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The antıoxıdant and antımutagenıc propertıes of dıfferent extracts of *Crataegus monogyna subsp. monogyna* collected from the Eastern Anatolıa regıon of Turkey

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In the present study, we aimed to investigate the genotoxic and anti-genotoxic potencies of fruits of *Crataegus monogyna subsp. monogyna* (C.M.) by using the micronucleus (MN) and sister chromatide change (SCE) tests in human blood cells. According to the results obtained from the MN and SCE tests, all extracts of C.M. have antimutagenic effect. The most effective extracts were in methanol and acetone. The 40-80 µg/mL doses of C.M. were more effective than other doses. In addition, we evaluated the antioxidant activity in order to clarify the possible mechanisms that may contribute to the anti-genotoxic activity of C.M. Similarly, all extracts displayed antioxidant activity; the most effective extracts were in methanol and acetone. The order of the antigenotoxic and antioxidant effect of the most effective extracts was as follows: CMA *(Crataegus monogyna* acetone*)* > CMM (*Crataegus monogyna* methanol) > CME (*Crataegus monogyna* ethanol*)* > CMW (*Crataegus monogyna* water).

Key words: *Crataegus monogyna subsp. monogyna*, antimutagenic, antioxidant, MN, SCE

INTRODUCTION

Crataegus monogyna is one of the most common species called "hawthorn" in traditional herbalism. The plant is native to Europe, nortwest Africa and western Asia. It has been introduced in many other parts of the world. The hawthorn is a shrub or small tree of 5-14 m height, with a dense crown. The leaves are 20 to 40 mm long, obovate and deeply lobed, sometimes almost to the midrib, with the lobes spreading at a wide angle. The hermaphrodite flowers are produced in carymbase of 5–25 together with five white petals, numerous red stamens and a single stem. Flowers are pollinated by midges, bees and insects and bear numerous haws. The haw is a small, oval, dark-red fruit about 10 mm long berry. Haws are important for wildlife in winter [1-3].

The plant parts used are usually both leaves and flowers or alternatively the fruit. Howthorn has been investigated in medicine for treating cardiac insufficency. On the other hand, *Creteagus monogyna* is a source of antioxidant phytochemicals, especially the extracts of howthorn leaves with flowers. The *Crataegus* species is well distributed in Turkey as a wild plant and is used as herbal medicine [4].

Earlier findings of *Crataegus monogyna* have shown its pharmacological properties such as antimicrobial, antioxidant, antitumor, antiviral, cardioprotective, neuroprotective, nephroprotective hepatoprotective and anti-inflammatory [3, 5]. On the other hand, although *Crataegus monogyna* species have been studied for its biological properties, its anti-mutagenic properties have not been reported up to the present.

Bernatoniene *et al*. [6] reported that extracts of hawthorn berries have antioxidant capacity and its ethanolic extracts have higher antioxidant activity than aqueous extracts. Mot *et al.* [7] suggested that *Crataegus monogyna* have pharmacological properties due to monoterpenes, sesquiterpenes, aliphatic and aromatic aldehydes (benzaldehyde, 3-pyridine carboxaldehyde), ketones, alcohols and esters (4-methoxybenzoic acid methyl ester). Leskovac *et al.* [8] showed that *in vitro* treatment of human peripheral blood lymphocytes with *Crataegus monogyna* fruit extract reduced micronuclei induced by gamma irradiation.

There are several reports on the antioxidant capacity of hawthorn species, due to the presence of different bioactive compounds, such as epicatechin, hyperoside and chlorogenic acid, epigallocatechin gallate, gallic acid, quercetin 7,4-dimethyl ether-3-O-rutinose, methyl (4-caffeoyl)-quinate, quinic acid, malvidin-3-O-(4‴coumaroyl)-rutinose-5-O-glucose, peonidin-3-O-(4‴coumaroyl)-rutinose-5-O-glucose, petunidin-3-O-(4‴coumaroyl)-rutinose-5-O-glucose, 8-Methoxy-Kaempferol-3-O-glucose.

