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Toxic potentials of ten herbs commonly used for aphrodisiac effect in Turkey

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Background/aim: Sexual dysfunction is a serious problem worldwide. In Turkey, herbal products are used by some people suffering from sexual dysfunction. Despite their therapeutic advantages, some constituents of herbs are potentially toxic and pose health risks because they can be bought from the market without a prescription. Therefore, we aimed to determine the safety of herbs possessing aphrodisiac effects, chosen on the basis of their frequency of medicinal use and commercial importance in Turkey.

Materials and methods: Ten herbs (*Anethum graveolens*, *Carthamus tinctorius*, *Citrus aurantium*, *Cocos nucifera*, *Glycyrrhiza glabra*, *Melissa officinalis*, *Nigella arvensis*, *Pinus pinea*, *Prunus mahaleb*, and *Zingiber officinale*) were extracted with water, methanol, and chloroform. The cyto- and genotoxic potentials of the extracts were assessed using an MTT test on a rat kidney cell line and an Ames assay in *Salmonella typhimurium* strains, respectively.

Results: In the cytotoxic evaluation, IC_{50} values were 1.51–31.4 mg/mL for the methanol and chloroform extracts, while the water extracts were not cytotoxic. In the genotoxic evaluation, it was revealed that the water extracts had more mutagenic activity than the chloroform and methanol extracts. Water extract of *M. officinalis* was shown to have the most genotoxic activities to TA100 (±S9) and TA98 (–S9).

Conclusion: These results might be useful in determining the toxic effects of herbs and lead to precautions being taken in regards to their consumption.

Key words: Herbal products, herbs, cytotoxicity, genotoxicity, MTT test, Ames assay

1. Introduction

Since ancient times, herbs have played an important role in the treatment of different diseases in many regions of world, largely for their expectorant, aphrodisiac, diuretic, diaphoretic, antispasmodic, stomachic, and sedative properties (1). The World Health Organization reported that 70%–80% of the global population relies on herbs for primary health care. In addition, because they are natural products, it is generally believed that herbs are essentially safe and free from side effects. Consequently, the consumption of nonprescription herbs has steadily increased over the past few decades (2).

Despite the therapeutic advantages of herbs, reports have indicated the potential toxicity of some herbal components. Herbs contain a variety of chemical substances that act upon the body. In addition, the quality of herbs can be affected by toxic contaminants originating from industry, agriculture, and private households, including toxins that can cause mutagenic and carcinogenic effects from long-term and widespread use (3–6). Moreover, herbal medicines are generally a mixture of a number of herbs in a single preparation and contain several active ingredients that induce various pharmacological effects (7,8). To date, there are few scientific studies on the safety and potential toxicity of herbs, despite growing concerns over the lack of both scientific evidence and quality control data regarding the safety and efficacy of herbs (9,10).

Sexual dysfunction is a condition that affects 15–30 million men worldwide, and it occurs in 10%–52% of men and 25%–63% of women (11,12). Many believe that sexual dysfunction is associated with the modern lifestyle. As a result, individuals turn to natural products, such as traditional herbs, that produce aphrodisiac effects for sexual enhancement. Aphrodisiac herbs are used to treat sexual dysfunction because they alter specific neurotransmitters or sex hormones. Specifically, herbs with aphrodisiac properties can induce vasodilatation that results in a sustained erection, and can cause irritation of genital mucosa that enhances sensory experience during coitus (13).

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The increased consumption of herbs readily available on the market without a prescription is a critical problem in Turkey, as well as in other countries. Because of its geographic location, climate, and the presence of nearly 10,000 natural plant species, Turkey is considered an important international floristic center (14,15). However, most of the herbs available in Turkey have not been subjected to chemical, toxicological, pharmacological, or clinical evaluations and have been ignored by national health authorities (16). Consequently, the goal of the current study was to investigate the cyto- and genotoxic potentials of ten herbs frequently used as aphrodisiacs in Turkey (Table 1). To investigate genotoxic activity, *Salmonella typhimurium* TA98 and TA100 strains were used to conduct the Ames assay, a bacterial mutation assay. In some instances, chemicals themselves are not mutagenic, but become mutagens after being metabolized in the liver. To mimic this in vivo activation process, the Ames assay was conducted in both the presence and absence of the S9 microsomal fraction metabolizing system, which is a mixture of mammalian liver enzymes. The MTT assay was used to evaluate the cytotoxicity of the herbs in the NRK-52E rat kidney cell line.

