Characterization and ameliorative effects of *Chenopodium murale* hydroethanolic extract against diethylnitrosamine-induced hepato-renal damage and malfunction

Howida S. Abou- Seif[a], Osama M. Ahmed[b], Samah A. El-Newary[c], Marwa E. Shabana[d], Mahitab I. EL-Kassaby[a], Ghada M. Elqattan[a], Hussein G. Sawie[a]

[a]Departments of Medical Physiology, [b]Department of Zoology, Faculty of Science, Beni-Suef University, [c]Medicinal and Aromatic Plants Research, [d]Department of Pathology, National Research Centre, Cairo, Egypt

Correspondence to Howida S. Abou- Seif, (PhD), Assistant Professor, Medical Physiology Department, Medical Research and Clinical Studies Institute, National Reserch Centre, Beni Suaif Governorate, Beni Suaif City, Post/ zip code: 2711860, Dokii, Cairo, Egypt. Mobile: +0128 846 2008; e-mail: drhoidaabouseif@gmail.com

Received: 9 May 2023
Revised: 21 June 2023
Accepted: 13 July 2023
Published: 26 December 2023

Journal of The Arab Society for Medical Research 2023, 18:149–160

Background/aim
Chenopodium genus has broad applications in folk medicine. *Chenopodium murale* (*C. murale)* exhibited several pharmacological benefits, including hypotensive, anti-inflammatory, analgesic, antifungal, antibacterial, phytotoxic, hepatoprotective and renoprotective effects. The principal objective unveils the preventive effects of *C. murale* against hepato-renal damage and malfunction induced by diethylnitrosamine (DEN).

Materials and methods
Forty rats were included in the present study divided into 4 groups, group 1, animals were given saline solution every day for 14 weeks. Group 2, animals were injected double times per week by intraperitoneal route with DEN at 150 mg/kg body weight for 2 weeks. Animals in group 3 were injected with DEN like in group 2 and subjected orally to *C. murale* hydroethanolic extract (500 mg/kg body weight) daily for 14 weeks. Animals in group 4 received the same dose of the hydroethanolic extract of *C. murale* for a similar period.

Results
DEN has injurious effects, associated with elevated liver enzyme activities (AST, ALT and ALP), urea and creatinine in serum. Also, lipid peroxidation and nitric oxide were elevated markedly. DEN lowered the hepatic and renal activities of endogenous antioxidants (CAT and SOD), reduced glutathione (GSH) level. Conversely, treatment with *C. murale* restored liver function biomarker activities, urea and creatinine levels as well as mitigated the oxidative damage induced by DEN. *C. murale* reflecting its ameliorative potential which diminished obviously the DEN-induced elevated hepato-renal levels of IL-1β and TNF-α (immuno-inflammatory indicators), also down regulated Bcl-2, NF-κB, and Nrf-2 (inflammatory mediators).

Conclusion
These findings proved that *C. murale* might protect and ameliorate DEN-induced hepato-renal damage through activation of antioxidant and anti-inflammatory systems.

Keywords:
antioxidants, diethylnitrosamine, inflammation, Nrf2, oxidative stress, phenolic content

J Arab Soc Med Res 18:149–160

1687-4293

Introduction

Hepatocellular carcinoma was responsible for 9.1% of mortality globally according to World Health Organization studies [1]. Diethylnitrosamine (DEN) which has cytotoxicity as well as carcinogenicity impacts is present in pharmaceutical preparations, tobacco smoke, crude and cooked meats and cosmetics [2,3]. Reactive electrophile species activated DEN through cytochrome P450 enzymes cause the production of free radicals, oxidative stress, cytotoxicity cell damage, altering DNA structure and carcinogenicity [4–6]. Also, DEN induces kidney injury through reactive oxygen species (ROS) overproduction or through depletion of endogenous antioxidants [7]. Renal dysfunction induced by DEN from free radicals' production and metabolization of its end product leading to oxidative stress and cell injury [8].

To achieve normal body physiology and to improve life expectancy, phytochemicals or phytonutrients have significant impacts on health [9]. *Chenopodium murale* (*C. murale*) is an annual herbaceous weed,

 DOI: 10.4103/jasmr.jasmr_7_23

planted in high nitrogenous habitats in the wheat field in Egypt and in the Mediterranean region. *C. murale* common names are Sowbane, Nettle-leaved Goosefoot, Salt-green and Australian-spinach belongs to the family *Chenopodiaceae*. Plant Arabic name is Rumram, Sentar, Mentab, Abu-Efein, Muntinab, and Zurbaih. The plant grows in Egypt and is known as Rumram (common name). *C. murale* which is rich in minerals as N, P, K, Se, and Mg considered as nutrient accumulator, its leaves are rich in vitamins A and C and calcium and oxalic acid [10,11].

Chenopodium genus has broad applications in folk medicine such as digestive disorders, peptic ulcers, dyspepsia, flatulence and hemorrhoids. Also, it has been used as laxative, abortifacient, antispasmodic, diaphoretic, anthelmintic, and emmenagogue. Additionally, it has been used to treat depression, general debility, ophthalmopathy, seminal weakness, pharyngopathy, in relieving migraine, amenorrhea pain and asthma catarrh, spleenopathy, cardiac disease, hepatopathy, anxiety, and hair loss [12].

C. murale contains alkaloids, phenolics (kaempferol, quercetin, sinapic acid, chlorogenic acid, gallic acid, and *p*-coumaric acid), herbacetin, and scopoletin. In addition, *C. murale* contains sterols, essential oils, flavonoids, tannins, saponins, steroidal estrogen-like substances, and coumarins [13,14]. *C. murale* exhibited several pharmacological benefits in preclinical trials, including hypotensive [14], anti-inflammatory, analgesic, diuretic [15], antifungal [16,17], antibacterial [18], phytotoxic [12], hepatoprotective [13] and renoprotective effects [19]. Phenolic compounds of *C. murale* include coumaric acid, cinnamic acid, vanillin, gallic acid, rutin, ellagic acid, chlorogenic acid, caffeic acid, methyl gallate, ferulic acid, syringic acid, pyro catechol, naringenin, daidzein, and quercetin. The flavonoids and phenolic components of *C. murale* making them outstanding drugs for medical and pharmacological use. *C. murale* exhibit immune response boosting effects, excellent antioxidative, antihypertensive, anticancer, antidiabetic, anti-inflammatory, protect the skin from the risky effects ultraviolet radiation, cardioprotective, and antibacterial [20–22].

Because, there is no biochemical investigation about the effects of *C. murale* on DEN-induced hepato-renal damage and on the levels of immuno-inflammatory indicators and inflammatory mediators. This study was designed to elucidate the protective and ameliorative effects of *C. murale* on DEN-induced hepato-renal damage by measuring the activity/amount of liver function and kidney biomarkers, oxidative stress parameters as well as inflammatory markers.

Materials and methods

Materials

Chemicals

Sigma-Aldrich provided the DEN and the Folin-Ciocalteu reagent (St. Louis, MO, USA). All of the chemicals and solvents used are of the analytical grade.

C. murale collection

In March 2021, aerial parts of *C. murale* were collected from fields in Sharkia Governorate, Egypt. Prof. Dr. Salwa Ali Kawashty, Department of Phytochemistry and Plant Systematics, Pharmaceutical and Drug Industries Division, National Research Centre, Giza, Egypt, authenticated the plant. A voucher specimen (1145) was also deposited in the National Research Centre's herbarium in Cairo, Giza, Egypt.

