RESEARCH ARTICLE

Potential antioxidant effects of *Narcissus tazetta* phenolic compounds against cadmium chloride–induced hepatotoxicity in Swiss albino mice

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Abstract

Narcissus tazetta (Amaryllidaceae) is a medicinal plant widely used for cut flowers and potted ornamental plant in Tunisia flora. The current study evaluated the phenolic composition and antioxidant properties of its flower extracts and investigated its potential protective activity against cadmium chloride ($CdCl_2$)–induced hepatotoxicity in mice. Mice were divided into six groups of six each: group 1, serving as negative controls, received by intraperitoneal way only distilled water; group 2 received by intraperitoneal way $CdCl_2$ (0.16 mg/kg bw); groups 3 and 4 received $CdCl_2$ at the same dose of group 2 and 100 or 200 mg/kg bw of *Narcissus tazetta* flower extracts via oral route; groups 5 and 6, serving as positive controls, received only *Narcissus tazetta* flower extracts. Polyphenolic compounds of the extract were analyzed by colorimetric and high-performance liquid chromatography-mass spectrometry (HPLC-MS) methods. Total antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential of the extract were estimated using colorimetric method. Results indicated that ethanolic flower extract contained high levels of total phenolic and flavonoid along with a strong total antioxidant and DPPH free radical scavenging activities. HPLC-MS analysis identified eight phenolic compounds, including rutin, kaempferol glycosides, and chlorogenic acids. The extract also exhibited marked hepatoprotective effects against $CdCl_2$ toxicity by reducing hepatic levels of malondialdehyde, advanced oxidation protein products, hydrogen peroxide, metallothioneins, and DNA degradation. Additionally, co-administration of *Narcissus tazetta* flower extracts lowered the plasma activities of transaminases, gamma glutamyl transpeptidase, and lactate dehydrogenase and increased hepatic levels of reduced glutathione, nonprotein thiols, vitamin C, and catalase activity. The hepatoprotective effects of the extract were demonstrated by histopathological improvement of liver disorders. The current study provided ethnopharmacological application of *Narcissus tazetta* flower extracts against $CdCl_2$-induced oxidative stress, suggesting its chemoprevention role of its phenolic compounds as a natural antioxidant.

Keywords Cadmium chloride · Mice · Hepatotoxicity · *Narcissus tazetta* L. · Polyphenols · Antigenotoxicity

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Introduction

Narcissus tazetta (monocotyledon, Amaryllidaceae family), commonly known as Narjes, is widespread as an ornamental plant in the Mediterranean regions (Grey-Wilson and Mathew 1981). It is cultivated for its bulb trade, as well as for its essential oil and volatile compounds extracted from its fragrant white flowers (Chun Chen et al. 2013; Ruíz-Ramón et al. 2014). Its bulb extract was used in the Traditional Persian Medicine to treat internal ulcer, burns, nerve injury, skin stain, and inflammation. This plant also showed diverse pharmacological properties including anti-inflammatory, anti-cholinesterase, and anti-malaria activities (López et al. 2002;

Sener et al. 2003). The study of Li et al. (2016) revealed an antioxidant activity of *Narcissus tazetta* var. *chinensis* bulb's flavonoid compounds against hydrogen peroxide–induced human SH-SY5Y neuroblastoma cell impairment. Recently, Rameshk et al. (2018) evaluated the potential wound healing properties of *Narcissus tazetta* bulb extract and demonstrated anti-inflammatory and antioxidant activities of this species. Besides, *Narcissus tazetta* was shown to accumulate high amounts of cadmium in its roots, suggesting the possible use of this species not only in phytoremediation programs but also for protective effects against heavy metal–induced toxicity (Homeira Soleimani et al. 2019).

Cadmium, along with arsenic, chromium, lead, and mercury, is among the most toxic metals, presenting human health risks even at low concentrations, and they are categorized as human carcinogen by the International Agency for Research on Cancer. Cadmium is introduced in the environment through the anthropogenic actions following rapid progress of modern technologies and industries (Satarug 2019). It is commonly used in industrial procedures, for example, to protect steel from corrosion, stabilize polyvinyl chloride, and fabricate nickel-cadmium batteries, and it is used as a pigment and a neutron absorber in nuclear power plants (Godt et al. 2006). In the biosphere, it can reach the human body from industrial and manufacturing activities or from food, water, or air contamination and many other potential sources (Madejczyk et al. 2015). This metal is a multi-target cumulative toxicant, which mainly enters the body by inhalation or ingestion, causing severe toxicity in different organs including the kidney, liver, and skeletal system (Genchi et al. 2020). It induced toxicity through the overproduction of reactive oxygen species (ROS), via the Fenton reaction. Additionally, the toxic effect of cadmium ions, with high affinity to cysteine, is due to its binding to biological molecules with thiol groups, such as metallothioneins and reduced glutathione (Matovic et al. 2015). These alterations contribute to the impairment of biological membrane phospholipids, leading to protein and DNA damages, which in turn affect intrinsic pathways of apoptosis (Genchi et al. 2020). In this context, phenolic compounds have the ability to bind cadmium ions, owing to their structural richness in free hydroxyl groups. In addition, several flavonoids (catechin, quercetin, naringenin, and hesperetin) and phenolic acids (ferulic acid and gallic acid protocatechuate), extracted from plants, were shown to be able to protect against cadmium-induced hepatotoxicity via inhibition of inflammation and the enhancement of the antioxidant defense system (Li et al. 2018; Mężyńska and Brzóska 2018).

The pharmaceutical and therapeutic aspects of flavonoids and other phenolic compounds from medicinal plants are increasingly considered in both developed and developing countries, for alleviating xenobiotic-induced oxidative stress damages. *Narcissus tazetta* is widely grown in Tunisia flora; although to the best of our knowledge, there is no reports on polyphenol composition and protective effects of this species. Thus, this study identified, for the first time, polyphenol compounds and evaluated antioxidant and hepatoprotective potentials of *Narcissus tazetta* ethanolic flower extracts on CdCl₂-intoxicated mice.

