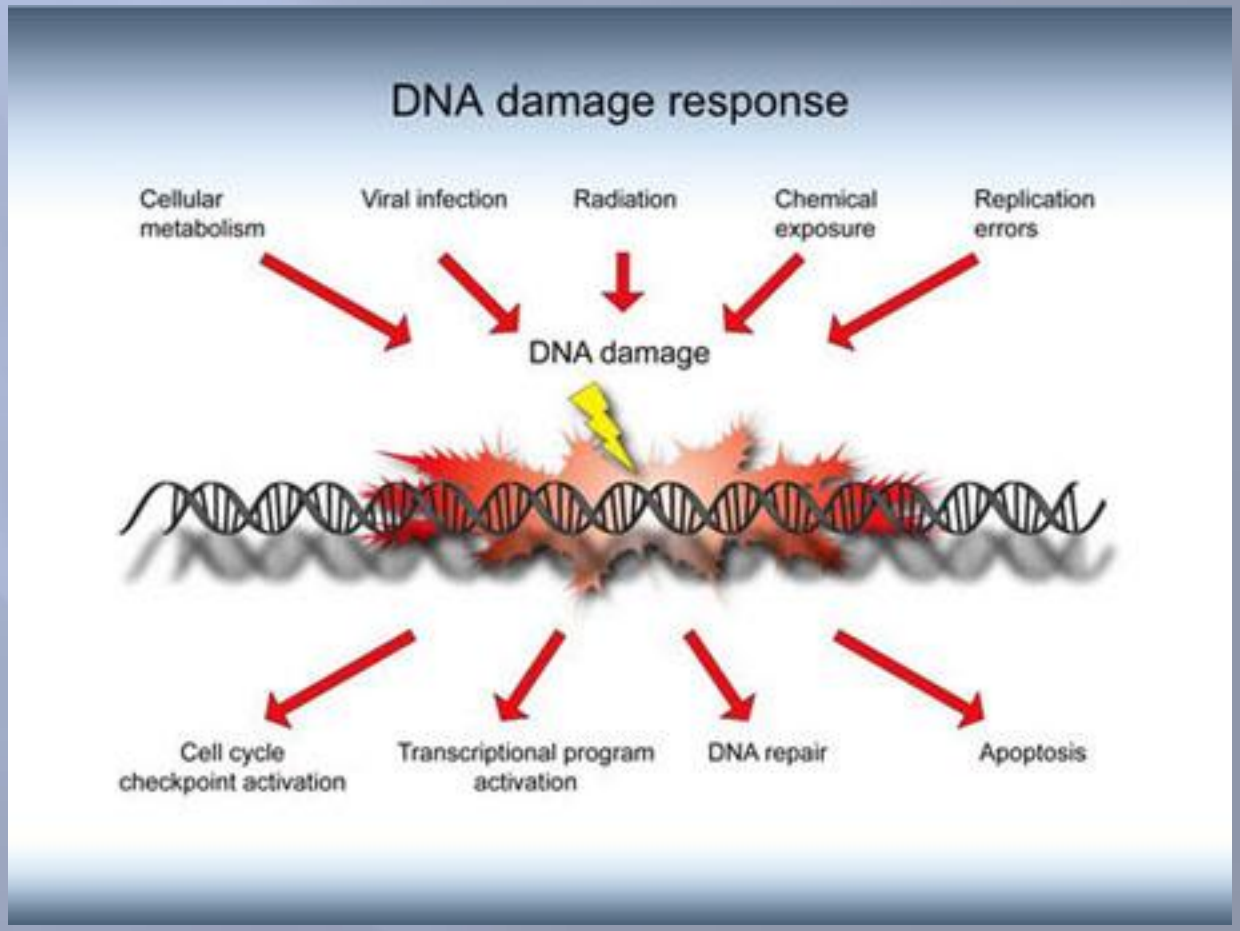
Evaluation of Genoprotective Effects of Hydroalcoholic and Polyphenolic Extracts of Quince by Comet Assay