Experimental animals

40 male Wistar albino rats 150–180 g, aged from 8 to 9 wks., obtained from the National Research Centre animal house, Giza, Egypt. In plastic cages and under 12 h light/dark cycle, 24±1 °C, and 45±5% humidity (standard laboratory conditions), rats were housed and fed. They were given a commercial chow meal and free access to water.

Ethical approval

All experiments were performed in line with the guidelines of ethical conditions approved by the Medical Research Ethics Committee (MREC) of The National Research Centre, El Dokki, Egypt of Experimental Animals, with approval number: 2020–20150.

Methods

C. murale extract preparation

The aerial components were dried at 50°C. By soaking, at room temperature; powdered material (1000 g) was thoroughly extracted with 70% ethanol. To obtain crude extract, the filtrate was evaporated under reduced pressure with a rotary evaporator. Resides extract (175 g) was lyophilized and stored at −20°C until it was incorporated into the bio-assay.

Identification of the phenolic composition of C. murale hydroethanolic extract using HPLC analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using the Eclipse C18 column (4.6 mm×250 mm i.e., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16–20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40°C.

Identification of lipid composition of C. murale hydroethanolic extract using GC analysis

The GC model 7890B from Agilent Technologies, Palo Alto, California (USA) was equipped with flame ionization detector at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Separation was achieved using a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 μm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.8 ml/min at a split-1 : 50 mode, injection volume of 1 μl and the following temperature program: 100°C for 3 min; rising at 2.5°C/min to 240°C and held for 10 min. The injector and detector (FID) were held at 250°C and 285°C, respectively.

Sample preparation

The sample was saponified with ethanol potassium hydroxide, unsaponifiable fraction extracted in petroleum ether.

Sample derivatization

The unsaponifiable fraction extracted in petroleum ether was mixed with 50 μL of bis (trimethylsilyl) trifluoroacetamide (BSTFA) +trimethylchloro-silane (TMCS) 99 : 1 salivation reagent and 50 μL pyridine for derivatization sample functional groups to trimethylsilyl groups (abbreviated TMS) prior to GC analysis.

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977 A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using Hydrogen as the carrier gas at a flow rate of 2.0 ml/min at a split less, injection volume of 2 μl and the following temperature program: 50°C for 5 min; rising at 5°C/min to 100°C and held for 0 min and rising at 10°C/min to 320°C and held for 10 min. The injector and detector were held at 280°C, 320°C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 25–700 and solvent delay 6 min. The mass temperature was 230°C and Quad 150°C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Experimental study

Acute oral toxicity assay (LD50)

Acute toxicity of the *C. murale* hydroethanolic extract was performed as per OECD guideline 425 [23] for acute oral toxicity –Up-and-Down-Procedure (UDP) using mice. Doses extracted started from 0.5 g and finished at 6 g/kg with the raised rate of 0.5 g/kg body weight. Mice (5 mice/group) received the extract orally using a stomach tube and control mice received saline justly. Animals were monitored and checked out for any changes and mortality for 48 h, remained mice was observed for two weeks. LD_{50}, the dose of the extract that killed 50% of animals, was calculated by the number of dead animals in each concentration during the first 48 h using the BioStat program (BioStat 2009 Build 5.8.4.3 # 2021 analyst Soft Inc., VA, USA). LD_{50} of *C. murale* hydroethanolic extract was 5 g/kg body weight.

Experimental protocol and drug administration

The present study included 40 rats were separated into four groups of 10 each at random:

(1) Group I (control): rats received saline solution (vehicles in which treatments are dissolved) daily for 14 weeks.
(2) Group II (DEN): by intraperitoneal injection, the animals received DEN [24] double times a week for two weeks with 150 mg/kg body weight.
(3) Group III (DEN + *C. murale*): rats injected double times a week for two weeks by intraperitoneal route with 150 mg/kg body weight of DEN concomitant with oral administration of 100 mg/kg body weight of *C. murale* hydroethanolic extract daily for 14 weeks [12].
(4) Group IV (*C. murale*): rats subjected to 100 mg/kg b.wt. of *C. murale* hydroethanolic extracts through oral tube daily for 14 weeks [12].

Blood and tissues sampling

Under light ether anesthesia, rats were sacrificed 24 hours after the treatments and blood samples were collected for serum preparation. After coagulation, the blood samples were centrifuged for 10 min at 3000 rpm (cooling centrifuge) to collect the serum.

The animals' livers and kidneys were excised and washed in ice-cold phosphate-buffered saline after being dissected. The liver and kidney samples were stored at 80°C and then homogenized in Tris-HCl (pH 7.4) buffer (10% w/v). The homogenate was centrifuged at 3000 rpm for 20 min to separate the clear supernatant for measurement of oxidative stress and antioxidant biomarkers.

Detection of liver and kidney functions markers

By Kind and King, Young [25,26] methods, serum samples were used to estimate kinetically alkaline phosphatase (ALP) activity, Egypt. According to Reitman and Frankel's method [27], Alanine and aspartate aminotransferase (ALT and AST) activities were estimated by Biodiagnostic kit, Cairo, Egypt. Regarding Patton and Crouch [28] and Bowers and Wong [29] methods respectively, urea and creatinine levels were estimated.

Estimation of inflammatory markers and mediator

The levels of proinflammatory cytokines and other proteins were measured by commercially available ELISA kits (SinoGeneClon Biotech Co., Ltd), including immuno- inflammatory indicators as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), inflammatory mediators as nuclear factor-kappa B (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf-2) and rat B-cell leukemia/lymphoma 2 (Bcl-2).

Measurement of oxidative stress and antioxidant biomarkers

Beutler and colleagues [30] method was used in measuring reduced glutathione level (GSH); Ohkawa and colleagues [31] method was applied for detecting lipid peroxidation (LPO) or malondialdehyde (MDA). Superoxide dismutase (SOD) and catalase (CAT) activities were determined using methods of Aebi [32] and Marklund and Marklund [33], respectively. Nitric oxide (NO) was estimated according to Grisham and colleagues reagent [34].

Histological examination

Rat's liver and renal tissues were immediately dissected and histopathologically analyzed after they were sacrificed under anesthesia. Hepatic and renal specimens were sectioned, fixed for 48 hours in 10% buffered formalin solution, dehydrated in ascending grades of alcohol, cleared in xylol, and embedded in paraffin blocks. Serial sections of 5 μm thickness were mounted on glass slides, washed in a water bath and dewaxed in an oven. The sections were then stained with hematoxylin and eosin. By using an Olympus CX 41 electrical light microscope, histological changes were examined. The photomicrographs were processed using Adobe Photoshop 8.0.

Statistical analysis

All data were presented as mean±standard error of the mean. When P less than or equal to 0.05, the significant level was set. The data were statistically analyzed using the SPSS program version 16, SPSS, Chicago, IL. Data were analyzed to compare groups, post-hoc using one-way analysis of variance and Duncan-Kramer methods were used.

Results

Lipidic and phenolic profiles of *Chenopodium murale*

The analysis of the lipidic profile of *C. murale* was performed via GC-MS analysis. All the identified fatty acids were assigned and presented in Table 1. The results revealed the identification of 18 fatty acids that categorized to unsaturated and saturated fatty acid in a percentage 48.3% and 51.7% respectively. Linolenic acid (20.51%), myristoleic acid (17.38%), tridecanoic acid (16.04%), and myristic acid (14.27%) represented the major identified fatty acids. All these compounds boosted the immune system and giving the plant its pharmacological and medical importance.

