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Evaluation of Genoprotective Effects of Hydroalcoholic and Polyphenolic Extracts of Quince by Comet Assay

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A B S T R A C T

Cydonia Oblonga Mill. (Quince) is a member of Rosaceae family that widely used in Iranian traditional medicine. This plant contains large amounts of flavonoids and polyphenols and has antioxidant, antibacterial, free radical scavenging and several other effects. This study was undertaken to evaluate the genotoxic and genoprotective effects of hydroalcoholic and polyphenolic extracts of Quince on HepG2 cells.
To evaluate genoprotective properties, cells were incubated by different concentrations of extracts for 2 hours followed by one-hour incubation period with genotoxic concentration (100 µM) of Methylmethane Sulfonate (MMS). In this research, comet assay as a sensitive, inexpensive and easy performance method was used. Tail length, percentage of DNA in tail and tail moment were measured.
The results showed that hydroalcoholic extracts of Quince at 1, 10, 100 and 500 µg/ml and polyphenolic extracts of Quince at 10, 50, 100 and 500 µg/ml has genoprotective properties .
The results revealed that all studied concentrations of both extracts had no genotoxic effect. Also genoprotective properties of these extracts increased in a dose-dependent manner.

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Introduction

Several factors can cause different patterns of DNA damage. DNA single- and double-strand breaks are the most frequent DNA defenses which can have an impressive role in the creation of cancers[1]. Double-strand breaks of DNA can cause more instability in its genomic content leading to important defenses such as chromosomal aberrations as the initial step of cancers and cell death cascade [2,3].Previous studies [4,5] have shown that a healthy diet can promote the genomic stability. Biomolecules found in fruits and vegetables are able to protect cells against development of diseases such as cancers [6]. A large number of phytochemicals such as phenolics and carotenoids are able to limit or slow down oxidative stress reactions [7]. Polyphenols as effective free radical scavengers have antimutagenic, anticarcinogenic, antioxidant, and anti-inflammatory properties which results in the stability of genomic content [8]. The relationship between consumption of polyphenols in daily diet and prevention of cancer, heart disease and osteoporosis has been proved in previous studies [9].

Cydonia oblonga Mill., known as Quince, is a member of Rosaceae family which contains several flavonoids such as 3-5-O-dicaffeoylquinic acid, quercetin-3-O-galactoside, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, and 3-O-, 4-O-, and 5-O-caffeoylquinic acids [10-12]. Different parts of Quince possess various therapeutic activities including carminative, expectorant, anti-cancer, antibacterial, antidiabetic, antioxidant, and free radical scavenging effect. In Iranian traditional medicine, fruits and seeds of quince have been widely used to treat conjunctivitis, cough, bronchitis, constipation, cystitis, diarrhea, dysentery, cardiovascular diseases, renal diseases, headache, inflammatory bowel disease, migraine, nausea, common cold, hepatitis, vomiting, hemorrhoids and cancer [12].

Methylmethane sulfonate (MMS) is a well-known genotoxic agent exerting its effect by alkylation of purines in DNA strains [13-15]. MMS has been frequently used as a genotoxic agent in various studies [4,16,17]. The single cell gel electrophoresis (comet assay) is a rapid and sensitive method used for detecting single strand DNA breaks in mammalian individual cells [13,18-23]. This method was developed in 1984 by Ostling and Johanson for determination of DNA damage in single cells and also in many kinds of eukaryotic cells. Due to their active metabolic system and metabolic potency, HepG2 cells (human hepatoma cell) were used in the current study [24].The results of this test were analyzed by a computer program to maintain higher sensitivity [19].

In the present study, the genoprotective effects of hydroalcoholic and polyphenolic extracts of Quince on HepG2 cells against genotoxicity of MMS were assessed using the comet assay method. The genotoxic effects of each individual extract were also evaluated.

Material and methods

Ethanol, EDTA (ethylene diamine tetra acetic acid), H_2O_2, NaCl, NaOH, NaH_2PO4, Tris and Triton X-100 were purchased from Merck Co. (Germany). Low melting point agarose (LMA), Na_2HPO4, KCl, and ethidium bromide were procured from Sigma Co. (USA). Normal melting point agarose (NMA) was supplied by Cinnagen Co. (Iran). RPMI-1640, Fetal bovine serum (FBS), and penicillin and streptomycin were purchased from PAA Co. (Australia). Human hepatoma cell line (HepG2 cells) was provided by Pasture Institute (Iran).

