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Original article

Nephroprotective, cytotoxic and antioxidant activities of *Euphorbia paralias*

Hanan M. Al-Yousef [a,*], Ali S. Alqahtani [a], Afaf S.A. Ghani [b], Sayed A. El-Toumy [c], Wagdi I.A. El-Dougdoug [d], Wafaa H.B. Hassan [b], Hanaa M. Hassan [e]

[a] *Pharmacognosy Department, College of Pharmacy, King Saud University, POBox 2457, Riyadh 11451, Saudi Arabia*
[b] *Department of Pharmacognosy Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt*
[c] *Chemistry of Tannins Department, National Research Center –Dokki- Cairo, 12622, Egypt*
[d] *Department of Chemistry, Faculty of Science, Benha University, 13518 Benha, Egypt*
[e] *Pharmacy Department Benha Educational Hospital, 13518 Benha, Egypt*

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ABSTRACT

Objective: To investigate the effect of the ethyl acetate fraction of the aerial parts of *E. paralias* L. F. *Euphorbiaceae* on nephroprotective, cytotoxic, and antioxidant.
Methods: different spectroscopic and spectrophotometric methods were applied to identify phytoconstituents. The nephroprotective potential of *E. paralias* ethyl acetate fraction (*Ep* EtOAc) was evaluated in male rats with thioacetamide-induced kidney injury, as wll as cytotoxic activity was evaluated using a viability assay, and the antioxidant activity was evaluated using the DPPH method. Results: quantitative estimation of total phenolics and flavonoids of *E. paralias* was performed using unique spectrophotometric methods. The polyphenolic compounds gallic acid **(1)**, ellagic acid **(2)**, kaempferol-3-*O*-(6″-*O*-galloyl-*β*-*D*-glucopyranoside) **(3)**, quercetin-3-*O*-*β*-*D*-glucopyranoside **(4)** and quercetin-3-*O*-*β*-*D*-arabinoside **(5)** were isolated from the ethyl acetate fraction of the aerial parts of *E. paralias*. The thioacetamide administration resulted in marked nephrotoxicity, but pretreatment with *Ep* EtOAc significantly attenuated the nephrotoxicity through alteration of kidney biomarkers, thereby improving the redox status of the tissue and restoring serum biochemical parameters nearly to normal levels. This study revealed a significant cytotoxic and strong antioxidant effect. Conclusion: we conclude that the *Ep* EtOAc may be used in the future as nephroprotective, cytotoxic, and antioxidant agent derived from a natural source.
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1. Introduction

With its remarkable diversity of natural compounds, the plant kingdom has merited particular interest (Lewinsohn et al., 2009). Among plant extracts, polyphenolic compounds have received much development and research attention (Harborne and Williams, 2000; Kesarkar et al., 2009; Buer et al., 2010). They have many beneficial biological activities such as antioxidative (Ammar et al., 2009; Ghasemzadeh et al., 2010), anticarcinogenic (Seelinger et al., 2008), antimicrobial (Zhou et al., 2007; Pereira et al., 2007), antimutagenic (Liverio et al., 1994), anti-inflammatory (Ueda et al., 2002), antiallergic (Mastuda et al., 2002), and antiobesity (Kamisoyama et al., 2008) properties. Compared with other organs, the kidney is greatly sensitive to toxicity due to its high blood flow and its functional and anatomical complexity. The kidney also has an essential role in blood pressure regulation and blood filtration. Most toxic substances are filtered through the kidneys and expelled in the urine. Nephrons concentrate toxic materials via filtration and transport them through the tubular cells (Nouri et al., 2019). *Euphorbia paralias* (*Ep*) belongs to the plant family *Euphorbiaceae* and is mainly distributed on sandy beaches from the North Sea, along the coasts of the Atlantic Ocean, and on Mediterranean beaches (James and Harden, 2008). Phytochemical investigations of *Ep* have revealed the isolation and identification of compounds such as diterpenes, triterpene, and flavonoids (Rizk et al., 1974; Sayed et al., 1980; Abdelgaleil et al., 2001; Barile et al., 2007; Barile and Lanzotti, 2007; Laila, 2011; Abdel Ghani et al., 2020a).

* Corresponding author.
E-mail address: halyousef@ksu.edu.sa (H.M. Al-Yousef).
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In the present study, total phenolics and flavonoids from fractionation of this plant were estimated. The ethyl acetate fraction of *E. paralias* (*Ep* EtOAc) was subjected to chromatographic investigation, leading to the isolation of five polyphenolic compounds, including two phenolic acids and three flavonol glycosides. Nephroprotective, cytotoxic and antioxidant activities were evaluated at the first time.

2. Materials and methods

2.1. General materials and equipment

Evaporation of the solvents was achieved using a Buchi rotary evaporator. A UV lamp was used for paper chromatocraphy (PC) visualization: UVP, GL-58 (λ_{max} 254 and 366 nm). A circulating hot-air oven, WT-binder 7200 (Germany), was used in this study. A Shimadzu UV-1700 spectrophotometer (Japan) was used for UV spectral analysis using NaOMe, $AlCl_3$, HCl, NaOAc, and boric acid as a shift reagent for UV analysis of flavonoids. PC was carried out on sheets of Whatman No. 1 filter paper for analytical PC and Whatman No. 3 filter paper for PPC. Infrared (IR) spectral analysis was recorded in potassium bromide disks on a Pye Unicam SP 3000 and an IR spectrophotometer (FT/IR-460 plus; Jasco). An ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) in negative mode was performed on a XEVO-TQD triple-quadruple instrument (Waters Corporation, Milford, MA, USA) mass spectrometer; Column, ACQUITY UPLC BEH C18 1.7 μm, 2.1 × 50 mm; column flow rate, 0.2 mL/min; solvent system consisted of (A) water containing 0.1% formic acid, (B) methanol containing 0.1% formic acid (Ain Shams University, Cairo, Egypt). Total estimations of phenolics and flavonoids were performed in the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. A polyamide 6 column was used and Sephadex LH-20 in the fractionation of ethyl acetate fractions. Nuclear magnetic resonance (NMR) analysis experiments were performed on a Bruker AMX 400 MH_Z for ^1H NMR and with standard pulse sequences operating at 100 MHz for ^{13}C NMR. Chemical shifts are given in δ values (ppm) by using tetramethylsilane (the internal standard); DMSO d_6 (solvent at room temperature) was of a spectroscopic grade for spectral analysis. Solvent systems for PC were as follows: solvent system 1, 15% HOAc; solvent system 2, n butanol: acetic acid: water (BAW) (4:1:5) (the upper layer).