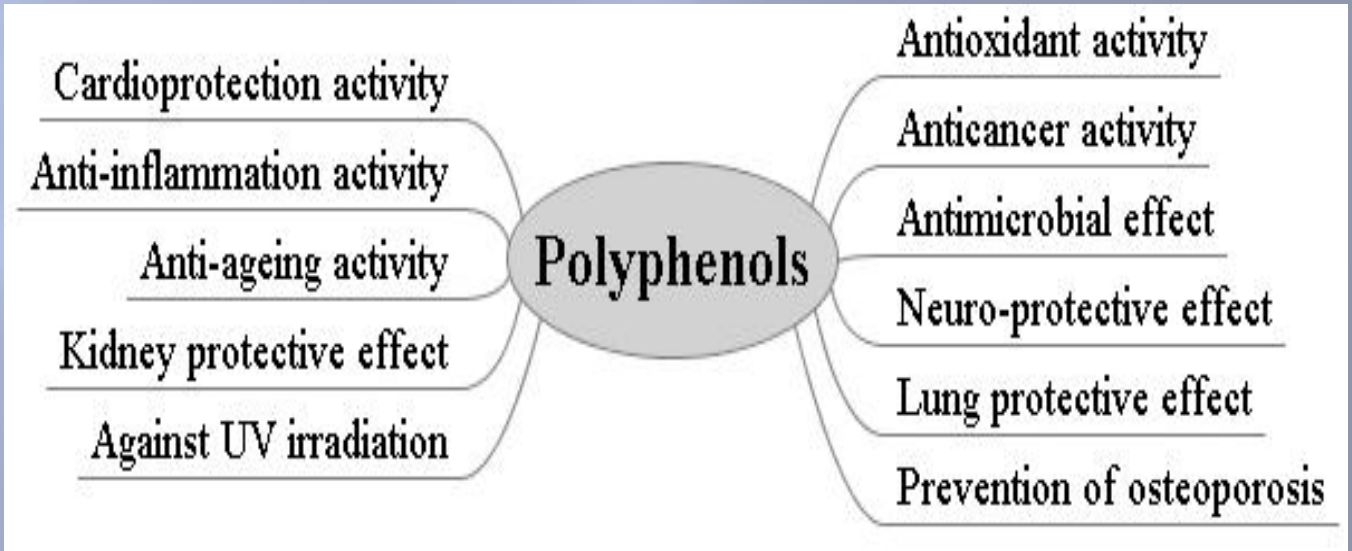
BY : Dr. khadijeh mousavi

Patterns of DNA damage

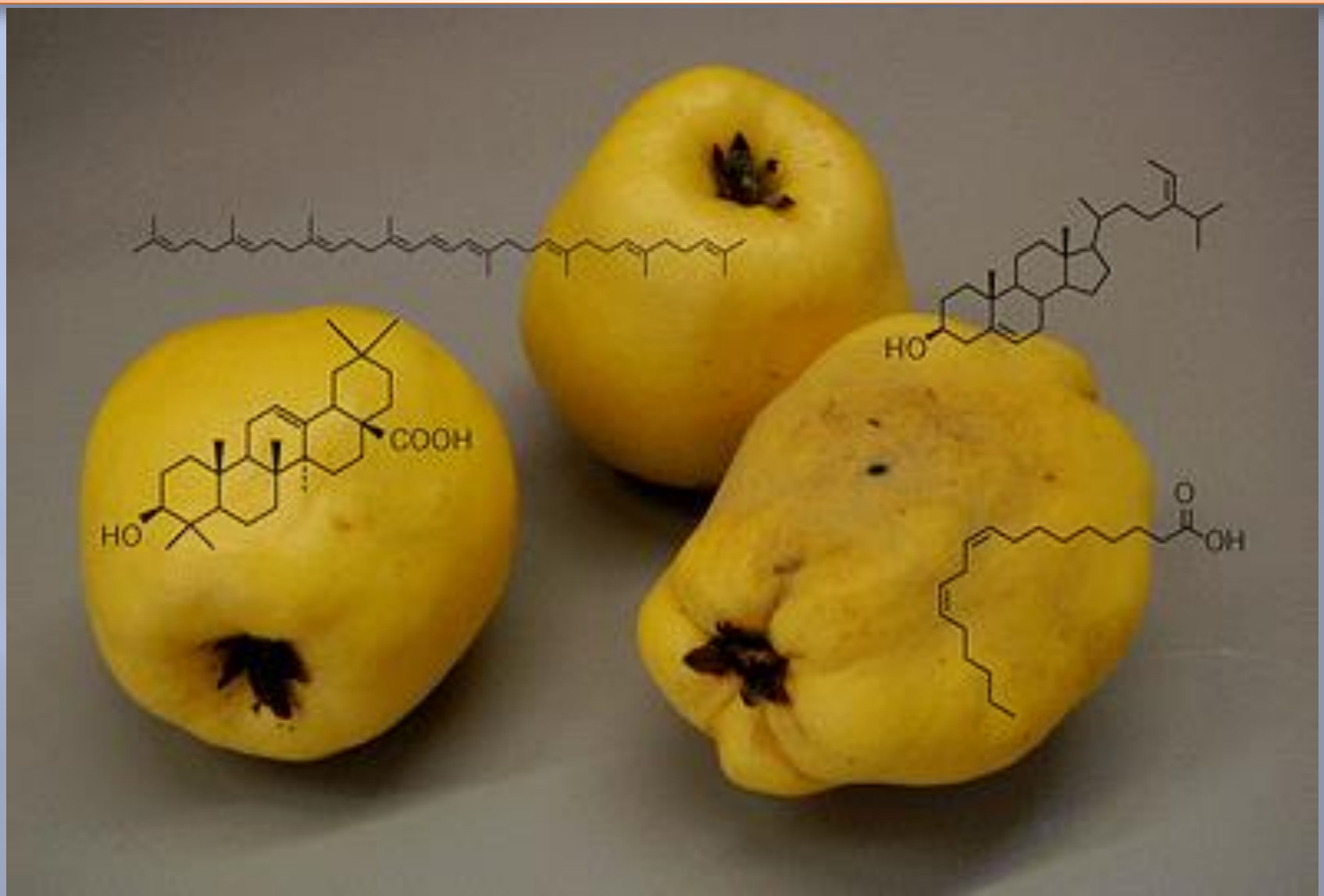


Cydonia oblonga Mill.

- member of Rosaceae family
- contains several polyphenols



Cydonia oblonga Mill.



- In Iranian traditional medicine, fruits and seeds of quince have been widely used to treat cough, bronchitis, constipation, diarrhoea, dysentery, cardiovascular diseases, renal diseases, headache, inflammatory bowel disease, migraine, nausea, common cold, hepatitis and cancer

Bioactivity

- Antiradical activity
- Antidiabetic effects
- Kidney protecting effects
- Antihemolytic activity
- Antiallergic activity
- Antiproliferative activity

- 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 genoprotective effects of each individual extract were also evaluated

HepG2 Cells

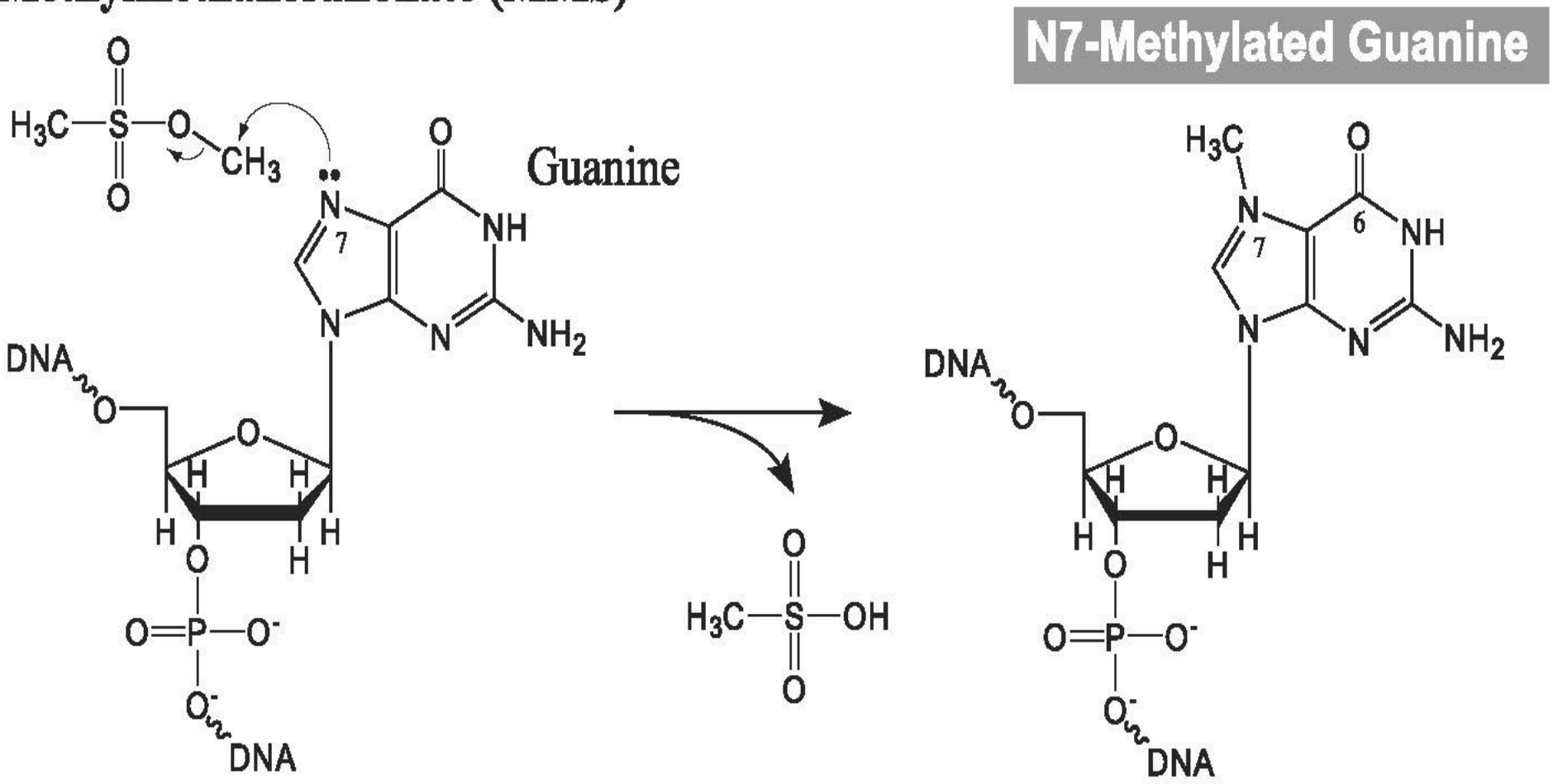
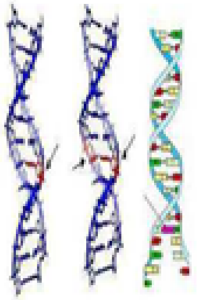


Figure 4. DNA alkylation by methylmethanesulfonate (MMS). Nucleophilic attack from the guanine N7 (*left*) generates a methyl adduct at position 7 of guanine.

ALKALINE COMET ASSAY



COMETCHIP

Detection of single-strand breaks
Detection of double-strand breaks
Detection of cross-links with DNA or protein
Detection of oxidative base damage

Detection of genotoxicity of various chemical and physical agents
Detecting the protective effect of several antoxidants against genotoxicity

APO/NECRO COMET ASSAY

Detection of apoptosis and necrosis due to double-strand breaks

Detecting and distinguishing two forms of cell death

NEUTRAL COMET ASSAY

Detection of DNA fragmentation in sperm cells

The degree of sperm DNA fragmentation can predict outcomes of in vitro fertilization

Materials and Methods

- Extract preparation
- Phenol content measurements
- Cell culture
- Cell treatment
- comet assay

