Research Article

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In vivo protective effects of vitamin C against cyto-genotoxicity induced by *Dysphania ambrosioides* aqueous extract

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Abstract: *Dysphania ambrosioides* (L.) holds a prominent place in Moroccan folk medicine due to its therapeutic attributes. Despite its widespread use, instances of inadvertent intoxication linked to its consumption have been reported. This study aims to evaluate the potential cytogenotoxic effects of *D. ambrosioides* leaf aqueous extract (DAAE) and explore the prospective protective role of vitamin C (L-ascorbic acid) through the micronucleus test conducted on (1) *Vicia faba* root-tip meristem and (2) mouse bone marrow cells. In addition, antioxidant enzyme activities, specifically superoxide dismutase (SOD) and catalase (CAT), were evaluated in *V. faba* treated with DAAE. After a 7-day daily administration of DAAE to mice, serum biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, and uric acid) were measured, and histological examination of liver and kidney tissues was performed. The results indicated that DAAE had dose-dependent cytotoxic and genotoxic effects on both biological models. Furthermore, *V. faba* treated with DAAE showed significant increases in the activities of SOD and CAT enzymes. Mice treated with DAAE exhibited significant elevations in serum biochemical parameters compared to the control group. Histological examination of liver and kidney tissues revealed hepatic degeneration, glomerular shrinkage, and distinct vacuolated tubular epithelial cells. The cotreatment with vitamin C demonstrated a significant protective effect against DAAE-induced cytogenotoxicity. These findings underscore the importance of vitamin C as a protective agent against oxidative stress and cytogenotoxicity induced by DAAE and recommend its use in any DAAE-based preparation.

Keywords: *Dysphania ambrosioides*, micronucleus, *Vicia faba*, oxidative damage, mice

1 Introduction

Throughout human history, plants have played a significant role in folk medicine, providing remedies for various ailments. In today's world, the appeal of traditional medicine seems to be on the rise, driven by the belief that herbal products are inherently safe and entail fewer side effects than synthetic drugs. This trend is particularly evident in developing countries where medicinal plants are abundant and affordable. However, like synthetic drugs, herbal medicine is not exempt from adverse effects. The factors contributing to such reactions are varied and may encompass the presence of toxic principles in certain plants, instances of overdose, interactions between conventional drugs and herbal remedies, as well as idiosyncratic responses such as allergies reactions [1]. It is crucial to approach herbal medicine with a balanced perspective, acknowledging both its potential benefits and associated risks.

In Morocco, a plethora of plants continues to be harnessed for their therapeutic properties in home remedies,

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illustrating the rich tradition of herbal medicine. One such plant is *Dysphania ambrosioides* (L.) Mosyakin & Clemants, formerly known as *Chenopodium ambrosioides*, originally native to Central and South America. Locally referred to as "M'khinza," the leaves of this plant hold significance for their analgesic and antipyretic properties, making them valued for addressing various health concerns, such as typhoid, gastrointestinal diseases, and dysentery [2]. Typically, these leaves are used as poultices or prepared as a juice, the latter is often combined with orange juice to mitigate the bitter taste of the plant.

Previous investigations into the pharmacological activities of *D. ambrosioides* have revealed an interesting bioactivity profile, showcasing its potential as an anthelmintic, nematocidal, antileishmanial, anti-inflammatory, antipyretic, analgesic, and molluscicidal agent [3–9]. However, alongside its therapeutic properties, several cases of intoxication subsequent to the ingestion of *D. ambrosioides* have been reported, including toxic encephalopathy, severe dehydration in acute gastroenteritis, acute toxic effects on the kidneys, and, in some unfortunate cases, even death [10–12]. Nevertheless, despite the substantial body of literature detailing the pharmacological activities of *D. ambrosioides*, our understanding of its cytogenotoxic potential remains limited. *In vitro* studies have evaluated the cytotoxic and genotoxic activities of *D. ambrosioides* using human lymphocyte cell cultures [13,14]. Despite the insights gained from these *in vitro* investigations, there remains a notable gap in our understanding, as no *in vivo* studies have been carried out to investigate the genotoxic effects of this plant.

To the best of our knowledge, the present study highlights the need for inaugural exploration of the *in vivo* cytotoxic and genotoxic effects of the aqueous extract derived from *D. ambrosioides* leaves. This research endeavors to bridge the existing knowledge gap and provide a more comprehensive understanding of the potential biological impact of *D. ambrosioides*. Furthermore, considering that in Moroccan traditional medicine, *D. ambrosioides* is often consumed with orange juice, a natural source high in vitamin C that plays an important role in protecting against oxidative damage, we have delved into the exploration of a potential modulatory effect of vitamin C (L-ascorbic acid, $C_6H_8O_6$) on the cytotoxic and genotoxic damage induced by *D. ambrosioides*. This investigation not only expands our understanding of the physiological interactions associated with *D. ambrosioides* but also sheds light on potential avenues for mitigating its adverse effects through dietary modulation.

2 Materials and methods

2.1 Plant sample

The studied plant was collected in October 2021 from the Rhamna region, located approximately 55 km from Marrakesh, Morocco. The identification process was conducted by Prof. Ouhammou A., a plant taxonomist affiliated with the Department of Biology, Faculty of Sciences Semlalia, Cadi Ayyad University, Morocco. A voucher specimen (No 14015) was deposited at the herbarium of the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco.

2.2 Preparation of the plant extract

D. ambrosioides leaf aqueous extract was prepared following the Moroccan traditional medicinal method. Fresh leaves of *D. ambrosioides* (60 g) were thoroughly crushed in a blender with 1.5 L of distilled water. The extraction was replicated three times. The obtained extract underwent a centrifugation step lasting 15 min at 3,000 rpm. Thereafter, the supernatant was collected, filtered through No. 1 Whatman filter paper, evaporated, and stored at 4°C until use. The percentage yield of the crude extract was 10.65%. The extract was prepared by dissolving it in distilled water to the required concentrations.

2.3 Phytochemical screening

Qualitative phytochemical screening of the aqueous leaf extract of *D. ambrosioides* was carried out to determine the chemical nature of the active ingredients responsible for its activities. The following classes of major bioactive constituents were screened: flavonoids (using cyanidin reaction, and by TLC with Neu's reagent), alkaloids (using the Dragendorff test), saponins (using the Froth test), terpenoids (using the Liebermann–Burchard test), tannins

(using Ferric test), and coumarins (by TLC with Antimony(III) chloride reagent). All these tests, procedures, and reagents are described elsewhere [15–18].

