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Evaluation of the Genoprotective Effect of Polyphenolic, Hydroalcoholic and Aqueous Extracts of Red Apple

Mahmoud Etebari[1*], Behzad Zolfaghari[2], Abbas Jafarian Dehkordi[1] and Vahid Pourbarkhordar[1]

[1]Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran; etebari@pharm.mui.ac.ir
[2]Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Apple has a tremendous supply of minerals, vitamins C, B Group and phenolic compounds. It has antioxidant properties and maintains genetic content against oxidative stress. In animal experiments, methyl methanesulfonate (MMS) has demonstrated genotoxic effects. In this study, the genoprotective effects versus the MMS genotoxicity of red apple extracts were investigated. Red apples (Red Delicious cultivar) were chopped in thin slices and three types of extracts consisting polyphenolic, hydroalcoholic and aqueous were taken. Lymphocytes were pre-incubated with various concentrations of extracts and then were exposed to 10µM of MMS. This was made possible by using alkaline comet assay and analysis of comet images. The total phenolic content (TPC) for polyphenolic, hydroalcoholic and aqueous extracts were obtained 127.74 ± 4.26, 118.12 ± 4.47 and 84.79 ± 4.26 mg/g, respectively. The extracts consisted of 48.92 ± 1.02, 42.09 ± 3.36, 29.08 ± 3.23 mg/g of total flavonoid (TF). The results illustrated that in genoprotective concentrations of 1, 10 and 100µg/ml for all these extracts, the tail length and tail moment variables were significantly lower than the positive control (10µM of MMS) ($p< 0.05$). In this study, results revealed that these extracts were able to protect lymphocytes against MMS. By considering the TPC and TF values, the polyphenolic and hydroalcoholic extracts had more genoprotective activity compared to the aqueous extract. The polyphenolic extract was proved to be the most protective extract of all.

Keywords: Comet Assay, DNA Damage, Methyl Methanesulfonate, *Malus domestica*

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1. Introduction

In living organisms, cells are constantly exposed to multifarious substances which can be detrimental for their genetic material and its components. They can change the chemical structure of the DNA and its sequence authenticity which accordingly is known as the DNA damage factors. If the sequence of the DNA changes, they can be described as mutagen factors. Main DNA damage factors include exogenous factors such as UV radiation, X-ray, human made alkylating agents like aromatic compounds and endogenous factors[1]. Major endogenous factors include Reactive Oxygen Species (ROS) in oxidative stress, aldehydes derived from lipid peroxidation, several estrogen metabolites with direct or indirect damage effects and methylating agents such as 7-methylguanine, 3-methyladenin and O^6-methylguanine. In these methylating agents, O^6- methyl guanine is the most mutagenic agent[1].

The mechanism of DNA damage depends on the type of mutagen. UV light can damage DNA by producing thymine dimers, which are cross-links between pyrimidine bases. Oxidants such as hydrogen peroxide produce multiple forms of damage, including double strand breaks, point mutations like deletions, insertions as well as chromosomal translocations. Double strand

* *Author for correspondence*

breaks are the most dangerous DNA damage, as these are difficult to repair[2,3]. In addition, most intercalators (the mutagens fit into the space between two adjacent base pairs) are aromatic and planar molecules such as ethidium bromide, acridines, daunomycin, and doxorubicin[4].

Consuming fresh fruits and vegetables is important because of their preventive and protective effects on DNA due to the significant presence of phytochemicals which inhibit oxidative damage caused by ROS and stimulate intracellular mechanisms that counteract with oxidative stress and inflammation. Polyphenolic substances are substantial components ofphytochemicals[5]. They are an important class of secondary metabolites in plants and are divided into many subgroups such as: hydroxyl cinnamic acids, benzoic acids, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones, dihydroflavonols and anthocyanins[6]. Polyphenolic substances and vitamins in fruits and vegetables can obviously diminish the risk of many chronic ailments like cardiovascular diseases and cancer which is directly related to DNA damage[7].

