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Research Article

Genotoxic And Antigenotoxic Effects Of Corilagin In *In Vitro* Human Lymphocyte Cultures

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Abstract

It has been reported in many researches that natural compounds synthesized by plants as secondary metabolites has protective effects against oxidative stress caused by free radicals and genetic damage mediated by them. Among these natural compounds, polyphenols are known to have particularly strong antioxidant activities. Corilagin, a tannin found in many plant species such as *Euphorbiaceae, Geraniaceae* and *Lythracea* families has various pharmacological effects. In this study, the geno/antigenotoxic effects of corilagin was investigated in *in vitro* human peripheral blood lymphocyte cultures using micronucleus (MN) and chromosomal aberrations (CA) tests to determine the safe concentrations of corilagin. 10-100 µg/ml corilagin were applied to the cells alone and simultaneously with 0.2 µg/ml MMC and all analyzes were performed in 3 repititions. According to the results of the analyzes, it was assessed that corilagin did not cause a statistically significant increase in the formation of MN and CA at 10, 25, 50 µg/ml concentrations, and it even provided a statistically significant decrease in the increased MN frequency and CA caused by MMC. On the other hand, it was determined that this effect was concentartion dependent and increased DNA damage at the highest concentration of 100 µg/ml. This is thought to be due to the concentration-dependent prooxidant activity seen in many potent antioxidants. As a review of the study low concentrations of corilagin have an antigenotoxic effect against genetic damage caused by genotoxic agents such as MMC and shows that it can be used against the side effects of chemotherapy.

Keywords: Corilagin, Antioxidant, Antigenotoxicity, Micronucleus, Chromosome Aberration

Korilaginin *In Vitro* İnsan Lenfosit Kültürlerindeki Genotoksik ve Antigenotoksik Etkileri

Öz

Bitkiler tarafından ikincil metabolitler olarak sentezlenen doğal bileşiklerin, serbest radikallerin neden olduğu oksidatif strese ve bunların aracılık ettiği genetik hasara karşı koruyucu etkilerinin olduğu birçok araştırmada bildirilmiştir. *Euphorbiaceae, Geraniaceae* ve *Lythracea* familyaları gibi birçok bitki türünde bulunan bir tanen olan korilagin, çok çeşitli farmakolojik etkilere sahiptir. Bu çalışmada, korilaginin güvenli konsantrasyonlarını belirlemek için *in vitro* insan periferik kan lenfosit kültürlerinde mikroçekirdek (MÇ) ve kromozom anormallikleri (KA) testleri kullanılarak genotoksik/antigenotoksik etkileri araştırıldı. 10-100 µg/ml korilagin hücrelere tek başına ve 0.2 µg/ml MMC ile eş zamanlı olarak uygulandı ve tüm analizler 3 tekrarlı olarak gerçekleştirildi. Analizlerin sonuçlarına göre korilaginin 10, 25, 50 µg/ml uygulama konsantrasyonlarında MÇ ve KA oluşumunda istatistiksel olarak anlamlı bir artışa neden olmadığı hatta MMC uygulaması ile artan MÇ frekansında ve kromozom anormalliklerde istatistiksel olarak önemli oranda azalma sağladığı belirlenmiştir. Diğer yandan bu etkinin konsantrasyona bağlı olduğu ve 100 µg/ml'de DNA hasarını artıcı etki gösterdiği belirlenmiştir. Bu durumun birçok güçlü antioksidanda görülen konsantrasyona bağlı prooksidan aktivite nedeniyle olduğu düşünülmektedir. Çalışma sonuçları korilaginin düşük konsantrasyonlarının MMC gibi genotoksik ajanlar tarafından oluşan genetik hasara karşı antigenotoksik etkisinin olduğunu, kemoterapinin yan etkilerine karşı kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Korilagin, Antioksidan, Antigenotoksisite, Mikroçekirdek, Kromozom Anormallikleri