2.4 Ultra-high-performance liquid chromatography with a diode array detector coupled to electrospray ionization tandem mass spectrometry (UHPLC-DAD-ESI/MS) analysis of polyphenol compounds

The DAAE samples were analyzed using UHPLC-DAD-ESI/MS according to the method of Zefzoufi et al. [19]. We utilized an Ultimate 3000 system (Dionex, CA, USA), which is equipped with a quaternary pump (HPG 3400 RS), an auto-sampler (WPS 3000 TSL), and a column oven (TCC 3000). For the proposed method, we employed a Kinetex C18 reversed-phase column (250 mm × 4.6 mm, 2.6 µm particles) provided by Thermo Fisher Scientific (CA, USA). For gradient separation, we used 0.1% formic acid in an aqueous solution as solvent A and methanol as solvent B.

Mass spectrometry was employed for the identification of phenolic compounds using TSQ-Endura (Thermo Fisher Scientific, CA, USA) equipped with heated electrospray ionization in negative mode. The full scan range was set from 100 to 1,000 *m*/*z*, and the fragmentation energy was maintained at 50 V.

2.5 Cytogenotoxicity assay

2.5.1 *Vicia faba* plant

The cytogenotoxicity assay was conducted in accordance with the methodology outlined by Marcato-Romain et al. [20]. *V. faba* seeds, purchased from Commercial Seeds for Farm, Greenhouse, and Garden Growing, were selected, and their surface was sterilized with sodium hypochlorite (1%, v/v) for 10 min. Then, they were soaked in water for 24 h and germinated between two layers of filter paper moistened with distilled water after removing the seed coats. After 4 days, the primary root tips, which had grown to approximately 2–3 cm in length, were trimmed by 2 mm to promote the emergence of secondary roots. The seedlings were then transferred to a hydroponic system. *D. ambrosioides* aqueous extract (DAAE) at concentrations ranging from 0.02 to 5.12 mg/mL was tested alone or in combination with vitamin C (1 mM). Distilled water was used as the negative control, and cyclophosphamide (CP) (1 mg/mL) served as the positive control. The experiment was replicated five times for each treatment, and the secondary roots were collected after a 4-day exposure period when they became visible, reaching a length of approximately 1 cm.

The secondary root tips were harvested and left to incubate overnight in Carnoy's fixative solution (glacial acetic acid/ethanol 1:3, v/v). The samples were then washed with distilled water for 10 min and stored in ethanol (30%). The root tips underwent several additional rinses with distilled water and were subjected to hydrolyzation using 1 N HCl at 60°C for 6 min. Afterward, they were stained with aceto-orcein (1%) at 60°C for 3 min. Approximately 1–2 mm of the stained secondary root tips were squashed onto a slide, and a cover slip was carefully placed to avoid air bubbles. The slides were observed under a digital light microscope (Optika B-290TB) equipped with a tablet at 400× magnification. A minimum of 1,000 cells were counted per treatment, and three replicates were processed for each treatment. The frequency of micronuclei (MN) and the mitotic index (MI) were determined as outlined below:

$$\mathrm{MI}(\%) = \frac{\text{Number of cells in mitosis}}{\text{total number of the observed cells}} \times 100,$$

$$\mathrm{MN}(‰) = \frac{\text{Number of MN}}{\text{total number of the observed cells}} \times 1{,}000.$$

The MN test was conducted solely on root tips exhibiting an MI surpassing 2% to prevent the underestimation of MN frequency caused by a compromised cell proliferation rate [21].

2.5.2 Reactive oxygen species (ROS) scavenging enzyme activity assay

2.5.2.1 Plant material and bioassays

Healthy and homogeneous *V. faba* seeds were selected, and their surface was sterilized with sodium hypochlorite (1%, v/v) for 10 min. Afterward, the plants were rinsed with tap water and soaked in the dark for 18 h. The seeds were germinated in Petri dishes at 22°C in the dark on filter paper moistened with distilled water. After germination, the seedlings were selected for uniformity and transferred to plastic vials containing Hoagland's nutrient solution (five plants per vial). The solutions were renewed daily to maintain a constant nutrient composition and pH. The plants were subdivided into six groups:

1. Untreated Plants (control group);
2. Plants treated with 1 mM of vitamin C;

3. Plants treated with 0.3 mg/mL DAAE;
4. Plants treated with 5 mg/mL DAAE;
5. Plants cotreated with 0.3 mg/mL DAAE + 1 mM vitamin C; and
6. Plants cotreated with 5 mg/mL DAAE + 1 mM vitamin C.

The plants were grown in a growth chamber with day/night temperatures of 24/22°C and a photoperiod of 16/8 h (light/dark). *V. faba* plants were harvested after 2 weeks and washed with distilled water. The experiment was repeated three times per treatment.

2.5.2.2 Extraction and estimation of antioxidative enzymes

Briefly, 0.5 g of fresh leaves was ground in an ice bath with 3 mL of 0.1 M cold phosphate buffer (pH 7, containing 1 mM 1,4-dithioerythritol, 1 mM phenylmethylsulfonylfluoride and 1% (w/v) polyvinylpyrrolidone). The homogenate was centrifuged for 15 min at 12,000 rpm at −4°C. The obtained supernatant was used for assessing enzyme activities.

Superoxide dismutase (SOD) activity was measured according to Lin et al. [22]. The reaction mixture (3 mL) consisted of 0.05 M phosphate buffer, pH 7.8, 10 µM EDTA, 10 µM riboflavin, 13 mM methionine, 75 µM nitro blue tetrazolium (NBT), and the enzyme extract. SOD activity was recorded at 560 nm. One unit (U) of SOD activity is expressed as the enzyme concentration required for 50% inhibition of NBT reduction. The activity was expressed as U per mg of fresh material.

Catalase (CAT) activity was determined following the method of Rao et al. [23]. The reaction volume of 3 mL consisted of potassium phosphate buffer (50 mM, pH 7.0), enzyme extract, and H_2O_2 (0.3%). The absorbance was monitored at 240 nm for 3 min and CAT activity (U per mg fresh material) was calculated using the molar absorptivity of 39.4 M^{-1} cm^{-1}.

2.5.3 Animals

Fifty adult male Swiss mice (25–30 g) were supplied by the central animal care facility of the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco. Five mice were housed per cage at a constant room temperature of 22 ± 2°C, following a 12-h dark/light cycle. The mice had free access to standard diet pellets and water. All animals received treatment in compliance with the Moroccan Ethics Committee of the Moroccan Society for Ethics and Animal Research (Ref. UCA-FSSM-07/2023). All efforts were made to reduce the number of animals used and to minimize any animal suffering.