Table 1 The lipidic composition of *Chenopodium murale* as identified by GC–MS analysis

Peak	RT	Name	Area Sum %
1	8.05	Caprylic acid	0.22
2	14.743	Undecanoic acid	0.16
3	17.532	Lauric acid	11.24
4	20.736	Tridecanoic acid	16.04
5	23.719	Myristic acid	14.27
6	25.115	Myristoleic acid	17.38
7	26.622	Pentadecanoic acid	0.18
8	29.576	Palmitic acid	8.17
9	30.88	Palmitoleic acid	1.15
10	32.34	Margaric acid	0.15
11	35.113	Stearic acid	0.93
12	35.943	Oleic acid	3.37
13	37.664	Linoleic acid	5.06
14	39.822	Linolenic acid	20.51
15	45.242	Behenic acid	0.16
16	47.079	*cis*-13,16-Docosadienoic acid	0.43
17	49.849	Lignoceric acid	0.33
18	51.841	DHA	0.25
Saturated fatty acids		Total %	51.69
Unsaturated fatty acids		Total %	48.31
		Monounsaturated%	26.25
		Polyunsaturated%	22.06

Table 2 and Fig. 1 illustrate HPLC analysis *of C. murale* hydroethanolic extract. The results revealed the presences of 15 phenolic compounds including 11 phenolic acids and/or compounds along with 4 flavonoids. From all the characterized compounds, pyro catechol and vanillin were the major components. Additionally, the four flavonoids were identified as rutin, naringenin, daidzein, and quercetin. The results revealed that all the identified flavonoids are minor components.

Chenopodium murale restores liver and kidney markers in DEN-intoxicated group

The obtained results demonstrated that, in comparison to the control group, the activities of AST, ALT, and ALP significantly increased ($P \leq 0.05$) after DEN injection compared with normal group. As opposed to the DEN-intoxicated group, animals given *C. murale* concurrently with DEN injection displayed a significant decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) activities (Table 3). Additionally, DEN-treated rats showed higher levels of urea and creatinine when compared with the control group. Rats orally administered *C. murale* concomitant with DEN injection significantly corrected this tendency ($P \leq 0.05$) compared with DEN-intoxicated group (Table 3).

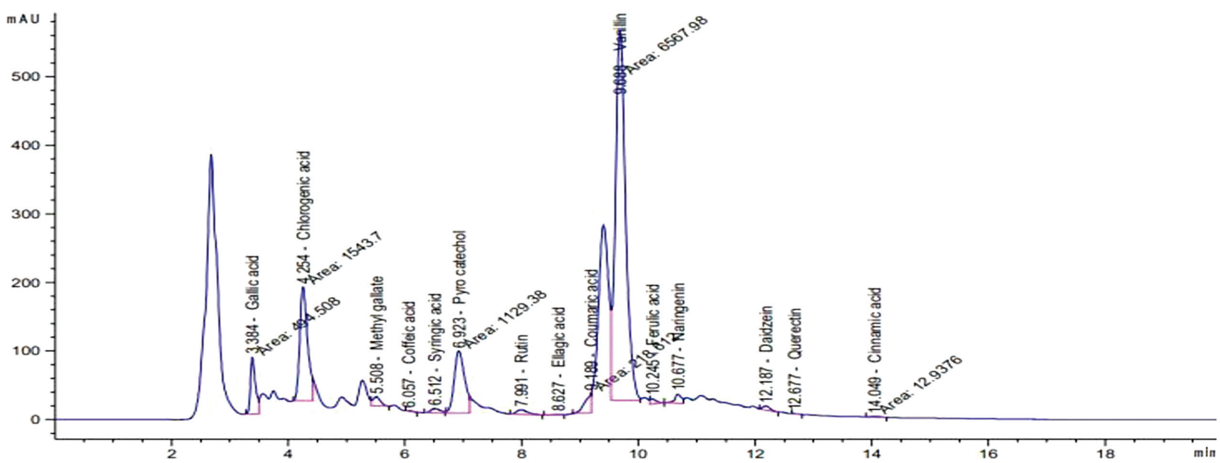
Chenopodium murale inhibits oxidative stress in DEN-intoxicated group

According to the current study, DEN injection significantly ($P \leq 0.05$) elevated MDA and NO levels of in liver and kidney homogenates when compared with the control group. As opposed to the DEN-intoxicated group, animals given *C. murale* plus DEN injection displayed a noticeable improvement in NO and MDA levels. GSH concentration was significantly lowered ($P \leq 0.05$) following DEN injection compared with the control group, along with a decline in CAT and SOD activity. As opposed and compared with the DEN-intoxicated group, rats given *C. murale* concurrent with DEN injection displayed a substantial elevation ($P \leq 0.05$)

Table 2 Phenolic composition and flavonoids of *Chenopodium murale* hydroethanolic extract identified by HPLC analysis

No.	Polyphenols	Area	µg/g extract	No.	Flavonoids	Area	µg/g extract
	Gallic acid	494.51	1319.60	1.	Rutin	91.16	242.01
	Chlorogenic acid	1543.70	7034.58	2.	Naringenin	113.47	470.35
	Methyl gallate	121.57	253.76	3.	Daidzein	51.31	116.81
	Caffeic acid	5.52	15.30	4.	Quercetin	2.39	9.72
	Syringic acid	74.48	237.76				
	Pyro catechol	1129.38	5399.28				
	Ellagic acid	3.88	42.54				
	Coumaric acid	218.61	259.96				
	Vanillin	6567.98	12191.16				
	Ferulic acid	55.42	146.39				
	Cinnamic acid	12.94	9.09				

Figure 1



HPLC analysis of *Chenopodium murale* hydroethanolic extract.

Table 3 Impact of DEN toxicity on liver and kidney functions and the protective role of daily dose of *Chenopodium murale* for 14 weeks

	G1 Control	G2 DEN	G3 DEN+ *C. murale*	G4 *C. murale*
AST (U/l)	61.44±1.79 [a]	113.33±6.82 [c]	93.78±8.93 [b]	60.35± 2.7 [a]
ALT (U/l)	26.00±2.03 [a]	42.00 ±1.64 [b]	37.13±1.84 [b]	26.38±1.77 [a]
ALP (U/l)	32.21±3.82 [a]	45.15±1.87 [b]	33.09±1.29 [a]	30.38±2.65 [a]
Urea (mmol/l)	25.09±1.12 [a]	37.91±1.37 [b]	31.27±1.61 [b]	25.03±1.39 [a]
Creatinine (mmol/l)	1.23 ±0.114 [ac]	1.58±0.122 [b]	1.45±0.089 [bc]	1.01±0.067 [ac]

All values are represented as mean±SE. All values with different letters are significant at *P* less than or equal to 0.05, using ANOVA test.

Table 4 Impact DEN toxicity on liver and kidney MDA, GSH, NO, CAT and SOD and the protective role of *Chenopodium murale* after 14 weeks of treatment

	G1 Control	G2 DEN	G3 DEN+*C. murale*	G4 *C. murale*
Liver				
MDA (nmol/g)	9.29±1.36 [a]	20.64±2.47[b]	12.59±2.45 [a]	9.07 ±1.03 [a]
NO (μmol/g)	0.540±0.057 [a]	0.992±0.067 [b]	0.603±0.162 [a]	0.543± 0.1 [a]
GSH (mmol/g)	20.31±1.28 [b]	15.35±0.71 [c]	17.3±0.85 [a]	21.66± 1.0 [b]
Catalase (U/g)	69.57±2.44 [a]	58.05±3.97 [b]	66.21±1.79 [a]	72.49±2.27 [a]
SOD (U/g)	5.38±1.44 [a]	3.75±0.81 [b]	5.30±1.15 [a]	5.31±1.17 [a]
Kidney				
MDA (nmol/g)	7.87±2.51 [a]	11.48±1.53 [bc]	9.69±3.21 [ac]	7.31±1.6 [a]
NO (μmol/g)	0.294±0.087 [a]	0.578±0.126 [bc]	0.326±0.068 [ab]	0.288±0.1 [a]
GSH (mmol/g)	29.43±4.23 [a]	22.77±3.59 [b]	25.56±3.46 [c]	30.58±4.0 [a]
Catalase (U/g)	47.81±8.81 [a]	26.24±5.94 [c]	37.85±5.68 [b]	48.08±6.78 [a]
SOD (U/g)	10.9±1.86 [a]	6.59±1.09 [c]	9.73±1.59 [a]	9.54±2.12 [a]

All values are represented as mean±SE. All values with different letters are significant at *P* less than or equal to 0.05, using ANOVA test.

in GSH level, CAT, and SOD activities in liver and kidney homogenates (Table 4).