Materials and methods

In vitro study

Plant material collection

The flowers of *Narcissus tazetta* were harvested in February 2019 near Djedeida, Manouba (region located in the Medjerda valley at 25 km west of Tunis). Botanical identification was carried out by Chokri Messaoud, a professor at the National Institute of Applied Sciences and Technology. The plant was deposited at the herbarium of the Faculty of Tunis El-Manar; Tunisia, and its voucher specimen is not yet ready until now. The plant material was stored at room temperature and placed under dry conditions prior to use.

Preparation of *Narcissus tazetta* flower extracts

Five grams of air-dried flowers were extracted with 50-ml ethanol/water (4V/1V) under continuous stirring at room temperature for 30 min. The obtained aqueous ethanol extract was filtered under vacuum with Whatman filter paper (No. 1). The filtrate was concentrated to dryness under reduced pressure with a rotary evaporator (Stwartte RE 300) at 50 ± 1 °C. Residual aqueous fraction of the extract was further evaporated under nitrogen gas. The dried extract was kept at 4 °C prior to analysis.

Total phenolic content

The total phenolic content was determined using the Singleton and Rossi method (1965), modified by Dewanto et al. (2002). Briefly, an aliquot (5 µl) of flower extract (5 mg/ml) or standard solution of gallic acid was mixed with 20 µl of distilled water and 5 µl of Folin-Ciocalteu's phenol reagent. After shaking, 52 µl of Na_2CO_3 (7%) and 41 µl of distilled water were added to the mixture. After an incubation period of 90 min at room temperature, the absorbance was measured at 760 nm with a microplate spectrophotometer reader (Multiskan Go Thermo, Life Sciences). The results were expressed as milligram gallic acid equivalent per gram of dry weight (mg GAE/g DW), through the calibration curve of gallic acid (0–500 mg/l).

Total flavonoid contents

The total flavonoid content was measured based on the aluminum chloride colorimetric analysis (Zhishen et al. 1999). An aliquot of plant extracts (16 µl) or standard solution of catechin was mixed with 75 µl of $NaNO_2$ (7%). The mixture was shaken for 6 min before adding 10 µl of $AlCl_3$ (10%). After 5 min, 33 µl of NaOH (1M) and 100 µl of distillated water were added. The absorbance of the mixture was determined at 510 nm, using a microplate spectrophotometer reader (Multiskan Go Thermo, Life Sciences). Using the calibration curve of catechin (0–500 mg/l), the results were expressed as milligram catechin equivalent per gram dry weight (mg CE/g DW).

Total antioxidant activity

The total antioxidant activity was determined using a colorimetric method as described by Prieto et al. (1999). In short, an aliquot of flower extracts (100 µl) was mixed with 1-ml reagent solution (sulfuric acid (H_2SO_4, 0.6 M), sodium phosphate ($NaH_2PO_4H_2O$, 28 mM), and ammonium heptamolybdate ($NH_4Mo_7O_{24}4H_2O$, 4 mM)). After an incubation period of 90 min at 95 °C, the absorbance was measured at 695 nm. Using the calibration curve of gallic acid (0–500 mg/l), the results were expressed as mg GAE/g DW.

DPPH scavenging activity assay

The DPPH free radical scavenging activity was determined following the method of Hatano et al. (1988). The sample was diluted in the solvent of extraction at different concentrations (10, 20, 100, and 200 µg/ml), and then 20 µl of sample was added to 180 µl of DPPH (0.2 mM/l) methanolic solution. After incubation in dark at room temperature for 30 min, the absorbance was read at 517 nm. The ability to scavenge DPPH radical was calculated using the following equation: DPPH= $(A_0\text{-}A_1)/A_0 \times 100$, where A_0 and A_1 stand for the absorbance at 30 min of control and sample, respectively. The scavenging activity was expressed as IC_{50} (µg/ml), which is the antiradical dose required to cause a 50 % inhibition.

Identification of phenolic compounds using HPLC-MS

The phenolic compounds present in *Narcissus tazetta* ethanolic flower extract were carried out using HPLC (Jasco-LC-Net II ADC; LC-2000Plus Series) equipped with a diode array detector and coupled with mass spectrometry (MS-ZMD4, Micromass, Waters Inc., Manchester, UK). A C18 reverse-phase analytical column (25 cm 202 length × 4.6 mm i.d., 5 µm particle size; Teknokroma, Barcelona, Spain) was used. Samples were eluted with a linear gradient of solvents A (0.1% aqueous formic acid solution) and B

(0.1% formic acid in acetonitrile) at a flow rate of 1 ml/min at 30°C (Hamdi et al. 2017).

The MS was recorded in the 100 to 1000 m/z range within negative- and positive-electrospray ionization (ESI), which were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode). For analysis, the ESI MS parameters were as follow: capillary voltage (3 Kv), dissolving temperature (200 °C), source temperature (100 °C), and extractor voltage (12 V). Results were calculated from the means of three replicates. Phenolic compound structures were proposed by comparison of the retention time, UV spectrum, and product ion spectra in negative [M-H]$^-$ and positive [M+H]$^+$ mode, with those of authentic standards.

Standards of rutin and chlorogenic acid were purchased from Sigma-Aldrich Quimica (Madrid, Spain; CAS Number: 153-18-4 and 9-97-327, respectively). Nicotiflorin and narcissin were purchased from Extrasynthese (Genay, France) with CAS Number 17650-84-9 and 604-80-8, respectively. All solvents were of HPLC grade purity (Romyl and Teknokroma, Barcelona, Spain); ethanol (CAS Number: 5-17-64), formic acid (96%) (CAS Number: 64-18-6), and acetonitrile (CAS Number: 75-05-08) were purchased from Sigma Chemical Co. (St. Louis, MO).

Quantification of individual phenolic compounds were expressed as mg/kg crude extract, through the calibration curve of authentic standards (0–250 µg for the flavonoids and 0–100 µg for phenolic acids). When standards were not available, tentative characterization was carried out by comparison of the experimental mass spectra with data from the scientific literature.

In vivo study

Animals and experimental design

Female Swiss albino mice, weighing approximately 30 g at their reception, were obtained from the Central Pharmacy (SIPHAT, Tunisia). They were maintained in a controlled condition: temperature (22 ± 2°C), humidity (40%), and photoperiod (12 h light-dark cycle). A commercial standard pellet diet (SNA, Sfax, Tunisia) and drinking water were provided to mice ad libitum. Animal care and all the experimental procedures were conducted with strict adherence to the Ethical Committee of Sciences Faculty of Sfax, with ethics approval (date: 18.05.2017, no: 1204), and were in accordance with the International Guidelines for Animal Care.