2.5.3.1 Experimental design

DAAE was administered orally (gavage) to the animals for seven days. The mice were allocated into ten treatment groups, with five mice in each group, and received different treatments as follows: group 1, control group (distilled water); groups 2–4, administered DAAE at doses of 250, 500, and 1,000 mg/kg b.w., respectively; groups 5–7, administered by intraperitoneal injection (i.p.) 100 mg/kg b.w. of vitamin C 1 h before the doses of DAAE to investigate the possible protective effect; group 8 was administered i.p. 100 mg/kg b.w. of vitamin C for 7 consecutive days; group 9 was administered 100 mg CP/kg given i.p. 30 h before animals were killed (positive control); and group 10 was administered i.p. 100 mg/kg b.w. of vitamin C 1 h before the dose of CP.

2.5.3.2 MN test

The animals were euthanized by cervical dislocation resulting in luxation of the cervical vertebrae without crushing, and then, bilateral femurs were separated. Bone marrow preparations were performed according to the protocol outlined by Schmid [24] with slight adjustments as documented by Agarwal and Chauhan [25]. In brief, the bone marrow was flushed out from the femurs in Hank's buffered salt solution containing 1% (w/v) bovine serum albumin and 0.15% (w/v) ethylenediaminetetraacetic acid at pH 7.4. After centrifugation, the flushed marrow was smeared onto slides, air-dried, and fixed by immersion in absolute methanol for 5 min. Finally, the slides were stained using the May Grunwald/Giemsa protocol and examined under a digital light microscope (Optika B-290TB) equipped with a tablet. To prevent any potential subjective errors, a minimum of 2,000 polychromatic erythrocytes per animal were evaluated to assess MN induction, and 1,000 erythrocytes per group were examined from randomly selected slides to determine the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCE/NCE).

2.5.3.3 Biochemical analysis of serum

Blood samples were collected in sterile vials without anticoagulants to facilitate serum separation. The serum biochemical parameters were analyzed using an automatic biochemistry analyzer (Abbott Architect Ci4100, Japan). The parameters analyzed included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and uric acid.

2.5.3.4 Histopathological studies

Liver and kidney tissues of mice were immediately fixed in 10% buffered formalin solution for 48 h. Histological analyses were performed at the Immunohistochemistry Laboratory, Department of Pathological Anatomy, Mohammed VI University Hospital Center, Marrakech, Morocco. The paraffin-embedded organs were cut into 3–4 µm thick sections using a rotary microtome (Leica Biosystems, RM 2245). The tissue sections were then processed in graded alcohol and stained with hematoxylin and eosin. To assess any potential toxic effects, the tissue sections were examined under a Leica DMD108 digital microimaging system.

2.6 Data analysis

The data are presented as the mean ± standard deviation (SD). Differences between controls and treatment groups were determined using one-way analysis of variance followed by Tukey's post hoc test. p values <0.05 were considered statistically significant. IBM SPSS Statistics (version 26.0) was used.

3 Results

3.1 Phytochemical screening

D. ambrosioides leaf aqueous extract underwent preliminary phytochemical screening, allowing the qualitative evaluation of its secondary metabolites. The results revealed the presence of coumarins, flavonoids, saponins, and alkaloids. The extract was negative for terpenoids and tannins.

3.2 UHPLC-DAD-MS analysis of polyphenol compounds

The UHPLC-DAD-ESI/MS chromatogram of DAAE enabled the identification of various compounds (Table 1, Figure 1). The results showed the presence of several phenolic acids, notably 4-caffeoylquinic acid, chlorogenic acid, *p*-coumaric acid, and 4,5-*O*-dicaffeoylquinic acid. In addition, flavonoid-*O*-glycosides, such as luteolin-4′-*O*-glucoside and kaempferol-3-*O*-beta-glucoside, were identified, along with the flavonoid rutin and dihydroquercetin.

Table 1: Identification of main compounds of *D. ambrosioides* aqueous extract by UHPLC-DAD-MS

Peak	Rt (min)	([M−H]$^-$) (*m/z*)	UHPLCDAD λ max (nm)	Proposed compound	Molecular formula	Molecular weight
1	4.2	447.1	260	Luteolin-4′-*O*-glucoside	$C_{21}H_{20}O_{11}$	448.38
2	5.4	353.9	267	4-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354.31
3	7.84	447.1	280	Kaempferol-3-*o*-beta-glucoside	$C_{21}H_{20}O_{11}$	448.38
4	14.06	353.1	317	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31
5	18.34	163.1	310	*p*-Coumaric acid	$C_9H_8O_3$	164.16
6	18.75	303.1	280, 323	Dihydroquercetin	$C_{15}H_{12}O_7$	304.1
7	19.52	515.6	313	4,5-*O*-dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.4
8	21.88	609.1	266, 356	Rutin	$C_{27}H_{30}O_{16}$	610.52

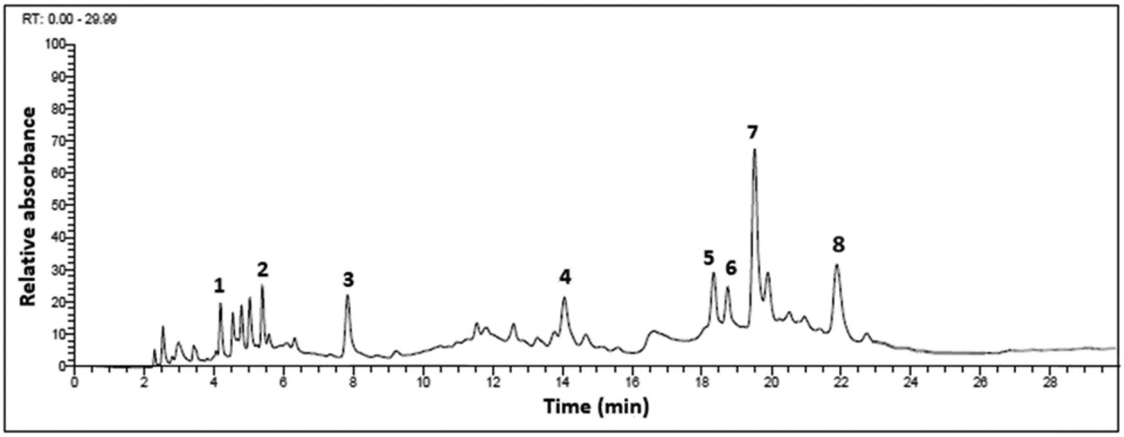

Figure 1: Chromatographic profiles of *D. ambrosioides* leaf aqueous extract recorded at 280 nm. Peak numbers are represented in Table 1.

