988) tekniğinde bazı modifikasyonlar yapılarak uygulanmıştır (Ünal, Saygılı ve Akbaş, 2022: 313, 340). Lenfositler, ayırma ortamı kullanılarak sağlıklı ve gönüllü donörlerden elde edilen kan örneklerinden izole edilmiştir. İzole lenfositler, hem borik asitin dört farklı konsantrasyonu (0,25; 0,5; 1 ve 2,5 µg/mL) ile tek başına (BA) ve hem de dört farklı konsantrasyon BA + H₂O₂ (100 µM) (BA+H₂O₂) ile eş zamanlı şekilde, 37°C'de bir saat muamele edilmiştir. Uygulamadan sonra, lenfositlerin hücre canlılığı tripan mavisini dışlama testi kullanılarak ≥%97 olarak tespit edilmiştir. Deneylerde, bir negatif (distile su) ve bir de pozitif (100 µM H₂O₂) kontrol grupları da bulundurulmuştur. İnkübasyon sonrasında, santrifüjden geçirilen hücreler PBS ile yeniden süspanse edilmiştir. Lenfositler, düşük erime ısıyla agar ile karıştırılarak, önceden yüksek erime ısıyla agar ile kaplanmış lamlara yayılmıştır. Lamlar buzdolabında 1 saat lizis solüsyonunda bekletilmiştir. Takiben elektroforez tamponunda 20 dakika bekletilen lamlara, 25 V, 300 mA'de 20 dakika elektroforez uygulanmıştır. Elektroforez sonrası nötralizasyon tamponuna daldırılan lamlar EtBr ile boyanarak incelenmiştir.

Hücreleri incelemek için X400 büyütmede floresan mikroskobu (Olympus BX51) (546 nm filtre ve 590 nm bariyer filtresi ile) kullanılmıştır. DNA hasarını belirlemek, her kontrol ve uygulama konsantrasyonundan toplam 300 komet (her donör için 100 komet; her lamdan 50 komet) özel bir Görüntü Analiz Sistemi (Comet Assay IV, Perceptive Instruments Ltd., UK) kullanılmıştır. Bu program ile komet kuyruk uzunluğu (µm), kuyruk yoğunluğu (%) ve kuyruk momenti değerlendirilmiştir. Komet görüntüleri, aynı mikroskopta, X400 büyütmede, bir CCD kamera ile fotoğraflanmıştır.

3.2.4. İstatistiksel analizler

Bu tez çalışmasında, IBM SPSS 23.0 Statistics One-Way ANOVA (post-Tukey) kullanılarak uygulama ve kontrol gruplarından elde edilen anormal hücre frekansı, KA/Hücre, mitotik indeks, KKD/Hücre, MN/Hücre, nükleer tomurcuk, nükleoplazmik köprü (NPB), kuyruk uzunluğu, kuyruk yoğunluğu ve kuyruk momenti için doz-etki ilişkisi ortaya konmuştur. Replikasyon indeksi, sitokinez blok proliferasyon indeksi ve nükleer bölünme indeksi sonuçları z-testi ile değerlendirilmiştir.

4. ARAŞTIRMA BULGULARI

4.1. Borik Asitin Genotoksik ve Mitomisin-C ile İndüklenen Genetik Hasara Karşı Antigenotoksik Etkilerinin Belirlenmesi

4.1.1. Kromozom aberasyonu testi sonuçları

İnsan lenfositlerinde, borik asitin dört farklı konsantrasyonu (0,25; 0,5; 1 ve 2,5 µg/mL) ile tek başına ve BA + Mitomisin-C (0,20 µg/mL)'in 24 ve 48 saat süreyle eş zamanlı uygulamaları sonucunda gözlenen anormal hücre frekansı, hücre başına düşen kromozom anormalliğinin düzeyi ve kromozom anormalliği tipleri Çizelge 4.1'de verilmiştir (sayfa 36).