2. Materials and methods

2.1. Materials

The Ames microplate fluctuation (MPF™) 98/100 kit was purchased from Xenometrix (Allschwil, Switzerland). Positive controls (2-nitrofluorene, 4-nitroquinoline N-oxide, 2-aminoanthracene) and the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent were purchased from Sigma (USA). The cell culture materials were purchased from Multicell-Wisent Inc. (Canada). The other chemicals were obtained from various sources (Biomatik, Canada; Merck, Germany; Fluka, Switzerland).

2.2. Herbal extraction

Herbs (*Anethum graveolens*, *Carthamus tinctorius*, *Citrus aurantium*, *Cocos nucifera*, *Glycyrrhiza glabra*, *Melissa officinalis*, *Nigella arvensis*, *Pinus pinea*, *Prunus mahaleb*, and *Zingiber officinale*) were purchased from local markets in Turkey. The taxonomic identity of each herb was confirmed by the Department of Pharmaceutical Botany, İstanbul University. Herbs were selected based on their availability and traditional usage frequency. The herb ingredients and their usage based on previous studies are indicated in Table 1.

Table 1. Ingredients of ten herbs investigated in the study.

Plant	Family	Ingredients	Reference
A. graveolens	Apiaceae	Carvone, bylimonene, apiole, linoleic acid, anethole, p-anisaldehyde, myristicin, etc.	(17)
C. tinctorius	Asteraceae	Myristic, palmitic, stearic, arachidic, oleic, linoleic acids, etc.	(18–20)
C. aurantium	Rutaceae	Limonene, octanal, neodiosmin, etc.	(21)
C. nucifera	Arecaceae	Minerals, vitamins, dietary fibers, sugars, organic acids, fatty acid composition, proline, valine, leucine, serine, alanine, etc.	(22–24)
G. glabra	Fabaceae	Glycirisin, glabranin, sitosterol, coumarin stigmasterol, herniarin, umbelliferon, etc.	(25,26)
M. officinalis	Lamiaceae	Rutoside, caftaric, caffeic, p-cumaric and ferulic acids, luteolin, apigenin, etc.	(27,28)
N. sativa	Ranunculaceae	Phytosterols, saponins, metarbin, melantin, lipase, α-pinen, nigellon, thymoquinone, thymohydroquinone, thymol, etc.	(29,30)
P. pinea	Pinaceae	1-Limonene, β-caryophyllene, germacrene, abienol, oleic, stearic, abietic, isopimaric, levopimaric and dehydroabietic acids, etc.	(31,32)
P. mahaleb	Rosaceae	Phenolic acids, o-coumaric acid, quercetin, anthocyanins, coumarin, etc.	(33–35)
Z. officinale	Zingiberaceae	Oleoresin, camphen, β-phellandrene, sineol, geranial, limonen, myrcen, α-pinen, borneol, citronellol, linalool, zingiberene, etc.	(36,37)

Local herbalists generally advise consumers to take the herbs as a 5%–10% decoction. To evaluate the different ingredients of each herb, polar and nonpolar compounds were extracted using chloroform, methanol, and water. We extracted 2.5 g of dried and mixed ground herbs in both 25 mL of chloroform and methanol, separately, in a sonication bath (25 °C) for 30 min. The extracts were concentrated in a rotary evaporator and dried under a gentle stream of nitrogen at 40 °C to yield a solid residue. The solid residues were dissolved in 1 mL of dimethyl sulfoxide (DMSO). For extraction with water, 2.5 g of dried and mixed ground herbs were extracted by boiling in 25 mL of water for 30 min. The extracts were then filter sterilized using 0.45-µm filters. The stock concentrations were 2500 mg/mL for the chloroform and methanol extracts and 100 mg/mL for the water extracts. For cyto- and genotoxicity assays, the extracts were further diluted to a final concentration of 0.75–75 mg/mL and 0.78–25 mg/mL, respectively, prior to use.