3.3 Cytogenotoxicity

3.3.1 Cytogenotoxicity of DAAE on *V. faba* plant assay

MN analysis was carried out to assess the genotoxic effect of DAAE on *V. faba* root meristem cells (Table 2). The positive control (CP, 1 mg/mL) induced a significant increase in MN frequency, 7.18-fold above that of the negative control. DAAE, at concentrations starting from 0.16 mg/mL, exhibited a dose-dependent induction of MN, ranging from 2.99- to 6.39-fold above the negative control.

To evaluate the potential cytotoxicity of DAAE, the MI was determined (Table 2). Concentration 0.04 mg/mL and higher, as well as the positive control, exhibited toxicity to the root meristem cells of *V. faba* compared to the negative control. Furthermore, the MI exhibited a decreasing trend with increasing DAAE concentration.

Table 3 summarizes the MI and MN data for the cotreatment of *V. faba* roots with vitamin C (1 mM) alongside each tested concentration of DAAE or CP. Co-administration of vitamin C with CP led to reduced genotoxicity to *V. faba* root meristem cells compared to that of CP alone, resulting in a 2-fold decrease in MN frequency. Cotreatment with various concentrations of DAAE alongside vitamin C reduced significantly the MN frequency compared to roots treated with DAEE alone, except for the concentrations of 2.56 and 5.12 mg/mL. Similarly, the combination of vitamin C with CP resulted in reduced cytotoxicity, leading to an increase in MI from 5.23 to 6.53%. In terms of cytotoxicity assessment, cotreatment of vitamin C with DAAE demonstrated a

Table 2: Results of the micronucleus assay of *V. faba* root-tip cells treated with *D. ambrosioides* aqueous extract (DAAE)

Samples	Concentrations (mg/mL)	Mitotic index	Micronucleus frequency
NC	0	8.73 ± 1.14	1.67 ± 0.58
CP	1	5.23 ± 0.45*	12.00 ± 1.00*
DAAE	0.02	7.67 ± 1.20	2.00 ± 1.00
	0.04	7.03 ± 0.68*	2.67 ± 0.58
	0.08	6.17 ± 0.38*	3.33 ± 0.58
	0.16	6.13 ± 0.42*	5.00 ± 0.00*
	0.32	5.50 ± 0.46*	7.33 ± 0.58*
	0.64	5.00 ± 0.89*	8.33 ± 1.15*
	1.28	3.93 ± 0.31*	9.33 ± 0.58*
	2.56	3.23 ± 0.15*	10.00 ± 1.00*
	5.12	2.47 ± 0.12*	10.67 ± 0.58*

NC: negative control; CP: cyclophosphamide; each value represents the mean ± SD of three independent experiments.
*$p < 0.05$ significant difference from the control.

Table 3: Results of the micronucleus assay of *V. faba* root tip cells cotreated with vitamin C and *D. ambrosioides* aqueous extract (DAAE)

Samples	Concentrations			Mitotic index	Micronucleus frequency
	CP (mg/mL)	Vit C (mM)	DAAE (mg/mL)		
NC	—	—	—	8.73 ± 1.14	1.67 ± 0.58
CP	1	—	—	5.23 ± 0.45*	12.00 ± 1.00*
Vit C	—	1	—	9.97 ± 0.35	3.67 ± 0.58*
Vit C + CP	1	1	—	6.53 ± 0.32*	6.00 ± 1.00*
DAAE + vitamin C	—	1	0.04	9.73 ± 0.25	1.67 ± 0.58
	—	1	0.08	9.27 ± 0.93	1.67 ± 0.58
	—	1	0.16	8.67 ± 0.60	2.00 ± 0.00
	—	1	0.32	7.77 ± 0.81	2.33 ± 0.58
	—	1	0.64	6.97 ± 0.15*	2.67 ± 0.58
	—	1	1.28	6.30 ± 0.46*	3.00 ± 0.00
	—	1	2.56	5.73 ± 0.15*	3.33 ± 0.58*
	—	1	5.12	5.17 ± 0.60*	4.33 ± 0.58*

NC: negative control; CP: cyclophosphamide; each value represents the mean ± SD of three independent experiments.
*$p < 0.05$ significant difference from the control.

mitigating effect on the cytotoxicity of *D. ambrosioides*. Notably, DAAE appeared to be significantly cytotoxic at concentrations equal to or higher than 0.64 mg/mL instead of 0.04 mg/mL. Likewise, the combination of vitamin C with CP resulted in reduced cytotoxicity, leading to an increase in MI from 5.23 to 6.53%.

3.3.2 ROS scavenging enzyme activity

Figure 2 presents the analysis of SOD and CAT antioxidant enzymatic activities in *V. faba* plants treated with DAAE alone or in combination with vitamin C. Treatment of *V. faba* with 0.3 mg/mL of DAAE led to increases in both SOD and CAT activities by 46 and 238%, respectively, compared with those in the control. At a concentration of 5 mg/mL, the effects were more pronounced, with a 145% increase in SOD activity and a 492% increase in CAT activity.

Treatment of *V. faba* with vitamin C and 0.3 mg/mL of DAAE significantly decreased CAT activity by 16% compared to that in plants treated with DAAE alone. However, a non-significant ($p < 0.05$) increase in SOD activity was observed for the same treatment. The effect of vitamin C was more pronounced in cotreatment with a concentration of 5 mg/mL, demonstrating a significant decrease in SOD and CAT activities by 23 and 42%, respectively, compared to plants exposed to DAAE alone.

3.3.3 Cytogenotoxicity analysis of bone marrow damage in mice

Table 4 shows the frequency of micronucleated polychromatic erythrocytes (MNPCEs) per 2000 PCE in mouse bone marrow cells. Administration of DAAE at different doses, namely 250, 500, and 1,000 mg/kg b.w., induced a genotoxic effect in a dose-dependent manner. This was evident through a significant increase in the frequency of MNPCEs by 64, 136, and 468% for the respective doses of DAAE compared with the control group. Co-administration of DAAE and vitamin C to mice resulted in a significant reduction in the number of MNPCEs compared to those treated solely with DAAE (Table 4), ranging from 15.2 to 43.4%.

The assessment of the PCE/NCE ratio in mice treated with DAAE unveiled a noteworthy decrease compared to the control group, indicating the cytotoxic effect of DAAE on the proliferation of bone marrow cells (Table 4). However, in groups treated with vitamin C + DAAE, an increase in the PCE/NCE ratio was observed compared to groups treated only with DAAE.