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1. Introduction

Many studies have been conducted for many years to identify compounds that can protect humans from DNA damage and its consequences. In order to find the most effective antigenotoxic substances, especially natural products, medicinal plants and edible plants are studied. Phenolic compounds isolated from plants are mostly used in studies to determine the antigenotoxic effects of antioxidants. An important group of these compounds is tannins, which are an important part of the human diet and are widely found in nature, especially in herbal products. Tannins are phenolic compounds that can be found in various organs of plants such as fruits, seeds, leaves and roots and have a role in plant development. Tannins have anti-inflammatory, anticarcinogen, antiviral, antibacterial and antiparasitic effects based on their antioxidant and anti-radical activities [1, 2, 3]. Ellagitannins are hydrolyzable tannins with numerous biological properties that can be found in berries, muscadine grapes, walnuts, pomegranates, and tea. Ellagitannins have significant effects on human health and nutrition [3, 4].Corilagin, a member of the ellajitannin family, is a natural product containing glucose and has been found in various medicinal plants such as *Phyllanthus urinaria* L., *Lumnitzera racemose, Canarium album* L., *Phyllanthus reticulatus* [5]. Studies have shown that corilagin has many biological effects such as antioxidative, anti-inflammatory, antitumor, antimicrobial, and antiviral [5-8]. Studies have shown that corilagin inhibits the formation of proinflammatory cytokines, also reduces ROS production, free radical formation and lipid peroxidation *in vitro*. The first bioactivity of corilagin to be found is its inbitory effct on reverse transcriptase activity of RNA tumor viruses. In later studies, it was observed that corilagin has various pharmacological properties including antihypertensive, antiatherogenic, antiviral, hepatoprotective, neuroprotective effects and it is effective in controlling type II diabetes mellitus [9, 10]. *In vivo* drug metabolism of corilagin leads to poor pharmacokinetic models, greatly contributes to the effectiveness and safety of the compounds. In addition, the metabolite profile is important in explaining the effectiveness, safety and further development of drugs [11,12].

Mitomycin-C (MMC) is a natural product of an Actinobacteria species, Streptomyces caespitosus, which is found in soil. MMC shows strong bioreductive alkylation and inhibits DNA synthesis under hypoxic conditions. Therefore it is highly activated in oxygen-poor cells. MMC has shown efficacy in a wide variety of cancers as an antitumor agent but it has a large side effect profile so its widespread use is prohibited. MMC has a large variety of genotoxic effects including the inhibition of DNA synthesis, clastogenesis and mutagenesis [13-15].

Although there are studies on the biological activity of corilagin in the literature, there are no studies on its genotoxic and/or antigenotoxic activity to our knowledge. In line with all these data, in this study the potential genotoxic effects of corilagin and also its antigenotoxic effects against genetic damage induced by mitomycin-C (MMC), a crosslinking chemotherapeutic agent, were investigated. Both genotoxic and antigenotoxic effects of corilagin were tested in human peripheral blood lymphocytes using cytokinesis-blocked micronucleus (CBMN) assay and chromosome aberration tests as widely used genotoxicity tests to determine clastogenic and aneugenic activities.

2. Materials and Methods

2.1 Chemical Substances

As the chemicals used in the experiments, corilagin (CAS number: 23094-69-1), mitomycin-C (CAS number 50-07-7), disodium hydrogen phosphate dihydrate (Na2HPO4.2H2O), acetic acid (Glacial, 99%), potassium dihydrogen phosphate (KH2PO4), ethyl alcohol (ethanol), methyl alcohol (methanol) (99%), cytochalasin B, and colchicine were purchased from Sigma, formaldehyde from Tekkim, dimethyl sulfoxide (DMSO), giemsa, chromosome medium from Merck, nitric acid (HNO3) from VWR and potassium chloride (KCl) from Isolab.

Corilagin

Corilagin (β-1-O-galoyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose) is a gallotannin, an off-white crystalline powder which is easily soluble in water, methanol, acetone, ethanol, and dimethyl sulfoxide (DMSO) [10]. It was isolated for the first time from the plant *Caesalpinia coriaria* in 1951 by Schmidt et al [16]. The chemical formula of corilagin is $C_{27}H_{22}O_{18}$ and its molecular weight is 634.45 g/mol. The structure of corilagin is given in Figure 1.