Extract preparation

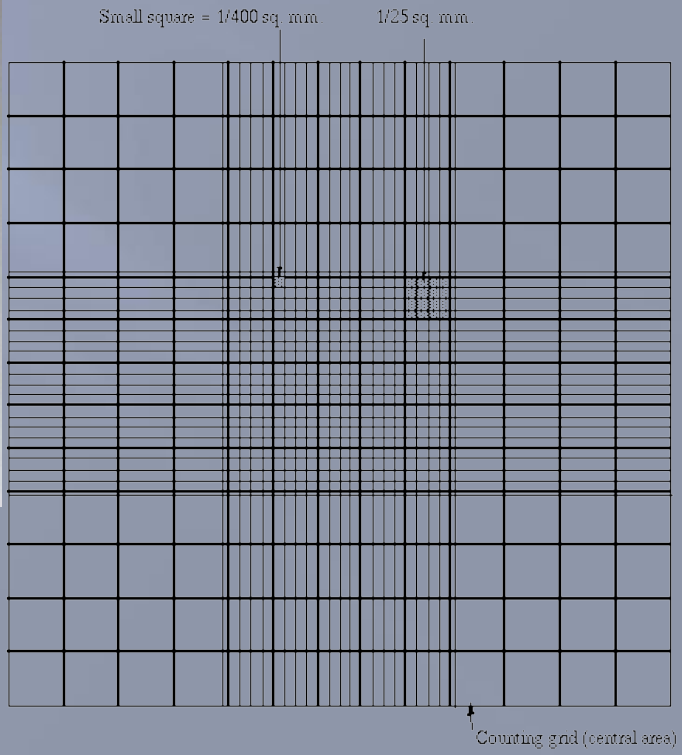
Extract preparation

Phenol content measurements

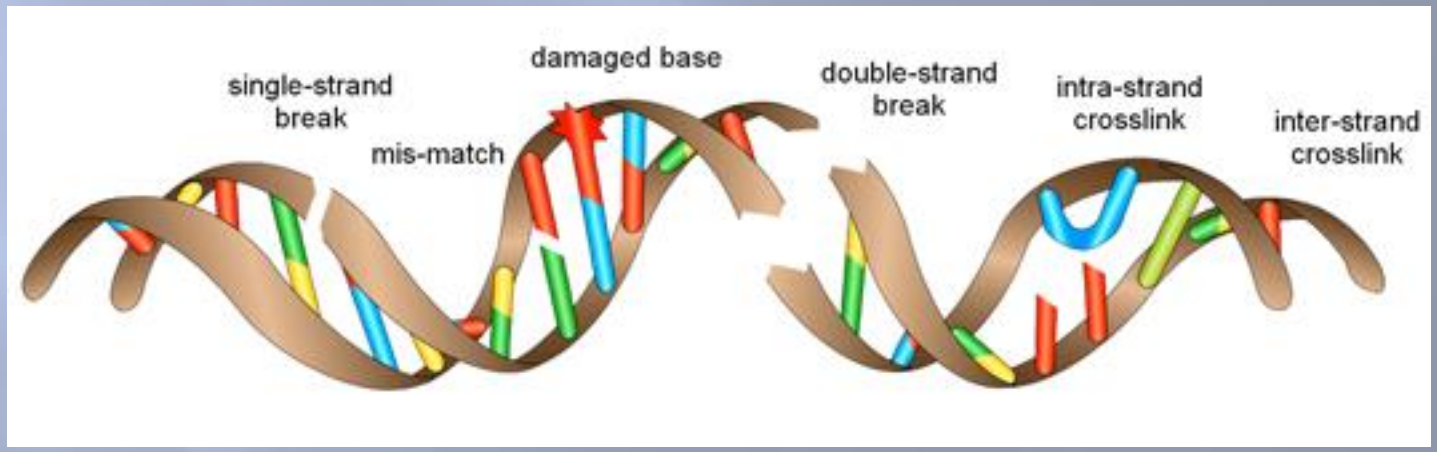
Cell culture

Cell culture

Cell counting



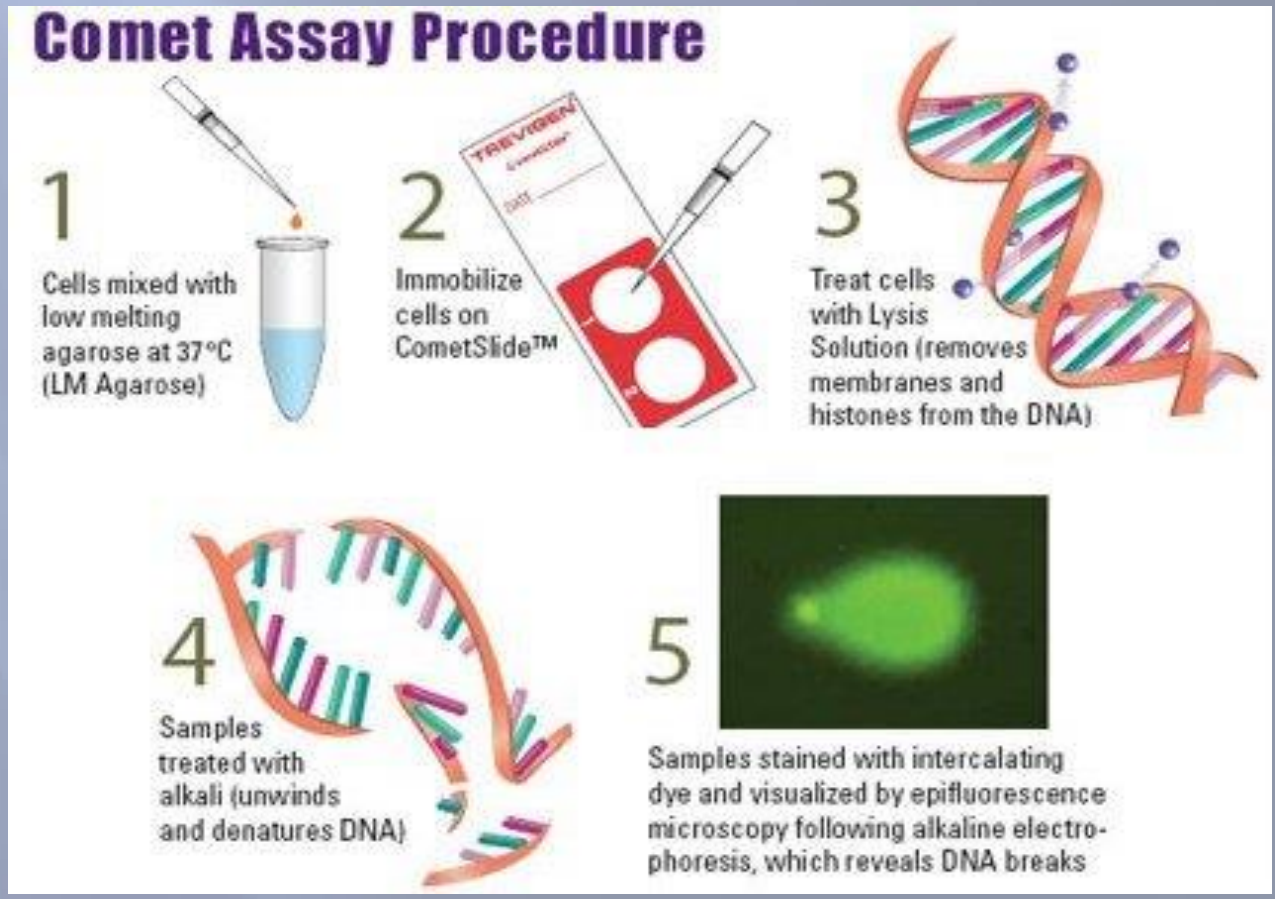
Cell treatment



Cell treatment

Comet Assay

- Preparation of Slides for the Comet Assay
- Lysing



Comet Assay

- Electrophoresis

Comet Assay

- Observation

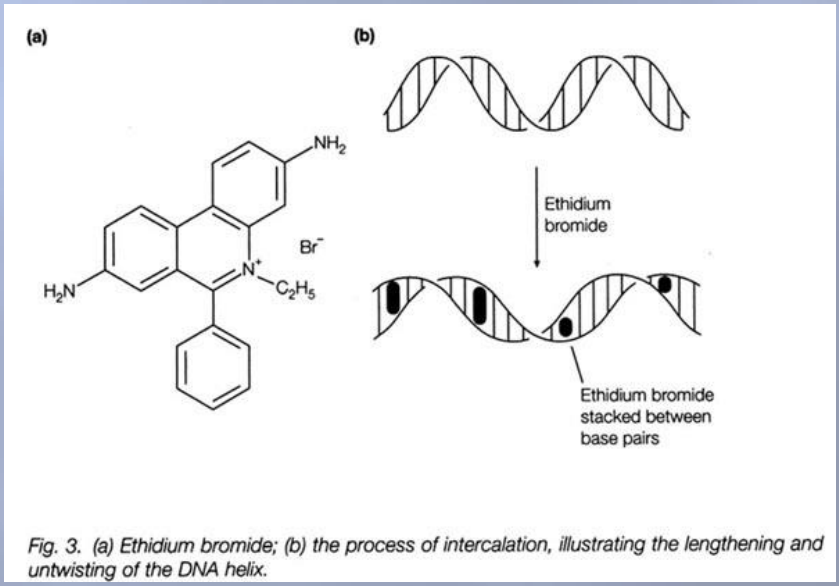