Apple (*Malus domestica* .Borkh) is a member of Rosaceae family originated in mid-Asia, middle-east and Europe. However, It is now cultivated all around the world. Apple consumption is associated with reducing the cancer risk in lungs, breast, ovary and GI tumors[8]. The protective effects of apples are primarily associated with their anti-oxidant characteristics. Apples contain vitamin C (mean 12.8mg/100g). However, it only includes 11% of the total expected anti-oxidant activity. Phytochemicals found in apples possess over 80% of apple's anti-oxidant activity. Polyphenolic substances in apples can indirectly protect genetic content through antiradical, antioxidant and antiproliferation activities. They can enhance the expression of glutathione S-transferases($GSTT_2$) that can help protect the cells against oxidative stress induced by peroxides during peroxidation of lipids or arachidonic acid[9]. Apple flavonoids which is a subgroup of polyphenolic compounds can modulate the genes involved in the biotransformation of xenobiotics such as $GSTP_1$, $GSTT_2$, $MGST_2$ (microsomal glutathione S-transferase), CYP_3A_7, CYP_4F_3, $CHST_5$, $CHST_6$ and $CHST_7$ (carbohydrate sulfotransferase)[10]. Apples have the second highest phenolic content after cranberries[11]. Major polyphenolic materials in apples are hydroxycinnamic acids (mostly quinic esters, such as chlorogenic acid), flavanols (catechins and procyanidins), and dihydrochalcones[12].

Genotoxicity tests are designed to recognize the substances that induce genetic damage through various mechanisms. They also make hazard identification with respect to DNA damage and its possible fixation. The general features of a standard approach in genotoxicity studies is first the assessment of mutagenicity in a bacterial reverse gene mutation test (Ames test). This test detects relevant genetic changes and the majority of genotoxic carcinogens. After that, genotoxicity should be evaluated in mammalian cells in vitro and/or in vivo.

Some compounds do not provide additional suitable information through in vivo tests. They include compounds that are not absorbed systemically such as radioimaging agents, aluminum based antacids, some compounds given by inhalation and some topically applied pharmaceuticals. So in vitro tests can be useful for these compounds. Several in vitro mammalian cell assay systems exist to determine chromatid and chromosome gaps, chromosome and DNA breaks, chromatid deletions, fragmentation, translocation and complex rearrangements. They include metaphase chromosome aberration assay, micronucleus assay, mouse lymphoma L5178Y cell *Tk* (thymidine kinase) gene mutation assay (MLA) and etc. When combined with other genotoxicity tests, these assays are appropriate for the measurement of chromosomal damage and chromatid aberrations[13].

Comet assay is suitable for detecting DNA damage at individual eukaryote cell level. Firstly developed by Östling & Johansson in 1984and later modified by Singh et al., in 1988, it is utilizable for human biomonitoring and molecular epidemiology, ecogenotoxicology and fundamental research in DNA damage and repair. The widespread usage of the assay is due to its simplicity, sensitivity, versatility, speed, economical aspects and the number of relevant annual publications. It consists of the embedment of cells in a low-melting-point agarose suspension, the lysis of cells in neutral or alkaline (pH>13) solutions, and the electrophoresis of the residual lysed cells. The pattern of DNA migration through the electrophoresis gel resembles a comet; accordingly this test is entitled comet assay[14,15].

The ultimate objective of this study is the evaluation of the genoprotective effects of polyphenolic, hydroalcoholic and aqueous extracts obtained from red apple versus the genotoxic effects of MMS. If this protective role is confirmed, red apple can be suggested as a herbal resource for prevention of cancer.

2. Materials and Methods

2.1 Materials

The RPMI-1640 medium and the fetal bovine serum (FBS) were purchased from Capricorn, Germany. Ficoll (lymphodex) was obtained from Inno-Train, Germany. PHA 1.5 % (phyto hemmagglutinine) m-form was purchased from Gibco, USA. Gallic acid, sodium nitrite, aluminum chloride, sodium hydroxide, sodium carbonate, Folin-Ciocalteu's phenol reagent, quercetin, NaCl, NaH_2PO_4, KCl, H_2O_2, tris and KH_2PO_4 were purchased from Merck, Germany. Triton- x100 and low melting agarose (LMA) and MMS were purchased from Sigma, USA. Normal melting agarose (NMA) was obtained from Cinnagen, Iran.