After 1 week of acclimatization, mice were randomly divided into six groups of six each: group 1, serving as negative controls, received by intraperitoneal way only distilled water; group 2 received by intraperitoneal way $CdCl_2$ (0.16 mg/kg bw); groups 3 and 4 received $CdCl_2$ at the same dose of group 2 and 100 or 200 mg/kg bw of *Narcissus tazetta* flower extracts via oral route; groups 5 and 6, serving as positive

controls, received only *Narcissus tazetta* flower extracts for 8 days. Cadmium chloride anhydrous (puriss. p.a. > 99.0%) was purchased from Sigma-Aldrich (CAS number: 10108-64-2 04-008-00-3; St. Louis, MO, USA). The dose of this metal (0.16 mg/kg bw, corresponding to 1/20 of LD_{50}), used in the present study, was selected on the basis of a previous study conducted by Smalinskiene et al. (2008).

At the end of the experimental period (8 days), animals of the aforementioned groups were killed by decapitation to avoid stress and suffering. The blood samples were immediately collected in heparinized tubes and centrifuged at 2200 × *g* for 10 min. Plasma samples were drawn and stored at −80 °C until analysis. Liver tissues were dissected out, cleaned from adipose tissue, and weighed. Some samples were homogenized in frozen Tris-HCl buffer (100 mM; pH 7.4). After centrifugation, the obtained supernatants were collected and maintained at −80 °C until biochemical analysis. Meanwhile, other samples were either fixed in 10 % of buffered formalin solution for histopathological studies or frozen at −80 °C for DNA assays.

Protein determination

Total protein content of the liver homogenates was measured according to Lowry et al. (1951), using bovine serum albumin as standard.

Liver malondialdehyde (MDA) measurement

The concentration of MDA, an index of lipid peroxidation, was determined according to the method described by Draper and Hadley (1990), and the results were expressed as nmol/mg protein.

Liver hydrogen peroxide (H_2O_2) measurement

The determination of H_2O_2 was carried out according to the method of Ou and Wolff (1996), using the ferrous ion oxidation xylenol orange reagent. The results were expressed as μmol/mg protein.

Liver advanced oxidation protein product content (AOPP)

Liver AOPP levels were determined according to the method of Witko et al. (1992), using the extinction coefficient 261 cm^{-1} mM^{-1}. The results were expressed as nmol/mg protein.

Assessment of DNA degradation

The extraction of the total DNA from liver tissue (50 mg) was carried out as previously described by the Clark and Melki (2002) method using cetyltrimethyl ammonium bromide buffer. After loading onto an agarose gel (1 %), the DNA content

in liver of each treatment was recorded at 260 nm according to Sambrook and Russell (2001). The DNA content was expressed as mg/g of tissue.

Liver non-enzymatic antioxidant levels

Reduced glutathione (GSH) levels were estimated based on the reduction of 5,5′dithiobis(2-nitrobenzoic acid) with glutathione (Ellman 1959; Jollow et al. 1974). The values of GSH were expressed as nmol/mg protein.

Nonprotein thiols (NPSH) levels were determined by the method of Ellman (1959). Absorbance of the colorimetric reaction mix was measured at 412 nm. NPSH content was expressed as nmol/mg protein.

Vitamin C (Vit C) levels were determined by the dinitrophenyl hydrazine method, following Jacques-Silva et al. (2001). The data were expressed as nmol/mg protein.

Metallothioneins (MTs) content in the liver was assayed according to Viarengo et al. (Viarengo 1997)) as modified by Petrovic et al. (2001). Absorbance was measured at 412 nm. The data were expressed as μmol/mg protein.

Liver enzymatic antioxidant activities

Superoxide dismutase (SOD) activity was determined at 560 nm by the Beauchamp and Fridovich method (1971), and the enzyme activity was expressed as U/mg protein.

Catalase (CAT) activity in liver tissue was measured at 240 nm by the Aebi method (1984), and the values were expressed as μmol H_2O_2 consumed/min/mg protein.

Glutathione peroxidase (GPx) activity was estimated according to the method described by Flohe and Günzler (1984). Enzyme activity was expressed as nmol GSH oxidized/min/mg protein.

Biomarkers of liver toxicity in plasma

Plasma activities of hepatic enzymatic markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (γGT) were estimated using the commercially available diagnostic kits (Biomaghreb, Ariana, Tunisia, Ref. 20046, 20042, and 20021). The plasma activities of AST, ALT, and γGT were expressed as IU/l.

Determination of lactate dehydrogenase in plasma and liver

Plasma and liver activities of lactate dehydrogenase (LDH) were measured using the commercially available diagnostic kit (Biomaghreb, Ariana, Tunisia, Ref: 20011), and the results were expressed as IU/l and IU/g of tissue, respectively.

Table 1 Total phenolic and flavonoid contents and antioxidant activities of *Narcissus tazetta* ethanolic flower extracts

	Total polyphenols (mg GAE/g DW)	Total flavonoids (mg CE/g DW)	Total antioxidant activity (mg GAE/g DW)	DPPH scavenging activity (IC$_{50}$, µg/ml)
Ethanolic flower extract	64.15 ± 1.00	70.79 ± 1.5	134.57 ± 5.89	29.00 ± 2.00

Means of three replicates in each parameter ± standard deviation

Histological examination

The samples of liver tissue were fixed in a 10 % formalin solution, dehydrated through a graded series of alcohol (70 to 90 %), cleaned in toluene, and then embedded in paraffin. A Leica Microsystems microtome was used to prepare tissue sections, at a thickness of 5 µm. Each tissue section was deparaffinized with toluene, rehydrated using a series of alcohol, and lastly stained with hematoxylin-eosin (Suvarna et al. 2013). Each liver slide was examined under a light microscope. Liver sections were examined and assigned for severity of changes using scores on a scale of none (−), mild (+), moderate (++), high (+++), and severe (++++) damages.