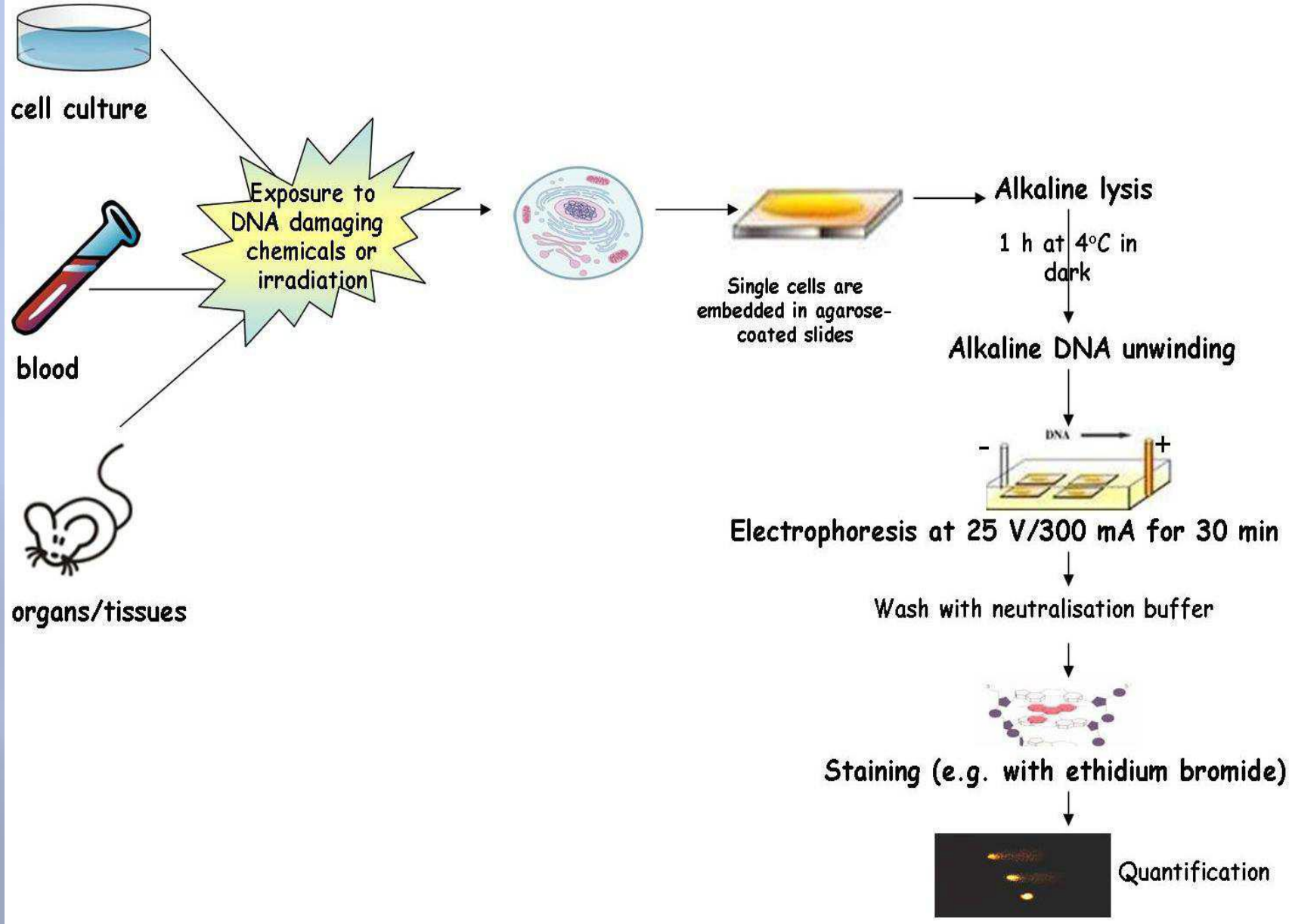


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

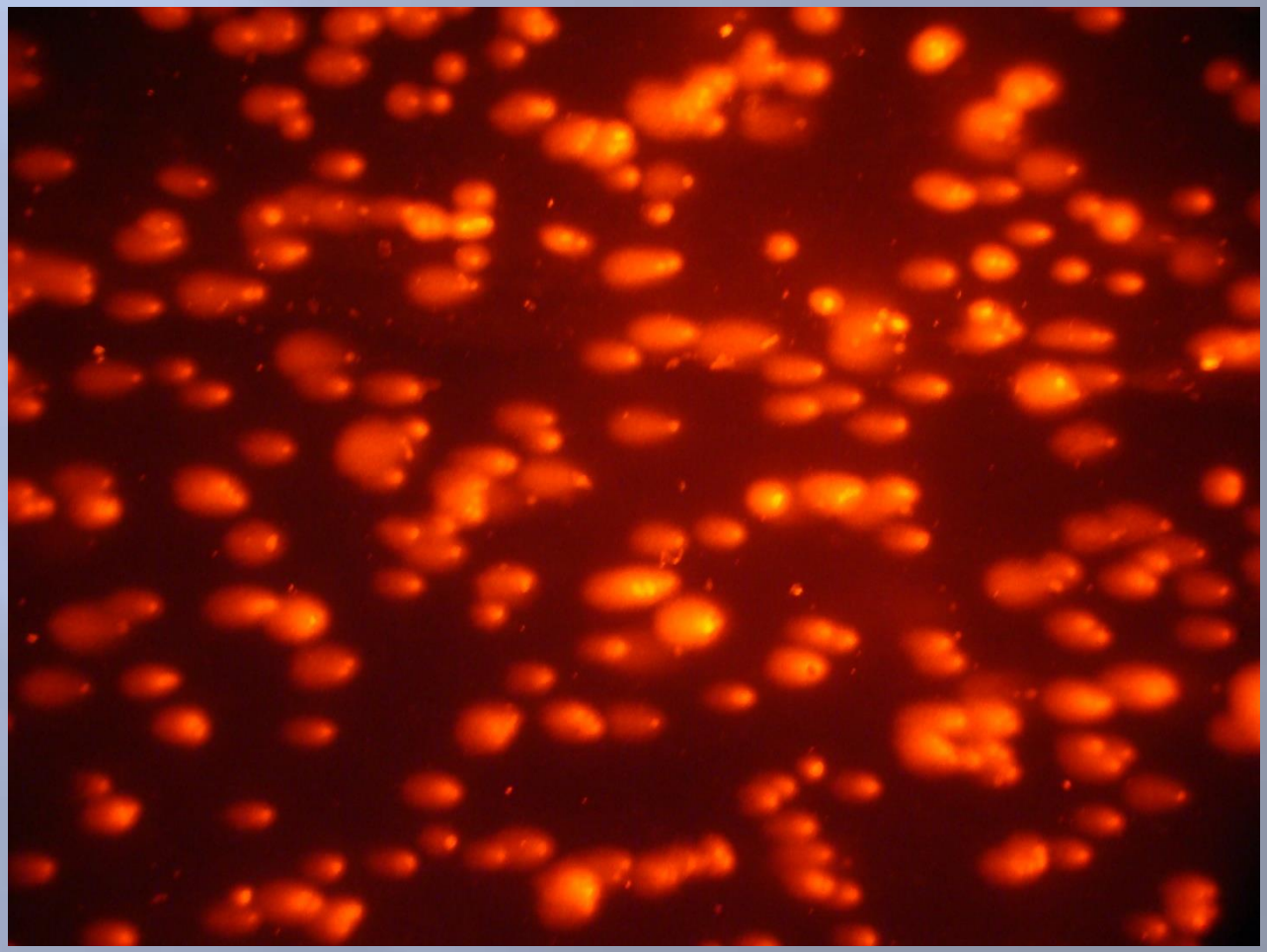

cell culture
blood
organs/tissues
Exposure to DNA damaging chemicals or irradiation
Single cells are embedded in agarose-coated slides
Alkaline lysis
1 h at 4°C in dark
Alkaline DNA unwinding
DNA
-
+
Electrophoresis at 25 V/300 mA for 30 min
Wash with neutralisation buffer
Staining (e.g. with ethidium bromide)
Quantification