C. murale improves inflammatory markers and mediators in DEN-intoxicated group

In the current obtained results and compared with the control group, DEN intoxicated group showed significant rise ($P \leq 0.05$) in the levels of TNF- α, IL-1β (inflammatory markers) and NF-κB, Bcl-2 and Nrf2 (mediators). Excitingly, co-administration of *C. murale* with DEN successfully (Table 5) caused remarkable ($P \leq 0.05$) down regulation in both inflammatory markers (TNF- α, IL-1β) and inflammatory mediators (NF-κB, Bcl-2 and Nrf2) compared with DEN-intoxicated group.

Histopathological changes

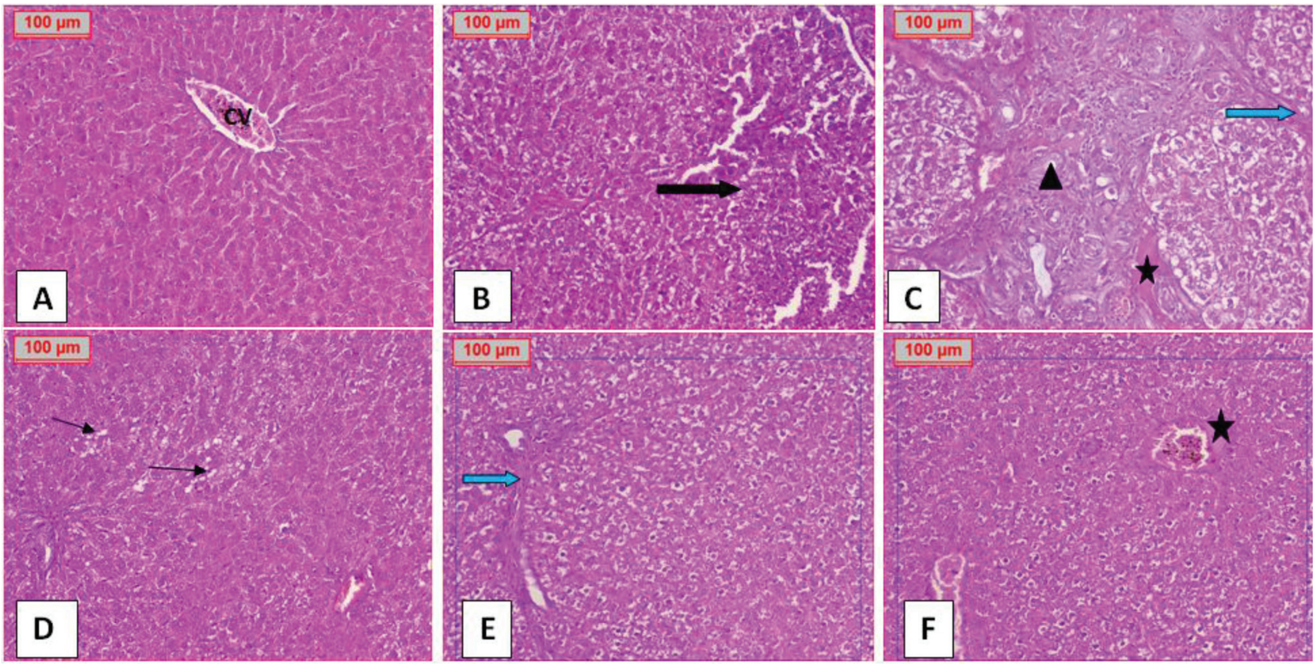
Liver tissues from each group were subjected to H and E staining and their histological morphology are compared. Normal hepatic architecture appeared in control group with radiating hepatic cord that surrounded the normal central vein and normal sinusoidal spaces (Fig. 2a). Administration of DEN cause severe changes in liver architecture, in the form

Table 5 Impact of DEN toxicity on the levels of protein expression of TNF-α, IL-1β, Bcl-2, Nrf-2 and NF-κB in liver and kidney tissues and the protective role of *Chenopodium murale* after 14 weeks of treatment

	G1 Control	G2 DEN	G3 DEN+ *C. murale*	G4 *C. murale*
Liver				
TNF-α (ng/g)	149.0±0.8 [a]	491.1±0. 9[c]	253.0±1.1 [b]	144.0±0.6 [a]
IL-1β (ng/g)	119.0±0.2 [a]	352.0±0.8 [c]	255.0±0.4 [b]	117.0±0.4 [a]
Bcl-2 (ng/g)	34.9±0.1 [b]	128.8±0.2 [c]	64.1±0.1 [a]	35.5±0.2 [b]
Nrf-2 (Pg/g)	809.2±4.9 [a]	2063±12.8[c]	1148±8.6 [b]	774. 3±5.3 [a]
NF-κB (ng/g)	147.7±0.8 [a]	402.7±2.6 [c]	204.0±1.9 [b]	144±0.8 [a]
Kidney				
TNF-α (ng/g)	74.8±0. 7 [b]	176.6±1.1 [c]	109.6±0.6 [a]	78 .4±0.6 [b]
IL-1β (ng/g)	102.1±0. 7 [c]	194.6±0.3 [a]	127.9±0.5 [b]	100.5±0.2 [c]
Bcl-2 (ng/g)	25.3±1.0 [a]	67.8±0.8 [c]	37.4±0.7 [c]	25.1±0.4 [a]
Nrf-2 (Pg/g)	228.6±1.8 [c]	2443.2±10.3 [a]	708.1±7.5 [b]	221.1±3.7 [c]
NF-κB(ng/g)	113.4±0.8 [c]	252.7±1.8 [a]	159.1±1.4 [b]	117.2±0.4 [c]

All values are represented as mean±SE. All values with different letters are significant at *P* less than or equal to 0.05, using ANOVA test.

Figure 2



Photomicrograph of hepatic tissue show A) normal control appeared with normal liver architecture, radiating hepatocytes around central vein and normal sinusoidal spaces B,C) DEN treated group show disrupted liver architecture with focal area of dysplastic hepatic cells with hyper-chromatic nuclei, increase nuclear cytoplasmic ratio (thick arrow), distorted liver architecture with lobular cirrhotic pattern, ballooning degenerated hepatic cells, increase fibrosis (blue arrow), increase collagen deposition (star) and marked bile duct proliferation (arrow head). D) *C. murale* treated group show normal hepatic tissue with few scattered fat cells (thin arrow) E, F) *C. murale* plus DEN treated group show ballooning of hepatic cells, minimal fibrosis (blue arrow) and collagen deposition (star) around dilated congested central vein (H and E, x200).

appearance of focal area of dysplastic cells with hyperchromatic nuclei and increase pleomorphism, loss of hepatocyte cells, ballooning of hepatocytes, marked steatosis, bridging fibrosis dividing liver into lobules (early cirrhosis was noticed), marked collagen deposition and marked bile duct proliferation (Fig. 2b, c). *C. murale* treated group has mild effect on normal hepatic tissue looks like mild steatosis (Fig. 2d). DEN groups that treated with *C. murale* showed improvement of liver architecture with minimal fibrosis, collagen deposition and mild steatosis (Fig. 2e, f).