2.3. Cytotoxicity test (MTT test)

The NRK-52E rat kidney cells, obtained from the American Type Culture Collection (CRL-1571™, ATCC, USA), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 1% streptomycin and penicillin at 37 °C in a 5% CO_2 and 95% O_2 humidified cell incubator. Cells were seeded in 96-well plates at 10^4 cells per well and incubated for 24 h. After incubation, various concentrations of the herbal extracts were added to each well. The exposure concentrations were determined as 0.75–75 mg/mL based on the maximum permissible concentrations of the test conditions. After incubation with the extracts for 24 h, the cytotoxicity of each extract was evaluated by MTT tests measuring mitochondrial activity in the cell line.

The assay principle is that, in the presence of an electron-coupling reagent, the yellow water-soluble tetrazolium salt MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, is reduced to an insoluble purple formazan product by mitochondrial succinate dehydrogenase, which belongs to the mitochondrial respiratory chain and is only active in viable cells (38). The optical density (OD) of formazan product was read at 590 nm against the reference wavelength of 670 nm using a microplate spectrophotometer system (Epoch, Germany).

In every test, negative (untreated, culture medium) and solvent (1% DMSO) controls were used. For each extract, eight concentrations (0.75, 1.50, 3.00, 6.00, 12.00, 25.00, 50.00, and 75.00 mg/mL) were tested in triplicate, and each test was performed in duplicate.

For cytotoxicity assays, the 50% inhibition concentration (IC_{50}), expressed as the concentration of sample that caused a 50% inhibition of enzyme activity in the cells, was applied. To calculate the IC_{50}, the absorbance value of the blank was subtracted from the absorbance values of each sample, and the results were compared with the absorbance values of the solvent controls. In the MTT test, the IC_{50} value was calculated as the percentage of solvent controls according to the formula below:

Inhibition (%) = 100 – [(corrected mean OD_{sample} × 100)/corrected mean $OD_{solvent\ control}$].

2.4. Genotoxicity test (Ames assay)

The Ames MPF™ 98/100 assay kit, a modified liquid microplate version of the traditional Ames assay, was used for the genotoxicity assays. The strains used for the Ames assay were the TA98 frame shift mutation and the TA100 base-pair substitution strains of *S. typhimurium*. The strains were obtained from the manufacturer's kit, preserved in 15% glycerol, and stored at –80 °C until use.

Lyophilized Aroclor 1254 induced rat liver S9 microsomal fractions were also purchased from Xenometrix. Just prior to use, S9 mix was freshly prepared for metabolic activation system. The S9 mix included 30% of S9 microsomal fractions. S9 mix was 0.083 mL 1.00 M KCl, 0.080 mL 0.25 M $MgCl_2.6H_2O$, 0.063 mL 0.20 M glucose-6-phosphate, 0.250 mL 0.04 M NADP, 1.270 mL 0.20 M NaH_2PO_4 buffer, and 0.750 mL S9 microsomal fraction (39).

The mutated TA98 and TA100 *S. typhimurium* strains are incapable of synthesizing the amino acid histidine. However, strains can produce histidine and grow if a reversion of the mutation occurs. The presence of mutagenic compounds capable of inducing reversions can cause an increase in the number of revertant colonies relative to background levels. In the assay, the catabolic activity of revertant cells causes a reduction in the pH of the solution, which results in a color change from purple to yellow (40).