2.2 Preparation of Red Apple Extracts

Some fruit samples were purchased from the suggested local markets and they were confirmed as the "Red Delicious" cultivar by Isfahan agricultural organization. Apples were washed and sliced, then dried in free air away from sunlight. The dried pieces were grinded into powder. To prepare hydroalcoholic extract, the apples powder was presoaked in ethanol (70 % v/v) for 48 hours. The mixture was filtered by Büchner funnel under vacuum condition and was freeze-dried. Polyphenolic extract was obtained in two steps. First, the apple powder was presoaked in ethanol 90 % (v/v) and then in ethanol 50 % (v/v) for 12 hours in each step. The mixtures from these two steps must remain washy, so more solvent could be added if necessary. Two mixtures were blended and evaporated to the 1/3 of the original volume. Aqueous phase was extracted by chloroform and the extract was dried in the oven in order to completely eliminate the chloroform. The finished product was filtered by Büchner funnel under vacuum condition and was freeze-dried. In order to prepare aqueous extract, apple juice was taken and was freeze-dried[16].

2.3 Determining the Total Phenolic Content (TPC) and Total Flavonoid (TF) of Extracts

The total phenolic content and total flavonoid for extracts were measured on the basis of gallic acid and quercetin calibration curves, respectively. Threefold repetition TPC and TF evaluations were implemented for each extract. 10mg of gallic acid and 10mg of quercetin were weighed and the stock solvents were prepared with distilled water (1mg/ml). For estimating TPC, various concentrations of Gallic acid (0.01, 0.02, 0.03, 0.04 and 0.05mg/ml) and extracts (0.1 and 1mg/ml) were provided. Different concentrations of quercetin or extracts (2, 4, 6, 8, 10µg/ml) were prepared for determination of TF.

Total phenolic content was determined by 10 fold diluted Folin-Ciocalteu's phenol reagent. 500 microliter of extract concentrations (0.1 and 1mg/ml)were blended with 2.5ml of diluted Folin-Ciocalteu's phenol reagent and 2ml of 7.5% sodium carbonate in glass tubes. The mixtures were covered by parafilm and put away for 30 minutes in room temperature, then they were placed in spectrophotometer and their absorbance in 760 nm were read.

To evaluate total flavonoid1ml of stock solution or extract was merged with 4ml distilled water and 0.3ml of 5%$NaNO_2$. After 5 minutes, 0.3ml of 10%$AlCl_3$ and 2ml of 1M NaOH were added and the total volume was brought to10mlwith distilled water. The sample was placed in spectrophotometer and the absorbance was noted in 510 nm[17].

2.4 Cell Culture

This paper investigates the PBMC (peripheral blood mononuclear cells) including lymphocytes (90%) and monocytes (10%) in terms of the genoprotective effects. 5ml human blood was taken and added into a 50ml falcon tube containing 30-40 microliter heparin (5000 IU/ml); then the phosphate buffer saline was subjoined to the same volume of blood sample. The mixture of the diluted blood with PBS was carefully added to glass tube in which 5ml of ficoll was previously added. Careful consideration was taken in order to avoid mixing the ficoll with the mixture. These tubes were spun horizontally at 2800 rpm for 30 minutes with no brake for deceleration. The top layer consists of plasma followed by a milky layer of PBMC (lymphocytes and monocytes) and the bottom layer is made up of polymorphonuclear cells (such as neutrophils and eosinophils) and RBCs. The PBMC layer was isolated and washed twice with PBS[18,19]. Lymphocytes were cultured by RPMI 1640 medium, with10 % FBS and 1.5% PHA in 12-wells plates.

2.5 Comet Assay

In this study, comet assay was performed in alkaline conditions (pH >13). For cell incubation at least 10^6 cells per mlwere needed with at least 90 percent viability.