2.2. Plant material and extraction process

Aerial parts of *E. paralias* L. F. *Euphorbiaceae* were collected in the flowering stage on May 2015 from the north coast of Alexandria, Egypt. Identification of the plants was kindly verified by Dr. Ahmed Abdel-Razik, lecturer of Plant Taxonomy, Department of Botany Faculty of Science, Banha University, Egypt. The voucher specimen (no. S303) was deposited in the National Research Centre, Dokki, Egypt. The plant material was air-dried and grounded by an electric mill into a moderately fine powder. Solvents used in this work, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), and methanol, were of the analytical grade for chromatography and crystallization. The air-dried powdered plant material (3 kg) was extracted by maceration with 70% methanol until complete exhaustion. Total methanolic extract was dried under reduced pressure at 45 °C. The greenish-brown viscous residue (500 g) was dissolved in the least amount of MeOH/H_2O (1:9) and subjected to fractionation by CH_2Cl_2 (avoided) and EtOAc to yield the ethyl acetate fraction (39.5 g).

2.3. Quantitative estimation of the total phenolics and flavonoids

Spectrophotometric determinations were done using the Folin–Ciocalteu method for phenolic estimation and the aluminum chloride colorimetric method for flavonoid estimation as previously described **(**Ghasemzadeh et al., 2010**).**

2.4. Isolation of compounds (1–5) from the ethyl acetate fraction

The *Ep* EtOAc (18 g) was fractionated on Polyamide 6 CC. The column was eluted with water and then gradually decreased in polarity using methanol. Six fractions (500 mL each) were collected, concentrated under reduced pressure, and subjected to PC followed by screening with solvents 1 and 2. The important fractions were applied on a Sephadex LH 20 column. Five compounds **(1–5)** were isolated in the following sequence: compounds 1 and 2 (20% MeOH in H_2O), compound 3 (40% MeOH in H_2O), and compounds 4 and 5 (60% MeOH in H_2O).

Compound (1), Gallic acid (17 mg), appeared as faint yellow needles with a melting point (m.p.) of 260 °C. It gave a brown color with ammonia vapors and turned blue upon exposure to $FeCl_3$. It was soluble in methanol. R_f values were 0.78 and 0.56 within solvent systems 1 and 2, respectively. UPLC-ESI-MS/MS in negative mode showed a molecular ion peak at *m/z* 169 $[M-H]^+$ and a daughter ion at *m/z* 125 $[M-H-COO]^+$. The UV spectral data of **(1)** exhibited absorption bands (λ_{max} 217, 272 nm). ^1H NMR (400 MHz, DMSO–d_6) displayed a singlet signal at δ_H 6.98 (2H, s, H-2/6) and a broad signal at δ_H 9.17 (–OH). ^{13}C NMR (100 MHz, DMSO–d_6) spectrum showed δc 145.5 (C-3/C-5), 138.1 (C-4), 120.6 (C-1), 108.8 (C-2/6), and 167.6 (C-7).

Compound (2), Ellagic acid (20 mg), appeared as yellowish-white needles in visible light, changed to brown with ammonia vapors, became blue when sprayed with $FeCl_3$, was soluble in methanol, and its m.p. was 360 °C. R_f values were 0.48 and 0.72 in solvent systems 1 and 2, respectively. UPLC-ESI-MS/MS in negative mode showed a molecular ion peak at *m/z* 301 $[M-H]^+$. The UV spectral data of **(2)** exhibited absorption bands (λ_{max}) 353, 367 nm. ^1H NMR (400 MHz, DMSO–d_6) δ_H 7.42 (2H, 5, 12), broad singlet at δ_H 10.62 (brs, 4H, 3, 4, 10,11, –OH), ^{13}C NMR (100 MHz, DMSO–d6) δc 159.6 (C-7 and C-14), 148.56 (C-4 and C-11), 140.02 (C-3 and C-10), 136.84 (C-2 and C-9), 112.77 (C-6 and C-13), 110.7 (C-5 and C-12), and 108.13 (C-15 and C-16).

Compound (3), Kaempferol–*3–O–(6″–O–*galloyl–*β–D–*glucopyranoside*)* (14 mg), was a yellow amorphous powder with R_f 0.52 (solvent system 2), purple florescent spot under UV light, and gave a yellow color with ammonia vapors. UV λ_{max} values were as follows: (MeOH) 256, 346 nm; (MeOH + NaOMe) 256, 403 nm; (MeOH + $AlCl_3$) 246, 394 nm; (MeOH + $AlCl_3$ + HCl) 246, 394 nm; (MeOH + NaOAc) 272, 360 nm; (MeOH + NaOAc + H_3BO_3) 271, 356 nm. IR ν_{max} (KBr) cm^{-1} occurred at 3473, 3286 and 3120, 2898, 1730, 1660, and 1568. UPLC-ESI-MS/MS in negative mode yielded *m/z* 599 $[M-H]^+$, 447 $[M-H-galloyl]^+$, 285 $[M-H-galloylglucosyl]^+$ (100%), and 151 [aglycone fragment of Ring-B]$^+$. Complete acid hydrolysis yielded kaempferol, gallic acid, and glucose as sugar moieties, which were all chromatographed with authentic samples. ^1H NMR (400 MHz, DMSO–d_6): δ_H 7.93 (d, *J* = 10.4 Hz, H-2′,6′), 6.77 (d, *J* = 9.2 Hz, H-3′,5′), 6.41 (d, *J* = 2 Hz, H-8), 6.20 (d, *J* = 2 Hz, H-6), δ 5.45 (1H, d, *J* = 7.5 Hz, H-1″), 6.93 (2H, s, 2‴, 6‴), 4.27 (1H, dd, *J* = 12, 5.5 Hz, H-6a″), 4.17 (1H, dd, *J* = 12, 2.4 Hz, H-6b″) and 3.90–3.10 (m, 4H, remaining sugar protons). ^{13}C NMR (100 MHz, DMSO–d_6): 177.7 (C-4), 164.8 (C-7), 161.7 (C-5), 160.4 (C-4′), 157.1(C 2), 156.9 (C-9), 133.71(C-3), 131.32 (C-2′ and 6′), 121.2 (C-1′), 115.62 (C-3′ and 5′), 104.45 (C-10), 99.39 (C-6) and 94.3 (C-8). The glucose carbons were at δ_c 101.9 (C-1″), 76.6 (C-3″), 74.6 (C-5″), 72.79 (C-2″), 70 (C-