3.3.4 Serum biochemical parameters

The administration of DAAE or CP to mice led to significant ($p < 0.05$) increases in the serum AST, ALT, and ALP activities compared to those in the control group. Treatment with vitamin C + DAAE resulted in significantly decreased values of these biochemical parameters compared to the group treated with only DAAE (Table 5).

Renal function was assessed by measuring serum creatinine and uric acid levels. Except for mice treated with 1,000 mg/kg b.w. of DAAE, no significant changes were observed in the creatinine levels of the treated animals compared to the control group. Regarding uric acid levels, a significant increase was observed in mice treated with

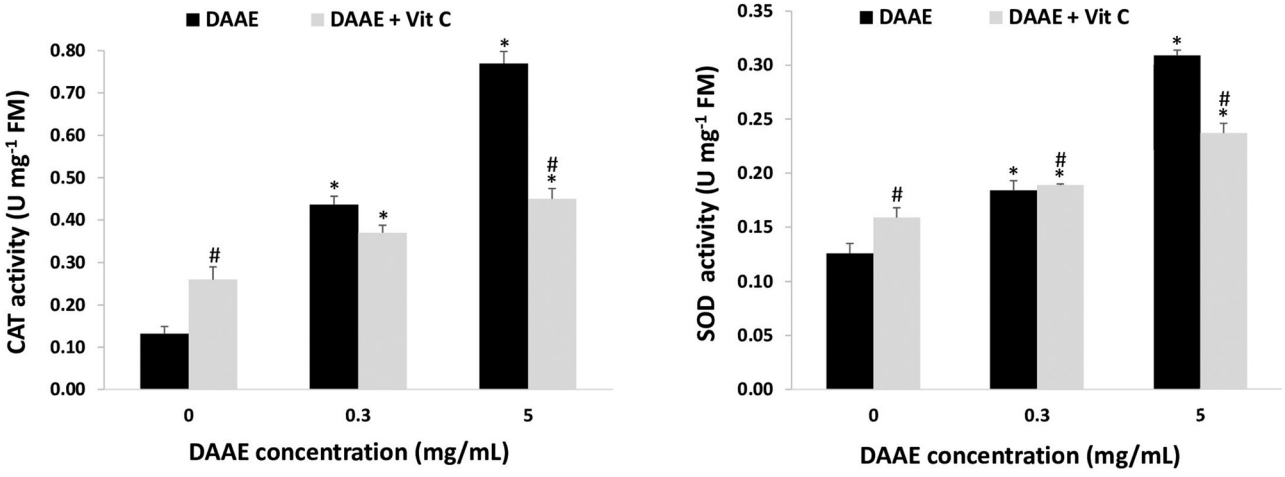

Figure 2: The effects of *D. ambrosioides* aqueous extract (DAAE) alone and combined with vitamin C on enzyme systems in *V. faba* plants. SOD, superoxide dismutase; CAT, catalase. *$p < 0.05$ significant difference from the control; #significant difference between plants co-treated with vitamin C compared to those treated with DAAE alone.

Table 4: Frequency of micronuclei and mitotic index in bone marrow cells of mice treated with DAAE alone and in combination with vitamin C

Groups (n = 5)	CP (mg/kg b.w.)	DAAE (mg/kg b.w.)				Vit C (100 mg/kg b.w.) combined with				
						DAAE (mg/kg b.w.)				CP (mg/kg b.w.)
	100	0	250	500	1,000	0	250	500	1,000	100
MNPCE/2000 PCE	14.2 ± 1.79*	5.6 ± 1.14	9.2 ± 1.3*	13.2 ± 1.30*	31.8 ± 0.84*	8.6 ± 1.14*,#	7.8 ± 1.30	10.8 ± 1.30*	18 ± 0.89*,#	10.6 ± 1.34*,#
Mitotic index	0.96 ± 0.15*	1.58 ± 0.10	1.05 ± 0.05*	1.02 ± 0.05*	0.76 ± 0.04*	1.26 ± 0.07*,#	1.36 ± 0.09*,#	1.18 ± 0.08*	0.93 ± 0.03*	1.09 ± 0.06*

Values are mean ± SD.
*$p < 0.05$ significant difference from the control.
#Significant difference between groups co-treated with vitamin C and DAAE/or CP compared to those treated with DAAE/or CP alone.

DAAE or CP compared to the control group. However, the administration of vitamin C resulted in a significant reduction in uric acid levels in the group treated with a dose of 250 mg/kg b.w. of DAAE compared to the control group (Table 5).

3.3.5 Histopathological observation

The histological examination results for the control group, the groups administered DAAE, and DAAE + vitamin C are depicted in Figure 3 for liver samples and Figure 4 for kidney samples. Histopathological assessment of liver tissues from the negative control group revealed normal hepatic cell architecture with granulated cytoplasm and intact nuclei. These tissues showed no alterations, abnormalities, degenerative changes, or infiltrative lesions (Figure 3). In contrast, the livers of mice treated with CP exhibited histopathological changes such as hepatic degeneration, steatosis, cytoplasmic degeneration, necrotic foci, and inflammatory granuloma. In the mice treated with 250 mg/kg b.w. DAAE, hepatocyte damage was evident and was characterized by cytoplasmic degeneration, hepatocyte ballooning, and steatosis. This toxicity in the liver increased in a dose-dependent manner. Liver tissues from the group treated with 500 mg/kg b.w. exhibited symptoms such as necrosis, hepatocyte apoptosis, loss of cellular outline and lobular architecture, cytoplasmic degeneration, and steatosis. Administration of a dose of 1,000 mg/kg b.w. further intensified these toxic effects, resulting in necrosis, hepatocyte apoptosis, and inflammatory infiltrate cells. However, livers of mice treated with a combination of DAAE + vitamin C showed a significant reduction in DAAE-induced liver toxicity. The main observable changes included moderate hepatocyte ballooning, necrosis, and steatosis, as illustrated in Figure 3.