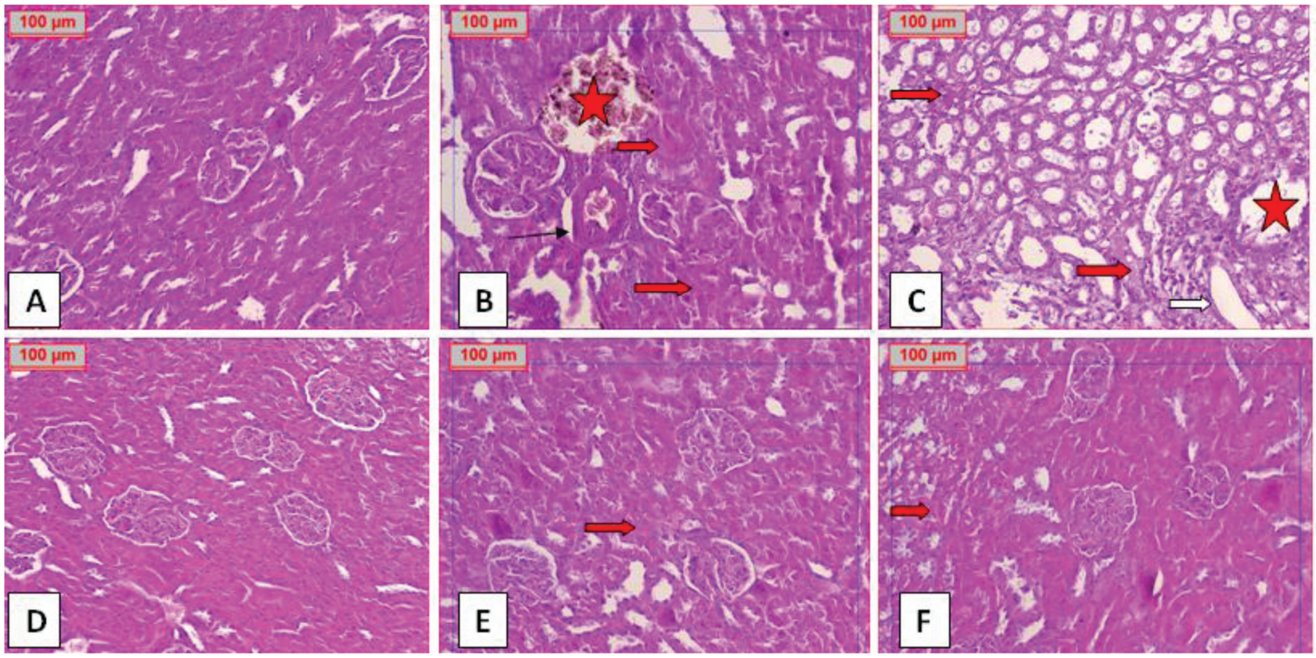
Renal tissues from each group were subjected to H and E staining and their histological morphology are compared. Control group displayed normal glomeruli with normal tubular architecture and normal renal tissue (Fig. 3a). DEN group tissue showed histological abnormalities as increase number of abnormal glomeruli as congestion, shrunken, tubular epithelial degeneration and presence of protein cast (amyloid) in tubular lamina (Fig. 3b, c). C. *murale* treated group show normal renal tissue (Fig. 3d). DEN groups that treated with *C. murale* displayed mild protein cast deposition with renal tubules and glomeruli pronounced regeneration (Fig. 3e, f).

Discussion

The third most common kind of cancer-related death worldwide is hepatocellular carcinoma (HCC), the fifth most common malignancy Devi and colleagues [35]. Because of delayed diagnosis and generated drug resistance, HCC therapies such as surgical resection, ablation, radiation, and chemotherapy might have unfavorable side effects or have little effectiveness Tawfik and colleagues [36]. Renal cell carcinoma (RCC), on the other hand, accounts for 1-3% of all human cancers and 75-80% of adult renal malignancies Athanazio and colleagues [37]. There were an estimated 51 190 new instances of kidney tumors in 2007, with 12 890 fatalities, considered about 2.3% of all cancer fatalities in the United States of America [38]. As a result, novel natural-source chemotherapeutic drugs are desperately needed to prevent or halt the progress of HCC and RCC.

DEN is a strong hepatocarcinogen in both animals and humans that can be found in fried meals, cigarettes, alcoholic drinks, makeup, medication metabolism, as well as farming or industrial contamination Chen [39]. DEN is the most frequently often utilized chemical motivated tumor growth in rodents. The efficacy and efficiency of DEN

Figure 3



Photomicrograph of kidney sections show A) normal control appeared with normal histological structure of renal parenchyma, glomeruli. B, C) DEN intoxicated group show distorted glomeruli (star), scattered glomerular and tubular amyloid deposition (red arrow), disturbed tubular epithelium (white arrow) and thickened vascular wall (arrow). D) *C. murale* treated group show normal renal glomerular and tubular histological structure. E, F) *C. murale* plus DEN treated group show normal appearance of kidney (red arrow) except for tiny amyloid deposition (H and E, x200).

application at various concentrations and to animals of varying ages vary Memon and colleagues [40].

C. murale Linn (*C. murale)* is a yearly erect plant native to parts of Asia, Europe, and Northern Africa, however it is besides found in warm and subtropical areas. The plant possesses antifungal, antibacterial, anti-inflammatory, analgesic, antihypertensive, anthelmintic, cytotoxic, hepatoprotective and antioxidant effects and is known as 'nettle leaf goosefoot' Ahmed and colleagues [41].

In this study, we discovered that serum concentrations of ALT, AST, and ALP in DEN-treated animals were two-fold greater than the control group by utilizing the release of these enzymes in serum as an indication of liver injury. This might be related to cytosolic enzyme leakage and translocation into the circulatory system caused by hepatic damage following DEN administration. This is the start of hepatocellular damage caused by liver hypofunction and a disruption in the synthesis of such serum marker enzymes, as well as alterations in the liver cell membrane's permeability Singh and colleagues [42]. Histopathologies of the liver verified the liver damage.

The hydroethanolic extract of *C. murale* reduced the high concentrations of liver function enzyme activities when compared with the DEN group. This is congruent with Saleem and colleagues [13] results, who obtained similar outcomes in mice with aqueous methanol extracts of *C. murale*. Flavonoids, saponins, and terpenoids are all found in *C. murale* Panda and colleagues [43]. According to Chen [39] and El-Denshary and colleagues [44], the flavonoids quercetin and naringenin may be the source of the protective impact on the liver since quercetin obstructs the rise of hepatic enzyme activities in rats by preserving the liver's internal anatomy. Naringenin, like quercetin, reduced DEN-induced hepatic damage, revealing that it may safeguard the liver by stabilizing the cell wall Ahmed and colleagues [45]. These findings are consistent with Al-Harbi's [46]. *C. murale* also β-Sitosterol and Stigmasterol, both of which have been shown to have antihepatotoxic properties Patra and colleagues [47].

The activities of enzymatic antioxidants (SOD and CAT), as well as nonenzymatic antioxidants (GSH) in liver and kidney tissues, were reduced in DEN-treated rats. This weakening of the antioxidant protection system was linked to an increase in oxidative stress indicators, including higher liver NO levels along with an increase in LPO. These findings are congruent with those of Ghosh and colleagues [48].