These compounds are reported to have many pharmacological effects [1, 9].

However, to the best of our knowledge, there has not been any attempt to evaluate the anti-

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mutagenic activities of different extracts of *Crataegus monogyna subsp. monogyna* species until now. Therefore, the aim of this study was to investigate the antioxidant capacity of the antimutagenic activity different extracts of *C. monogyna subsp. monogyna*.

MATERIALS AND METHODS

Fruit samples of *C. monogra species* were collected from different locations in the vicinity of Erzurum, located in eastern Anatolia, Turkey (the taxonomic identification of the plant materials was confirmed by a senior plant taxonomist, Dr. Meryem Sengul, Department of Biology, Atatürk University, Erzurum, Turkey). The collected fruit materials - the fruits - were ground in a grinder with 2 mm mesh size.

Preparation of the extract

The fresh fruits (100 g) were extracted with 1 L of methanol, ethanol, aceton or pure water using a Soxhlet extractor (ISOPAD, Heidelberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper (no. 1), and then concentrated *in vacuo* at 60°C using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland). Plant extracts were then lyophilized and kept in the dark at + 4°C until being tested.

Antimutagenicity assay

Peripheral blood lymphocytes were taken from four nonsmoking healthy donors between the ages of 22 and 25. Lymphocyte cultures were set up by adding 0.5 mL of heparinized whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated fetal calf serum, 100 IU/mL streptomycin, 100 IU/mL penicillin and 1% L-glutamine. Lymphocytes were stimulated to divide by 1% phytohemagglutinin. Aflatoxin B_1 (AFB_1; 5 μM), *Crataegus monogyna* acetone (CMA), *Crataegus monogyna* ethanol (CME), *Crataegus monogyna* methanol (CMM) and *Crataegus monogyna* water (CMW) (in concentrations of 5; 10; 20; 40 and 80 μg/mL) were added to the cultures just before incubation.

For sister chromatide exchange (SCE) demonstration, the cultures were incubated at 37 °C for 72 h, and 5-bromo 2-deoxyuridine (BrdU) at 8 mg/mL was added at the initiation of cultures. All cultures were kept in darkness. Next, 0.1 mg/mL of colcemide was added 3 h before harvesting to arrest the cells at metaphase. The cultures were centrifuged at 1200 rpm for 10 min. The supernatants were used for enzyme analysis. Cells were harvested and treated for 28 min with hypotonic solution (0.075 M KCl) and fixed in a 1:3 mixture of acetic acid/methanol (v/v). BrdU incorporated metaphase chromosomes were stained by fluorescent plus Giemsa technique as described by Perry and Evans [10]. In SCE study, by selecting 60 satisfactory metaphases, the results of SCE are shown in Table 2. For each treatment condition, well-spread second division metaphases containing 42–46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell [11].

In the MN test system, cytochalasin B (3 μg/mL) was added to the whole blood samples at 44 h incubation. After 72 h incubation, the cells were harvested by centrifugation (1000 rpm, 10 min), and the supernatant was removed. 6 mL of 0.05 M KCl was added to the pellet containing lymphocyte cells, vortexed and incubated at 37 °C for 7 min. After the incubation period, the lymphocyte cells were harvested by centrifugation (1000 rpm, 10 min) and the supernatant was removed. 6 mL of fresh fixative solution (acetic acid and methanol (1:3)) was added dropwise to the pellet. The fixation procedure was repeated three times and the tube was centrifuged (1000 rpm, 10 min). The cell pellet was re-suspended in 1 mL of fresh fixative solution, and then the suspension was dropped on to clean labeled microscope slides and incubated at room temperature for 72 h. After the incubation period, the slides were stained with 5% giemsa dye solution for 10 min and excess giemsa dye was removed with distilled water. The slides were air-dried and only bi-nucleated cells were scored for MN analysis. For each experimental group, approximately 1000 bi-nucleated cells were analyzed for the presence of MN [12].