Statistical analysis

All the data presented the mean values of three and six repeats in plant extracts and different animal groups, respectively. The data were represented by means ± standard deviation, and one-way analysis of variance (ANOVA, CoStat software, version 6.4, CoHort Software, Monterey, CA) was applied. Statistical assessments of differences between mean values were analyzed using Duncan's multiple range test at a probability of $P \leq u0.05$.

Results

Evaluation of levels of total polyphenols, flavonoids, antioxidant, and free radical scavenging activities

In this study, phytochemical characterization of *Narcissus tazetta* flower ethanolic extracts was evaluated, relative to its total phenolic and flavonoid contents. Results showed that the extracts contained total polyphenols at 64.14 mg GAE/g DW and flavonoids at 70.79 mg CE/g DW (Table 1). In addition, the results revealed that the flower extracts of this species exhibited 134.57 mg GAE/g DW of the total antioxidant activity. Its IC$_{50}$ values to quench DPPH radical were 29 µg/ml, indicative of an important aptitude for its phenolic fraction to scavenge free radicals (Table 1).

HPLC-MS identification of polyphenolic compounds

A typical HPLC-MS profile of polyphenol constituents of *Narcissus tazetta* flower ethanolic extracts was presented in Figure 1. The analysis showed the presence of three phenolic acids (two mono-caffeoylquinic acid isomers (1 and 2) and one chlorogenic acid (5-O-caffeoylquinic acid (5-CQA)) and five flavonols (kaempferol-di-O-glucoside, rutin (quercetin-3-O-rutinoside), kaempferol-3-O-rutinoside, nicotiflorin (kaempferol-3-O-rutinoside), and narcissin (isorhamnetin-3-O-rutinoside)) (Fig. 1).
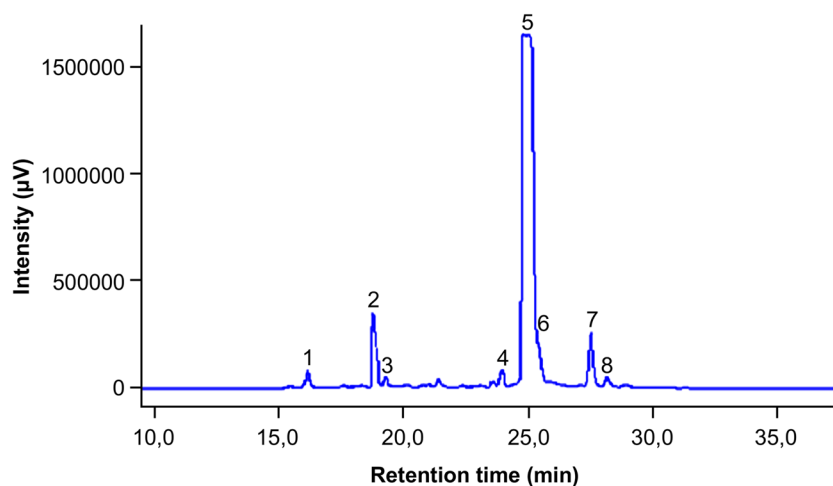
Fig. 1 HPLC-MS profile of phenolic compounds in *Narcissus tazetta* ethanolic flower extracts: chlorogenic acid 1 (1), chlorogenic acid 2 (2), chlorogenic acid 3 (3), kaempferol-di-O-glucoside (4), rutin (5), kaempferol-3-O-rutinoside (6), nicotiflorin (7), narcissin (8)

Table 2 HPLC-MS analysis of phenolic compound of *Narcissus tazetta* ethanolic flower extracts

Peak[a]	Phenolic compounds	Rt (min)[b]	λ_{max} (nm)	Ions (ESI+) (m/z)	Ions (ESI⁻) (m/z)	ID[c]	Content (mg/kg crude extract)
1	Chlorogenic acid isomer 1	16.20	220, 264sh, 324	355, 193, 181, 175	353, 191, 179, 173	Willems et al. (2016)	1766.50 ± 12.63[d]
2	Chlorogenic acid isomer 2	18.84	220, 264sh, 320	355, 193, 181, 175	353, 191, 179, 173	Willems et al. (2016)	8307.50 ± 19.82[b]
3	Chlorogenic acid isomer 3	19.28	220, 264sh, 328	355, 193, 181, 175	353, 191, 179, 173	std	257.70 ± 14.15[g]
4	Kaempferol-di-O-glucoside	23.97	252, 300sh, 348	611, 449, 287	609 , 447, 285	Li et al. (2015)	515.93 ± 4.95[e]
5	Rutin	25.23	256, 280sh, 352	611, 465, 303	609, 463, 301	std	47109.50 ± 12.76[a]
6	Kaempferol-3-O-rutinoside	25.40	264, 280sh, 348	595, 449, 287	593, 447, 285 ⁻	-	91.60 ± 4.26[h]
7	Nicotiflorin	27.55	264, 280sh, 344	595, 449, 287	593, 447, 285	std	2046.70 ± 12.00[c]
8	Narcissin	28.15	252, 304sh, 352	625, 479, 317	623 , 477, 215	std	384.36 ± 17.70[f]

[a] Numbered according to Fig. 1; [b] *Rt*, retention time; [c] *ID*, std, standard compound; *ref*, reference; *ESI*, electrospray ionization

The quantitative analysis revealed that rutin (47109.5 mg/kg crude extract) was the most abundant flavonol, followed by nicotiflorin (2046.7 mg/kg crude extract), kaempferol-di-O-glucoside (515.9 mg/kg crude extract), narcissin (384.3 mg/kg crude extract), and kaempferol-3-O-rutinoside (91.6 mg/kg crude extract). Concentrations of chlorogenic acid isomers 1 and 2 and those of chlorogenic acid (5-O-caffeoylquinic acid (5-CQA)) were 1766.5, 8307.5, and 257.7 mg/kg crude extract, respectively (Table 2).