Histological examination of kidney tissues from the control group revealed normal renal tubules and glomeruli, as illustrated in Figure 4. In contrast, kidney tissues from mice treated with CP showed degeneration and swelling of the lining epithelial cells, focal tubular necrosis, and inflammatory infiltration. Kidney tissues from mice treated with DAAE at concentrations of 250 and 500 mg/kg b.w. exhibited mild degeneration of renal epithelial cells compared to the control group. More pronounced changes were observed in kidney tissues from mice treated with DAAE at 1,000 mg/kg b.w., with evident shrinkage of the glomeruli and distinct vacuoles. In animals administered both DAAE (or CP) + vitamin C, degenerative changes were noted in some renal tubular epithelia. Kidney tissues from mice treated with DAAE at 500 mg/kg b.w. + vitamin C

Table 5: Serum biochemistry of mice treated with DAAE alone and in combination with vitamin C (AST, ALT, ALP, creatinine, and uric acid)

Groups (n = 5)		AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (µmol/L)	Uric acid (µmol/L)
Negative control		231.59 ± 5.38	38.06 ± 1.37	37.40 ± 2.37	26.15 ± 1.55	198.50 ± 5.17
CP (100 mg/kg)		736.39 ± 4.36*	129.01 ± 3.65*	76.19 ± 3.24*	30.54 ± 1.91	285.63 ± 5.64*
DAAE (mg/kg b.w.)	250	375.48 ± 5.95*	70.42 ± 1.81*	56.25 ± 1.15*	29,28 ± 1.76	220,25 ± 2.27*
	500	616.31 ± 3.8*	113.40 ± 2.19*	67.14 ± 3.25*	29.98 ± 1.83	240.25 ± 2.27*
	1,000	>913.00	184.68 ± 5.23*	86.15 ± 1.33*	35.50 ± 2.39*	288.30 ± 4.08*
Vit C (100 mg/kg b.w.)		295.58 ± 7.50*	41.82 ± 2.08	44.92 ± 3.07	28.18 ± 0.67	204.98 ± 6.12
Vit C (100 mg/kg b.w.) + DAAE (mg/kg b.w.)	250	324.20 ± 5.78*,#	45.49 ± 3.27#	44.13 ± 2.50#	28.46 ± 1.81	208.82 ± 4.15
	500	444.92 ± 7.34*,#	82.16 ± 4.89*,#	55.43 ± 1.40*,#	29.63 ± 1.01	236.93 ± 3.41*
	1,000	>913.00	107.39 ± 4.72*,#	73.32 ± 2.91*,#	31.21 ± 1.67*	264.26 ± 3.43*,#
CP (100 mg/kg) + Vit C (100 mg/kg)		639.40 ± 4.70*,#	79.49 ± 7.90*,#	60.22 ± 4.71*,#	28.69 ± 1.32	223.99 ± 5.00*,#

Values are mean ± SD.

*p < 0.05 significant difference from the control.

#Significant difference between groups co-treated with vitamin C and DAAE/or CP compared to those treated with DAAE/or CP alone.

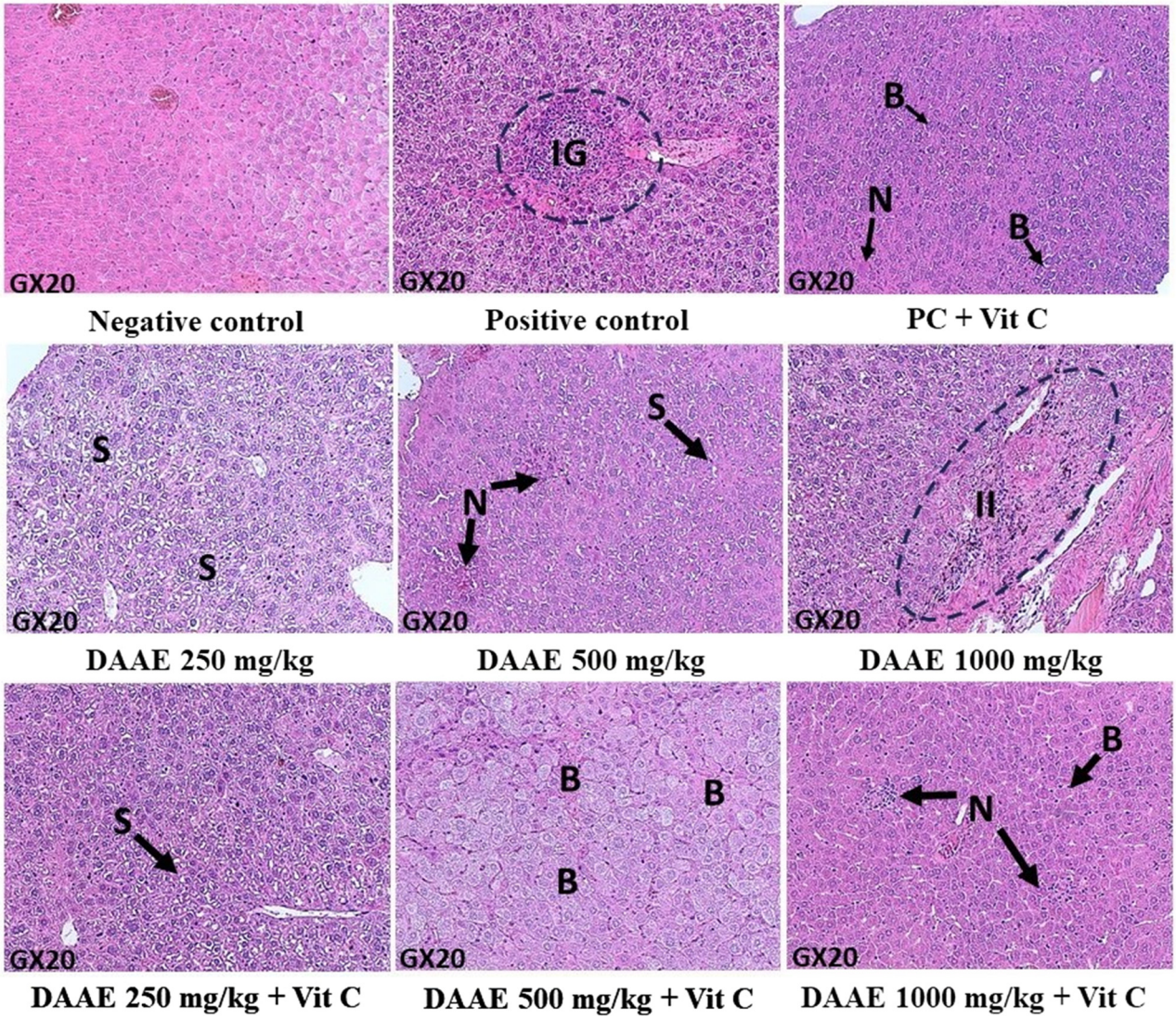

Figure 3: Histopathological observations of mouse livers for the negative control (distilled water), positive control (cyclophosphamide), and groups treated with 250, 500, and 1,000 mg/kg b.w. of *D. ambrosioides* aqueous extract (DAAE), as well as the combination of different concentrations of DAAE with vitamin C. B: ballooned hepatocytes; IG: inflammatory granuloma; N: necrosis; S: steatosis; II: inflammatory infiltrate.