Antioxidant status assays

DPPH scavenging activity. The scavenging activity of the samples was measured according to the method described by Barku *et al.* [13] with some modification. Extracts of different concentrations were added to 0.5 mL of a methanolic DPPH solution (0.1 mmol). The estimated time of reaction (30 min) was determined by considering the reduction of the absorbance at 517 nm. The absorbance was measured at room temperature, in darkness, against a blank. The absorbance of the control (3 ml of methanol in 0.5 mL of DPPH solution) was measured. All assays were conducted in triplicate. The percentage activity for the DPPH technique was calculated as follows:

$$\% \text{ inhibition activity} = 100 \times ((A_0-A_1)/A_0)$$

where A_0 is the absorbance of the control samples

and A_1 is the absorbance of the test compound. The results are expressed as sample concentration in the extract causing a decrease in DPPH radical solution. Concentrations are expressed in μg/mL.

RESULTS

The extracts of the fruits of *C. monogyna subsp. monogyna* showed significant anti-mutagenic activity in the study groups (Tables 2 and 3).

The most effective extracts of CM were CMA and CMM. The effects of the extracts of CM on the frequencies of SCE and MN were related to their concentrations. The 40-80 μg/mL doses of CM were more effective than other doses. The various extracts of *C. monogyra* have different antioxidant activity as shown in Table 1. The acetone and methanol extracts of CM displayed higher antioxidant activity than other extracts. These findings show that MN and SCE are parallel.

DISCUSSION

The antigenotoxic activities of CM were assessed by evaluating MN and SCE frequencies as shown in Tables 2 and 3. All extracts of CM were found to have antigenotoxic and antioxidant activities. Although the biological activities of *C. monogyna* such as antimicrobial, antioxidant, antitumor, antiviral, cardioprotective, neuroprotective, nephroprotective, hepatoprotective and anti-inflammatory are thoroughy studied, up to present there are limited investigations about their antimutagenic effects [3, 5, 14].

The present study focused on assessing the mutagenic effects of different extracts of *C. monogyna* and the antigenotoxicity mechanisms of CM are associated with its antioxidant nature. The results obtained from the mutagenicity assays showed that the four *C. monogyna* extracts have no mutagenic activity in any concentration. In the antimutagenicity assays, it was shown that all extracts of *C. monogyna* species have antimutagenic activity on human lymphocytes *in vitro* at all concentrations.

Table 1. DPPH free radical-scavenging activity of the different extracts

Plant species	Used solvent	Abbreviation	Mean % inhibition
Crataegus monogyna subsp. *monogyna*	Acetone	CMA	91.252
Crataegus monogyna subsp. *monogyna*	Ethanol	CME	88.109
Crataegus monogyna subsp. *monogyna*	Methanol	CMM	90.034
Crataegus monogyna subsp. *monogyna*	Pure water	CMW	52.144

Table 2. The effects of AFB_1 and extracts of *Crataegus monogyna subsp. monogyna* (CM) on SCE

Test Items	Concentrations	SCE Frequency ± S. E (CMA)	SCE Frequency ± S. E (CME)	SCE Frequency ± S. E (CMM)	SCE Frequency ± S. E (CMW)
Control		6.27 ± 0.7^{a}	6.27 ± 0.7^{a}	6.27 ± 0.7^{a}	6.27 ± 0.7^{a}
AFB_1	5 μM	14.00 ± 0.60^{e}	14.00 ± 0.60^{e}	14.00 ± 0.60^{e}	14.00 ± 0.60^{e}
CM	20 μg/mL	6.29 ± 0.53^{a}	6.31 ± 0.30^{a}	6.30 ± 0.77^{a}	6.33 ± 0.49^{a}
AFB_1 + CM	5 μM + 5 μg/mL	11.51 ± 0.78^{d}	12.98 ± 0.40^{e}	11.97 ± 0.81^{d}	13.30 ± 0.66^{e}
AFB_1+ CM	5 μM + 10 μg/mL	10.18 ± 0.38^{c}	10.90 ± 0.21^{cd}	10.20 ± 0.93^{c}	12.43 ± 0.76^{de}
AFB_1+ CM	5 μM + 20 μg/mL	8.34 ± 0.33^{b}	8.93 ± 0.33^{b}	8.61 ± 0.47^{b}	10.73 ± 0.41^{c}
AFB_1 + CM	5 μM + 40 μg/mL	7.01 ± 0.25^{a}	7.74 ± 0.18^{ab}	7.18 ± 0.57^{a}	8.99 ± 0.51^{b}
AFB_1 + CM	5 μM + 80 μg/mL	6.35 ± 0.88^{a}	6.81 ± 0.65^{a}	6.51 ± 0.29^{a}	6.97 ± 0.63^{a}