4″), 63.16 (C-6″). The galloyl carbons were at δc 166.1 (C-7), 146.02 (C-3 and C-5), 138.8 (C-4), 119.79 (C-1) and 109.01 (C-2 and C-6).

Compound (4), Quercetin-*3-O-β-D*-glucopyranoside (12 mg), a yellow amorphous powder, gave purple fluorescent spot and yellow color with ammonia vapor. UV λ_{max} values were as follows: (MeOH) 259, 359 nm; (MeOH + NaOMe) 268, 418 nm; (MeOH + $AlCl_3$) 269, 427 nm; (MeOH + $AlCl_3$ + HCl) 268, 398 nm; (MeOH + NaOAc) 274, 376 nm; (MeOH + NaOAc + H_3BO_3) 273, 385 nm. IR ν_{max} (KBr) cm^{-1}: 3260–3320, 2876, 1661, 1608, 1563, 1514, and 1460. UPLC-ESI-MS/MS in negative mode showed a molecular ion peak at *m/z* 463 (100%) $[M-H]^+$, and daughter ion peak at *m/z* 301 $[M-H-glucose]^+$. ^1H NMR (400 MHz, DMSO–d6) δ_H 7.76 (1H, s, H-2′); δ 7.53 (1H, d, *J* = 2 Hz, H-6′); and 6.80 (1H, d, *J* = 8.6 Hz, H-5′), two doublets at δ 6.40 and 6.20 ppm with *J* = 1.8 Hz (H-8 and H-6), 5.37 (1H, d, *J* = 8 Hz, H-1″), 3.90–3.10 (6H, m, remaining sugar protons). ^{13}C NMR (100 MHz, DMSO–d_6): 177.7 (C4), 164.82 (C7), 161.62 (C5), 156.74 (C9), 156 (C2), 148.79 (C4′), 146.3 (C3′), 133.9 (C3), 122.34 (C6′), 121.51 (C1′), 116.4 (C5′), 115.54 (C2′), 104.26 (C10), 99.08 (C6), 93.93 (C8), and 101.2 (C1″).

Compound (5), Quercetin-*3-O-β-D*-arabinopyranoside (13 mg), a yellow amorphous powder, gave a dark purple fluorescent spot that turned yellow on PC with ammonia vapor. UV λ_{max} was as follows: (MeOH) 265, 365 nm; (MeOH + NaOMe) 261, 424 nm; (MeOH + $AlCl_3$) 273, 438 nm; (MeOH + $AlCl_3$ + HCl) 268, 394 nm; (MeOH + NaOAc) 278, 376 nm; and (MeOH + NaOAc + H_3BO_3) 273, 387 nm. IR ν_{max} (KBr) cm^{-1} were at 3476, 3372, 3189, 2902, 1655, 1615, 1510 and 1469. UPLC-ESI-MS/MS in negative mode showed a molecular ion peak at *m/z* 433 $[M-H]^+$ and a daughter ion at *m/z* 301 $[M-H-arabinosyl]^+$. ^1H NMR (400 MHz, DMSO–d6): δ 7.67 (1H, s, H-2′); δ 7.57 (1H, d, *J* = 2 Hz, 6′), 6.83 (1H, d, *J* = 8.6 Hz, H-5′), 6.43 (1H, d, *J* = 1.8 Hz, H-8), 6.20 (1H, d, *J* = 1.8 Hz, H-6), and 5.38 (1H, d, *J* = 8 Hz, H-1″), ^{13}C NMR (100 MHz, DMSO–d6): δ_C 177.96 (C-4), 164.77 (C-7), 161.67 (C-5), 156.75 (C-2), 156.71 (C-9), 149.07 (C-4′), 145.45 (C-3′), 134.19 (C-3), 122.53 (C-1′), 121.36 (C-6′), 116.22 (C-5′), 115.83 (C-2′), 104.34 (C-10), 101.87 (C-1″), 99.17 (C-6), 93.99 (C-8), 72.12 (C3″), 71.2 (C2″), 66.5 (C4″), and 64.8 (C5″).

2.5. Nephroprotective analyses

2.5.1. Animals

Male Sprague–Dawley rats (220 ± 6 g body weight) were purchased from Theodor Bilharz Institute (Giza, Egypt). The animals were housed in the animal facility of the Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, Cairo, Egypt. They were fed with standard diet pellets (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and were allowed free access to water. The animals were kept at room temperature (25 °C ± 3 °C) and natural humidity (55% ± 6%) with a 12 h light/12 h dark cycle. The experiments were conducted per the ethical guidelines for investigations in laboratory animals and comply with the guidelines for the care and use of laboratory animals. Approval was awarded by the ZU-IACUC committee with approval number ZU-IACUC/3/F/145/2019.