C. murale stigmasterol reduces lipid peroxidation in the liver and kidney while increasing catalase, superoxide dismutase, as well as reduced glutathione values Panda and colleagues [43]. This is in accordance with our findings, which indicated that treatment with a hydroethanolic extract of *C. murale* caused a reduction in lipid peroxidation in the liver and kidney and an increase in CAT, SOD, and GSH values. This also may be attributed to the flavonoids quercetin and naringenin, which can suppress free radical production induced by DEN, suggesting that they have substantial antioxidant action against ROS/ RNS in addition to their free radical scavenging characteristics Jagetia and Rajanikant [49]. It was hypothesized that naringenin can eliminate free radicals due to its 4-hydroxyl group in the b-ring having electron-giving capabilities and being a radical objective that prevents free radical attack on the membrane, hence preserving the membrane Seyoum and colleagues [50]. These results are compatible with Mershiba and colleagues [51].

In addition, the plant possessed a heat-stable SOD enzyme that protects against oxidative damage Khanna-Chopra and Sabarinath [52]. This is also consistent with the former studies of Rashed and colleagues [53] and Jadon and colleagues [54], which found quercetin and gallic acid to have hepatoprotective effects Tsuda and colleagues, Sudjaroen and colleagues [20,21]. The change for the better of the antioxidant protection system and inhibition of oxidative stress in the liver due to management with the hydroethanolic extract of *C. murale*, as well as the absence of HCC cells and precancerous lesions and a reduction in tumor indicators in blood, reveal the antioxidant properties of the hydroethanolic extract of *C. murale* role in the avoidance of hepatic damage. This hypothesis is consistent with Ahmed's [55] findings that antioxidant properties-containing agents have the capacity to inhibit carcinogenesis Liu and colleagues [56].

In our study, we observed that the mean blood levels of urea and creatinine in DEN-treated group were greater than those in the control group. This is in accordance with the results of Liu *et al.* Batra and Sharma [57], who reported that the levels of urea and creatinine were significantly elevated in rats given DEN. They ascribed this to the fact that DEN attaches to the target cell, which is anion phospholipids, and begins the aberration into the various intracellular metabolism, cell organelles, and kidney activities, causing injury to the kidney proximal tubular, resulting in acute renal failure Baeuerla [58].

NF-κB (the nuclear factor-kappa B) transcription factor family consists of five members: p50, p52, p65 (Rel A), c-Rel, and Rel B, all of which contain an N-terminal Rel homology domain that is accountable for DNA binding Ju and colleagues [59]. In cancer, it regulates cellular activities such as cell viability and inflammation Schett [60]. Inflammatory stimuli, free radicals, tumor promoters, carcinogens, cytokines, UV light, endotoxins, radiography and c-radiation all make NF-κB active. It activates NF-κB target genes involved in apoptosis suppression, cellular progression and alteration, metastasis, invasion, radio resistance, inflammation and chemo resistance Parameswaran and Patial [61]. Tumor necrosis factor (TNF-α) and IL-6 are thought to be major inflammatory mediators of proinflammatory cytokines secreted by monocytes and macrophages throughout inflammation Hashemzaei and colleagues [62]. TNF-α has a critical function in inflammatory reactions and apoptotic initiation Crespo and colleagues [63]. It is a primary regulator of the cellular discharge of other cytokines, chemokines and inflammatory mediators, rendering it an effective target for the treatment of inflammatory disorders and cancers caused by inflammation Nair and colleagues [64].

Hepatic damage beginning due to DEN demonstrated its effects on Kupffer cells of the liver via stimulating NF-κB and controlling the numerous inflammatory mediators (IL-6 and TNF- α) in the circulatory system Singh and colleagues [42]. The administration of DEN raised the concentrations of IL-1β, NF-κB, and TNF-α; this may lead to chronic inflammation caused by DEN-induced ROS generation, which activates oxidative stress-sensitive transcription factors for instance NF-κB and pro-inflammatory transcription factors, which in turn increase the production of numerous cytokines Mershiba and colleagues [51].

The reduction in IL-1β, NF-κB, and TNF-α levels after treatment with *C. murale* hydroethanolic extract may be owing to its flavonoids, particularly quercetin, as flavonoids could prevent one or more phases in the NF-κB signaling pathway, such like suppression of the most upstream growth factor receptors that cause the NF-κB signalling pathway to be active, nuclear translocation of NF-κB, DNA binding of the dimers or interactions with the basal transcriptional apparatus Schett and colleagues [60]. This is consistent with Crespo and colleagues [63], who stated that quercetin has been proven to block NF-κB-evoked cell survival pathways and diminish pro-inflammatory cytokine production, which eventually causes cancer development. Nair and colleagues [64], who discovered that quercetin reduces the generation of TNF-α, a significant pro-inflammatory chemical implicated in persistent inflammation-related illnesses that can lead to malignancies. The decrease of TNF-α by quercetin leads to the production of anti-inflammatory cytokines via the suppression of NF-κB activation Hastak and colleagues [65]. Flavonoids have an inhibiting effect on NF-κB target genes such as Bcl-2 and Bclx (L), cyclin D1, matrix metalloproteinases (MMP), and Vascular endothelial growth factor (VEGF) Li and colleagues, El Khazendar and colleagues [66,67]. These results are also consistent with the results of Hashemzaei and colleagues [62], who found that quercetin can inhibit tumor development and enhance animal lifespan (regardless of how it affects the onset of apoptosis).

C. murale polyphenols, such as ferulic acid, have anti-inflammatory and antioxidative properties that are mediated by free radical scavenging, preventing apoptosis and lipid peroxidation under physiologically typical and stressful circumstances by modulating Nrf2, p38, MMP and mTOR, and suppressing IL-1β and TNF-α Elwakkad and colleagues [68]. The mitochondrial pathway of apoptosis may be modulated by pro-apoptotic and anti-apoptotic effectors and it is commonly known that the preponderance of the pro-apoptotic protein (Bax) over the anti-apoptotic protein (Bcl-2) induces apoptosis Hsu and Yen [69]. The IL-1b mRNA expression in the group administered naringenin also declined; confirming its anti-inflammatory activity in modulating immune response and this may be related to the inhibition of early inflammatory events such as inhibition of production or effects of ROS and NF-κB activation Al-Rejaie and colleagues [70]. Chlorogenic acid (CGA) promoted the expression of protective genes such as antioxidant by up-regulating the signal pathway of Nrf2. In addition, the possible explanation for the antioxidant property of CGA could be attributed to its special molecular structure. It contains five active hydroxyl groups that react easily with free radicals and eliminate the hydroxyl radicals and superoxide anions, thus playing a strong antioxidant role. These antioxidant effects of CGA are the key factor that promotes the recovery of hepatic damage in aflatoxin B1-challenged mice Cheng and colleagues [71].

The hydroethanolic extract of *C. murale* decreased the elevated level of Bcl-2 if contrasted with the DEN group. This is consistent with Hashemzaei and colleagues [62]. This might be due to quercetin, which can produce proapoptotic effects through a variety of mechanisms, including antioxidant effects and the inhibition of the Bcl-2 protein and p53 gene. The inhibition of Bcl-2 gene transcription reduces the deterrent consequences of the BAD protein in the mitochondria, which is thought to be the trigger of apoptosis for the intrinsic pathway Vargas and Burd [72].