Following the manufacturer's protocol, the *S. typhimurium* TA98 and TA100 strains in semisolid form were homogenized in 200 µL of growth medium. Next, 25 µL of each suspension was added to a mixture of 10 mL of growth medium and 10 µL of ampicillin (50 mg/mL). A negative control, lacking only the bacterial strains, was also prepared. The culture tubes were incubated in a shaker at 37 °C and 250 rpm for 14–16 h. The overnight cultures were diluted in growth medium (1:10), and the absorbance was measured at 600 nm. The assay was continued if the absorbencies of the culture and negative controls were approximately 0.25 and 0.005, respectively. To perform the assay, 1 mL of the overnight culture was added to 3 mL of growth medium and incubated in a shaker at 37 °C and 250 rpm for 90 min. After incubation, the absorbance of the culture was measured at 600 nm. The assay was continued if the absorbance of the culture was approximately 1.5–1.9.

The mutagenic potential of extract was assessed in the presence and absence of the S9 metabolic activating system, at a final concentration of 4.5% (v/v) in sterile medium. The positive controls used included 2-nitrofluorene (2 μg/mL) and 4-nitroquinoline N-oxide (0.1 μg/mL) without S9, and 2-aminoanthracene (5 μg/mL) with S9. DMSO was used as the negative control.

The extracts were transferred to individual wells of 24-well plates containing culture medium and incubated for 90 min at 37 °C in the presence or absence of the S9 mix. The exposure concentrations were determined as 0.78, 1.56, 3.13, 6.25, 12.50, and 25.00 mg/mL based on the maximum permissible concentrations of the test conditions.

After incubation for 90 min, indicator medium was added to each well in the 24-well plates. The mixture from each well of the plate was then distributed into 48 wells of a 384-well microtiter plate and incubated at 37 °C in a dry incubator for 48 h. The indicator medium contained a pH indicator dye that changed from purple to yellow in the presence of bacterial growth. After incubation at 37 °C for 48 h, the plates were scored by visual inspection for the number of yellow wells.

The number of positive (yellow) wells out of the 48 wells in triplicate were counted and compared with the negative control. The criteria used to evaluate the results of the Ames assay were the fold increase in number of positive wells over the solvent control baseline and the dose dependency. The fold increase of revertants relative to the solvent control was determined by dividing the mean number of positive wells for each dose by the solvent control baseline. The solvent control baseline was defined as the mean number of positive wells in the solvent control plus one standard deviation. All solvent controls from an experiment with identical conditions (same day, bacterial culture, solvent, and incubation conditions) were combined.

A 2-fold or greater increase relative to the baseline was considered positive for a given dose. Furthermore, when positive responses were ≥2-fold relative to the baseline at more than one dose and a dose-response was observed, the test sample was classified as positive. A test sample was classified as negative when no responses were ≥2-fold relative to the baseline and no dose-response was observed. Student's t-test (1-sided, unpaired) was used to evaluate dose response and $P < 0.05$ was considered statistically significant. Each experiment was repeated in duplicate.

3. Results

In the current study, water, chloroform, and methanol extracts obtained from ten herbs commonly used as aphrodisiacs were screened for their cyto- and genotoxic potentials using MTT cytotoxicity tests in a rat kidney cell line (NRK-52E) and bacterial mutation assays with *S. typhimurium* TA98 and TA100 strains, with or without the S9 metabolic activating system, to evaluate genotoxicity. Different extraction methods were used because herbal ingredients are effectively isolated from organic solvents, despite the fact that most herbs are used in the aqueous decoction form. The concentrations used were established based on the maximum permissible concentrations of the test conditions.

The IC_{50} values resulting from the MTT test ranged from 1.51 to 51.97 mg/mL for the ten herbal extracts prepared using methanol and chloroform, but the water extract did not exhibit any cytotoxic activity. Among the herbs tested, *N. avensis* was the most cytotoxic, with IC_{50} values of 1.51–2.44 mg/mL for the methanol and chloroform extracts (Table 2).