2.5.2. Chemicals

Silymarin (Sil), a fine yellow powder that dissolved in normal saline, and thioacetamide (TAA), a white powder with a purity of 98.1% that dissolved in normal saline, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.5.3. Experimental design

Twenty-four rats were divided into four groups (n = 6). Group 1 (control) rats were given normal saline daily (2 mL/kg body weight, orally) for four consecutive weeks. Group 2 (TAA) rats were injected i.p. with TAA (100 mg/kg body weight) (Ahmad et al., 2018) dissolved in saline two times per one week. Group 3 (Sil + TAA) rats were pretreated orally with Sil (100 mg/kg body weight) ((Nouri et al., 2019) daily (one dose) for four consecutive weeks and then with TAA two times per one week (Ahmad et al., 2018). Group 4 (*Ep* + TAA) rats were pretreated orally with *Ep* ethyl acetate fraction (200 mg/kg body weight) (Nouri et al., 2019; Ahmad et al., 2018) daily (one dose) for four consecutive weeks and then with TAA. *Ep* ethyl acetate fraction and Sil were given to the animals orally by gastric intubation for four weeks (Nouri et al., 2019; Ahmad et al., 2018).

2.5.4. Serum and tissue preparations

The rats were lightly anesthetized under an atmosphere of ether. Blood samples collected from the *retro*-orbital plexus were centrifuged at 1000 × *g* for 15 min at 4 °C to obtain serum for measuring biochemical parameters. The serum was then transferred into prelabeled non-heparinized tubes and stored on ice. Then, the animals were weighed and euthanized by decapitation under light ether anesthesia; kidneys were weighed, dissected, washed in saline, blotted between two dry filter papers, and fixed in 10% formalin for histopathological examination.

2.5.5. Biochemical analysis

Sera separated from blood samples were used for the determination of kidney enzymes creatinine (CR), urea (UR), albumin (Alb), total protein (TP). Kidney weight/body weight ratios (Kid W/BW) were calculated, and the oxidative enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were quantified from the supernatant of kidney tissue homogenate after centrifugation at 5,000 rpm for 10 min (Ahmad et al., 2018; Ramakrishman, 2012; Balahoroğlu et al., 2008). All tests were performed using colorimetric spectrum Biodiagnostics™ and Diamond™ kits (Cairo, Egypt).

2.5.6. Histopathological examination

Histopathological assessment of kidney tissues was performed by standard methods (Balahoroğlu et al., 2008; Banchroft et al., 1996). Kidney tissues from each group were immediately fixed in 10% formalin for 24 h. All samples were embedded in paraffin, and sections (5 μm thickness) were cut by rotary microtome (Amarican Optical, Buffalo, NY, USA). Sections were stained using hematoxylin and eosin (H&E) or Masson's trichrome (MT). Photomicrographs of stained kidney sections were captured and examined for pathological alterations in the tissues.

2.5.7. Statistical data analysis

All data are presented as means ± SEM. One-way analysis of variance (ANOVA) was used to calculate the total variation in a set of data. Probability values (p) ≤ 0.05 were considered statistically significant.

2.6. Evaluation of cytotoxic activity

The cytotoxicity of the ethyl acetate fraction on $HepG_2$ human liver cancer cells (Elaasser et al., 2011; Mosmann, 1983) was evaluated by viability assay. The percentage of inhibition and IC_{50} values with their standard deviation concentrations were reported.

2.7. Evaluation of the antioxidant activity

The radical scavenging effect of *Ep* was determined using the DPPH assay (Gomha et al., 2015) for the first time for ethyl acetate fraction and was calculated by the following formula: Radical scavenging % = $[(A_{control} - A_{sample})/A_{control}] \times 100$ (Gomha et al., 2015; Carocho et al., 2014). The investigation was performed in the

Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

3. Results

3.1. Quantitative estimation of total phenolics and flavonoids

Total phenolic contents of the total extract of *Ep* was determined by the Folin–Ciocalteu method and expressed as gallic acid equivalent (GAE), and the total flavonoid contents of the same extracts were determined by aluminum chloride colorimetric method and expressed as rutin equivalents (RE) (Ghasemzadeh et al., 2010). The total phenolic content was 54.16 mg GAE/g, and the total flavonoid content was 95.38 mg RE/g from the total methanolic extract of *Ep* (Fig. 1) (see Fig. 2).

3.2. Chemical investigation

Compound (1): Based on the spectroscopic data of compound **(1)** (UV, IR, ESI-MS/MS, ^{1}H NMR, and ^{13}C NMR) in comparison with published data (Mabry et al., 1970; Harborne, 1973; Nawwar et al., 1994) and co-PC with the authentic sample, compound **(1)** was identified as gallic acid.

Compound (2): The spectral data of compound **(2)** (UV, IR, ESI-MS/MS, ^{1}H NMR, and ^{13}C NMR) were compared with the published data (Mabry et al., 1970; Harborne, 1973; Nawwar et al., 1994) co-PC with the authentic sample. It was clear that compound **(2)** was ellagic acid.

Compound (3) was identified as kaempferol-3-*O*-(6″–*O*–galloyl–*β*–*D*–glucopyranoside) by comparison of its spectral data (IR, UV, ESI-MS/MS, ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC), which were consistent with the data reported in literature (Mabry et al., 1970; Harborne, 1973; Nawwar et al., 1994; Ochoa-Pacheco et al., 2017; Dilek et al., 2005).

Compounds (4) and (5): Based on their spectral data (IR, UV, ESI-MS/MS, ^{1}H NMR, and ^{13}C NMR), which matched their respective published data (Metwally et al., 2010; Vedenskaya et al., 2004), these compounds were identified as quercetin 3-*O*-*β*-*D*-glucopyranoside **(4)** and quercetin 3-*O*-*β*-*D*-arabinopyranoside **(5)**. Compounds 1–3 and 5 were isolated from *E. paralias* from Egypt for the first time while compound 4 was previously isolated from the same species from Egypt (Safwat et al., 2018).