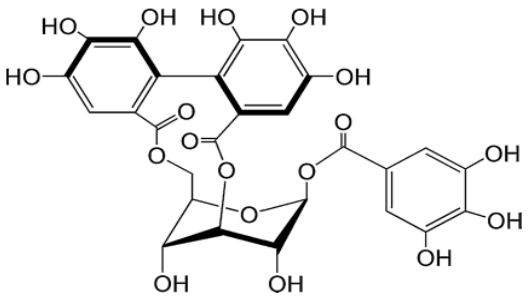

Figure1. Structure of corilagin [10]

Corilagin has been found in 16 plant families and a total of 50 species. There are 20 species in the *Euphorbiaceae* family, 10 species in the *Geraniaceae* family and 7 species in the *Combretaceae* family. Apart from these, studies have shown that corilagin is also present in *Phyllanthus niruri* L., *Phyllanthus emblica* L., *Phyllanthus urinaria* L., *Geranium sibiricum* L. and *Terminalia catappa* L. species. It has been observed that it is easier to isolate plants from above-ground parts, including leaves, flowers, fruits, and seeds [10].

In this study, we purchased and used 99% pure commercial form of corilagin as active ingredient. Test concentrations of corilagin were chosen on the basis of literature data [17-19] and preliminary tests. In line with the data in the literature, it has been determined that it is appropriate to use corilagin at increasing concentrations up to 100 µg/ml in cell culture in terms of cell viability and biological activity. MN test and CA test protocols were followed to examine both genotoxic and antigenotoxic effects against MMC by applying 10, 25, 50, 100 µg/ml corilagin concentrations to human lymphocyte cultures prepared in ethanol-sterile distilled water (1:4) mixture.

2.2 Cell cultures and treatments

Human peripheral blood lymphocyte cultures were prepared for both micronucleus (MN) and chromosome aberration (CA) methods. Blood samples was obtained from 3 healty female donors (aged 20-25 years) who do not smoke, do not use alcohol or drugs, and did not have any health problems and no history of exposure to genotoxic agents. Blood samples were used to obtain biological material from healthy volunteers and there is no interventional clinical practice. While the study project was being prepared for this stage, an approval from the Uludağ University Faculty of Medicine Ethics Committee (2019-11/17) was obtained. Blood samples were taken into heparin tubes then 0.25 ml of heparinized peripheral blood was added into tubes with 2.5 ml of chromosome medium including fetal bovine serum, heparin, antibiotics and phytohaemagglutinin (PHA) under sterile conditions. Then, the total incubation time of the cells in the culture was followed for 72 hours (3 cell divisions). Corilagin concentrations (10, 25, 50, 100 μg/ml) and MMC (0,2 μg/ml) were added to the medium 24 hours after the beginning of the culture, allowing it to remain in the culture medium for 48 hours. During the culture, the tubes were gently inverted and shaken 2-3 times a day.