For the Ames assay, concentrations of 0.78, 1.56, 3.13, 6.25, 12.50, and 25.00 mg/mL were used for the methanol, chloroform, and water extracts. We took into consideration that herbs are usually consumed several times per day as tea and that 1%–5% (v/w) proportions are suggested for a single intake. The results of our analyses indicated that some herbal extracts possessed mutagenic potential, and that the herbal extracts prepared using water showed higher mutagenic activity than the chloroform and methanol extracts. We also noted that the TA98 strain was more sensitive to the extracts than the TA100 strain, and that the mutagenic potentials of the herbs were generally concentration-dependent (Table 3). The mutagenic activities of all of the herbs evaluated are indicated as either a positive or negative result in Table 3, while the extracts that exhibited mutagenic properties are detailed in Table 4.

For the chloroform extractions, *C. nucifera* and *N. avensis* extracts displayed mutagenic potentials in the absence of S9 (2.5–6.5- and 2.17–4.24-fold, respectively). A possible explanation for the results obtained is that *C. nucifera* and *N. avensis* extracts could cause a base-pair substitution and a frame shift mutation, respectively. For the methanol extractions, *A. graveolens* extract displayed mutagenic potential with the TA98 strain in the presence of S9 (2.0–8.0-fold). For the water extractions, *A. graveolens*, *C. tinctorius*, and *C. aurantium* extracts exhibited mutagenic activities only with the TA98 strain and in the absence of metabolic activation (2.25–4.5-, 2.14–5.82-, and 2–2.15-fold, respectively). The *Z. officinale* extract demonstrated mutagenic potential with the TA100 strain (4.15–7.32-fold) while *P. mahaleb* extract displayed mutagenic potential with both strains (2.45–9.94-fold) with S9 at concentrations higher than 3.13 mg/mL. At similar concentrations, the *M. officinalis* extract exhibited the highest level of genotoxicity of all of the extracts evaluated. Further, the *M. officinalis* extract showed

Table 2. Results of cytotoxic activity with MTT test in NRK-52E cell line exposed to the extracts of ten herbs.

Plant	Extract	IC_{50} value (mg/mL)	Plant	Extract	IC_{50} value (mg/mL)
	Methanol	3.09		Methanol	-
A. graveolens	Chloroform	3.30	*M. officinalis*	Chloroform	6.18
	Water	-		Water	-
	Methanol	5.20		Methanol	2.44
C. tinctorius	Chloroform	0.75	*N. arvensis*	Chloroform	1.51
	Water	-		Water	-
	Methanol	11.80		Methanol	-
C. aurantium	Chloroform	0.50	*P. pinea*	Chloroform	51.97
	Water	-		Water	-
	Methanol	18.49		Methanol	31.41
C. nucifera	Chloroform	-	*P. mahaleb*	Chloroform	-
	Water	-		Water	-
	Methanol	-		Methanol	-
G. glabra	Chloroform	-	*Z. officinale*	Chloroform	9.08
	Water	-		Water	-

Table 3. Results of mutagenic activity with Ames MPF™ 98/100 assay by using bacterial strains TA98 and TA100 exposed to the extracts of ten herbs with/without metabolic activation.

	TA98						TA100					
	Chloroform		Methanol		Water		Chloroform		Methanol		Water	
	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+	S9-	S9+
A. graveolens	?*	?*	—	+	+	—	—	—	—	?*	—	—
C. tinctorius	—	—	—	—	+	?*	—	—	—	—	—	—
C. aurantium	?*	—	—	—	+	?*	—	—	—	—	—	—
C. nucifera	+	—	—	—	—	?*	—	—	—	—	—	?*
G. glabra	—	—	—	—	—	—	—	—	—	—	—	?*
M. officinalis	—	—	—	—	+	—	—	—	—	—	+	+
N. arvensis	—	—	—	—	—	—	+	—	—	—	—	—
P. pinea	—	—	—	—	—	—	—	—	—	—	—	—
P. mahaleb	—	?*	—	?*	—	+	—	—	—	—	?*	+
Z. officinale	—	—	—	—	—	+	—	—	—	—	—	—

?* = Mutagenic activities only at the highest concentration (25 mg/mL).
+ = Mutagenic activities at the studied concentrations (0.78–25 mg/mL).