After 1 hour of incubation, cells were mixed with 2ml Low Melting Agarose (LMA) and were located on pre-coated slides with Normal Melting Agarose (NMA). Coverslips were placed on LMA-cells mixture, then the slides were put in a refrigerator (4° c) for 10 minutes. The coverslips were removed and the slides were submerged in lysis buffer for 40 minutes. This was done because it removes nucleus membrane and histone molecules from chromatin network. After the lysis stage, the slides were washed three times with deionized water. Then, they were immersed in alkaline buffer for 40 minutes. This stage makes the DNA breaks more distinctively and causes the DNA to unwind. In the next stage, slides were located in electrophoresis tank and the buffer from the previous step was added. The electrophoresis was performed for 40 minutes at the fixed voltage of 25 V, and the current of 300 mA. Then, the slides were put in a neutralizing buffer for 10 minutes. Afterwards, they were located on an arid surface and were dried completely. Slides were observed by a fluorescent microscope and the photos of these slides were taken[20,21]. Threefold repetitions of comet assay were performed for each step of the experiment and the analysis was implemented on at least 100 cells in the obtained photos.

In the present study MMS (an alkylating agent) and red apple extracts (polyphenolic, hydroalcoholic and aqueous) were genotoxic and genoprotective agents, respectively. First, least genotoxic concentration of MMS was examined, then genotoxic concentrations of the extracts were investigated. Both of these stages were implemented through incubating lymphocytes with MMS or the extracts for1hour. To determine the genoprotective effects of the extracts, lymphocytes were pre-incubated with various safe concentrations of the extracts for 1 hour and after being washed, were exposed to least genotoxic concentration of MMS for 1 hour. Least genotoxic concentration of MMS was examined in different concentrations (1, 5, 10, 100, 200 and 500μM of MMS). Afterwards, the genotoxic effects of polyphenolic extract were investigated in various concentrations (1, 10, 100, 500μg/ml and1, 10mg/ml). Genotoxic effects of the hydroalcoholic extract were surveyed in various concentrations (1, 10, 100, 500μg/ml and1, 10mg/ml). Then, the genotoxic effects of aqueous extract were investigated in various concentrations (100, 500μg/ml and 1, 10mg/ml). After determining the genotoxic consequences, genoprotective effects of the extracts were determined. In the end, the taken photos of comet assay slides were analyzed with a particular comet assay software (Comet score, TriTek Corp).

2.6 Statistical Analysis

The crude numerical results that were obtained from comet score software were imparted as means± SD (standard deviation) and three continuous quantitative variables (tail length, DNA percentage in tail and tail moment) were analyzed; the post hoc test (Tukey) was performed on the crude results till significant differences between the concentrations were clarified. Statistical analysis was accomplished by IBM-SPSS software v21, USA.

3. Results

TPC for the polyphenolic, hydroalcoholic and aqueous extracts were obtained as127.74 ± 4.26, 118.12 ± 4.47 and 84.79 ± 4.26 mg/g, respectively. Also, including the TF the results were48.92 ± 1.02, 42.09 ± 3.36, 29.08 ± 3.23 mg/g, respectively.

In terms of the least genotoxic concentration of MMS, the statistical results showed significant differences between the concentrations of 10, 100, 200 and 500μM of MMS with negative control (p<0.05). So the 10μM was selected as the least genotoxic concentration of MMS for determination of genoprotective effects of extracts(Table1).

Table 1. The genotoxic effects of MMS

Variables	Concentrations						
	Negative control	1 μM	5 μM	10 μM	100 μM	200 μM	500 μM
Tail length (pixel)	0.16 ± 1.61	1.43 ± 1.91	9.67 ± 2.73	96.01 ± 3.03*	106.53 ± 4.03=*	165.25 ± 7.94*	239.81 ± 11.65*
% of DNA in tail	0.00074	0.19 ± 0.23	0.93 ± 1.52	11.9 ± 0.66*	14.28 ± 1.25*	22.23 ± 1.36*	31.02 ± 2.32*
Tail moment (pixel)	1.3636E-05	0.001	0.17 ± 0.3	11.93 ± 0.64*	14.35 ± 1.95*	36.78 ± 2.92*	74.23 ± 5.08*

Various concentrations of MMS (mean ± SD) in comparison to negative control which included lymphocytes without exposure to any particular chemical substances, significant differences (p < 0.05) have been shown by (*)mark.