24 saatlik uygulama

MMC ile muamele edilen lenfositlerde anormal hücre frekansı ve KA/Hücre oranı, negatif kontrole kıyasla anlamlı şekilde artmıştır (Çizelge 4.1). Ancak, borik asitin tek başına uygulaması, anormal hücre frekansı ve KA/hücre düzeyinde, negatif kontrole kıyasla anlamlı olmayan bir artış meydana gelmiştir. Diğer yandan, borik asidin MMC ile eş zamanlı olarak uygulanması, pozitif kontrole kıyasla anormal hücre frekansı ve KA/Hücre düzeyinde (2,5 BA+MMC hariç) anlamlı düşüş tespit edilmiştir. Bu azalmalar, konsantrasyon artışıyla düşük düzeyde bağlantılı olup, sırasıyla $r=-0,46$ ve $r=-0,49$ 'dur (Çizelge 4.1).

MMC beş farklı yapısal kromozom anormalliği oluşturmuştur. Bunlar kromatid ve kromozom kırığı, fragment, disentrik kromozom ve kardeş kromatidlerde birleşmedir. Ayrıca bir tip sayısal anormallik (poliploidi) oluşturmuştur. Kromatid kırığı, en yaygın gözlenen yapısal anormallik tipidir. Borik asitin tek başına muamelesi sonucunda yedi tip yapısal anormallik oluşmuştur. Kromatid kırığı, en yaygın oluşan anormallik tipidir. Bunu takiben, fragment, kromozom kırığı, kardeş kromatidlerde birleşme, disentrik kromozom, kromatid değişimi (sadece 1 µg/mL'de) ve halka kromozom (sadece 0,25 µg/mL'de) oluşmuştur (Çizelge 4.1). Sayısal anormalliklerden poliploidi de BA'nın tüm konsantrasyonlarında gözlenmiştir (Çizelge 4.1). BA+MMC eş zamanlı uygulamasında, altı farklı yapısal anormallik gözlenmiştir. Bu anormallik tipleri kromatid ve kromozom kırığı, disentrik kromozom, fragment, kromatid değişimi ve kardeş kromatidlerde birleşmedir (sadece 0,25 BA+MMC'de). Poliploidi uygulanan tüm konsantrasyonlarda gözlenirken,

endoreduplikasyon sadece 0,5 BA+MMC'de tespit edilmiştir. Negatif kontrolde ise oldukça az sayıda kromatid ve kromozom kırığı, fragment ve kardeş kromatidlerde birleşme gözlenmiştir (Çizelge 4.1).

48 saatlik uygulama

MMC, 48 saatlik uygulamada, anormal hücre frekansı ve hücre başına düşen anormallik sayısını negatif kontrole göre anlamlı düzeyde artırmıştır. Borik asitin tek başına uygulamaları, tüm konsantrasyonlarda anormal hücre frekansı ve KA/Hücre sayısını hafifçe artırmıştır ancak bunların hiçbiri anlamlı değildir. Diğer taraftan, MMC ile eş zamanlı uygulamada BA'nın tüm konsantrasyonları, anormal hücre frekansı ve KA/Hücre sayısını pozitif kontrole kıyasla önemli ölçüde azaltmıştır. Buradaki azalma, konsantrasyon artışı ile düşük bir korelasyon göstermiştir (sırasıyla $r=-0,46$ ve $r=-0,43$) (Çizelge 4.1).

Mitomisin-C, kromatid ve kromozom kırığı, kardeş kromatidlerde birleşme, disentrik kromozom, fragment, kromatid değişimi ve halka kromozom olmak üzere yedi farklı yapısal kromozom anormalliği ve tek tip sayısal anormalliğe (poliploidi) neden olmuştur (Çizelge 4.1). Borik asidin tek başına uygulaması da kromatid ve kromozom kırığı, fragment, kardeş kromatidlerde birleşme ve disentrik kromozom olmak üzere beş çeşit yapısal anormalliğe neden olurken, sayısal bir anormallik olan poliploidi 2,5 $\mu\text{g/mL}$ hariç diğer tüm konsantrasyonlarda gözlenmiştir (Çizelge 4.1). Borik asit ile MMC'nin eş zamanlı uygulamasında da beş çeşit yapısal anormallik gözlenmiş olup, kromatid kırığı en sık görülen anormallik olmuş, bunu kromozom kırılması, fragment, kardeş kromatid birleşmesi ve halka kromozom (sadece 1 BA+MMC'de) izlemiştir.