Table 4. The revertant numbers obtained with Ames MPF™ 98/100 assay for the extracts that possessed mutagenic activity.

Extracts	Plants	Concentrations (mg/mL)	Revertants' fold increase over baseline*			
			TA98		TA100	
			S9-	S9+	S9-	S9+
Methanol	*A. graveolens*	0.78		2.00		
		1.56		2.50		
		3.13		2.00		
		6.25		3.00		
		12.50		8.00		
		25.00		5.50		
Chloroform	*C. nucifera*	0.78	-			
		1.56	6.50			
		3.13	3.50			
		6.25	2.50			
		12.50	3.60			
		25.00	3.70			
	N. avensis	0.78			-	
		1.56			2.33	
		3.13			2.17	
		6.25			2.45	
		12.50			4.24	
		25.00			3.50	
Water	*A. graveolens*	0.78	4.50			
		1.56	-			
		3.13	3.00			
		6.25	2.25			
		12.50	3.67			
		25.00	4.12			
	C. tinctorius	0.78	-			
		1.56	2.14			
		3.13	-			
		6.25	2.73			
		12.50	3.45			
		25.00	5.82			
	C. aurantium	0.78	2.00			
		1.56	2.00			
		3.13	2.50			
		6.25	2.50			
		12.50	2.10			
		25.00	2.00			

*The results were statistically significantly mutagenic ($P < 0.05$) as indicated in evaluation of Ames assay. The values are means.

Table 4. Continued.

Extracts	Plants	Concentrations (mg/mL)	Revertants' fold increase over baseline*			
			TA98		TA100	
			S9-	S9+	S9-	S9+
	M. officinalis	0.78	-		-	-
		1.56	-		-	-
		3.13	4.00		3.21	2.56
		6.25	2.50		2.50	3.21
		12.50	9.00		11.00	4.30
		25.00	9.50		9.00	16.00
	P. mahaleb	0.78		-		-
		1.56		-		-
		3.13		2.45		2.65
		6.25		4.14		2.87
		12.50		6.21		7.02
		25.00		9.94		9.35
	Z. officinale	0.78		-		
		1.56		-		
		3.13		6.15		
		6.25		4.21		
		12.50		4.15		
		25.00		7.32		
DMSO			1.35	2.03	1.56	2.08
Positive controls			13.28	12.90	16.04	17.52

*The results were statistically significantly mutagenic ($P < 0.05$) as indicated in evaluation of Ames assay. The values are means.

mutagenic activity with both the TA100 and TA98 strains independent of metabolic activation (2.50–16.00-fold and 2.5–9.5-fold, respectively).

4. Discussion

The use of herbs as aphrodisiacs to enhance libido, improve potency and fertility, and increase sex drive, endurance, and energy levels, and as sexual enhancers for purposes of seduction, has increased throughout history (13). Because they are natural products, herbs are assumed to be safe for long-term use and are thought to elicit no side effects. This assumption of safety and lack of side effects could prove to be potentially hazardous. The lack of available supporting safety data and the unregulated use of herbal products by the general population calls for studies to investigate their side effects and toxicity (41). Nonetheless, controlled studies on herbal plants are still lacking, and the risks associated with their consumption remain unknown. To date, only the data obtained from a limited number of in vitro, in vivo, and human studies are available for use as the basis for risk assessments and the establishment of recommended herbal doses (42).