Results

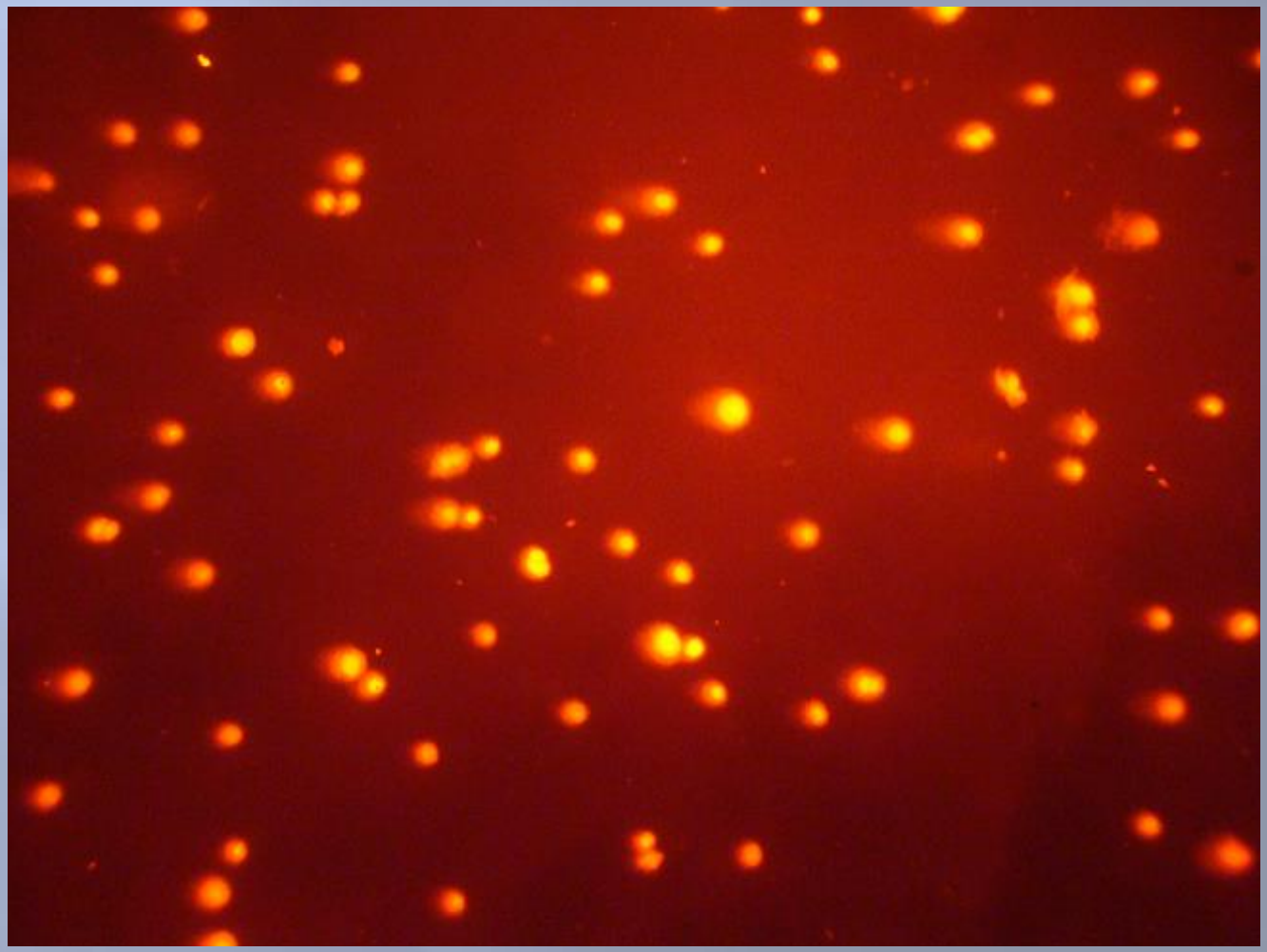
Phenolic and Polyphenolic contents

	total phenolic content
Hydroalcoholic extracts	67.7 mg GAE/g
polyphenolic extracts	118.7 mg GAE/g