Regarding the genotoxicity of polyphenolic extract, the concentrations higher of 100µg/ml showed significant difference in comparison with negative control (p<0.05). So these concentrations (500µg/ml and1, 10mg/ml) were genotoxic (the DNA was damaged) and the concentrations of 1, 10, 100µg/ml were safe, no genotoxic effect was observed. To investigate the genoprotective effects of polyphenolic extract, in terms of the tail length and tail moment variables, safe concentrations showed DNA protective effects in comparison with positive control (p< 0.05)(Table2).

Genotoxicity of hydroalcoholic extract was also investigated and the concentrations of 1, 10mg/ml (all three variables) and only the tail length of 500µg/ml had significant difference with negative control (p<0.05), so500µg /ml and higher concentrations were genotoxic and100µg /ml and the lower concentrations (1 and 10µg/ml) were safe. About the genoprotective effects of hydroalcoholic extract, the results showed significant differences between these concentrations and the positive control (10µM of MMS)i.e., they mentioned that these concentrations were decreased the DNA damage of MMS and they had genoprotective effects against MMS(p< 0.05) (Table3).

Results of the genotoxic effects of aqueous extract showed significant differences between concentrations of1(tail length and DNA percentage in tail) and10 mg /ml in comparison with negative control (p<0.05). So these concentrations were genotoxic and the lower concentrations (100, 500µg/ml) were safe without any harmful effects on the DNA structure. Last but not least, the genoprotective effects of the aqueous extract were surveyed in various concentrations(1, 10 and 100µg/ml). The consequences indicated that these concentrations had genprotective effects in comparison with positive control (p< 0.05) (Table 4).

Table 2. The genotoxic and genoprotective effects of the polyphenolic extract

Genotoxicity							
Variables	**Concentrations**						
	Negative control	**1 µg/ml**	**10 µg/ml**	**100 µg/ml**	**500 µg/ml**	**1 mg/ml**	**10 mg/ml**
Tail length (pixel)	0.141	1.22	2.58 ± 2.48	3.14 ± 0.05	27.45 ± 2.34*	41.27 ± 2.21*	74.92 ± 4.07*
% of DNA in tail	0.0008	0.003	0.006	0.06 ± 0.06	3.06 ± 1.39*	3.32 ± 1.51*	8.95 ± 2.13*
Tail moment (pixel)	2.54444E-05	3.06778E-05	0.0001	0.004 ± 0.04	0.79 ± 0.13*	1.41 ± 0.3*	6.82 ± 2.2*
Genoprotection							
Variables	**Concentrations**						
	1 µg/ml	**10 µg/ml**	**100 µg/ml**	**Positive control**			
Tail length (pixel)	83.69 ± 5.47#	71.01 ± 5.87#	69.15 ± 2.77#	109.09 ± 8.56			
% of DNA in tail	11.42 ± 1.28	8.21 ± 1.25#	7.47 ± 1.87#	12.72 ± 1.93			
Tail moment (pixel)	9.35 ± 2.85#	5.87 ± 2.62#	5.11 ± 2.23#	13.76 ± 4.95			

Various genotoxic concentrations of the polyphenolic extract (mean ± SD) were compared to negative control which included lymphocytes without exposure to any particular chemical substances; significant differences (p < 0.05) have been shown by (*)mark. Various genoprotective concentrations of polyphenolic extract were compared to positive control (10µM of MMS); significant differences (p < 0.05) have been shown by (#)mark.