2.3 Cytokinesis-blocked micronucleus (CBMN) Assay

For CBMN assay, at the 44th hour of culture, cytochalasin-B (6 μg/ml) was added to all tubes to block cytokinesis. Then, the tubes were wrapped with foil to prevent light exposure. At the end of 72 hours, the culture was terminated and the tubes were centrifuged at 1000 rpm for 10 minutes, and the supernatant was discarded with a pasteur pipette, being careful not to remove the cells at the bottom. The 0.5-0.7 ml remaining in the tubes was homogenized by using vortex and then 5 ml of the cold 0.075 M KCl hypotonic solution, was appended slowly, drop by drop, then kept at 4°C for 5 minutes. The tubes were centrifuged at 1000 rpm for 10 minutes, and after the supernatant was discarded, 5 ml of cold fixative prepared from 3:1 methanol: acetic acid was added to the tubes and kept at 4°C for 15 minutes. The fixation process was applied 3 times. After the first fixative, the same procedure was applied two more times and the tubes were kept in the refrigerator for 5 minutes after each fixative. 1% formaldehyde was included into the third fixative. After the last centrifugation, the supernatant in the tubes was removed, and the remaining cell solution was slowly homogenized with a pipette. The prepared slides were left in a dust-free place at room temperature for 24 hours to dry. Air-dried slides were stained for 15 minutes with 5% Giemsa (pH=6.8) prepared with Sorensen buffer. Micronuclei were scored in 1,000 binucleated cells per donor (totally 3,000 binucleated cells per concentration). Only binuclear cells are counted during MN examination because these cells are the cells that divide after adding the chemical substances whose effects are examined. The micronuclei observed in mononuclear cells were micronuclei that were originally present in those cells, not due to the addition of test substance. In each treatment group, 1000 binuclear cells (total 3000 cells from 3 donors) are examined for the presence or absence of micronuclei (Figure 1). MN frequency was determined as MN number per cell (MN/cell) using the formula 1x(1MN)+2x(2MN)+3x(3MN+4MN)/N (N total number of cells examined).

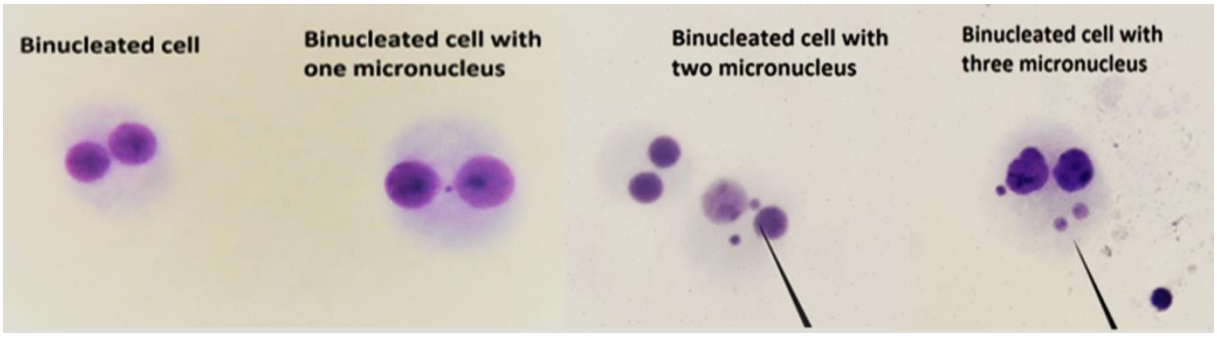

Figure 1 Micrograhs of giemsa stained binucletaed cells without and with different number of micronuclei

2.4 Chromosome aberrations (CA) test

For CA test, at the 70th hour of the culture, colchicine (0.06 µg/ml) was added to the tubes. The cells were then kept in an incubator at 37°C for 2 hours. At the end of the culture period, the tubes were centrifuged at 1200 rpm for 10 minutes and the supernatant was discarded. The 0.5-0.7 ml portion at the bottom of the tube was mixed in a vortex, and the remaining liquid was homogenized. Afterwards, 5 ml of 0.075M KCl hypotonic solution at 37ºC was added to tubes drop by drop using a vortex. The tubes were kept at 37ºC for 30 minutes. Then, the tubes were centrifuged at 1200 rpm for 10 minutes and after the supernatant was removed, 5 ml of cold fixative (3:1 methanol:acetic acid) was slowly added to the tubes in a vortex. After adding the first fixative, the tubes were kept at +4°C for 1 hour. The fixative process was repeated two more times to ensure complete clarification of the remaining contents in the tube. After each fixative process, the tubes were centrifuged, and the supernatant was removed, leaving 0.5-0.7 ml at the bottom after the last fixative application. After discarding the supernatant, the remaining content in the tube was homogenized by pipetting. Then, the cell suspension was dropped from a height of 15-20 cm on cold glass slides that were previously cleaned in 1N HNO3 and taken into 70% alchol. Then air-dried slides were stained with 5% Giemsa dye prepared in Sorensen buffer (pH 6.8) for 15-20 min. For each treatment group at least 100 metaphases were analyzed per donor (totally 300 metaphases per concentration).