Liver oxidative stress biomarkers and DNA degradation

In CdCl2-treated mice, MDA, H2O2 and AOPP levels, and DNA degradation increased by 52, 80, 159, and 58 % respectively, as compared with controls (Table 3). Co-administration of *Narcissus tazetta* flower extracts significantly reduced the levels of studied oxidative stress marks and repaired DNA degradation relative to CdCl2-treated mice. There was no

Table 3 Liver oxidative stress (MDA, H2O2, AOPP, and DNA degradation) and non-enzymatic antioxidative (GSH, NPSH, Vit C, and MTs) biomarkers in control and treated mice with CdCl2, CdCl2 with *Narcissus tazetta* ethanolic flower extracts (100 or 200 mg/kg), or *Narcissus tazetta* flower extracts (100 or 200 mg/kg), during 8 days

Parameters and treatments	Control	CdCl2	CdCl2 + *Narcissus tazetta* (100 mg/kg)	CdCl2 +*Narcissus tazetta* (200 mg/kg)	*Narcissus tazetta* (100 mg/kg)	*Narcissus tazetta* (200 mg/kg)
MDA (nmol/g tissue)	2.05 ± 0.101[c]	3.13 ± 0.025[a]	2.04 ± 0.172[c]	2.15 ± 0.012[b]	2.04 ± 0.17[c]	2.14 ± 0.014[b]
H2O2 (µmol/mg protein)	0.088 ±0.002[d]	0.162 ±0.007[a]	0.116 ±0.004[b]	0.108±0.003[c]	0.093 ±0.006[d]	0.092 ±0.005[d]
AOPP (nmol/mg protein)	0.023 ±0.003 [d]	0.059 ±0.005[a]	0.034 ±0.003[b]	0.029 ±0.003 [bc]	0.026 ±0.002 [cd]	0.023 ±0.002 [d]
DNA degradation (µg/g)	1736.67 ±29.06[b]	2740.00 ±202.24[a]	1980.00±178.98[b]	1786.67± 74.24[b]	1026.67±48.07[c]	690.00±15.28[c]
GSH (nmol/mg protein)	0.28 ±0.018[a-b]	0.14 ±0.012[c]	0.24 ±0.012[ab]	0.23 ±0.014[b]	0.29 ±0.025[a]	0.26 ±0.027[ab]
NPSH (nmol/mg protein)	18.40 ± 1.154[b]	11.73 ± 0.840[c]	13.86 ± 0.692[c]	20.09 ± 1.204[ab]	19.71 ± 0.927a[b]	21.75 ± 0.545[a]
Vit C (nmol/mg protein)	0.75 ±0.050[a]	0.49 ±0.077[b]	0.78 ±0.061[a]	0.75 ±0.054[a]	0.83 ±0.042[a]	0.82 ±0.079[a]
MTs (µmol/mg protein)	0.054 ±0.001[e]	0.103 ±0.004[a]	0.069 ±0.002[b]	0.064 ±0.001[c]	0. 058 ±0.002[d]	0.053±0.003[e]

Means of six animals in each group ± standard deviation. For each parameter, means followed by different letters are significantly different according to Duncan's multiple range test at P ≤ 0.05

differences between controls and mice treated only by *Narcissus tazetta* flower extracts (Table 3).

Non-enzymatic antioxidant status in the liver

The levels of non-enzymatic antioxidants such as GSH, NPSH, Vit C, and MTs were determined in the liver of experimental animals (Table 3). Results showed that CdCl$_2$ treatment decreased significantly the levels of GSH (−44 %), NPSH (−36 %), and Vit C (−34 %), while the liver MTs content was increased by 87 % after CdCl$_2$ intoxication, relative to control. These alterations were corrected following co-administration of *Narcissus tazetta* flower ethanolic extracts, compared to CdCl$_2$-treated mice (Table 3).

Enzymatic antioxidant stress biomarkers

The CdCl$_2$ treatment increased the activities of SOD and GPx by 47 and 40 %, respectively, and decreased those of CAT by 42 % as compared to controls (Fig. 2). Co-administration of *Narcissus tazetta* flower ethanolic extracts restored the activities of SOD and GPx (Fig. 2A and C) and upregulated those of CAT (Fig. 2B), near to the control values, relative to those of CdCl$_2$-treated mice.

Plasma and liver biochemical parameters

Plasma AST, ALT, and γGT activities increased in CdCl$_2$-treated mice by 59, 90, and 180 %, respectively, when compared to controls. In addition, LDH activity increased by 140 % in the plasma and decreased by 42 % in the liver of CdCl2-treated mice, in comparison with controls. Co-administration of *Narcissus tazetta* flower extracts decreased the leakage of these hepatic enzymes (Table 4).

Histopathological analysis

Histological analysis showed normal structure of liver in controls and in those treated only by *Narcissus tazetta* flower extracts (Fig. 3A, E, and F). After 8 days of CdCl$_2$ treatment, there was marked disorganization of hepatic architecture. In fact, central and portal veins were dilated and surrounded with leukocytes infiltration (Fig. 3B$_1$ and B$_2$). Most of the hepatocytes appeared fused together or extensively deteriorated with necrosis. Cadmium chloride induced also the progress of numerous micro-vesicular steatosis (Fig. 3B$_3$) and the appearance of granulomatous inflammation (Fig. 3B$_4$). In animals co-administered with *Narcissus tazetta* flower extracts, the central and portal veins regained their normal structure. Inflammatory cells progressively decreased with the flower extracts mainly at 200 mg/kg bw. The hepatocytes appeared to regain their normal organization and structure with the disappearance of the micro-vesicular steatosis (Fig. 3C and D).
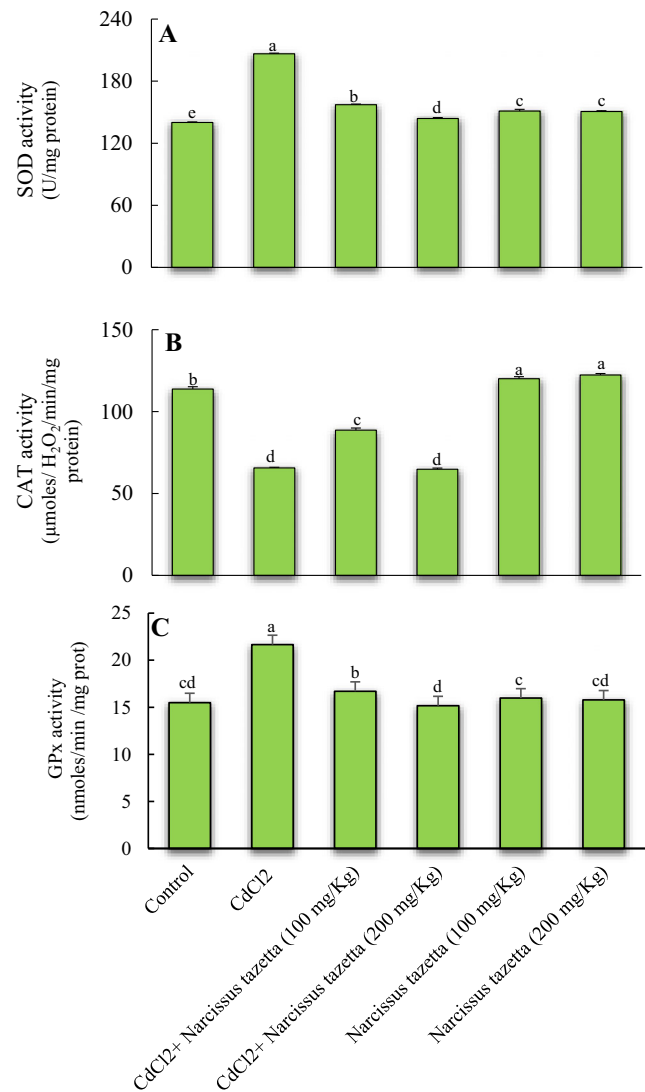
Fig. 2 Enzymatic antioxidant activities of superoxide dismutase (**A**), catalase (**B**), and glutathione peroxidase (**C**) in the liver of control and treated mice with CdCl$_2$, CdCl$_2$ with *Narcissus tazetta* ethanolic flower extracts (100 or 200 mg/kg), or *Narcissus tazetta* flower extracts (100 or 200 mg/kg), during 8 days. Means of six animals in each group ± standard deviation. Bars labeled with different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$