Table 3. The genotoxic and genoprotective effects of the hydroalcoholic extract

Genotoxicity					
Variables	**Concentrations**				
	Negative control	**100 µg/ml**	**500 µg/ml**	**1 mg/ml**	**10 mg/ml**
Tail length (pixel)	1.25 ± 1.10	5.38 ± 1.29	21.26 ± 2.13*	46.12 ± 2.45*	72.66 ± 2.52*
% of DNA in tail	0.0007	0.25 ± 0.23	0.89 ± 0.88	3.61 ±1.67*	12.48 ±1.56*
Tail moment (pixel)	5.65657E-06	0.02 ± 0.04	0.25 ± 0.24	2.13 ± 1.01*	10.16 ± 1.11*
Genoprotection					
Variables	**Concentrations**				
	1 µg/ml	**10 µg/ml**	**100 µg/ml**	**Positive control**	
Tail length (pixel)	78.65 ± 4.05#	69.61 ± 5.61#	67.27 ± 5.01#	102.82 ± 6.74	
% of DNA in tail	11.95 ± 0.84#	11.22 ± 2.28#	7.51 ± 1.23#	19.08 ± 2.27	
Tail moment (pixel)	9.58 ± 3.29#	7.89 ± 2.24#	5.52 ± 1.07#	19.15 ± 4.49	

Various genotoxic concentrations of the hydroalcoholic extract (mean ± SD) were compared to negative control which included lymphocytes without exposure to any particular chemical substances; significant differences (p< 0.05) have been shown by (*)mark. Various genoprotective concentrations of the hydroalcoholic extract were compared to positive control (10µM of MMS); significant differences (p < 0.05) have been shown by (#)mark.

Table 4. The genotoxic and genoprotective effects of aqueous extract

Genotoxicity					
Variables	**Concentrations**				
	Negative control	**100 µg/ml**	**500 µg/ml**	**1 mg/ml**	**10 mg/ml**
Tail length (pixel)	1.28 ± 0.14	4.16 ± 1.2	5.85 ± 1.34	40.08 ± 0.86*	74.1 ± 4.73*
% of DNA in tail	0.001	0.0007	0.06 ± 0.03	4.45 ± 0.83*	10.9 ± 2.27*
Tail moment (pixel)	0.0003	7.57576E-06	0.31 ± 0.02	1.89 ± 0.48*	8.49 ± 1.95*
Genoprotection					
Variables	**Concentrations**				
	1 µg/ml	**10 µg/ml**	**100 µg/ml**	**Positive control**	
Tail length (pixel)	79.08 ± 3.34#	76.13 ± 3.95#	70.66 ± 3.87#	101.78 ± 7.94	
% of DNA in tail	13.95 ± 2.84#	12.52 ± 2.66#	10.12 ± 1.21#	18.16 ± 3.62	
Tail moment (pixel)	10.9 ± 2.14#	9.58 ± 1.61#	7.58 ± 2.14#	18.36 ± 3.32	

Various genotoxic concentrations of the aqueous extract (mean ± SD) were compared to negative control included lymphocytes without exposure to any particular chemical substances; significant differences ($p < 0.05$) have been shown by (*)mark. Various genoprotective concentrations of the aqueous extract were compared to positive control (10µM of MMS); significant differences ($p < 0.05$) have been shown by (#)mark.

4. Discussion

Plant resources, especially fruits and vegetables have a considerable contribution in human diet in most countries. Apples are one of the most frequently consumed fruits and a source of sugars, acids, vitamin C and B group. There are various biologically active substances in apples, especially in their peels such as phenolic compounds and flavonoids[22].

Studies have shown a significant relationship between the occurrence of cancer and the frequency of DNA damages. The relevance of the DNA damage to the generation of cancer (carcinogenesis) became evident when it was recognized that all the agents that cause cancer (carcinogens) also cause DNA damage and change its sequence; so they are mutagens[23].

Now in order to prevent the adverse effects of carcinogens on the DNA, it is going to be proved that apples are a good solution. Regarding the above point, genoprotective and anticancer effects of apple can occur in various ways. Some of these procedures consist of antioxidant activity and reduction of oxidative stress, scavenging free radicals and decreasing level of ROS (genotoxic agents), induction of chemoprotective enzymes such as detoxification liver enzymes (Phase I and II), anti-invasive property and modulation of cancer biomarkers, antiproliferation of tumor cell lines andetc.[9,24-26]

In vitro and animal studies have demonstrated that apples have high antioxidant activity, can inhibit cancer cell proliferation and decrease lipid oxidation. In numerous epidemiological studies, apples have been associated with a decreased risk of chronic diseases such as cancer. These studies also regard apples as the source of a wide variety of phytochemicals including phenolic compounds and flavonoids which have strong antioxidant and anticancer activity[27].