Aflatoxin B_1 (AFB_1) was used as a positive control for human blood cells. Values of SCE ($^{a, b, c, d, e}$) are significantly different compared to negative control ($P < 0.05$).

Table 3. The effects of AFB_1 and extracts of *Crataegus monogyna subsp. monogyna* (C.M.) on MN

Test Items	Concentrations	MN Frequency ± S. E (CMA)	MN Frequency ± S. E (CME)	MN Frequency ± S. E (CMM)	MN Frequency ± S. E (CMW)
Control		4.44 ± 0.84^{a}	4.44 ± 0.84^{a}	4.44 ± 0.84^{a}	4.44 ± 0.84^{a}
AFB_1	5 µM	9.50 ± 0.90^{e}	9.50 ± 0.90^{e}	9.50 ± 0.90^{e}	9.50 ± 0.90^{e}
CM	20 µg/mL	4.51 ± 0.44^{a}	4.37 ± 0.99^{a}	4.47 ± 0.72^{a}	4.46 ± 0.55^{a}
AFB_1 + CM	5 µM + 5 µg/mL	8.17 ± 0.79^{de}	8.71 ± 0.77^{e}	8.42 ± 0.45^{e}	8.91 ± 0.69^{e}
AFB_1+ CM	5 µM + 10 µg/mL	7.13 ± 0.36^{c}	7.78 ± 0.60^{d}	7.44 ± 0.39^{cd}	7.85 ± 0.76^{d}
AFB_1+ CM	5 µM + 20 µg/mL	6.08 ± 0.47^{b}	6.46 ± 0.41^{c}	6.12 ± 0.42^{bc}	6.59 ± 0.38^{c}
AFB_1 + CM	5 µM + 40 µg/mL	5.04 ± 0.66^{a}	5.54 ± 0.53^{b}	5.06 ± 0.95^{a}	5.86 ± 0.44^{b}
AFB_1 + CM	5 µM + 80 µg/mL	4.13 ± 0.61^{a}	4.89 ± 0.56^{a}	4.18 ± 0.70^{a}	4.97 ± 0.83^{ab}

Aflatoxin B_1 (AFB_1) was used as a positive control for human blood cells. Values of MN ($^{a, b, c, d, e}$) are significantly different compared to negative control ($P < 0.05$).

In the present study the antioxidant capacity of C.M. was determined. Our results showed that all C.M. extracts have antioxidant capacity, as well as antimutagenic activities. Previous studies reported that hawthorn fruit possesses potent antioxidant and free radical scavenging activities and these studies suggested that the antioxidant capacity is due to the presence of different bioactive compounds such as epicatechin, hyperoside, and chlorogenic acid [1, 15]. The results of our study indicate that the antigenotoxic effects of C.M. could be related to its antioxidant potential. However, the bioactive compounds in C.M. needed isolation and identification in order to understand the mechanism underlying its chemoprotective and antigenotoxic effects. Our future studies will be focused on the fractionation and isolation of the crude extract of *C. monogyna*. Nonetheless, we can suggest C.M. fruits as medicines with antioxidant and antimutagenic effect.

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