The histopathological changes are graded and summarized in Table 5.

Discussion

Based on their richness in bioactive components including polyphenols, medicinal and aromatic plants have been largely used in traditional medicine as remedies to treat heavy metal–induced liver damages. In this study, *Narcissus tazetta* flower extracts contained considerable amounts of total polyphenols and flavonoids compounds, confirming that bioactive molecules of the Amaryllidaceae family are not just limited to

Table 4 Plasma transaminases (AST and ALT), γGT, LDH activities, and liver LDH activity in control and treated mice with CdCl$_2$, CdCl$_2$ with *Narcissus tazetta* ethanolic flower extracts (100 or 200 mg/kg), or *Narcissus tazetta* ethanolic flower extracts (100 or 200 mg/kg), during 8 days

Parameters and treatments	Control	CdCl$_2$	CdCl$_2$ + *Narcissus tazetta* (100 mg/kg)	CdCl$_2$ + *Narcissus tazetta* (200 mg/kg)	*Narcissus tazetta* (100 mg/kg)	*Narcissus tazetta* (200 mg/kg)
Plasma						
AST (IU/l)	108.50 ± 3.38[d]	172.37 ± 4.44[a]	138.54 ± 3.14[c]	156.33 ± 3.40[b]	105.87 ± 2.05[d]	113.45 ± 2.87[d]
ALT (IU/l)	29.45 ± 1.05[c]	56.29 ± 2.57[a]	31.79 ± 1.05[c]	40.83 ± 1.95[b]	26.83 ± 1.33[c]	31.50 ± 1.10[c]
γGT (IU/l)	31.24 ± 2.82[c]	87.03 ± 2.99[a]	71.41 ± 4.46[b]	84.80 ±2.82[a]	33.47 ± 2.99[c]	35.70 ± 2.82[c]
LDH (IU/l)	154.58 ±5.38 [d]	370.42 ±8.35[a]	177.92 ±5.45[C]	242.08 ± 5.00[b]	157.50 ±6.39[d]	169.17 ±5.83[cd]
Liver						
LDH (UI/g tissue)	8.82 ±0.39[ab]	5.09 ±0.48[d]	7.97±0.40[bc]	7.08±0.26[C]	8.98±0.46[ab]	9.75±0.56[a]

Means of six animals in each group ± standard deviation. For each parameter, means followed by different letters are significantly different according to Duncan's multiple range test at $P \leq 0.05$

alkaloids, but they are able to synthesize a diverse range of polyphenols, with well-known antioxidant activities properties (Bati Ay et al. 2018; Benedec et al. 2018). Actually, the antioxidant capacity of plant extracts containing polyphenols might be evaluated through several techniques. Evaluation of this study revealed that *Narcissus tazetta* flower extracts exhibited a strong total antioxidant activity and a low IC$_{50}$ scavenging ability of DPPH, which can be explained by the presence of phenolic compounds in its flower extracts. Indeed, the scavenging properties of polyphenols are owned to the presence of one or more hydroxyl groups and phenolic ring, which are able to donate an electron and/or a hydrogen atom to free radicals (Erkan et al. 2011). These properties may be principally attributed to flavonols (rutin, nicotiflorin, narcissin, kaempferol-di-O-glucoside, and kaempferol-3-O-rutinoside) and/or chlorogenic acids derivatives (two mono-caffeoylquinic acid isomers and one 5-O-caffeoylquinic acid) identified in *Narcissus tazetta* ethanolic flower fraction. In the same context, Li et al. (2015) identified various glycosides

forms of quercetin, kaempferol, and isorhamnetin and one chlorogenic acid derivative in different cultivars of *Narcissus* extracts, suggesting that this genus of Amaryllidaceae plants would offer important biological activities. Benedec et al. (2018) reported also that Amaryllidaceae plants, including *Narcissus poeticus*, *Narcissus pseudonarcissus*, *Galanthus nivalis*, and *Leucojum vernum*, were rich in chlorogenic and p-coumaric acids, with important anti-staphylococcal and antifungal activities.