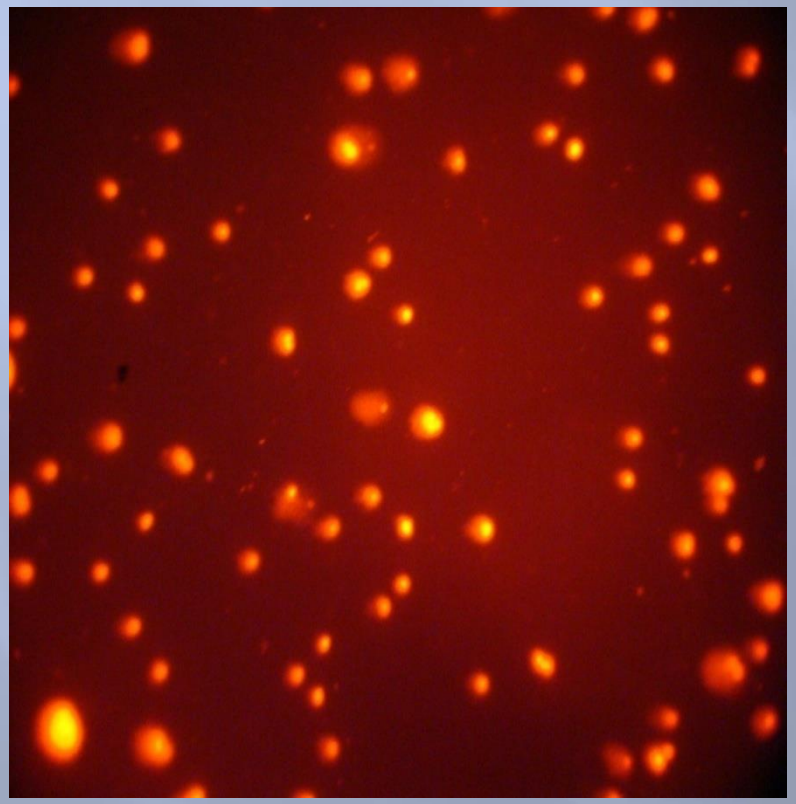
The comet assay results



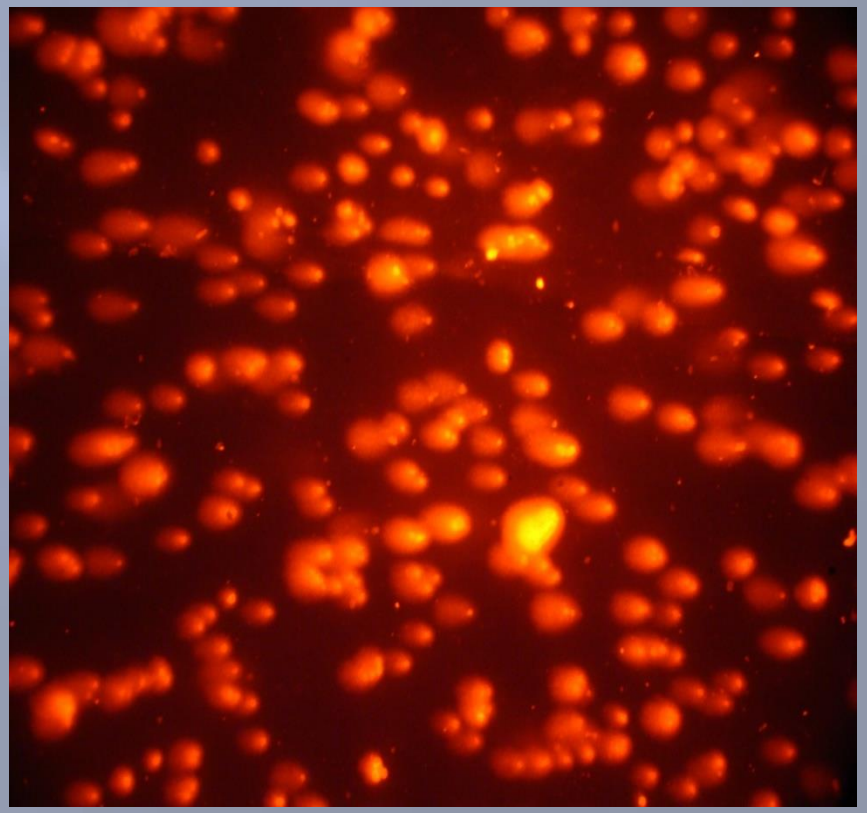
Negative



Hydroalcoholic extracts + MMS 100



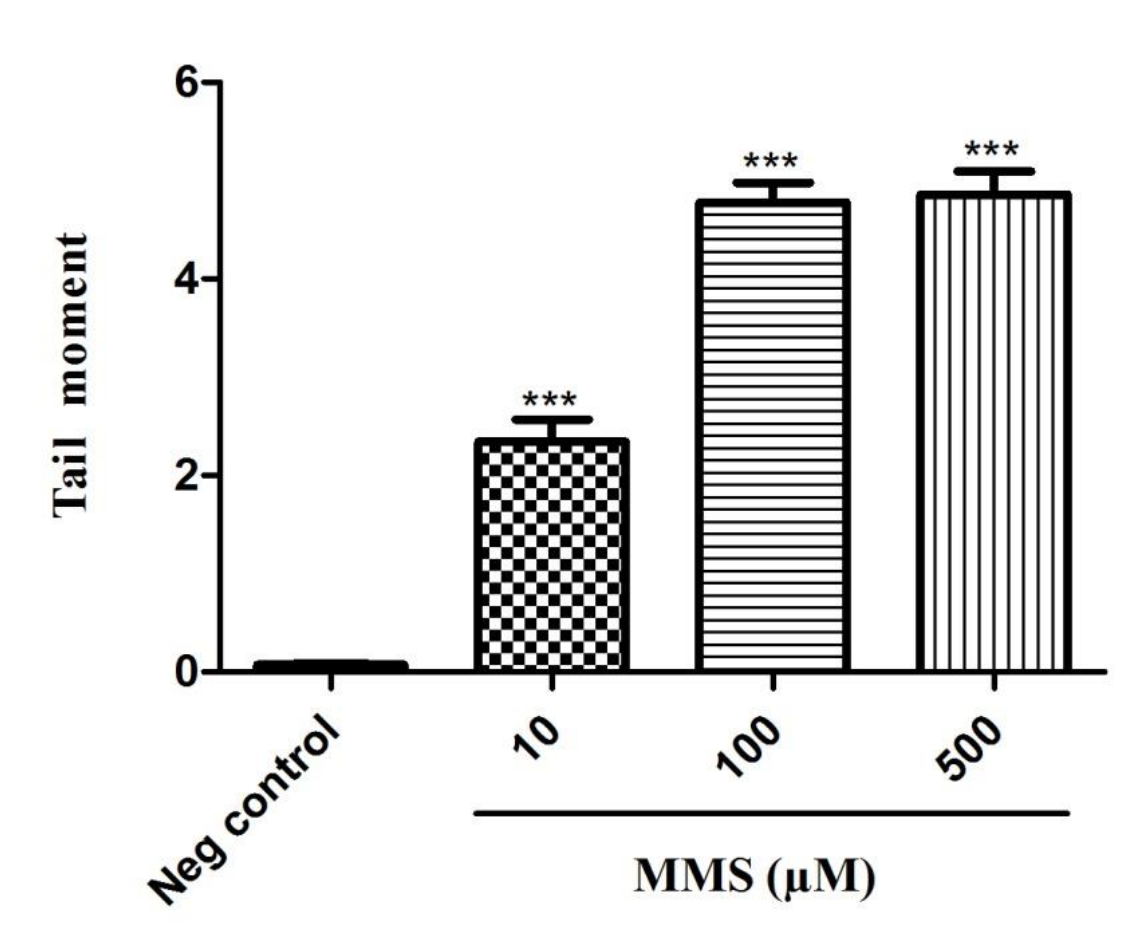
Positive control (MMS 100)



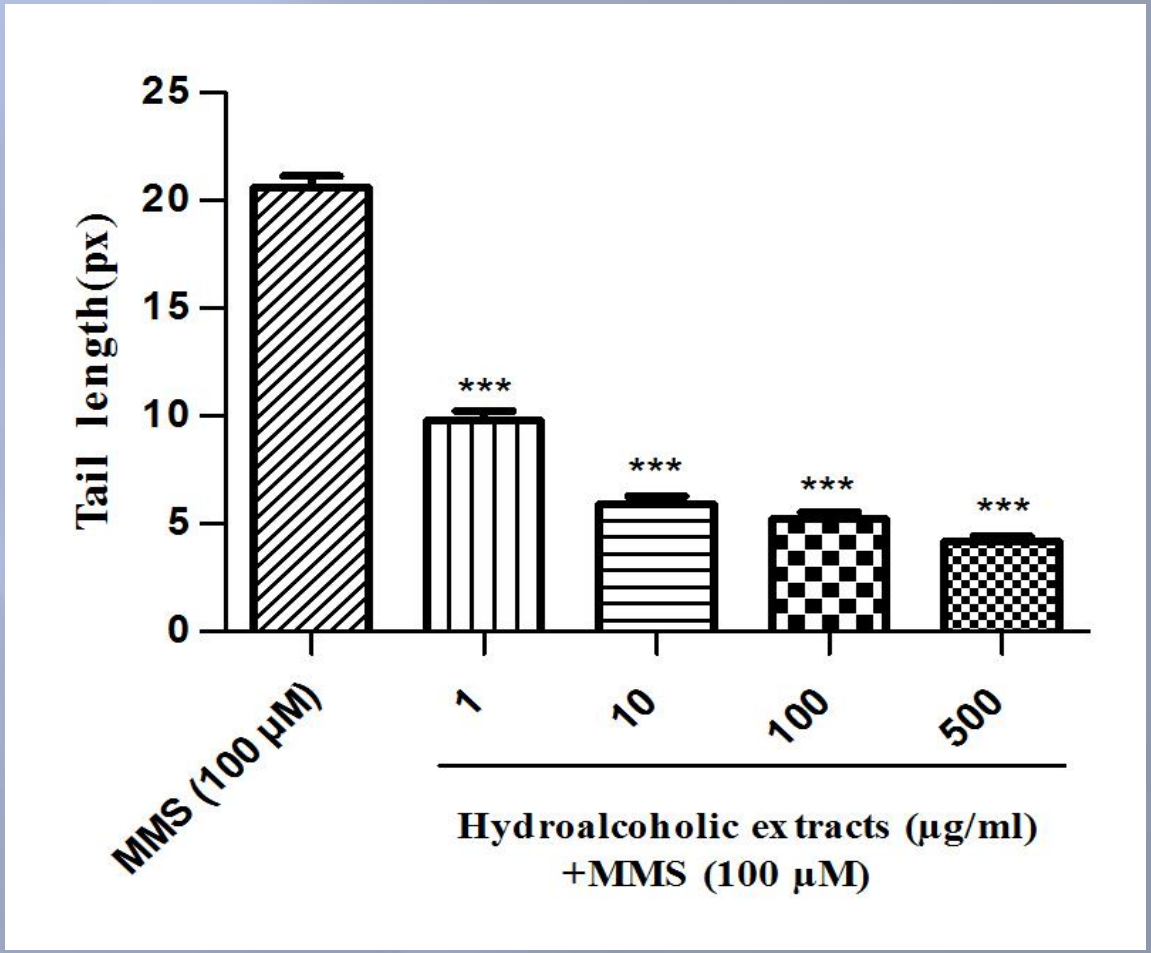
Genotoxic effect of extracts of quince

- Genotoxic properties of both hydroalcoholic and polyphenolic extracts of quince were assessed and compared to the negative group