The European Food Safety Authority (EFSA), the International Life Sciences Institute (ILSI), the Institute of Medicine (IOM)/National Research Council (NRC), the International Union of Pure and Applied Chemistry (IUPAC), and the European Medicines Agency (EMEA) have recently issued protocols and guidance documents on safety assessment and toxicity testing of herbal products used in both foods and medicines. From a regulatory perspective, a safety assessment could influence whether or not certain products should be restricted, removed from the market, or have augmented safety labeling information. In cases where little toxicity information exists on a specific herbal product or its ingredients, regulatory decisions regarding risk mitigation are likely

to be cautious until further information is obtained that could potentially elucidate the toxicity of herbal products and reduce the uncertainty related to risk assessments (42–45).

The ingredients of herbs are complex, which makes the fractionation of complex mixtures and chemical identification of their components difficult, and in some cases not feasible. Furthermore, the potential genotoxic and cytotoxic effects of herbal mixtures could possibly differ from the sum of the effects of individual components (8). Therefore, it was aimed to evaluate the toxic profiles of the herbal mixtures without analyzing the active substances of the herbs in the study.

The Ames assay is a useful tool for quantifying the genotoxicity of complex herbal mixtures and for predicting the genotoxic effects of herbal consumption on human health. Furthermore, the responses of the different *Salmonella* strains used in the assay could help identify the classes of genotoxic compounds present in the herbs (46). However, a positive result does not necessarily indicate that the substance is a carcinogen. The Ames assay only confirms whether the substance is mutagenic or not to the particular bacterial strain used and for the genetic endpoint tested (47).

In Turkey, consumers can buy herbal products without a prescription from the market, despite the fact that these herbs lack proper scientific evaluation as well as mandatory safety and toxicological studies. Accordingly, the present study was conducted to investigate the cytotoxic and genotoxic effects of ten herbs commonly used as aphrodisiacs in Turkey. The kidney is the route of excretion of most of the substances present in herbs. Kidney injury has been described in association with ingestion of several botanicals (48). Therefore, kidney cells were used in the study. Limited genotoxicity data were available for the herbs included in this study, with the exception of data from a small number of prior studies conducted to assess their antimutagenic/anticarcinogenic potentials (49–55).

Of the herbs evaluated, *M. officinalis* was both genotoxic and mutagenic, and it could have carcinogenic potential (56). The presence of phenolic compounds in *M. officinalis*, particularly caffeic acid derivatives, could be correlated to its apparent genotoxicity. The spectrum of mutations revealed a great trend for base substitutions, mainly in guanines and adenines (41). An ethanolic extract of *M. officinalis* also displayed antigenotoxic/antimutagenic properties, and its use in pretreatment could reduce the induction of DNA damage by an alkylating agent (50). Saraydin et al. (57) reported that *M. officinalis* exhibited cytotoxic activity by inducing an increase in annexin-positive cells and the expression of the caspase-7 protein. Likewise, Saraydin et al. (57) indicated that the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive cells was greater in rats treated with *M. officinalis*, while the expression of Ki-67 was decreased. In the present study, the water extracts of *M. officinalis* exhibited the highest level of genotoxicity among all of the extracts evaluated. *M. officinalis* possessed mutagenic activity with the TA100 strain independent of metabolic activation (2.50–16.00-fold), and with the TA98 strain in the absence of S9 (2.5–9.5-fold). However, the methanol and chloroform extracts of *M. officinalis* were not mutagenic.

Fukuoka et al. (58) reported that *A. graveolens* exhibited mutagenic activity in *S. typhimurium* TA98 and TA100 strains, while Alqasoumi et al. (59) observed that *A. graveolens* significantly increased the frequency of micronuclei in Swiss albino mice. Lazutka et al. (60) found that the essential oil extracted from *A. graveolens* was genotoxic in sister chromatid exchange tests and in chromosomal aberrations. The latter report (60) likewise indicated, based on unpublished data, that *A. graveolens* was also very toxic against *S. typhimurium* TA98 and TA100 strains. However, Morkunas (53) conducted a micronucleus test using *A. graveolens* and reported that the herb was not genotoxic in mouse bone marrow. In accordance with Fukuoka et al. (58), the results of the present study indicated that the *A. graveolens* water extracts incubated with the TA98 strain in the presence of S9, and the methanol extracts incubated with the TA98 strain in the absence of S9, exhibited mutagenic potential.