The anti-peroxidative properties of *Narcissus tazetta* flower extracts, at tested doses, on CdCl$_2$ toxicity was assessed by a significant decrease in MDA and H$_2$O$_2$ levels, remembering its richness in aforementioned flavonol and chlorogenic acid compounds. In particular, rutin, the most abundant compound identified in its flower extracts, was described as potential molecule to alleviate cell disorders induced by oxidative stress. This flavonol may interact with the polar head of membrane phospholipids, resulting in the improvement and

Table 5 Grading of the histopathological changes in the liver sections

Parameters and treatments	Control	CdCl$_2$	CdCl$_2$ + *Narcissus tazetta* (100 mg/kg)	CdCl$_2$ +*Narcissus tazetta* (200 mg/kg)	*Narcissus tazetta* (100 mg/kg)	*Narcissus tazetta* (200 mg/kg)
Dilatation	-	+++	++	-	-	-
Inflammatory leucocytes	-	++++	++	-	-	-
Micro-vesicular steatosis	-	+++	-	+	-	-
Granuloma inflammation	-	+	+	-	-	-
Necrosis	-	+++		-	-	-

Scoring was done as follows: none (−), mild (+), moderate (++), high (+++), and severe (+++) damages of CdCl$_2$

Fig. 3 Liver histological sections of control (**A**), treated mice with CdCl₂ (**B₁**, **B₂**, **B₃**, and **B₄**), CdCl₂ co-treated with *Narcissus tazetta* ethanolic flower extracts at 100 or 200 mg/kg (**C** and **D**, respectively), or treated only with *Narcissus tazetta* ethanolic flower extract at 100 and 200 mg/kg (**E** and **F**, respectively). Coloration hematoxylin and eosin. Magnification ×400. **B₁** and **B₂** Marked dilatation of center and portal veins surrounded with leucocytes infiltration; **B₃** extensive degeneration of hepatocytes with necrosis and micro-vesicular steatosis (black arrows); **B₄** marked granuloma inflammatory disorders (yellow arrows); **C** normal central and portal veins and mild organization of hepatocytes; **D** normal appearance of the liver histoarchitecture
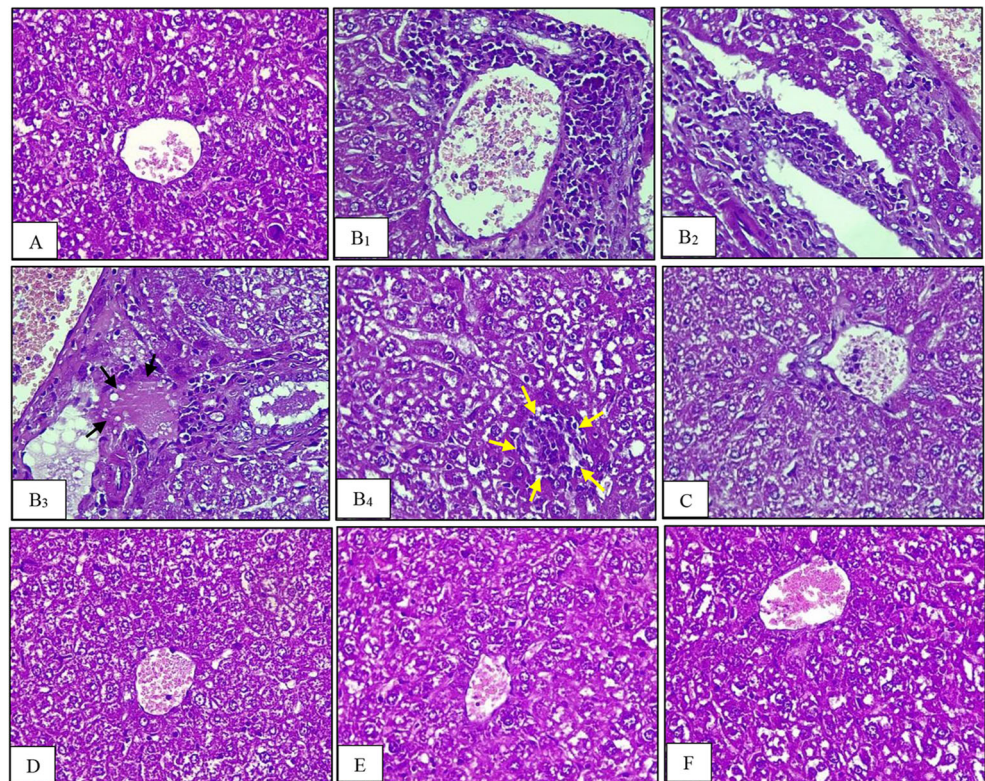
protection against oxidative stress–induced membrane damages (Erlejman et al. 2004). It may protect cells through attenuating H₂O₂-induced apoptosis (Gong et al. 2010). In addition, glycoside kaempferols, recognized in *Narcissus tazetta* flower extracts, might be involved in the alleviation of CdCl₂-induced hepatotoxicity in mice (Zang et al. 2017). For instance, nicotiflorin, the most abundant glycoside kaempferol of *Narcissus tazetta* flower extracts, has probably an important role in attenuating CdCl₂-induced liver injuries. In fact, according to Zhao et al. (2017), the extraction of this flavonol from *Nymphaea candida* has antioxidant activity on concanavalin A and D-galactosamine–induced liver injuries in mice via MDA level reduction. Chlorogenic acid constituents, identified in *Narcissus tazetta* flower extracts by HPLC-MS, was also revealed to have significant ability to mitigate carbon tetrachloride (CCl₄)–induced hepatotoxicity in rats (Shi et al. 2016). These properties would be owned to their one-electron oxidation products formed after interaction with free radicals, resulting in their broken down (Shibata et al. 1999).

Toxicity of CdCl₂ may also induce protein oxidation and damage to DNA structure, giving rise to mutations and/or cell death (Jadoon and Malik 2017). In this study, it is reasonable to conclude that *Narcissus tazetta* flower extracts could modulate liver AOPP levels and DNA degradation. This was again referred to the presence of rutin, as this compound was reported to prevent increased DNA fragmentation and inhibit inflammation and apoptosis (Gong et al. 2010). It is noteworthy to not exclude the importance of the other identified flavonols

and chlorogenic acid isomers in the protection of the liver against CdCl₂, through targeting pathway of ROS activation, protein oxidation, and DNA degradation of hepatocytes.