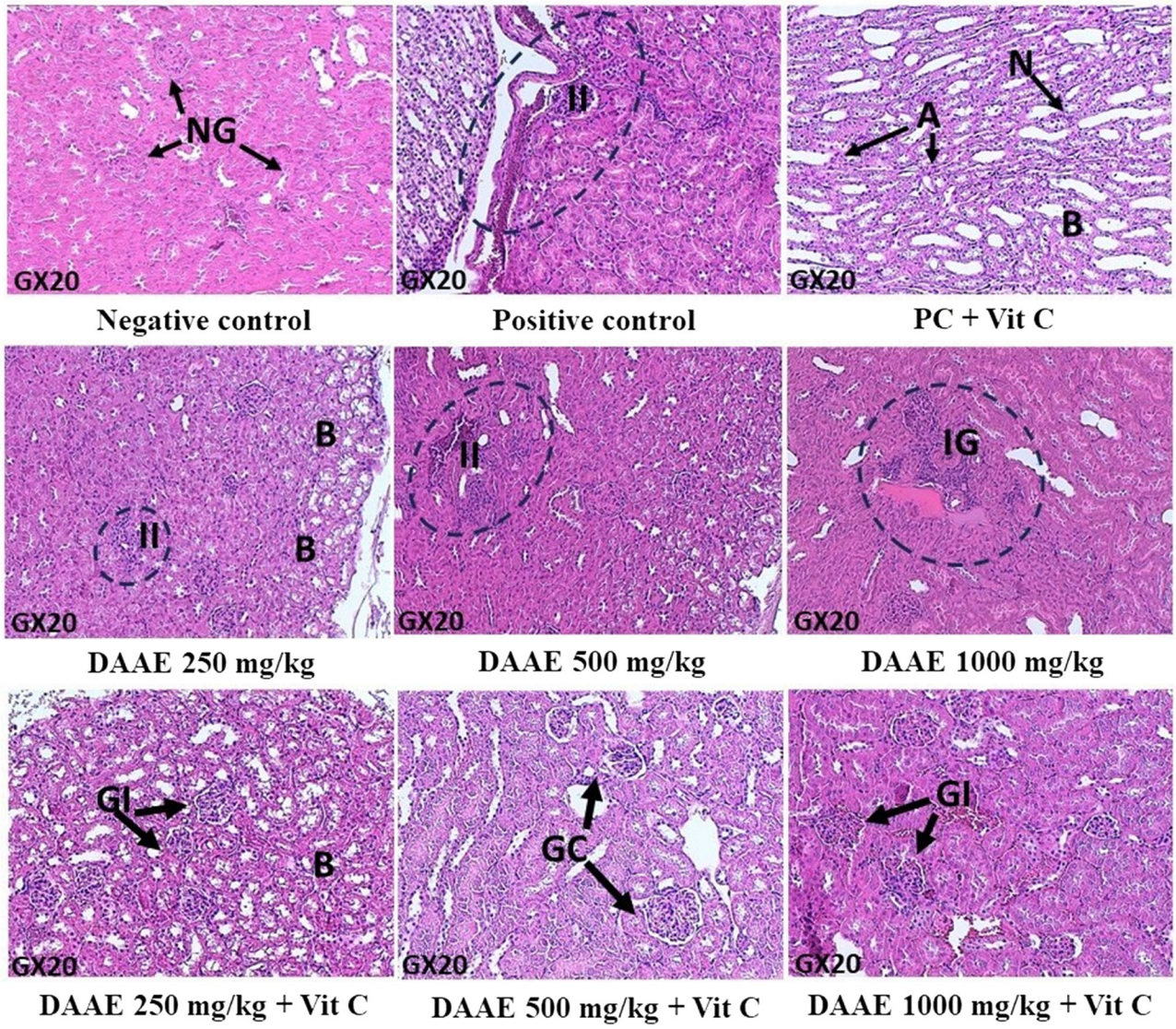

Figure 4: Histopathological observations of mouse kidneys for the negative control (distilled water), positive control (cyclophosphamide), and groups treated with 250, 500, and 1,000 mg/kg b.w. of *D. ambrosioides* aqueous extract (DAAE), as well as the combination of different concentrations of DAAE with vitamin C. A: apoptosis; B: ballooned tubules; GC: glomerular vascular cluster congestion; GI: glomerular inflammation; IG: inflammatory granuloma; N: necrosis; NG: normal glomeruli; II: inflammatory infiltrate.

exhibited glomerular vascular cluster congestion. Moreover, glomerular inflammations were observed in the group treated with DAAE at 1,000 mg/kg combined with vitamin C (Figure 4).

4 Discussion

The present study was conducted to assess the potential cytogenotoxic effects of *D. ambrosioides* leaf aqueous extracts. Furthermore, the potential protective effect of co-treatment with vitamin C was also investigated.

The *in vivo* MN assay is a highly successful and reliable test for assessing the genotoxicity of various substances, including natural products, chemicals, physical agents, and environmental samples. In this study, the *V. faba* MN assay was used as a preliminary test to examine the cytogenotoxic activity of DAAE. The endpoints evaluated were the MI and MN frequency. According to Rojas et al. [26], the decrease in the MI can be interpreted as cellular death or as the arrest of cell division at any stage during interphase, which could be attributed to the inhibition of DNA synthesis or the blocking of the cell cycle in the G2 phase [27]. An increase in the frequency of MN is indicative of

chromosome aberrations during cell mitosis. MN are small extranuclear chromatin bodies surrounded by a nuclear envelope. They can be generally formed in mitotic cells from chromosomal fragments or lagging chromosomes that are not integrated into the daughter nuclei during telophase [28]. The results obtained indicate a dose-dependent decrease in the MI and an increase in the frequency of MN in *V. faba* root meristematic cells treated with DAAE. A significant decrease was observed in the mitotic activity for concentrations higher than or equal to 0.04 mg/mL compared to the negative control, confirming the cytotoxic effect of DAAE. In addition, concentrations of 0.16 mg/mL and higher were found to be genotoxic. To determine if there are any differences in the mutagenic effects of DAAE on animal and plant cells, the MN assay was performed on the bone marrow cells of mice, after obtaining reliable results from the *V. faba* test. Our results revealed that continuous administration of DAAE for 7 days led to a significant dose-related decrease in the MI (PCE/NCE ratio). These findings indicate that DAAE has an impact on the normal proliferation of bone marrow cells, resulting in cytotoxicity. Moreover, an increased frequency of MNPCEs at the tested doses compared to the control group was observed, which indicates the occurrence of chromosomal damage caused by DAAE.