Some studies have reported that licorice (ethanol extracts of *G. glabra*), which contains glycyrrhizin and glycyrrhetic acid, showed negative results in the TA98 and TA100 strains (61,62). The genotoxic potential of *G. glabra* was investigated using the Ames II™, chromosomal aberration, and micronucleus test systems, and the results indicated that the herb possessed no mutagenic activities, either with or without metabolic activation (63,64). Martinez et al. (65) reported that TA98 was more sensitive to the mutagens present in the licorice water extract. The reported percentage of licorice extract cytotoxic activity was 63% at 0.24 mg/mL with greater percentages apparent as the concentrations of licorice were increased up to 4.8 mg/mL. Rathi et al. (66) reported that IC_{50} values of chloroform, methanol, and water extracts of *G. glabra* L. in the breast MCF7 cell line were 0.45, 0.99, and 1.29 μM, respectively. In the present study, the *G. glabra* water extract displayed mutagenic activity against the TA100 strain in the presence of metabolic activation, but only at the highest extract concentration.

Mothana et al. (67) observed that IC_{50} values of *C. tinctorius* were 100 μg/mL and greater than 1000 μg/mL for the methanol and water extracts, respectively. However, no prior studies have reported the genotoxic activity of *C. tinctorius*. In the current study, the water extracts of *C.*

tinctorius were mutagenic against the TA98 strain in the absence of the S9 metabolic activation.

In the present study, none of the aqueous decoctions of the ten herbs evaluated exhibited any cytotoxic activities. In accordance with our results, Sharififar et al. (68) reported similar outcomes for *N. avensis* and *Z. officinale* using a brine shrimp lethality assay. Conversely, Yang et al. (69) reported that *Z. officinale* had cytogenotoxic potential. Similarly, Wei et al. (70) indicated that the diarylheptanoids and gingerol-related compounds isolated from the *Z. officinale* rhizome were cytotoxic against human promyelocytic leukemia (HL-60) cells (IC_{50} < 50 μM), while Zaeoung et al. (71) reported that the IC_{50} of *Z. officinale* was higher than 39.2 μg/mL against breast (MCF7) and colon (LS174T) cell lines. Rong et al. (72) observed that administration of 2000 mg/kg *Z. officinale* led to slightly reduced absolute and relative testicular weights in rats. In a study conducted on the motility, grading, and morphological aspects of human sperm, Jorsaraei et al. (73) claimed that *Z. officinale* caused toxic effects. None of the prior studies, however, examined the genotoxic activities of *N. avensis* and *Z. officinale*. In the current study, the chloroform extracts of *N. avensis* exhibited mutagenic activity with the TA100 strain in the absence of the S9 metabolic activation, and the water extracts of *Z. officinale* were mutagenic with the TA98 strain in the presence of S9. Additionally, we found that the chloroform extracts of *C. nucifera* and the water extracts of *C. aurantium* were mutagenic with the TA98 strain in the absence of the S9 metabolic activation, while the water extracts of *P. mahaleb* were mutagenic against both strains in the presence of S9. However, the genotoxic activities of *C. nucifera* and *C. aurantium* have not been investigated.

In conclusion, our investigation into the active constituents of herbs used as aphrodisiacs may provide useful comparative information for future studies, despite the difficulty of identifying all of the compounds present in the herbs. In addition, it would be beneficial to evaluate the carcinogenicity of these plants in order to assess associated risks to human health. Quality control data and standardization of methods used to assess safety and efficacy are required in order to understand the potential risks associated with the use of herbal products. Contrary to the popular belief that herbs are safe because they are natural products, indications exist that herbs can cause significant toxic effects, drug interactions, and even morbidity or mortality. The purpose of the current study was not to introduce a bias against herbal products, but rather to raise the awareness of researchers and/or national authorities regarding the human use of herbal products.

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