Chemo-protective effects of natural antioxidant components were reported to regenerate and/or preserve intracellular non-enzymatic antioxidants to their normal levels. In particular, *Narcissus tazetta* flower extracts restored the hepatic GSH, NPSH, and Vit C levels of CdCl₂-treated mice, which became comparable to normal values. It may be suggested that phenolic constituents of this plant either acted synergistically or separately to prohibit the binding of Cd to –SH groups of GSH and NPSH and to increase Vit C content via activating the activity of gulonooxidase (Girja and Chandrat 1998). Consideration on rutin's role to regenerate GSH levels was reported by Gong et al. (2010), proposing that this molecule might rise up the intracellular GSH under oxidative stress conditions. According to Mirani et al. (2012), co-administration of rutin restored GSH and Vit C contents to normal values in the liver of cadmium-intoxicated mice. Related studies on other xenobiotic intensified liver injury confirmed that kaempferol (Xu et al. 2019; Zang et al. 2017) and chlorogenic acid (Shi et al. 2016) significantly restored liver glutathione consumption.

Metallothioneins act as antioxidants and free radical scavengers due to their (–SH) groups presents in their cysteine residues. They may play a protective role against the cytotoxicity of cadmium and other heavy metals through ROS detoxification under oxidative stress conditions (Jurczuk et al.

2006; Genchi et al. 2020). In our study, enhanced hepatic levels of MTs following CdCl$_2$ exposure contradicted with the aforementioned disturbances. In fact, these antioxidants would further increase cadmium retention in hepatocytes, exceeding their ability to bind with this metal. The reduction of its level after co-administration of *Narcissus tazetta* flower extracts might be accredited to phenolic compounds, essentially to rutin's ability to decrease cadmium absorption from the gastrointestinal tract (Genchi et al. 2020).

The promising therapeutic effects of plant extracts on liver injuries may be involved in the amelioration of antioxidative defense enzymes. For instance, enzymes like SOD, CAT, and GPx are crucial in the defense of living organs against ROS, and the alteration in their activities might restrict cell protection against oxidative damages (Chater et al. 2008). In our study, CdCl2 increased liver activities of SOD and GPx enzymes but reduced those of CAT. These results would be probably due to enhanced H2O2 production through SOD activity, resulting in the exhaustion of CAT detoxification capacity (Jurczuk et al. 2004). Co-administration of *Narcissus tazetta* flower extracts modulated SOD, CAT, and GPx activities, designating the involvement of its phenolic constituents, most likely rutin, in harmonizing activities of these enzymes. In this way, Mirani et al. (2012) assumed that rutin was able to restore hepatic SOD, CAT, and GST activities to normal values in cadmium-intoxicated mice. In other studies, rutin was proposed to restore the activities of these enzymes under nonalcoholic fatty liver disease and CCl$_4$ exposure (Liu et al. 2017; Ravi et al. 2018). Besides, kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside, isolated from *Carthamus tinctorius*, restored the SOD and CAT activities, indorsing great antioxidant properties of these compounds on CCl$_4$-induced oxidative liver injury (Wanga et al. 2018). According to Shi et al. (2016), restoration of hepatic activities of SOD and CAT was accredited to chlorogenic acid importance to increase expression levels of nuclear Nrf2 and Nrf2-regulated anti-oxidant genes in treated mice with CCl$_4$.

Liver injuries, including lipid peroxidation and imbalances in the antioxidant system, may induce the release of hepatic enzymes, an indicator of cell membranes integrity loss (Paul et al. 2013). In the current study, co-administration of *Narcissus tazetta* flower extracts reduced the release of AST, ALT, γGT, and LDH enzymes, proposing the involvement of flavonols and/or chlorogenic acids of this plant in the stabilization of hepatocyte membranes. Similarly, aqueous extracts of *Raphanus sativus* and *Monodora myristica* reduced serum activities of ALT, AST, LDH, and γGT in CdCl$_2$-induced liver damage in mice (Emmanuel Oyinloye et al. 2016; He et al. 2019). Shehab et al. (2015) highlighted that the presence of abundant rutin concentration in *Zygophyllum hamiense* prompted the downregulation of AST activity in blood of CCl$_4$-intoxicated mice. Kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside prevented CCl$_4$-induced elevation of AST and alkaline phosphatase activities in blood, restoring

therefore hepatic membrane integrity (Wanga et al. 2018). Isorhamnetin 3-O-glucoside extracted from *Atsumi-kabu* leaves significantly reduced plasma activities of ALT and AST, supported the effective role of this metabolite to alleviate CCl$_4$ induced liver injury in mice (Igarashi et al. 2008). In this regard, the tendency of liver enzymatic biomarker to return to near normalcy may indicate hepatocyte tissue regeneration as described in our study. Indeed, the non-expression of histological alterations after co-administration of *Narcissus tazetta* flower extracts was revealed mainly at the dose 200 mg/kg, suggesting that the chemopreventive role of its phenolic compounds was in a dose-dependent manner.

To this end, it might be hypothesized that phenolic constituents of *Narcissus tazetta* ethanolic flower extracts either acted synergistically or separately to inhibit lipid peroxidation; prevent GSH, NPSH, and Vit C depletion; and decrease transaminases, LDH, and GGT leakage to plasma, therefore playing a protective role against CdCl2-mediated liver injury. Additional investigations are required to understand the action mode of the identified phenolic compounds to challenge liver enzymes leakage and histoarchitecture disorders. Investigations, evaluating the impact of *Narcissus tazetta* flower extracts on cell membrane fatty acid composition, will be also of great importance due to its involvement to maintain membrane integrity.

Conclusion

The current study indicated that CdCl$_2$ induces hepatotoxicity resulting in the rise of oxidative stress markers and the disturbance of biochemical and histopathological parameters. Ethanol flower extracts of *Narcissus tazetta* revealed potential defensive effects against this toxicant. Indeed, HPLC-MS analysis identified many phenolic compounds including flavonols and chlorogenic acid isomers, which may have synergetic antioxidant, radical scavenging activity, and hepatoprotective properties. Our study on *Narcissus tazetta* flowers, collected from Tunisia flora, may be a prominent finding through offering new friendly alternative drug against CdCl$_2$-induced hepatotoxicity.

Data availability Not applicable

Declarations

Ethics approval and consent to participate The animals received humane care in accordance with the International Guidelines for Animal Care and with strict adherence to the Ethical Committee of Faculty of Sciences, Sfax, Tunisia, under the ethical approval no. 1204.

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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