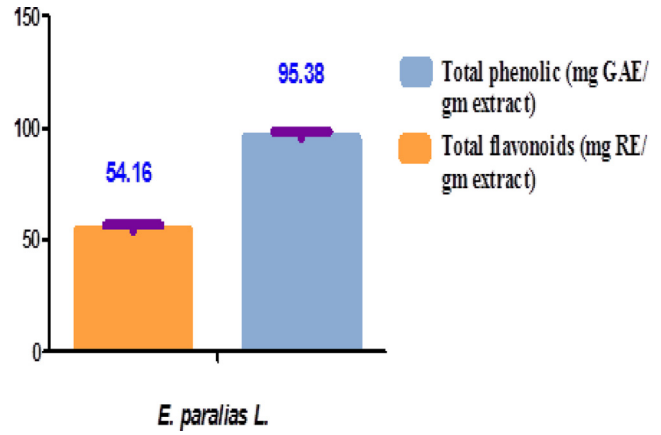

Fig. 1. Total content of phenolics and flavonoids (mg GAE and mg RE, respectively) of the total methanolic extract of the aerial parts of *Euphorbia paralias* L. GAE: gallic acid equivalent, RE: rutin equivalent.

3.3. Nephroprotective activity

TAA is a nephrotoxic agent known to induce acute or chronic kidney disease (fibrosis) in an experimental rat model (Dwivedi and Jena, 2018). In the present work, TAA was used as a potent nephrotoxic agent in rats. A dose of 100 mg/kg TAA administered intraperitoneally (i.p.) has been reported to cause nephrotoxicity due to increased oxidative stress (Balahoroğlu et al., 2008; Ahmed et al., 2018).

3.3.1. Evaluation of kidney biochemical parameters

Exposure of animals with the nephrotoxic agent TAA resulted in a significant ($p \leq 0.05$) increase in the serum kidney enzymes CR and UR, with a significant decrease in albumin (Alb), total protein (TP), kidney weight/body weight ratio (Kid W/BW), GSH, CAT, and SOD, indicative of nephron damage (Balahoroğlu et al., 2008). Pretreatment of experimental animals with *Ep* EtOAc reversed the TAA-induced nephrotoxicity and restored elevated levels of kidney biomarkers toward normality compared with the Sil-treated group. *Ep* and Sil caused a significant decrease in serum CR and UR (Fig. 3) and a significant increase in Alb, TP, Kid W/BW, GSH, CAT, and SOD (Figs. 3, 4, 5).

3.3.2. Histopathological results

Histopathological examination of the kidney sections of rats exposed to TAA showed glomerular and blood vessel congestion in addition to epithelial desquamation of tubular casts compared with the kidneys of control rats with healthy kidneys. Pretreatment with *Ep* EtOAc attenuated this nephron injury and demonstrated significant protection of the nephrotic cells from damage, as shown by almost complete normalization of the kidney tissues (Figs. 6, 7). These data demonstrate the nephroprotective potential of *Ep* EtOAc compared with Sil treatment.

3.3.3. Nephroprotective discussion

In this study, our histopathological findings of acute renal injury in rats treated with TAA concurrently manifested as a significant elevation of serum UR and CR and a decline in Alb, TP, CAT, SOD, GSH, Kid W/BW, and BW. Our histological findings agree with those of previous studies of TAA-induced renal damage (Ahmed et al., 2018) (Fig. 7A). In our study, TAA-induced deteriorations were partially ameliorated by Sil or *Ep* treatments, in agreement with prior investigations (Nouri et al., 2019). The renal protective effect of *Ep* EtOAc may be due to a high content of phenolics and flavonoids, leading to an elevated scavenging activity and a stabilizing effect on the plasma membrane and proteins against reactive oxygen species (Nouri et al., 2019).

3.4. In vitro cytotoxic activity

Evaluation the effect of *Ep* EtOAc on $HepG_2$ cells (Fig. 8) revealed significant activity represented by significant inhibition of cell proliferation, as concluded by the low IC_{50} value 26.4 ± 1.2 μg/mL compared with that of doxorubicin (a standard cytotoxic drug; IC_{50} = 0.39 μg/mL).

3.5. Antioxidant activity

As *Ep* EtOAc showed a high content of phenolics and flavonoids, it was evaluated for its DPPH radical scavenging ability (Balahoroğlu et al., 2008; Kosalec et al., 2004). Serial solutions of concentrations ranged from 10 to 1,280 μg in methanol. *Ep* EtOAc showed greater antioxidant activity than ascorbic acid (positive standard) with 50% DPPH scavenging concentration (SC_{50}) values of 13.1% and 14.2%, respectively (Fig. 9).

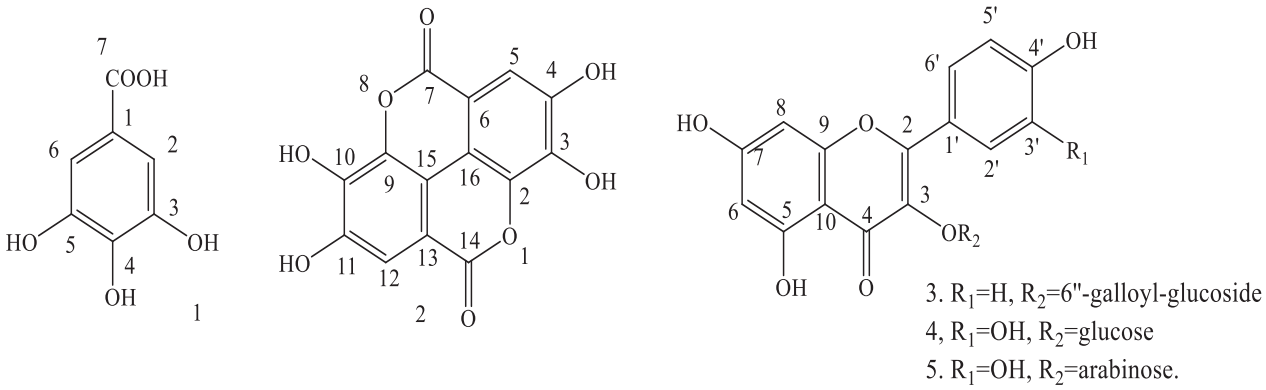

Fig. 2. Chemical structures of compounds **(1–5)** isolated from the total methanolic extract of the aerial parts of *Euphorbia paralias* L.