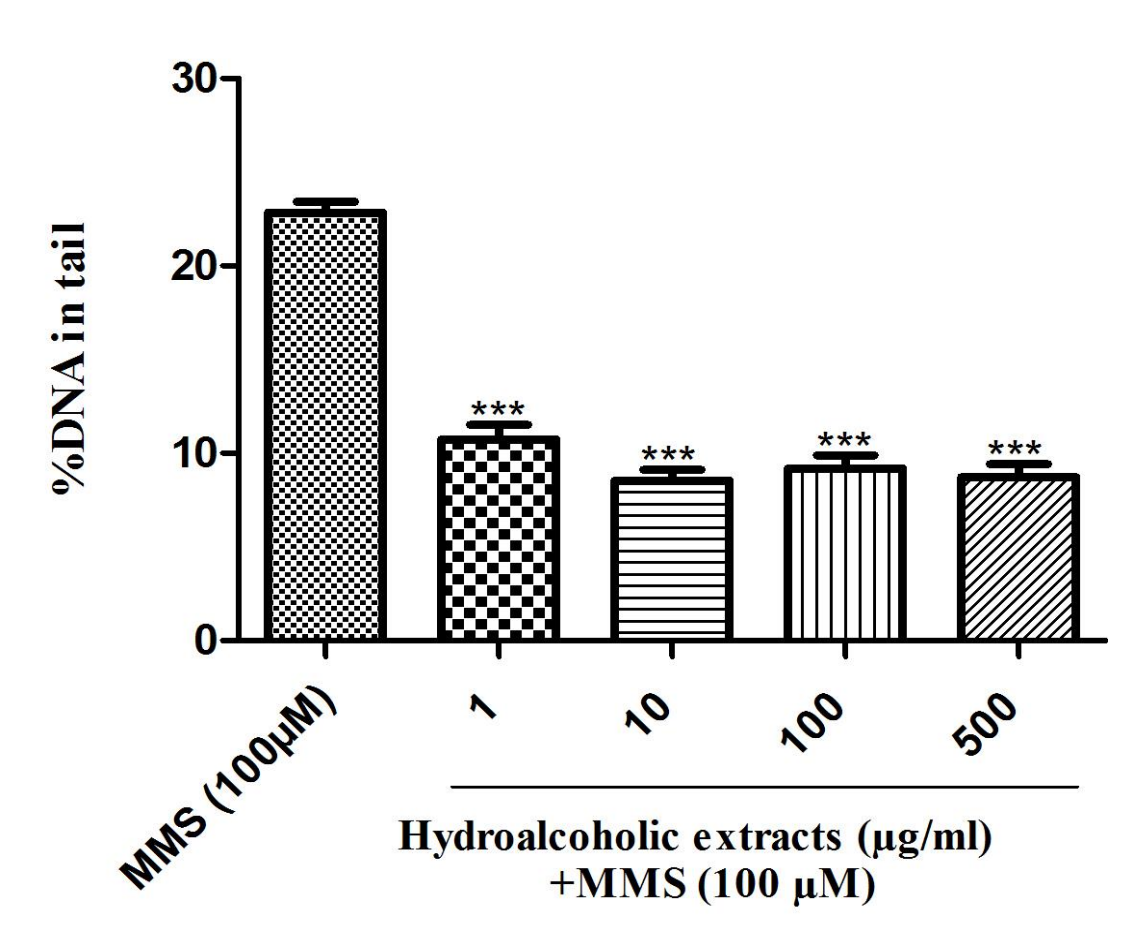
Determine the minimal genotoxic MMS concentration



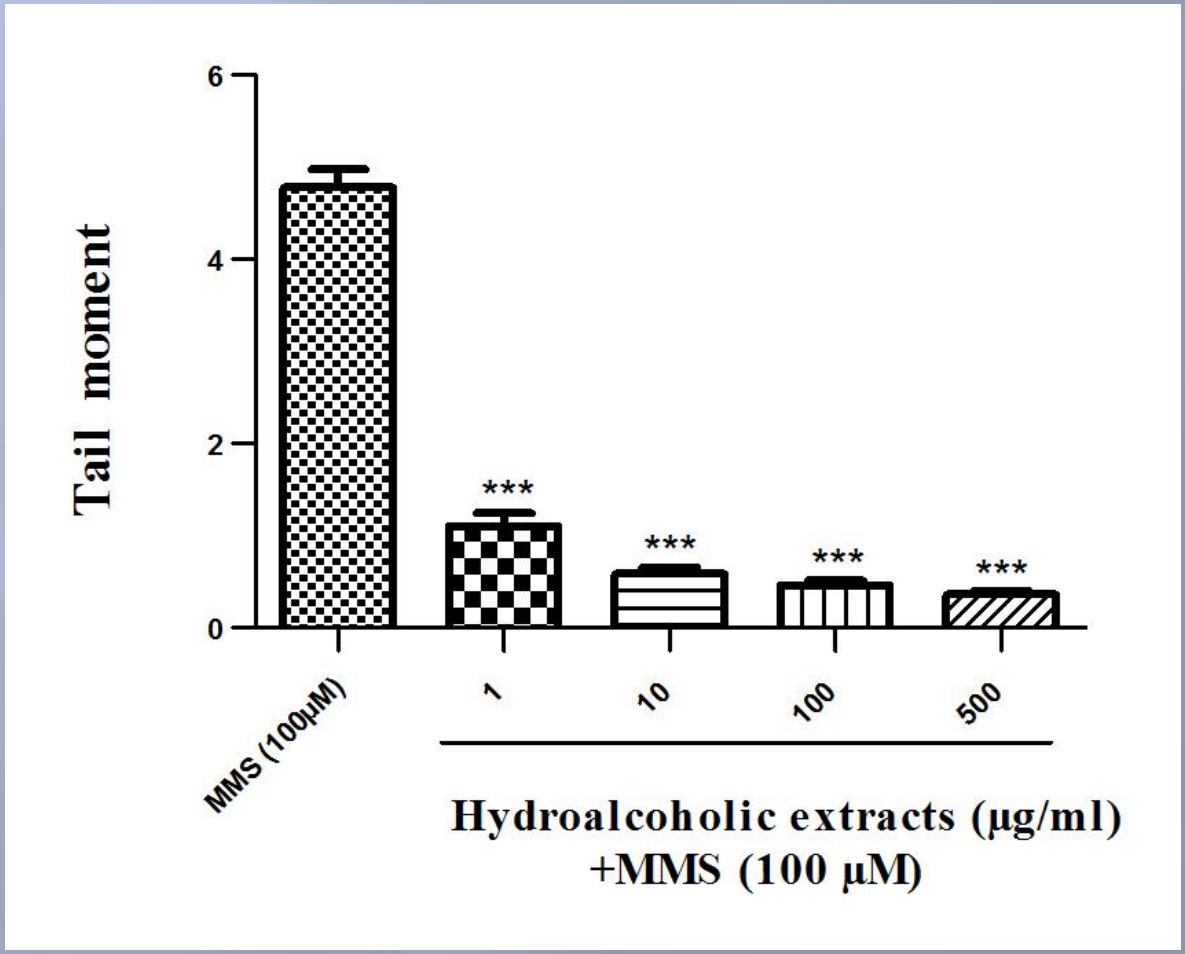
Genoprotective effect of hydroalcoholic extract of quince



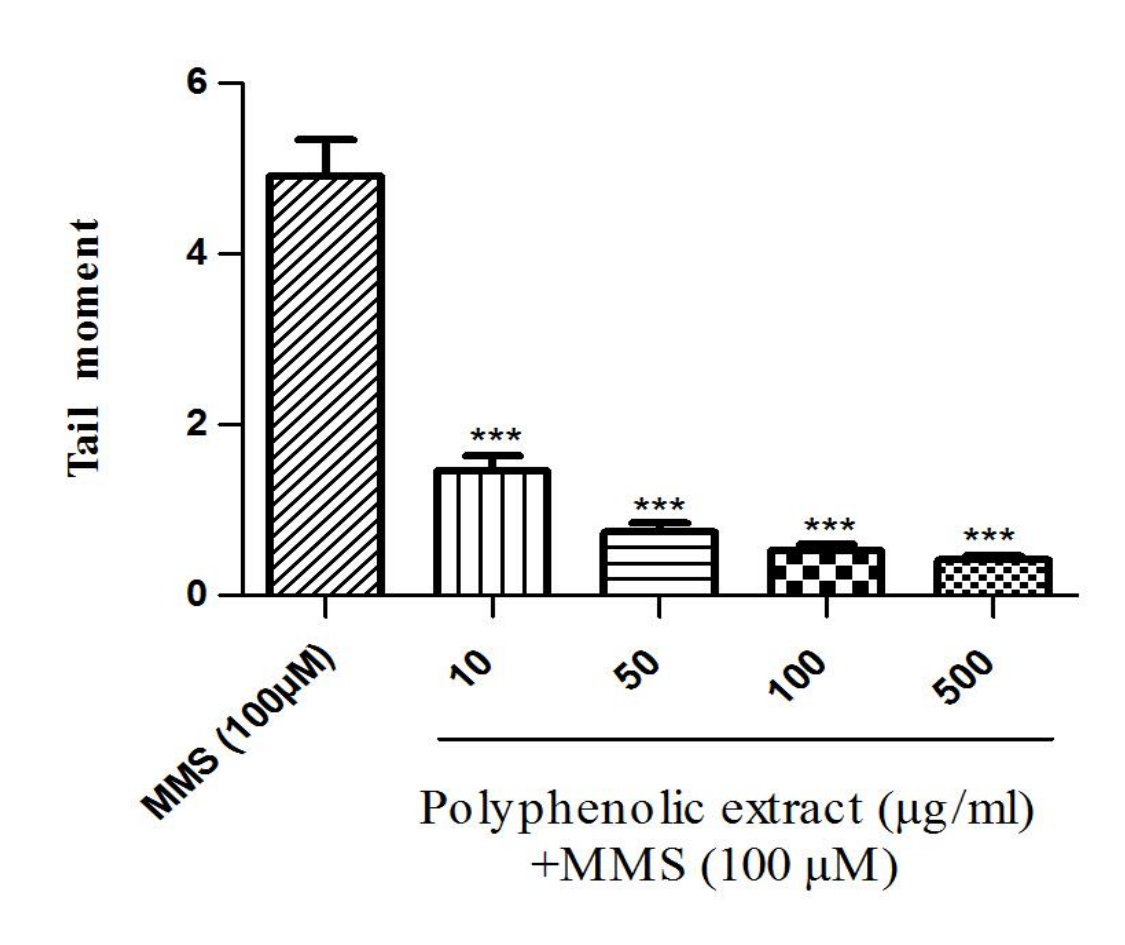
Genoprotective effect of hydroalcoholic extract of quince