4.1.2. Kardeş kromatid değişimi test sonuçları

Borik asitin dört farklı konsantrasyonu (0,25; 0,5; 1 ve 2,5 $\mu\text{g/mL}$) ile tek başına ve BA + Mitomisin-C (0,20 $\mu\text{g/mL}$)'in 24 ve 48 saat süreyle eş zamanlı uygulamaları sonucunda insan lenfositlerinde oluşturduğu kardeş kromatid değişimleri, mitotik indeks ve replikatif indeks üzerindeki etkileri Çizelge 4.2'de gösterilmiştir (sayfa 44).

24 saatlik uygulama

24 saatlik uygulamayı takiben, MMC, hücre başına düşen KKD frekansını negatif kontrole kıyasla anlamlı düzeyde artırmıştır (Çizelge 4.2). MMC, hücre başına 2-18 kardeş kromatid değişimi oluşturmuştur. Borik asitin tek başına uygulaması, insan lenfositlerinde 1-11 KKD/Hücre sayısı oluşturmuş ve KKD/Hücre düzeyini doz artışına bağlı olarak hafifçe artırmıştır. Ancak, bu artışların hiç biri, negatif kontrole kıyasla istatistiksel olarak anlamlı değildir (Çizelge 4.2). Buna karşılık, borik asit ve MMC'nin birlikte muamelesinde, pozitif kontrole kıyasla, uygulanan bütün konsantrasyonlarda KKD/Hücre'de istatistiksel olarak anlamlı bir azalma meydana gelmiştir. MMC tarafından oluşturulan KKD/Hücre düzeyinin azaltılmasında borik asitin en etkili konsantrasyonu 0,25 µg/mL olmuştur (Çizelge 4.2). Ancak, gözlenen azalmalar konsantrasyona bağlı değildir ($r=-0,32$). BA+MMC uygulamalarını takiben bazı lenfositlerde 14'e kadar varan KKD tespit edilmiştir.

Pozitif kontrol MMC, mitotik indekste, negatif kontrole kıyasla anlamlı düşüşe sebep olmuştur. Borik asitin tek başına uygulanan bütün konsantrasyonları, Mİ'i, negatif kontrole kıyasla bir miktar düşürmekle birlikte, bunlardan hiç biri anlamlı değildir. BA+MMC eş zamanlı uygulamaları da Mİ'i anlamlı şekilde değiştirmemiştir. Replikasyon indeksi de, ne MMC, ne BA ve ne de BA+MMC uygulamalarında istatistiksel açıdan anlamlı bir değişim oluşturmamıştır (Çizelge 4.2).

48 saatlik uygulama

Lenfositlerde, MMC negatif kontrolün aksine, KKD/Hücre sayısını anlamlı ölçüde artırmıştır (Çizelge 4.2). MMC hücre başına 4-31 KKD oluşumuna neden olmuştur. Diğer yandan, borik asitin tek başına uygulamasının ardından 0-17 arasında KKD oluşumu gözlenmiştir. Borik asit tarafından oluşturulan KKD/Hücre düzeyi, negatif kontrole kıyasla, 0,25, 0,5 ve 1 µg/mL'de hafifçe artarken, 2,5 µg/mL'de önemli bir artış göstermiştir. Ancak bu artış konsantrasyon artışına bağlı değildir ($r=0,20$) (Çizelge 4.2). BA+MMC'nin eş zamanlı uygulamasında, 20'ye kadar KKD oluşumu gözlenirken (Çizelge 4.2), pozitif kontrole kıyasla, KKD/Hücre oranı, tüm BA+MMC uygulamalarında anlamlı şekilde azalmıştır. Ancak bu düşüşler konsantrasyona bağlı değildir ($r=-0,25$). KKD/Hücreyi azaltmada en etkili borik asit dozu 0,25 µg/mL olmuştur (Çizelge 4.2).