Conclusion

It can be assumed from the data of the present study that DEN elevated liver and kidney biomarkers, induced oxidative stress and inflammation. The co-administration of *C. murale* restored hepatic and renal functions, attenuated inflammation and improved tissue morphology by enhancing the antioxidant defense mechanism. Therefore, administration of *C. murale* may be beneficial, easy as well as economical to protect humans exposed to DEN from its toxic impacts. Additional studies are necessary to evaluate *C. murale* antioxidant, anti-inflammatory, hepatoprotective and renoprotective effects as well as to apply it as a medicinal plant.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al.* Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136:359–386.

2 Arboatti A, Lambertucci F, Sedlmeier M, Monti J, de L Álvarez M, Francés D, *et al.* Diethylnitrosamine increases proliferation in early stages of hepatic carcinogenesis in Insulin-Treated type 1 diabetic mice. BioMed Research International 2018; 2018:1–10.

3 Dar K, Ali S, Ejaz M, Nasreen S, Ashraf N, Gillani S, *et al.* In vivo induction of hepatocellular carcinoma by diethylnitrosoamine and pharmacological intervention in Balb C mice using Bergenia ciliata extracts. Brazilian Journal of Biology 2019; 79:1–10.

4 Bansal AK, Trivedi R, Soni GL, Bhatnagar D. Hepatic and renal oxidative stress in acute toxicity of Nnitrosodiethylamine. Indian J Exp Boil 2000; 38:916–920.

5 Rajkapoor B, Murugesh N, Chodon D, Sakthisekaran D. Chemoprevention of N-nitrosodiethylamine induced phenobarbitol promoted liver tumors in rat by extract of Indigofera aspalathoides. Biol Pharm Bull 2005; 28:364–366.

6 Al-Rejaie S, Aleisa A, Al-Yahya A, Bakheet S, Alsheikh A, Fatani A, *et al.* Progression of diethylnitrosamine- induced hepatic carcinogenesis in carnitine-depleted rats. World J Gastroenterol 2009; 15:1373–1380.

7 Mahmoud AM, Ahmed RR, Soliman HA, Salah M. Ruta graveolens and its active constituent rutin protect against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress. J Appl Pharma Sci 2015; 5:16–21.

8 Abdel-Moneim AA, Ahmed OM, Fahim HI, Zaky MY. Ameliorative effects of quercetin and naringenin on diethylnitrosamine/2-acetyl aminoflourine-induced nephrotoxicity in male wistar rats. Am J Biochem 2016; 6:113–121.

9 Memariani Z, Farzaei MH, Ali A, Momtaz S. Nutritional and bioactive characterization of unexplored food rich in phytonutrients. Phytonut in Food. Book chapter, ElsevierScience Publishing CoInc (Imprint – Woodhead Publishing). 2020. 157–175.

10 Qasem JR. Nutrient accumulation by weeds and their associated vegetable crops. Journal of Horticultural Science, 1992; 67:189–195.

11 Ahmed OH, Hamad MN, Jaafar NS. Phytochemical investigation of Chenopodium murale (family: Chenopodiaceae) cultivated in Iraq, isolation and identification of scopoletin and gallic acid. Asian J Pharm Clin Res 2017; 10:70–77.

12 Ahmed B, Jan Q, Bashir S, Nasir M, Shaheen F, Ahmed M. Pharmacological and biological investigation of *Chenopodium murale* Linn. Asian Journal of Plant Sciences 2003; 2:1107–1111.

13 Saleem M, Ahmed B, Qadir MI, Mahrukh Rafiq M, Ahmad M, Ahmad B. Hepatoprotective effect of *Chenopodium murale* in mice. Bangladesh J Pharmacol 2014; 9:124–128.

14 Gohar AA, Elmazar MMA. Isolation of hypotensive flavonoids from *Chenopodium* species growing in Egypt. Phytotherapy Research 1997; 11:564–567.

15 Ibrahim LF, Kawashty SA, Baiuomy AR, Shabana MM, El-Eraky WI, El-Negoumy SI. A comparative study of the flavonoids and some biological activities of two *Chenopodium* species. Chemistry of Natural Compounds 2007; 43:24–28.

16 Javaid A, Amin M. Antifungal activity of methanol and n-hexane extracts of three *Chenopodium* species against Macrophomina phaseolina. Nat Prod Res 2009; 23:1120–27.

17 Mostafa RM, El Rahman RIA, Ghamry AA, Yassin MH, Essawy HS. Antibacterial, Antivirulence Activities and Phytochemical Screening of Some Wild & Wasts Plants in Egypt. Acad J Biolog Sci 2022; 14:65–76.

18 Ali N, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol 2001; 74:173–79.

19 Abdel-Wahhab MA, Ahmed HMS, El-Nekeety AA, Abdel-Aziem SH, Sharaf HA, Abdel-Aziz MS, *et al. Chenopodium murale* essential oil alleviates the genotoxicity and oxidative stress of silver nanoparticles in the rat kidney. Egypt J Chem 2020; 63:2631–2646.

20 Tsuda T, Watanabe M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T. Antioxidative components isolated from the seed of tamarind (Tamarindus indica L.) J Agric Food Chem 1994; 42:2671–2674.

21 Sudjaroen Y, Haubner R, Wuìrtele G. Isolation and structure elucidation of phenolic antioxidants from Tamarind (Tamarindus indica L.) seeds and pericarp. Food Chem Toxicol 2005; 43:1673–1682.

22 Komutarin T, Azadi S, Butterworth L. Extract of the seed coat of Tamarindus indica inhibits nitric oxide production by murine macrophages in vitro and in vivo. Food Chem Toxicol 2004; 42:649–658.

23 OECD. Test No. 425: acute oral toxicity: up-and-down procedure, OECD guidelines for the testing of chemicals, section 4. Paris: OECD Publishing; 2008.

24 de Luján AM, Cerliani JP, Monti J, Carnovale C, Ronco MT, Pisani G, *et al.* The in vivo apoptotic effect of interferon alfa-2b on rat preneoplastic liver involves Bax protein. Hepatology 2002; 35:824–833.

25 Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol 1954; 7:322–326.

26 Young DS. Effects of Drugs on Clinical Laboratory Tests. XX; 1990; 3:6–12. Third Edition

27 Reitman A, Frankel SA. Colorimetric method for the determination serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56–63.

28 Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Analytical Chemistry 1977; 49:464–469. doi:10.1021/ac50011a034

29 Bowers LD, Wong ET. Kinetic serum creatinine assays. II. A critical evaluation and review. Clinical Chemistry 1980; 26:555–561.

30 Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882–888.

31 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351–8.

32 Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121–126.

33 Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 1974; 47:469–474.

34 Grisham MB, Johnson GG, Lancaster JR. Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol 1996; 268, 237–246.

35 Devi AS, Sivakumar G, kumar GK. Overview on Diethylnitrosamine Induced Hepatocellular Carcinoma Animal Model for the Biological Screening of Anticancer Herbal Medicines. Indian Journal of Natural Sciences 2020; 10:18690–18696.

36 Tawfik NG, Mohamed WR, Mahmoud HS, Alqarni MA, Naguib IA, Fahmy AM, Ahmed OM. Isatin Counteracts Diethylnitrosamine/2-Acetylaminofluorene-Induced Hepatocarcinogenesis in Male Wistar Rats by Upregulating Anti-Inflammatory, Antioxidant and Detoxification Pathways. Antioxidants 2022; 11:699.

37 Athanazio DA, Amorim LS, da Cunha IW, Leite KRM, da Paz AR, Gomes RDPX, *et al.* Classification of renal cell tumors – current concepts and use of ancillary tests: recommendations of the Brazilian Society of Pathology. Surgical and Experimental Pathology 2021; 4:4.

38 Ng CS, Wood CG, Silverman PM, Tannir NM, Tamboli P, Sandler CM. Renal Cell Carcinoma: Diagnosis, Staging, and Surveillance. AJR 2008; 191:1220–1232.