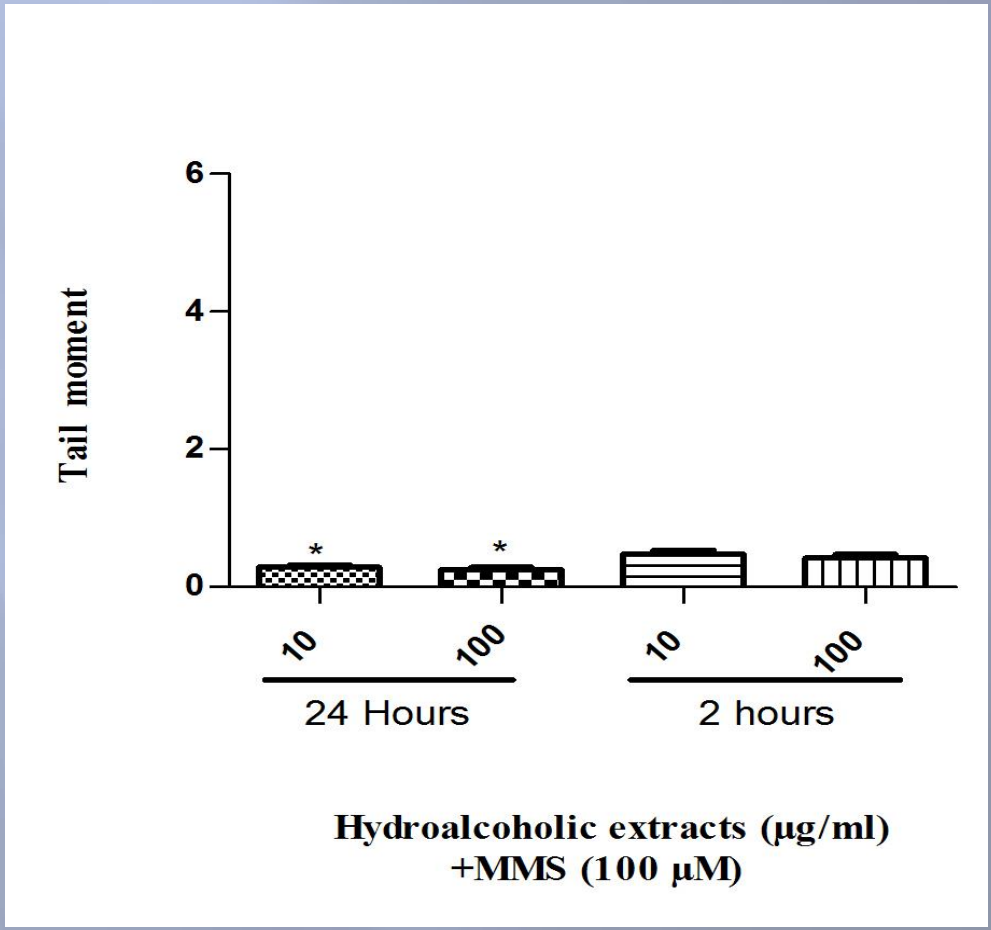
Genoprotective effect of hydroalcoholic extract of quince



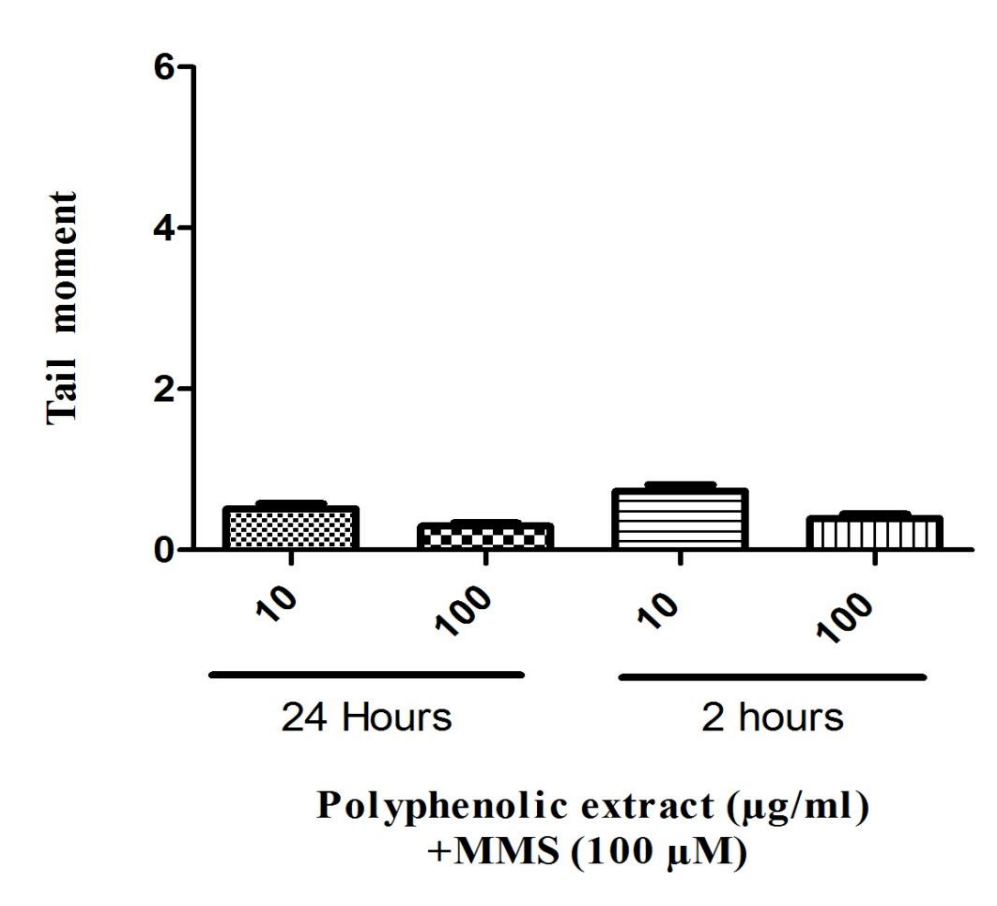
Genoprotective effect of polyphenolic extract of quince



Time-dependent protective effect of hydroalcoholic extract



Time-dependent protective effect of polyphenolic extract



Discussion

- The results of our investigation showed that hydroalcoholic and polyphenolic extracts of quince have no genotoxic effect on HepG2 cells under studied condition and different concentrations of hydroalcoholic and polyphenolic extracts of quince had genoprotective effects against genotoxicity of MMS

Discussion

- The free radical scavenging activities of the methanolic extracts of quince show the antioxidant activities (Magalhaes et al., 2009)
- Alesiani et al. (2010) investigated the antiproliferative activities of the isolated phytochemicals from quince peels against murine melanoma B16-F1 cells in which the most active phytochemical to inhibit the growth of melanoma cells.

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Khadijeh Mousavi mobarakeh[a], Mahmoud Etebari[a*], Behzad Zolfaghari[b], Abbas Jafarian-Dehkordi[a]

[a]Department of Pharmacology, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[b]Department of Pharmacognosy, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

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

أمیرالمؤمنین علی علیه السلام: الفرصة سریعة الفوت بطیئة العود

فرصت زود گذر است و دیریاب

مستدرک الوسائل ج۱۲ ص۹

با تشکر از توجه شما

امام صادق (ع) می فرماید:

- خوردن به چهره را نیکو کند و قلب را آرام سازد.
- بِه مایه ی قوت قلب و روشنایی ضمیر است.
- در بِه ، ویژگی ای است که در دیگر میوه ها نیست.
- بِه اندوه اندوهگین را از میان می برد، چنان که دست ، عرق پیشانی را می برد.
- خوردن بِه ، مایه توان قلب و بیداری دل است و ترسو را دلیر می کند.
- هر کس ناشتا یك بِه بخورد، نطفه اش پاکیزه می شود و فرزندش نیکو می گردد.