Pozitif kontrol MMC, mitotik indeksi, negatif kontrole kıyasla anlamlı şekilde düşürmüştür. Borik asitin tek başına uygulamaları, Mİ'i, negatif kontrole kıyasla bir miktar düşürmekle birlikte, bu düşüşlerin hiç biri anlamlı değildir. Benzer şekilde, BA+MMC eş zamanlı uygulaması da Mİ'te, pozitif kontrole kıyasla anlamlı bir değişiklik oluşturmamıştır. Rİ'te, pozitif kontrol, BA tek başına ve BA+MMC uygulamalarının hiç birinde anlamlı bir değişim gözlenmemiştir (Çizelge 4.2).

4.1.3. Sitokinez-blok mikronükleus sitom testi sonuçları

İnsan lenfositlerinde, borik asitin dört farklı konsantrasyonu (0,25; 0,5; 1 ve 2,5 µg/mL) ile tek başına ve BA + Mitomisin-C (0,20 µg/mL)'in 48 saat süreyle eş zamanlı uygulamaları sonucunda oluşturduğu mikronükleus, nükleoplazmik köprü (NPK), nükleer tomurcuk (NT), sitokinez blok proliferasyon indeksi ve nükleer bölünme indeksi üzerindeki etkileri Çizelge 4.3'te verilmiştir (sayfa 50).

MMC, negatif kontrole kıyasla MN frekansını anlamlı düzeyde ($p<0,05$) artırmıştır. Borik asitin tek başına uygulamaları, mikronükleuslu hücrelerin frekansında hafif bir artış oluşturmakla beraber, bunların hiçbiri negatif kontrole göre istatistiksel olarak anlamlı değildir. Diğer yandan, tüm BA+MMC konsantrasyonlarının eşzamanlı uygulamasında pozitif kontrole kıyasla MN frekansında istatistiksel olarak anlamlı bir azalma tespit edilmiştir. Bu azalma zayıf düzeyde konsantrasyona bağlıdır ($r=-0,54$).

Lenfositlerde, negatif kontrole kıyasla, MMC, NT frekansında önemli bir artışa neden olmuştur. Borik asit ile tek başına muamele, negatif kontrole kıyasla NT sıklığını belirgin şekilde artırmamıştır. Buna karşılık, BA+MMC uygulamaları, tüm konsantrasyonlarda, MMC'ye karşı NT frekansını azaltmıştır, ancak bunların hiçbiri anlamlı değildir. Bununla birlikte, ne MMC ne de MMC ile birlikte BA'nın tek ve eş zamanlı uygulaması nükleoplazmik köprü frekansı, nükleer bölünme indeksi ve sitokinez-blok proliferasyon indeksini etkilememiştir (Çizelge 4.3).

4.1.4. Komet testi sonuçları

Borik asitin dört farklı konsantrasyonu (0,25; 0,5; 1 ve 2,5 µg/mL) ile tek başına ve BA + H₂O₂ (100 µM)'in 1 saat süreyle eş zamanlı uygulamaları sonucunda insan lenfositlerinde,

DNA'da oluşturduğu hasarlar, üç farklı parameter kullanılarak tespit edilmiştir. Bu parametreler komet kuyruk yoğunluğu (%DNA), kuyruk uzunluğu (μm) ve kuyruk momentidir. Elde edilen sonuçlar Çizelge 4,4'te gösterilmiştir (sayfa 53).

Hidrojen peroksit (H_2O_2) tek başına uygulandığında, lenfositlerde, DNA hasarını, negatif kontrole kıyasla anlamlı düzeyde artırmıştır. Borik asidin 1 saatlik tek başına uygulaması, negatif kontrole kıyasla kuyruk yoğunluğunu (0,25 ve 0,5 $\mu\text{g/mL}$ hariç) ve kuyruk uzunluğunu anlamlı şekilde artırmıştır. Kuyruk momenti de negatif kontrole kıyasla hafif ama önemli ölçüde artmıştır. BA + H_2O_2 eş zamanlı uygulaması, borik asidin tüm konsantrasyonlarında, pozitif kontrole kıyasla, H_2O_2 tarafından indüklenen DNA hasarında kayda değer bir azalma oluşturmuştur. Lenfositlerde, her üç parametrede de (kuyruk yoğunluğu, kuyruk uzunluğu ve kuyruk momenti) azalma, anlamlı olmuştur. Bu azalma kuyruk yoğunluğu, kuyruk uzunluğu ve kuyruk momentinde konsantrasyona bağlı değildir (sırasıyla $r=-0,22$, $r=-0,25$ ve $r=-0,11$). (Çizelge 4.4).