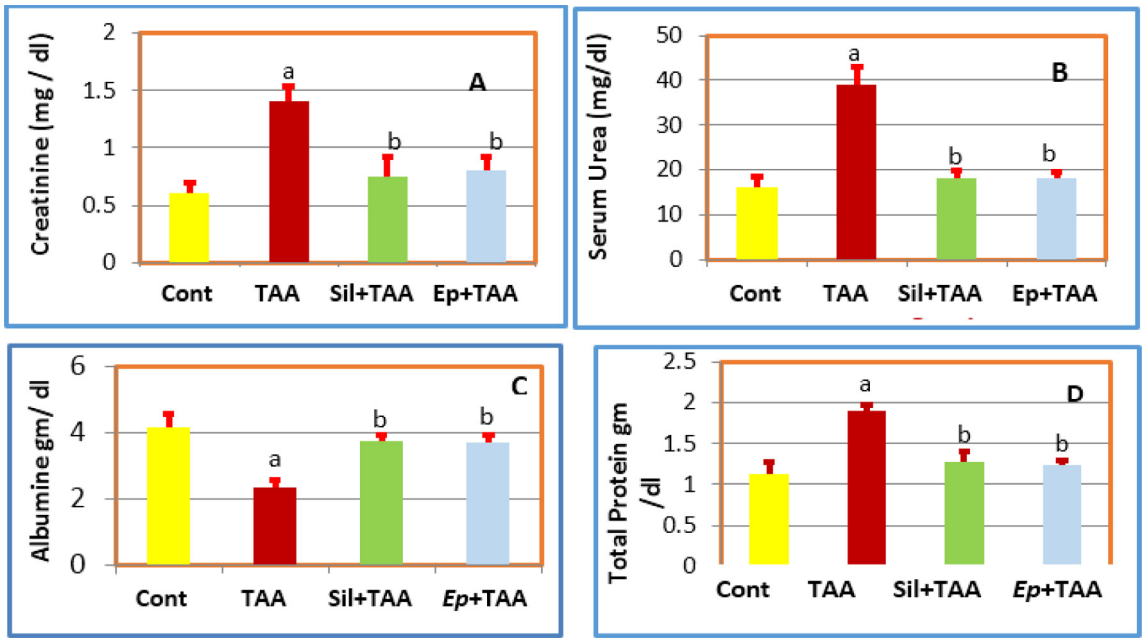

Fig. 3. Effects of pretreatment with *Ep* or Sil on kidney enzymes in rats with thioacetamide-induced kidney injury. Groups of Sprague–Dawley rats were pretreated orally with Sil (100 mg/kg body weight) or *Ep* ethyl acetate fraction (200 mg/kg body weight) daily for four consecutive weeks and then orally gavaged with saline (control) or TAA two times per week. The effects of pretreatment with *Ep* or Sil were analyzed by serum levels of (A) creatinine (CR), (B) urea (UR), (C) albumin (AL), and (D) total protein (TP). $p \leq 0.05$ was considered significant. [a]Compared with the control group. [b]Compared with the TAA group, n = 6. Control, saline-treated healthy control rats; Ep, *E. paralias* ethyl acetate fraction; Sil, silymarin; TAA, thioacetamide.

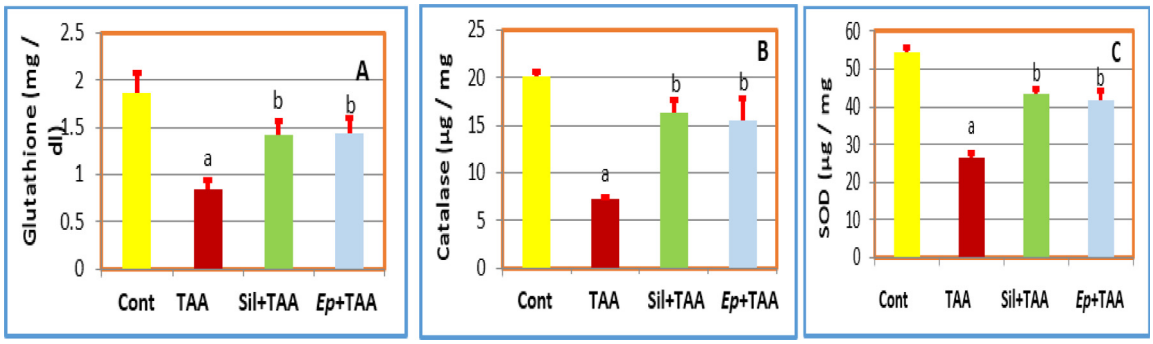

Fig. 4. Effects of pretreatment with *Ep* and Sil on enzymes related to oxidative stress in the kidneys of rats with thioacetamide-induced kidney injury. Sprague–Dawley rats were pretreated orally with Sil (100 mg/kg body weight) or *Ep* ethyl acetate fraction (200 mg/kg body weight) daily for four consecutive weeks and then orally gavaged with saline (control) or TAA two times per week. The effects of pretreatment with *Ep* or Sil on oxidative stress markers in the kidney were analyzed by measuring levels of (A) glutathione (GSH), (B) catalase (CAT), and (C) superoxide dismutase (SOD) in kidney homogenates. $p \leq 0.05$ was considered significant. [a]Compared with the control group. [b]Compared with the TAA group, n = 6. Control, saline-treated healthy control rats; Ep, *E. paralias* ethyl acetate fraction; Sil, silymarin; TAA, thioacetamide.