Comet assay is a precise, sensitive and economic method which is utilizable for genotoxicity testing and DNA damage studies. In vitro experiments in general and single cells in particular can be examined to determine the decrease or increase of DNA damage level[28].

The presence of phenolic compounds and flavonoids in apple was mentioned in many studies. On the other hand, these substances have antioxidant and anticancer properties. Carcinogenicity and DNA damage are closely related and the comet assay as a diagnostic method can detect these adverse effects; so genoprotective effects of phenolic compounds and flavonoids or their capability to decrease the DNA damage of genotoxic agents can be determined with the aid of comet assay.

In some researches, comet assay was used to detect genoprotective effects of polyphenols and flavonoids in apples. Distinct decrease of DNA damage was observed at incubation of Caco-2 cells with apple juice extracts (AE01 and APE03) and concentration-response curves were in minimum level at 100µg/ml of apple juice extracts. AE02 and AE04 were only weakly effective at 50 and 250µg/ml. Caco-2 cells were incubated with the oxidants menadione (Md) or tert-butylhydroperoxide (TBH)[29].

To investigate the influence of apple extract (AE) and synthetic polyphenol mixture (SPM) on protection against DNA damage, LT97 cells were incubated with128µg/ml of AE or SPM. The cells were harvested and challenged with different concentrations of Cumenehydroperoxide or H_2O_2. AE (128µg/ml) significantly reduced the CumOOH-induced DNA damage at 180µM of CumOOH by 23% of tail intensity ($p<0.05$) and at 300µM CumOOH by 26% of tail intensity ($p<0.01$) compared to the medium control[9].

Repair of mutagen-induced DNA lesions during transportation, storage and cultivation of PHA-stimulated lymphocytes against lesions induced by MMS and benzo[a]pyrenediolepoxide (BPDE) were investigated. The comet assay indicated the fast repair of MMS-induced damage during the first 18 hours of cultivation; however, removal of BPDE-induced lesions was slower[30].

As it was demonstrated, this study investigates the effects of various apple extracts on protection of DNA against MMS through the comet assay. Lymphocytes were used instead of cell lines because a lot of data could be obtained from them within a relatively short period of time in an inexpensive manner. Lymphocytes were easily collected in a non-invasive way and proved to be good surrogate cells of different cancer target organs[31].

In terms of amount of DNA damage decrease in the tail length variable of genoprotective concentrations, the hydroalcoholic extract positioned in first place followed by polyphenolic extract in second place and the aqueous extract in the last place (Tables 2-4, the genoprotectivity sections). Regarding the DNA percentage in the tail variable of the genoprotective concentrations, the polyphenolic extract showed the most decrease of DNA damages; the hydroalcoholic and aqueous extracts were in second and third places, respectively (Tables 2-4, the genoprotectivity sections). About tail moment variable, the polyphenolic extract showed the most decrease of DNA damages and the hydroalcoholic and aqueous extracts were positioned in next ranks, respectively (tables 2-4, the genoprotectivity sections).

By comparing numeric values of the variables, it can be concluded that the polyphenolic and hydroalcoholic extracts showed more DNA damage prevention compared to the aqueous extract. Moreover, when the polyphenolic and hydroalcoholic extracts were compared, the tail length of hydroalcoholic extract showed more decrease. However, in terms of the DNA percentage in tail and tail moment variables, the most effect belonged to polyphenolic extract. Therefore, through decreasing two of these variables, the polyphenolic extract had the most genoprotective effect. Moreover, polyphenolic extract showed the most TPC and TF values and could confirm these genoprotective results.

Consequently, red apple and their extracts could be used both as a protective edible resource and supplementary product against mutagenic and DNA damage effects of genotoxic materials which are used in laboratories and industries. All in all, more studies might be needed to confirm the mentioned effects.

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Abbreviations

MMS: methyl methanesulfonate; ROS: reactive oxygen species; PBMC: peripheral blood mononuclear cells including lymphocytes and monocytes (with 0.9 to 0.1 ratios); TPC: total phenolic content; TF: total flavonoid

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