These findings align with prior research exploring the cyto- and genotoxic properties of two plants belonging to the *Chenopodium* genus. Gadano et al. [13,14] reported that the aqueous preparation (infusion and decoction) of *C. ambrosioides* and *Chenopodium multifidum* aerial parts induced genetic damage by increasing chromosomal aberrations and sister chromatid exchanges in human lymphocytes cultured *in vitro*. Other studies also indicated a cytotoxic effect of both *C. ambrosioides* and *C. multifidum*, as evidenced by a decrease in the MI [14,29]. In contrast, neither genotoxic nor cytotoxic effects of *C. album* were observed using *in vitro* and *in vivo* assays [14,29].

The probable mechanism underlying the cyto- and genotoxic potential of DAAE may be attributed to the generation of oxidative stress. The current study revealed that DAAE increased the enzyme activities of SOD and CAT in *V. faba*. SOD is widely acknowledged as the primary defense barrier against ROS, thereby safeguarding cells from oxidative damage. It functions by catalyzing the dismutation of superoxide anion radicals ($O^{2\bullet-}$) into hydrogen peroxide (H_2O_2). Subsequently, H_2O_2 is further eliminated through subsequent reactions catalyzed by CAT, converting it into water (H_2O). The increase in antioxidant enzyme activity can be considered a normal cellular response to counteract the oxidative stress induced by DAAE. However, despite the increased activity of these enzymes, the cells were unable to effectively mitigate their effects.

The effect of DAAE on biochemical markers of liver and kidney function was assessed in mice treated with DAAE daily for 7 days. Sensitive indicators of hepatotoxicity, such as AST, ALT, and ALP activities, were measured, as well as the kidney function markers: creatinine and uric acid [30–32]. The activities of AST, ALT, and ALP increased after DAAE treatment. Elevated serum levels of these enzymes suggest that DAAE might cause a certain degree of damage to hepatic function in mice [33]. DAAE treatment also induced renal toxicity, as evidenced by elevated serum creatinine and uric acid levels. These parameters are usually associated with impaired kidney function [34]. Histopathology of kidney and liver tissues in mice administered DAAE revealed a reduction in glomerular size, accompanied by noticeable vacuolation in tubular epithelial cells and hepatic degeneration. In alignment with our findings, both acute and sub-chronic treatments of rats with the aqueous extract of *C. ambrosioides* leaves led to mild toxic effects in the liver and kidneys. These effects were associated with changes in serum biochemistry and notable histological irregularities, as reported by [35]. Another study underscored the nephrotoxicity in rats treated with *C. ambrosioides* leaf extract for 6 weeks, manifesting as evident necrosis in the kidney tubules [36].

This plant has been reported to contain secondary metabolites, such as coumarins, flavonoids, saponins, and alkaloids, which is consistent with our research findings [37]. In addition to essential oil, polar extracts have served as the primary source of compounds. The majority of isolated and characterized compounds are flavonoids and flavonoid glycosides [38].

The observed cytotoxic and genotoxic effects of *D. ambrosioides* are likely associated with the presence of these compounds. Several studies have reported the genotoxic effect of coumarins, which have been found to cause DNA damage in mammalian and plant cells [39–41]. Furthermore, among the isolated alkaloids from this species, piperine has been reported to inhibit the proliferation and survival of various types of cancer cells by influencing apoptotic signaling activation and inhibiting cell cycle progression [42]. However, the available information on the genotoxicity of piperine, especially *in vivo*, is deemed incomplete due to conflicting results. While piperine (up to 4 mg/kg b.w.) yielded negative results in the MN test in polychromatic erythrocytes of bone marrow in mice, it displayed positive outcomes in the *Allium cepa* MN test at the concentrations of 50, 100, and 200 µM [43,44]. This discrepancy raises a clear warning flag regarding the genotoxic potential of piperine. The genotoxic/antigenotoxic effects of saponin compounds have also been reported [45,46]. While phenolic compounds are widely recognized for their antioxidant

properties, it is important to note that they may also exhibit pro-oxidant effects, leading to the generation of reactive free radicals and inducing cytotoxic effects and DNA damage [47,48]. Among the phenolic compounds detected in DAAE, luteolin-4′-*O*-glucoside has been shown to have a pro-oxidant effect in human skin fibroblasts exposed to UVA [49]. In addition, luteolin has been shown to induce genotoxic effects in mouse bone marrow cells, as evidenced by the MN assay [50]. It also exhibited cytotoxicity, indicated by cell viability and cellular ATP measurements, and genotoxic potential in wild-type human lymphoblastoid TK6 cells, as determined by the alkaline comet assay and the high-throughput MN assay [51]. Furthermore, studies have demonstrated that kaempferol induces genotoxicity and DNA damage in human hepatoma Hep G2 cells [52] and mouse lymphoma L5178Y cells [53].

It is worth noting that the toxic and genotoxic activities of plant extracts may be modulated by interactions between carcinogens and protective compounds. Our cotreatment experiments revealed that vitamin C effectively mitigated the cytotoxicity and genotoxicity of DAAE. Vitamin C, a water-soluble antioxidant, is known to protect cells from oxidation by free radicals and to reduce the genotoxicity induced by recognized mutagens [54–59].

5 Conclusions

Although extensive studies have been conducted to explore the medicinal properties of *D. ambrosioides*, only a few have focused on its aqueous extract. After several reports of intoxication, concerns regarding the safety and toxicology of *D. ambrosioides* have arisen. The present study suggests that the aqueous leaf extract of *D. ambrosioides*, which is frequently used in Moroccan traditional medicine, exhibits dose-dependent cytogenotoxic effects. Therefore, precautions must be taken when using this extract as an alternative remedy in traditional healing. However, vitamin C has shown a significant protective effect against DAAE-induced cytogenotoxicity. Further studies should be conducted to determine the active compounds of *D. ambrosioides*, and their mechanisms of action, and to standardize the extract. It is also necessary to ascertain the optimal concentrations of the extract and vitamin C for the safe use of this plant.

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Conflict of interest: Authors state no conflict of interest.

Ethics approval: All animals received treatment in compliance with the Moroccan Ethics Committee of the Moroccan Society for Ethics and Animal Research (Ref. UCA-FSSM-07/2023).

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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