Extract preparation

The Quince fruits were purchased from Mobarakeh market (Isfahan province, Iran) in February 2013 and authenticated as *Cydonia oblonga* Mill by the Department of Pharmacognosy of School of Pharmacy at Isfahan University of Medical Sciences. The fruits were sliced, airdried, and powdered at room temperature. Hundred grams of the powder was extracted with 70% ethanol by maceration method[25]. For preparation of polyphenolic extract, 100 grams of the powder was extracted with ethanol: water (9:1). The residual was further extracted with ethanol:water (1:1) by maceration method. Two extracts were

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then filtered and mixed together to obtain polyphenolic extract. Both hydroalcoholic and polyphenolic extracts were concentrated, dried in a rotary evaporator, and freeze dried to afford crude dried extract.

Phenol content measurements

The total Phenolic content of the extracts was determined by Folin-Ciocalteau micro method already described by Waterhouse[26].

Cell culture

The human hepatoma cell line (HepG2) was cultured in RPMI medium (containing7 % FBS and 1% penicillin/streptomycin to avoid the growth of undesirable and pathogenic bacterial microorganisms) and incubated under 5 % CO2 at 37 °C in micro filter plates. Cells (0.25 × 10^6 cells per well) were incubated with different concentrations of MMS (10, 100 and 500 µM) for one hour to determine the optimized genotoxic concentration. In other events, cells were incubated with different concentrations of hydroalcoholic extract at 1, 10, 100, and 500 µg/ml or polyphenolic extract at 10, 50, 100, and 500 µg/ml for 2 h which was further incubated with selected concentration of MMS (100 µM) for one hour . The upper medium of wells was thrown away and cells washed with PBS. After trypsinization, 1 ml of medium was added to each falcon tube to be used for the comet assay[27,28].

Alkaline comet assay

The comet assay procedure has been described in detail in our previous studies [21,27-29]. Briefly, cell suspensions (1 × 10^6 cells/ml) were incubated and mixed with 1% LMP agarose at 37 °C, placed on the precoated slides (1 % NMP agarose), and covered by cover glasses for 5 minutes. The slides were incubated with lysis solution (pH = 10) for 40 minutes and rinsed with distilled water to remove excess lysis solution. Slides were then incubated with electrophoresis buffer (pH > 13) for 40 minutes. Electrophoresis was carried out for 40 minutes at 25 V with an electric current adjusted to 300 mA. Then, the slides were rinsed with distilled water to remove excess alkaline buffer and were placed in neutralization solution (pH = 7.5) for 10 minutes. The slides were covered by sufficient dye solution (20 µg/ml ethidium bromide) for 5 minutes and washed with distillated water. Finally comets were visualized under ×400 magnification using fluorescence microscope with an excitation filter of 510 - 560 nm and a barrier filter of 590 nm[30]. All stages of comet assay were performed at room temperature and in dark conditions. All solutions were freshly prepared and used cool [27,28].

Statistical analysis

Tail moment (% DNA in the tail × tail length), tail length, and percent of DNA in the tail were used for statistical analysis in this investigation[31,32]. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test was used to compare the results. The results assumed to be significant if $p<0.05$.

Results

Phenolic and Polyphenolic contents

The total phenolic content of hydroalcoholic and polyphenolic extracts was estimated (in terms of gallic acid equivalent) at 13.06 and 28.3mg GAE/g.

The comet assay results

As explained in previous section, to determine the minimal genotoxic MMS concentration, HepG2 cells were incubated at various concentrations of MMS for one hour and then observed by comet assay. The adopted concentration of MMS (100 µM) showed significant difference with the control group. Moreover, the number of incubated cells did not decline during incubation. Thus the concentration of 100 µM was selected for further investigation.