39 Chen X. Protective effects of quercetin on liver injury induced by ethanol. Pharmacognosy Magazine 2010; 6:135–141.

40 Memon A, Pyao Y, Jung Y, Lee JI, Lee WKA. Modified Protocol of Diethylnitrosamine Administration in Mice to Model Hepatocellular Carcinoma. Int J Mol Sci 2020; 21:5461.

41 Ahmed ZR, Uddin Z, Shah SWA, Zahoor M, Alotaibi A, Shoaib M, *et al.* Antioxidant, antidiabetic, and anticholinesterase potential of *Chenopodium murale* L. extracts using *in vitro* and *in vivo* approaches. Open Chemistry 2022; 20:1171–1186.

42 Singh D, Singh M, Yadava E, Falls N, Dangid DS, Kumar V, *et al.* Attenuation of diethylnitrosamine (DEN)-Induced hepatic cancer in experimental model of Wistar rats by Carissa carandas embedded silver nanoparticles. Biomedicine & Pharmacotherapy 2018; 108:757–765.

43 Panda S, Jafri M, Kar A, Meheta B. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia 2009; 80:123–126.

44 El-Denshary ES, Aljawish A, El-Nekeety AA, Hassan NS, Saleh RH, Rihn BH, *et al.* Possible synergistic effect and antioxidant properties of chitosan nanoparticles and quercetin against carbon tetrachloride-induce hepatotoxicity in rats. Soft Nanoscience Letters 2015; 5:36–51.

45 Ahmed OM, Ahmed AA, Fahim HI, Zaky MY. Quercetin and naringenin abate diethylnitrosamine/acetylaminofluorene-induced hepatocarcinogenesis in Wistar rats: the roles of oxidative stress, inflammation and cell apoptosis. Drug and Chemical Toxicology 2022; 45:262–273.

46 Al-Harbi MS. Hepatoprotective effect and antioxidant capacity of naringenin on arsenic-induced liver injury in rats. International Journal of Pharmcy and Pharmaceutical Sciences 2016; 8:103–108.

47 Patra A, Jha S, Murthy PN. Phytochemical and pharmacological potential of Hygrophila spinosa T. anders. Pharmacogn Rev 2009; 3:330–341.

48 Ghosh D, Choudhury ST, Ghosh S, Mandal AK, Sarkar S, Ghosh A, *et al.* Nanocapsulated curcumin: oral chemopreventive formulation against diethylnitrosamine induced hepatocellular carcinoma in rat. Chemico-Biological Interactions 2012; 195:206–214.

49 Jagetia GC, Rajanikant GK. Curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated c-irradiation. Antioxidants 2015; 4:25–41.

50 Seyoum A, Asres K, El-Fiky FK. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry 2006; 18:2058–2070.

51 Mershiba SD, Dassprakash MV, Saraswathy SD. Protective effect of naringenin on hepatic and renal dysfunction and oxidative stress in arsenic intoxicated rats. Molecular Biology Reports 2013; 40:3681–3691.

52 Khanna-Chopra R, Sabarinath S. Heat-stable chloroplastic Cu/Zn superoxide dismutase in *Chenopodium murale*. Biochem Biophys Res Commun 2004; 320:1187–1192.

53 Rashed KN, Chang CW, Wu LY, Peng WH. Hepatoprotective activity of Diospyros lotus fruits on acute liver injury induced by carbon tetrachloride and phytochemical analysis. Topcls J Herb Med 2013; 2:75–83.

54 Jadon A, Bhadauria M, Shukla S. Protective effect of Terminalia belerica Roxb and gallic acid against carbon tetra-chloride-induced damage in albino rats. J Ethnopharmacol 2007; 109:214–218.

55 Ahmed OM. Relationships between oxidative stress, cancer development and therapeutic interventions. Journal of Cancer Science and Research 2016; 1:104.

56 Liu Z, Mao H, Chu D, Wang J. Chemoprotective Effect of Ginsenoside Against Ferric Nitrilotriacetate Induced Renal Cancer via Inhibition of Inflammatory Mediators. Int J Pharmacol 2022; 18:133–142.

57 Batra P, Sharma AK. Anti-cancer potential of flavonoids: recent trends and future perspectives. Biotech 2013; 3:439–459.

58 Baeuerle PA. IκB-NF-κB structures: AT the interface of inflammation control. Cell 1998; 95:729–731.

59 Ju W, Wang X, Shi H, Chen W, Belinsky SA, Lin Y. A critical role of luteolin-induced reactive oxygen species in blockage of tumor necrosis factor-activated nuclear factor kappa B pathway and sensitization of apoptosis in lung cancer cells. Mol Pharmacol 2007; 71:1381–1388.

60 Schett G. Effects of inflammatory and anti-inflammatory cytokines on the bone, Eur J Clin Investig 2011; 41:1361–1366.

61 Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit RevTM Eukaryot Gene Exp 2010; 20:87–103.

62 Hashemzaei M, Far AD, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, *et al.* Anticancer and apoptosis-inducing effects of quercetin *in vitro* and *in vivo*. Oncology Reports 2017; 38:819–828.

63 Crespo I, García-Mediavilla MV, Gutiérrez B, Sánchez-Campos S, Tuñón MJ, González-Gallego J. A comparison of the effects of kaempferol and quercetin on cytokine-induced pro-inflammatory status of cultured human endothelial cells. Br J Nutr 2008; 100:968–976.

64 Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA, Kandaswami C. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappa beta system. Clin Vaccine Immunol 2006; 13:319–328.

65 Hastak K, Gupta S, Ahmad N, Agarwal MK, Agarwal ML, Mukhtar H. Role of p53 and NF-kappaB in epigallocatechin-3-gallate induced apoptosis of LNCaP cells. Oncogene 2003; 22:4851–4859.

66 Li Y, Kucuk O, Hussain M, Abrams J, Cher ML, Sarkar FH. Antitumor and antimetastatic activities of docetaxel are enhanced by genistein through regulation of osteoprotegerin/receptor activator of nuclear factor-B (RANK)/RANK ligand/MMP-9 signaling in prostate cancer. Cancer Res 2006; 66:4816–4825.

67 El Khazendar M, Chalak J, El-Huneidi W, Vinod A, Abdel-Rahman WM, Abu-Gharbieh E. Antiproliferative and proapoptotic activities of ferulic acid in breast and liver cancer cell lines. Tropical Journal of Pharmaceutical Research 2019; 18:2571–2576.

68 Elwakkad A, Ghoneum M, El-Sawi M, Mohamed SI, Gamal el Din AA, Pan D, Elqattan GM. Baker's yeast induces apoptotic effects and histopathological changes on skin tumors in mice. Cogent Medicine 2018; 5:1–18.

69 Hsu CL, Yen GC. Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. Mol Nutr Food Res 2008; 52:53–61.

70 Al-Rejaie S, Abuohashish H, Al-Enazi M, A Al-ssaf A, Parmar M, Ahmed M. Protective effect of naringenin on acetic acidinduced ulcerative colitis in rats. World Journal of Gastroenterology 2013; 19:5633.

71 Cheng K, Niu J, Zhang J, Qiao Y, Dong G, Guo R, *et al.* Hepatoprotective effects of chlorogenic acid on mice exposed to aflatoxin B1: Modulation of oxidative stress and inflammation. Toxicon 2023; 231:107–177.

72 Vargas AJ, Burd R. Hormesis and synergy: Pathways and mechanisms of quercetin in cancer prevention and management.Nutr Rev 2010; 68:418–428.