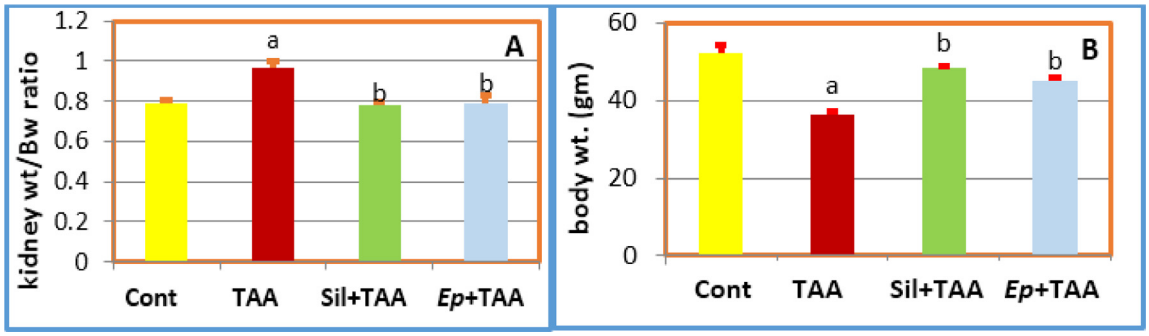

Fig. 5. Effects of pretreatment with *Ep* and Sil of TAA-treated rats on (A) kidney weight/body weight ratio (Kid W/BW) and (B) body weight (BW). $p \leq 0.05$ was considered significant. [a]Compared with the control group. [b]Compared compared with the TAA group, n = 6. Control, saline-treated healthy control rats; Ep, *E. paralias* ethyl acetate fraction; Sil, silymarin; TAA, thioacetamide.

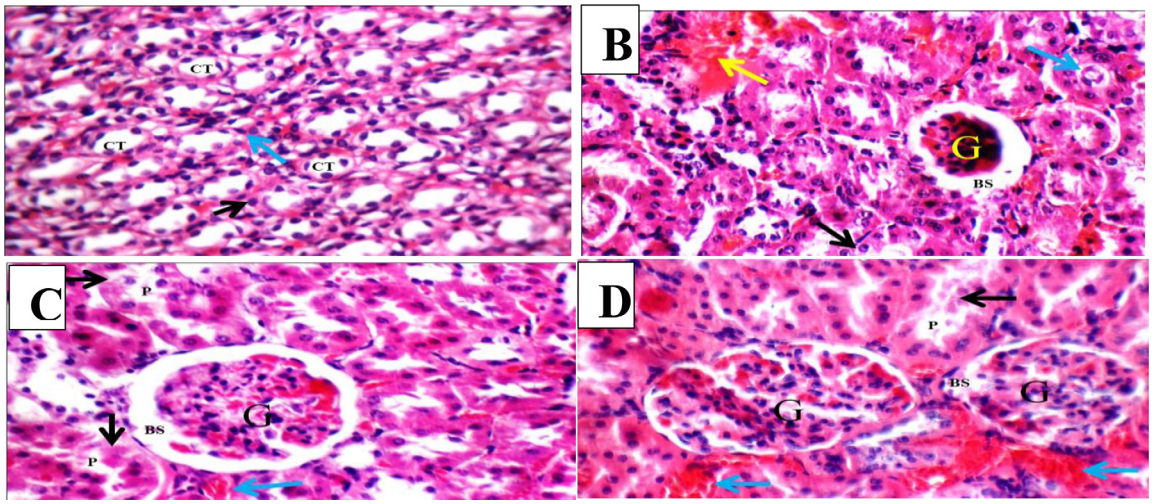

Fig. 6. Light photomicrograph showing the histopathology of kidneys from rats with thioacetamide-induced kidney injury (H&E, 400 ×). **A (Cont gp):** Renal medulla showing normal collecting tubules (CT), epithelial lining (black arrow), and interstitium (blue arrow). **B (TAA gp):** Small-sized glomerulus (G) with widened Bowman's space (BS), proximal tubules with edematous lining (black arrow) with intra-tubular debris (blue arrow), and areas of interstitial hemorrhage (yellow arrow). **C (Sil + TAA gp):** Congested edematous glomerulus (G), proximal tubules with edematous and apoptotic epithelial lining with partial loss of brush borders (black arrow), and congested blood vessels (blue arrow). **D (*Ep* + TAA gp):** Renal medulla showing collecting tubules (CT) with edematous epithelial lining (black arrow) and congested BV (blue arrow). Cont, saline-treated healthy control rats; Ep, *E. paralias* ethyl acetate fraction; Sil, silymarin; TAA, thioacetamide.