2.5 Statistical analysis

z test was performed for statistical analysis of the results of the percentage of MN, abnormal cells, CA/cell. All data was expressed as mean±SE.

3. Results and Discussion

The total number of micronuclei (MN) and MN frequency values obtained from 3 replicate studies in human lymphocyte cultures in which corilagin was administered alone or in combination with MMC are shown in Table 1. When the groups treated with corilagin concentrations were compared with the untreated negative control group, it was determined that corilagin (10-100 µg/ml) did not cause a statistically significant increase in the formation of MN. On the other hand in all groups treated with 0.2 µg/ml MMC, the MN% frequency increased significantly ($p<0.05$) compared to the control. When the groups in which corilagin was administered simultaneously with MMC were examined, a statistically significant decrease in MN frequency was observed in the groups in which 10, 25, 50 µg/ml concentrations of corilagin were applied compared to the group in which MMC was administered alone. In the group in which the highest concentration of corilagin (100 µg/ml) was applied with MMC, it was observed that the MN frequency did not decrease, even increased, compared to the MMC group.

Table 1. Micronucleus frequencies in human lymphocyte cultures treated with corilagin and MMC with corilagin.

Treatment groups	Cells scored	Distrubition of BN Cells according to the number of MN			Total MN	MN% ±SE
		(1)	(2)	(3)		
Negative Control	3000	11	-	-	11	0,37 ± 0,11
MMC (0,2 µg/ml)	3000	201	7	-	215	7,17 ± 0,47 a
10 µg/ml Corilagin	3000	7	1	-	9	0,30 ± 0,10 b
25 µg/ml Corilagin	3000	12	-	-	12	0,40 ± 0,11 b
50 µg/ml Corilagin	3000	14	-	-	14	0,47 ± 0,12 b
100 µg/ml Corilagin	3000	17	-	-	17	0,57 ± 0,14 b
10 µg/ml Corilagin+ 0,2 µg/ml MMC	3000	127	6	-	139	4,63 ± 0,38 a,b
25 µg/ml Corilagin+ 0,2 µg/ml MMC	3000	150	6	-	162	5,40 ± 0,41 a,b
50 µg/ml Corilagin+ 0,2 µg/ml MMC	3000	163	2	2	173	5,77 ± 0,43 a,b
100 µg/ml Corilagin+ 0,2 µg/ml MMC	3000	186	21	1	231	7,70 ± 0,49 a

a Significantly different at $p\leq0.05$ when compared with negative control (z test), b Significantly different at $p\leq0.05$ when compared with MMC group (z test). MMC Mitomycin-C, BN Binucleat, MN micronucleus, SE standard error

It is thought that this may be due to the prooxidant activities and additive effects at high concentrations observed in many of the potent antioxidant substances. Many studies have shown that corilagin is more effective at lower concentrations. However, in the literature, it is seen that most of the studies on corilagin are based on *in vivo* experiments carried out in experimental animals.The results of the CA test indicated as structural and numerical abnormalities, total abnormality number and total abnormal cells in human lymphocyte cultures in which 10, 25, 50, 100 µg/ml corilagin alone and in combination with 0.2 µg/ml MMC are

applied, are shown in Table 2 and 3. When the corilagin treated groups were compared with the negative control group, it was determined that it did not cause a statistically significant increase in the formation of chromosomal abnormalities at the concentrations (10-100 µg/ml) applied alone, that shows it did not have a clastogenic effect at studied concentrations. When the groups in which corilagin was administered simultaneously with MMC were examined, a statistically significant decrease in abnormal cell frequency and CA/cell values was observed at 10, 25, 50 µg/ml corilagin concentrations compared to the group in which MMC was administered alone. In the group in which the highest concentration of corilagin (100 µg/ml) was applied with MMC, it was observed that there was no decrease in DNA damage compared to the MMC group, even increased. It is seen that the results of micronuclei and chromosomal abnormality test applied at the same time are compatible with each other and cause similar results.