5. TARTIŞMA

Kanser, hücresel genomdaki değişikliklerin neden olduğu, küresel ölüm oranı yüksek, insan sağlığına yönelik ciddi bir tehdittir, ayrıca karmaşık ve multifaktöriyel bir hastalıktır. Kanser, hücrelerin hızlı ve kontrolsüz bir şekilde çoğaldığı ölümcül bir hastalıktır. Gelişmiş ülkelerde ikinci en yaygın ölüm nedeni olarak yer almaktadır. İnsan vücudunu etkilediği bilinen 200'den fazla farklı kanser türü ve 500'den fazla kanserle ilişkili gen bulunmaktadır (Pavlopoulou ve diğerleri, 2015; Cao, ve diğerleri, 2021).

Kanserin önlenmesi ve tedavisinde büyük ilerlemeler kaydedilmiş olmasına rağmen, birçok kanser türü hala tedavi edilemez ve ölümlü sonuçlanır. Cerrahi, kemoterapi ve radyoterapi en sık kullanılan kanser tedavi yöntemleridir. Ancak bu kanser tedavilerinin tümör hücrelerinin çoklu ilaç direncine (MDR) ek olarak baş dönmesi, bulantı, kusma ve saç dökülmesi gibi çok sayıda yan etkiye sahip olduğu bilinmektedir (Sağlık Bakanlığı, 2013). Özellikle radyoterapi ve kemoterapide kanserli hücrelerin yanı sıra normal hücrelerin de etkilendiği ve kanser hastalarında ikincil tümörlerin oluşmasına neden oldukları tespit edilmiştir (National Cancer Institute, 2010; Nussbaumer ve diğerleri, 2011; Saini ve diğerleri, 2020). Kemoterapide carmustine, altretamine, bendamustine, busulfan, carboplatin, cisplatin, chlorambucil, thiotepa, trabectedin, temozolomide, melphalan ve mitomycin-C gibi çeşitli ilaçlar yaygın olarak kullanılmaktadır. MMC, DNA hasarına neden olan alkilleyici bir kemoterapötiktir.

Bor (B), metal ile ametal arasında özelliklere sahip bir metaloiddir. İnsanların, hayvanların ve bitkilerin sağlığı için gereklidir. Çoklu reaksiyonların sentezinde ve metabolizmasında yer alır. B, çevrede borik asit, borat, üleksit, boraks ve borik oksit gibi çeşitli formlarda bulunabilen bir eser mineraldir. Borik asit (H_3BO_3) çeşitli amaçlarla kullanılan bor bileşiklerinden biridir. Özellikle gıda koruyucu, dezenfektan, gübre, alev geciktirici ve yalıtım malzemelerinde kullanılmaktadır (Lopalco ve diğerleri, 2020).

Borik asit, membran geçiş sinyallerini ve redoks süreçlerindeki hareketleri etkileyerek düzenleyici bir iyon olarak işlev görür. Ayrıca, beyaz kan hücrelerindeki (lökositler) bakterileri yok etmek için reaktif oksijen partiküllerini kullanan oksidatif patlama sürecini de kontrol eder. Bazı hipotezlere göre bu düzenleme, yakın dokulardaki reaktif oksijen partiküllerini aktive ederek bazı kollateralleri yıkımdan korur. Öte yandan, borik asit iltihaplı

hastalık riskini azaltır. Uygulanan konsantrasyona bağılı olarak ya tehlikeli etkilere neden olabilir ya da organizmaları çeşitli toksik ajanlara karşı koruyabilir. Bu nedenle, borik asidin zararlı hale geldiği maruziyet seviyelerinin ve ayrıca, koruyucu rolünün olduğu konsantrasyonlarının belirlenmesi çok önemlidir (Powell et al., 2019).