Genoprotective effects of Quince extracts

To study the protective effects of hydroalcoholic and polyphenolic extracts of Quince on DNA damage induced by MMS, HepG2 cells were

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incubated with different concentrations of either hydroalcoholic or polyphenolic extract for 2 hour followed by one hour incubation in the presence of 100 μM MMS. The results of comet assay after 2 hours incubation for both extracts were analyzed. One-way analysis of variance (ANOVA) for the tail length for both extracts was significant. Tukey's multiple comparison post hoc tests showed that at all concentrations of either extract, the tail length was significantly decreased as compared with that of cells were exposed only to MMS. (Fig. 1A and 2A) The results of the ANOVA for the percentage of DNA in the tail were also significant. Tukey's multiple comparison post hoc test revealed that percentage of DNA in the tail at all concentrations of either extract was significantly decreased compared to the MMS group (Fig. 1B and 2B). At all concentrations tested, the tail moment was significantly decreased as compared with that of MMS group (Fig. 1C and 2C).

Genotoxic effect of extracts of Quince

Genotoxic properties of both hydroalcoholic and polyphenolic extracts of Quince were assessed and compared to the negative group. The results of comet assay showed that the extracts did not exhibit genotoxic effect under studied concentrations.

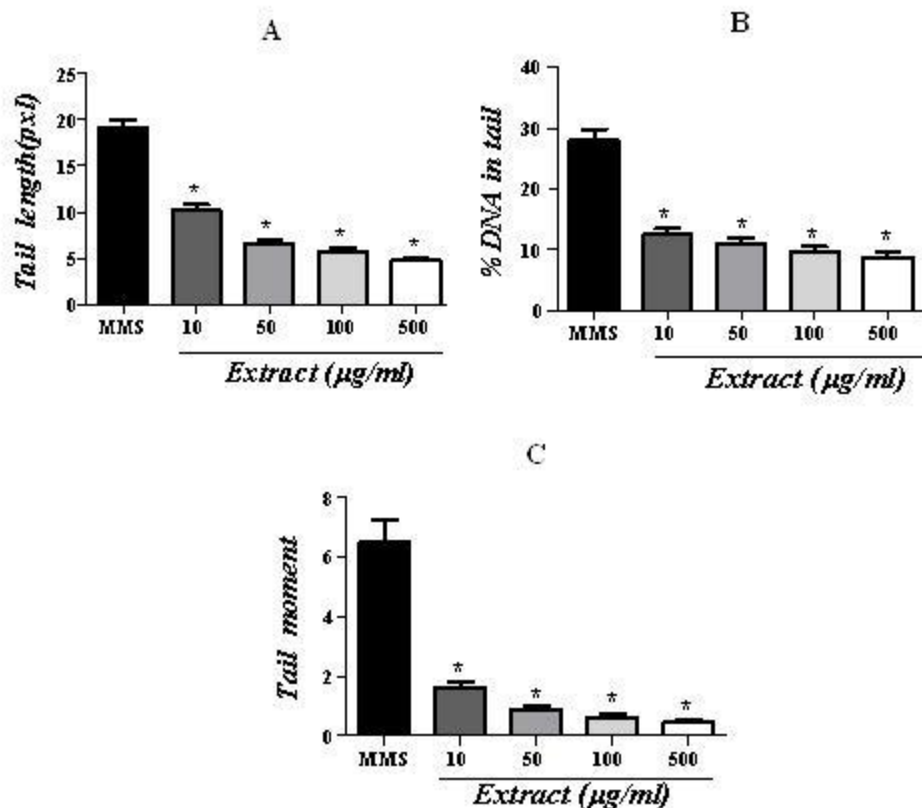

Fig. 1. Comparison of (A): Tail length, (B): % DNA in tail and (C): Tail moment of different concentrations of hydroalcoholic extract of Quince with the MMS group (100 μM). Each graph has been represented as Mean ± SEM. The sign (*) shows significant results (P<0.001) in comparison with the MMS group.