Table 2. Chromosomal abnormality types and abnormal cell numbers in human peripheral lymphocytes treated with corilagin and MMC

Treatment Groups	Total Cells Scored	Chromosomal abnormalities								
		ctb	csb	f	scu	dic	cte	p	Total	Abnormal cells
Negative control	300	8	-	1	-	-	-	-	9	9
MMC (0,2 µg/ml)	300	45	9	4	4	3	20	-	85	81
10 µg/ml Corilagin	300	7	-	-	-	-	-	1	8	8
25 µg/ml Corilagin	300	9	-	-	-	-	-	-	9	9
50 µg/ml Corilagin	300	8	2	1	-	-	-	-	11	11
100 µg/ml Corilagin	300	9	3	-	-	-	-	1	13	13
10 µg/ml Corilagin+ MMC	300	42	7	2	1	1	18	-	71	69
25 µg/ml Corilagin+ MMC	300	38	9	1	-	-	12	-	60	58
50 µg/ml Corilagin+ MMC	300	33	11	1	1	-	9	1	56	56
100 µg/ml Corilagin+ MMC	300	55	13	2	-	1	22	-	93	89

MMC Mitomycin-C, ctb chromatid break, csb chromosome break, f fragment, scu sister chromatid union, dic dicentric, cte chromatid exchange, p polyploidy, CA/cell Chromosome aberrations/cell, SE Standard Error

The ellajitannins found in pomegranate fruit are very potent antioxidants, and pomegranate juice has a stronger *in vitro* antioxidant effect than other common commercial juices [20]. Corilagin is an elajitanen found in different organs and tissues in many plant species, including pomegranate, and numerous studies have been conducted on its antigenotoxic and anticarcinogen activity. Prakash et al. [21] conducted *in vitro* and *in vivo* studies using the extract they isolated from the leaves of the *Punica granatum* L. plant and examined the effects of its components on oxidative stress and genomic damage. As a result of their studies, they reported that *Punica granatum* L. plant is an important chemoprotective agent thanks to the antioxidant effects of apigenin, luteolin, gallitanins and ellagitannins such as punicalin, punikalagin, corilagin and punicafolin.

Table 3. Frequency of abnormal cells and number of abnormalities per cell in human peripheral lymphocytes treated with corilagin and MMC.

Tretment Group	Cells Scored	Abnormal cells ± SE (%)	CA/ cells ± SE
Negative control	300	3,00 ± 0,985 b	0,030 ± 0,010 b
MMC (0,2 µg/ml)	300	27,00 ± 2,563 a	0,283 ± 0,026 a
10 µg/ml Corilagin	300	2,67 ± 0,93 b	0,027 ± 0,009 b
25 µg/ml Corilagin	300	3,00 ± 0,985 b	0,030 ± 0,010 b
50 µg/ml Corilagin	300	3,67 ± 1,086 b	0,037 ± 0,011 b
100 µg/ml Corilagin	300	4,33 ± 1,175 b	0,043 ± 0,012 b
10 µg/ml Corilagin+ MMC	300	23,00 ± 2,429 a,b	0,237 ± 0,025 a,b
25 µg/ml Corilagin+ MMC	300	19,33 ± 2,279 a,b	0,200 ± 0,023 a, b
50 µg/ml Corilagin+ MMC	300	18,67 ± 2,250 a,b	0,187 ± 0,023 a, b
100 µg/ml Corilagin+ MMC	300	29,67 ± 2,637 a	0,310 ± 0,027 a

a Significantly different at $p \leq 0.05$ when compared with negative control (z test), b Significantly different at $p \leq 0.05$ when compared with positive control (z test). MMC Mitomycin-C, CA Chromosome Aberrations SE standard error