Bu araştırmanın amacı, BA'in potansiyel genotoksik etkisini ve bir anti-kanser ilaca (MMC) ve bir de oksidan olan H_2O_2 'ye karşı potansiyel koruyucu etkisini ilk kez insan lenfositlerinde dört farklı genotoksisite (kromozom anormallikleri, kardeş kromatid değişimi, sitokinez blok mikronükleus sitom ve komet) testi ile incelemektir. Kromozomal aberasyon, mikronükleus ve komet testleri OECD kılavuzlarında standartları belirlenmiş ve genotoksisite değerlendirmelerinde kullanılması tavsiye edilen testlerdir. Diğer yandan, OECD kılavuzundan çıkarılmış olmasına rağmen, KKD testi diğer genotoksisite testlerinin bulgularını desteklemek için sıklıkla kullanılmaktadır (Yüzbaşıoğlu, Dilek, Erikel ve Ünal, 2023; Ünal, Akbaş ve Yüzbaşıoğlu, 2023).

Bu çalışmada, KA, KKD ve MN testlerinde, BA tek başına anormallik freansını anlamlı şekilde artırmazken, BA+MMC uygulaması, bütün dozlarda, anormalliklerin freansında anlamlı azalmaya sebep olmuştur. BA tek başına bazı parametrelerde DNA hasarında artışa sebep olmakla beraber, BA+ H_2O_2 uygulaması, bütün dozlarda, oluan tüm DNA hasarlarını anlamlı düzeyde düşürmüştür. Bütün bu sonuçlar, BA'in kullanılan konsantrasyonlarda zararlı olmadığını tam tersine MMC ve H_2O_2 'e karşı, antigenotoksik/koruyucu/kemoprenventif olduğunu ilk kez göstermiştir.

6. SONUÇ VE ÖNERİLER

Kanser, 2. derecede ölüm sebebi olan evrensel bir sağlık hastalığıdır. Kontrolsüz hücre bölünmeleriyle karakterize edilen kanser tedavisinde en yaygın kullanılan tedavilerden olan kemoterapi sonucunda bazı kanser hastalarında, ikinci bir kansere yakalanma riski ortaya çıkmaktadır.

Bazı ilaçlara karşı antioksidan koruma sağlayan doğal maddeler arasında bor ve türevleri de yer almaktadır. Alınan dozlara bağlı olarak, B, ilaçların ve toksik ajanların yan etkilerine karşı organizmalara zarar verebilir ya da onları koruyabilir. Bu araştırmada, alkilleyici ajan MMC ve oksidan ajan H_2O_2 'nin neden olduğu hasarlara karşı borik asidin iyileştirici etkisinden kaynaklanabilecek kromozom aberasyonu, kardeş kromatid değişimi, mikronükleus ve DNA hasarı sıklığında önemli bir azalma gözlenmiştir. MMC, özellikle hücre döngüsünün geç G1 ve S fazları sırasında DNA sentezini etkiler, yüksek konsantrasyonlarda hücre RNA ve protein sentezini baskılar ve apoptoz ve nekrozu indükler.

BA'nın koruyucu etkisi, MMC ve H_2O_2 'ye karşı kromozomal aberasyon, kardeş kromatid değişimi, MN frekansı ve komet testi parametrelerinde (kuyruk yoğunluğu, uzunluğu ve moment) bir azalmaya neden olmuştur. BA'nın antioksidan mekanizması hala tam olarak anlaşılamamış olsa da, enzimatik ve hücre zarı süreçlerinin iyi bilinen bir parçasıdır. Bu, doku antioksidan kapasitesini ve vücudun serbest oksijen radikallerine karşı doğal savunmasını artırma gücünün bir sonucu olabilir.

Sonuçta, bu çalışma, insan lenfositlerinde KA, KKD, CBMN-Cyt ve komet testleri kullanılarak borik asidin hem tek başına, hem de MMC ile eş zamanlı olarak hem genotoksik hem de antigenotoksik potansiyellerini araştıran ilk çalışmadır. Sonuçlar BA'nın *in vitro* olarak insan periferik lenfositlerinde MMC ve H_2O_2 'nin neden olduğu genomik hasarı önleme yeteneğine sahip olduğunu göstermiştir. Bununla birlikte, borik asidin antigenotoksik mekanizmasını tam olarak anlamak için ek *in vitro* ve *in vivo* çalışmalar yapılmalıdır.



Gazili olmak ayrıcalıktır