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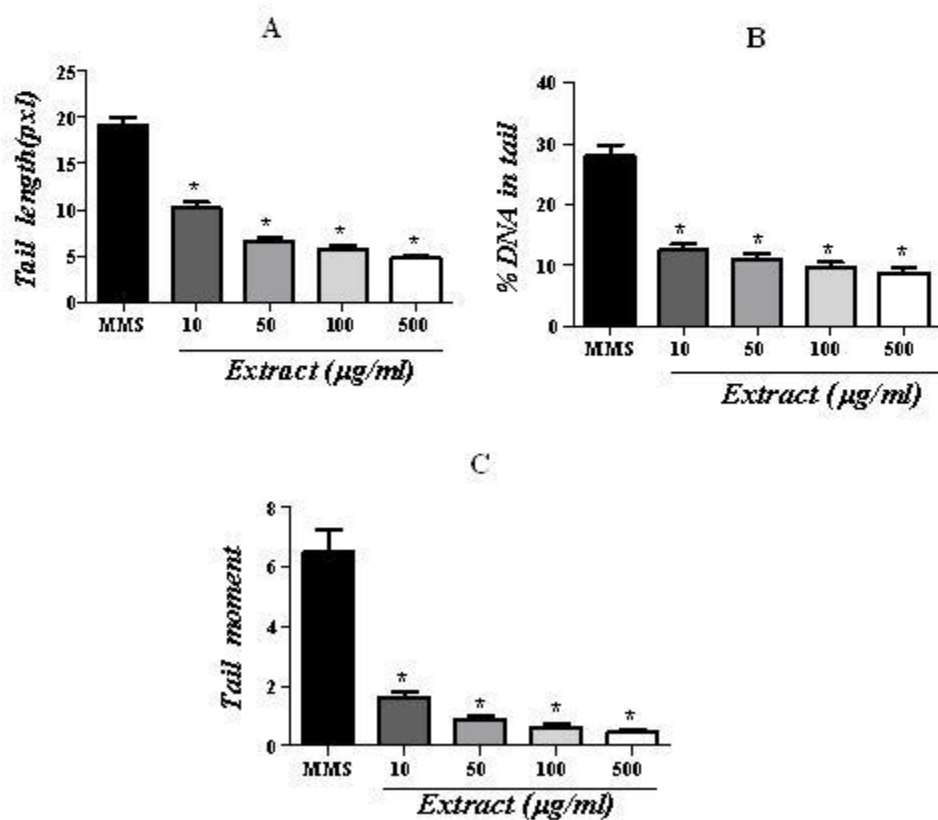

Fig. 2. Comparison of (A): Tail length, (B): % DNA in tail and (C): Tail moment of different concentrations of polyphenolic extract of Quince with the MMS group (100 μM). Each graph has been represented as Mean ± SEM. The sign (*) shows significant results (P<0.001) in comparison with the MMS group.

Discussion

With regard to the fact that DNA damages are likely to be one of the major primary causes of cancers, detection and prevention of these changes could be helpful to reduce the risk factors of this deadly disease. Single-strand DNA breaks measured by the comet assay are a reliable indicator of genotoxic effects of different factors. Previous studies have indicated that herbal extracts containing phenolic compounds such as caffeoylquinic acids, dicaffeoylquinic acid, quercetin-3-O-galactoside, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside and kaempferol-3-O-rutinoside with anti-genotoxic properties [10-12,33]. It has been shown that methanolic extract of Quince could prevent oxidative hemolysis of human erythrocytes in a dose dependent manner [6]. Previous studies have reported that limitation of inflammatory factors could reduce the risk factors of DNA damage and cancers [34]. Polyphenolic compounds and hot-water extract of Quince also have shown anti-inflammatory properties [10,35]. Several researches have indicated that metabolites of Quince have antioxidant, antiproliferative and genoprotective effects and could reduce the risk factors of cancers [7,36,37]. The results of an MTT analyzing study on Quince have indicated no toxicity on human normal cells [38].Quince and its jam are rich in antioxidant compounds [37]. We detect total phenolic content of extracts as GAE. Our results and previous studies shows that polyphenols increase the stability of genomic content and decrease genotoxic effects of internal and external genotoxic factors [8]. Quince has a large quantity of polyphenols [11]. Among available protocols, we use pretreatment method for evaluation of

chemopreventive activity. The reason of selection HepG2 was specialized liver function and comparable activities with human hepatocytes. Suitability of this cell line for assessing genotoxicity have shown repeatedly [24]. The results of the present study indicated that 2-hour incubation of HepG2 cells with different concentrations of hydroalcoholic and polyphenolic extracts of Quince had genoprotective effects against genotoxicity of MMS. The results of our investigation showed that hydroalcoholic and polyphenolic extracts of Quince have no genotoxic effect on HepG2 cells under studied condition.

Conclusion

The results of the current study indicated that while hydroalcoholic and polyphenolic extracts of Quince have no genotoxic effect on HepG2 cells, they possess genoprotective effects on the cells. Further studies are required to prove the observed effects in human beings.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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