Tong et al. [22] used tumor-free breast epithelium and breast cancer cell lines (MCF-7, SK-BR 3 and MDA-MB-231) and applied 40, 60, 80 µmol/L corilagin. They observed that corilagin inhibited breast cancer growth through reactive oxygen species-dependent apoptosis and autophagy. Rencüzoğulları et al. (2019) reported that 5, 10, 25 and 50 µM corilagin had an antitumoral effect on A549 lung cancer cells [23]. In a study, 30 male Balb/c mice, 6-8 weeks old and 19-25 g, and the RAW264.7 cell line, they determined that corilagin exerts anti-inflammatory effects by regulating TLR4 signaling molecules to ameliorate the excessive inflammatory state in sepsis [24]. When corilagin isolated from the plant *Phyllanthus emblica L.* was applied to esophageal cancer cell lines (ECA109, KYSE150) and normal esophageal epidermis cell line (HEEPIC) at doses of 0, 5, 10, 20, 40 and 60 µM, it was observed that it exerted an antitumor effect by activating mitochondrial apoptosis and endoplasmic reticulum stress signaling pathways [25]. Tong et al. (2018) showed that corilagin inhibited cancer growth in breast cancer cell lines via reactive oxygen species-dependent apoptosis and autophagy [26]. Guo et al. (2017) reported that corilagin reduced lung injury by suppressing oxidative stress and anti-apoptotic activity in their *in vivo* study [27]. Tan et al. (2022) reported that corilagin inhibited intracellular oxidative stress and decreased the inflammatory bone defect caused by LPS [28]. It was reported by Zhang et al. (2022) that corilagin induced apoptosis and inhibited HMBG1/PI3K/AKT signaling pathways in gastric cancer *in vivo* [29]. Huang et al. (2022) reported that corilagin improved doxorubicin induced cardiac function by reducing injury, inflammation and promoting apoptosis in rats [30]. Liu et al. (2021) reported that corilagin reduced NRF2 expression and induced apoptosis and autophagy [4]. In the literature, there are many *in vivo* and *in vitro* studies to examine the biological effects of corilagin on genotoxic molecules and cancer cells. Up to date, Yang et al. (2021) showed that corilagin could be a safe antiviral agent against COVID-19 and could be used in anti-virus hygienic products [9].

4. Conclusion

Studies with natural compounds to prevent or eliminate the genotoxic effect give positive results to a large extent. For this purpose, researches are carried out on secondary metabolites found in different organs of plants and especially polyphenolic compounds. Various antioxidant and anticarcinogenic effects of polyphenols found in plants have been investigated in studies carried out to date. In this study, the possible genotoxic and antigenotoxic effects of corilagin, which is one of the phenolic compounds of many plant species including pomegranate and accepted as a natural antioxidant, were investigated on human peripheral lymphocyte cells. As a result it was determined that corilagin had a reducing effect on the damage caused by MMC, when used at low concentrations, similiar with the *in vitro* and *in vivo* studies in the literature. Treatment concentrations of corilagin showed no genotoxic effects on healthy human peripheral lymphocyte cells even showed antigenotoxic effects against damage caused by MMC. These results suggest that it has the potential to be used as a therapeutic agent in reversing the damage caused by mutagens and chemotherapy drugs.

In this study, MN and CA tests were carried out in cell culture under *in vitro* conditions for the detection of DNA damage. Compared to *in vivo* studies, *in vitro* methods that both eliminate ethical problems and also provide cheap, easy, fast evaluation present important data as preliminary indicator studies. However, according to many researchers, data obtained from cell cultures and animal models may not fully reveal the effect in humans. For this purpose, it is recommended to carry out *in vivo* studies and epidemiological studies as a continuation of these studies, which are considered as the first step. In this respect, the genomic approach, which reveals the effects of different components, can be presented as a powerful tool to describe and predict the pharmaco-toxicological activities of medicinal plants.

Ethics in Publishing

In this study ethics committee approval (2019-11/17) was obtained from Uludag University Faculty of Medicine Ethics Committee for the use of human peripheral blood lymphocyte cultures.

Author Contributions

This study was completed by Elif Turan as a master's thesis. Study experiments, data evaluations, and manuscript writing were done jointly by both authors. Conceptualization, funding acquisition, project administration, methodology, writing - original draft was done by Gökçe Taner.

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