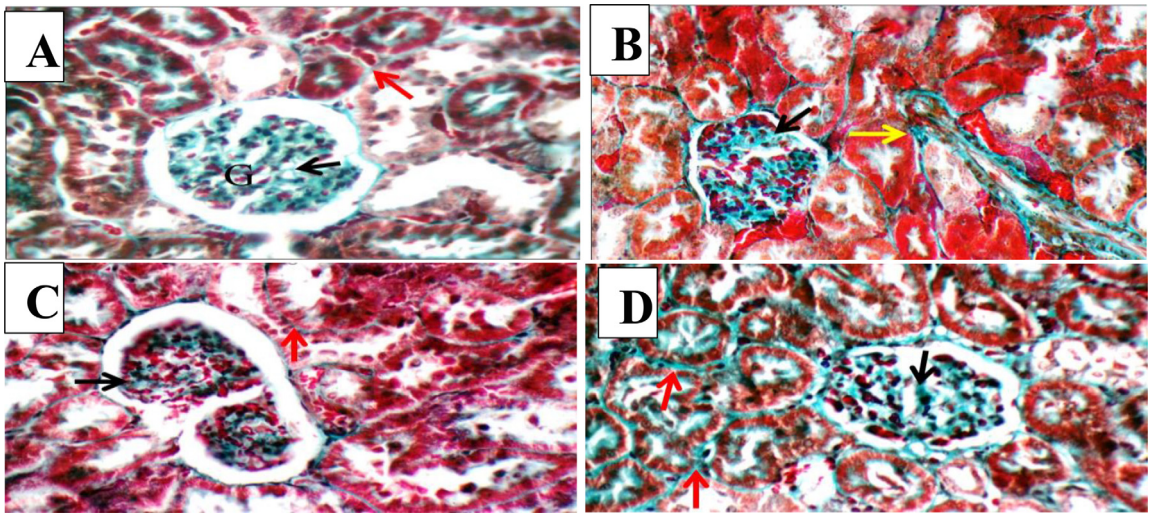

Fig. 7. Light photomicrograph showing the histopathology of kidneys from rats with thioacetamide-induced kidney injury (Masson's trichrome stain, 400 ×). **A (Cont gp):** Normal collagen distribution in glomeruli (black arrow) and around tubules (red arrow). **B (TAA gp):** Excess collagen is present in glomeruli (black arrow) and around tubules (yellow arrow). **C (Sil + TAA gp):** Normal collagen distribution in glomeruli (black arrow) and around tubules (red arrow). **D (Ep + TAA gp):** Excess collagen in glomeruli (black arrow) and around tubules (red arrow). Cont, saline-treated healthy control rats; Ep, *E. paralias* ethyl acetate fraction; Sil, silymarin; TAA, thioacetamide.

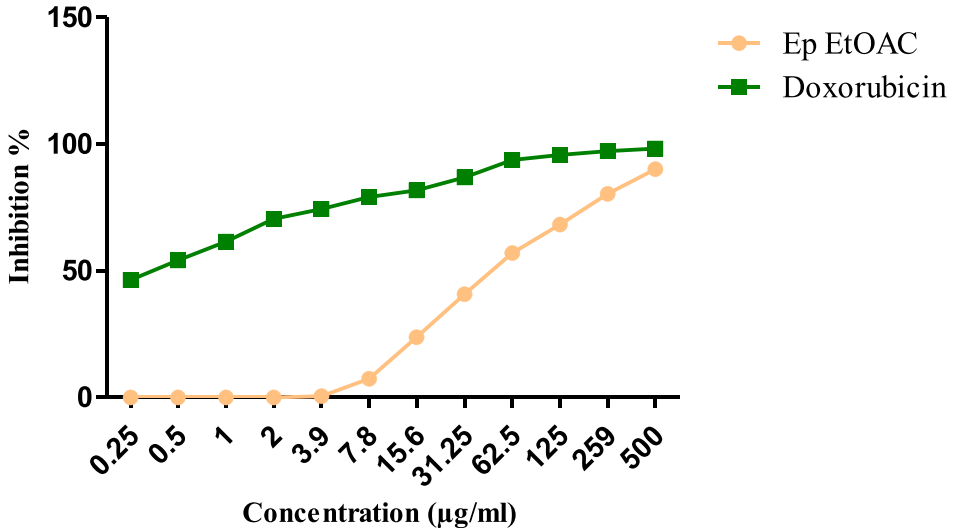

Fig. 8. Cytotoxic activity of *E. paralias* ethyl acetate fraction against the $HEPG_2$ liver cancer cell line in comparison with doxorubicin (standard cytotoxic drug).

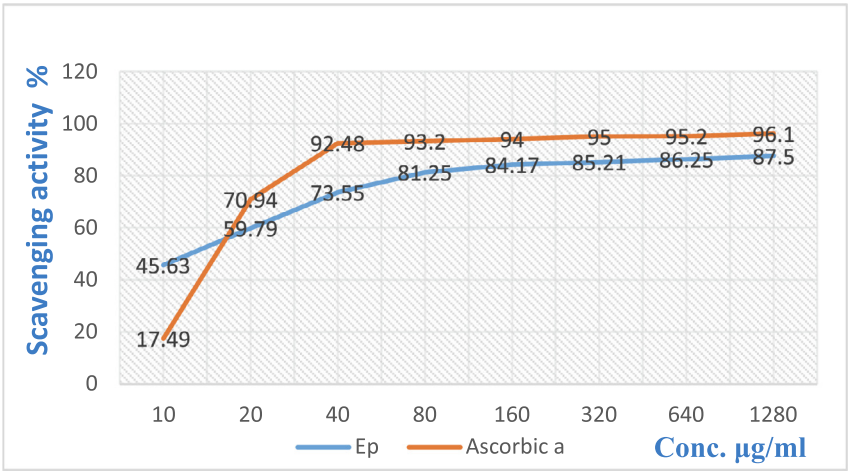

Fig. 9. Scavenging activity (%) of ethyl acetate fractions of *E. paralias* (Ep) and ascorbic acid. The radical scavenging effect of *Ep* was determined using the DPPH and was calculated by the following formula: Radical scavenging % = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

4. Discussion

Herein, we report a high content of phenolics and flavonoids in the total methanolic extract of *E. paralias*. Isolation of five polyphenolic compounds from the *Ep* EtOAc produced mainly tannins and flavonoid glycosides. Referring to another study (Abdel Ghani et al., 2020b) as revealed the identification of 32 polyphenolic compounds which explain strong hepatoprotictive activity of *E. paralias* and *E. geniculata* (in comparison) under the study and that's also explain heigh nephroprotictive activity of the plant in addition to antioxidant and cytotoxic activities. As the results of the study on kidney pretreated with TAA then *Ep* EtOAc showed strong significant nephroprotective effect of *Ep* EtOAc in comparison with silymarin (the positive control). In addition, cytotoxic and antioxidant activities exhibited by this fraction may be attributed to the high content of high polyphenolic contents. Another reseach was done on the decoction of *E. paralias* from Saudi arabia causing renal disfunction (Boubaker et al., 2013) that resulted from heigh content of the decoction of diterpenoidal esters (Abdelgaleil et al., 2001; Abdel Ghani et al., 2020a) which are toxic compounds identified from *E. paralias* so current study was done on ethyl acetate fraction only not total extract or methylene chloride fraction to avoid such toxic effect, in addition to safty marigin of EtOAc fractions of *E. paralias* reported by (Abdel Ghani et al., 2020b) using LD_{50} test.

5. Conclusion

These findings merit further examination with the potential for *Ep* EtOAc to become a nephroprotective, cytotoxic and antioxidant drug from a natural origin.

Author contributions

All authors made considerable contributions to the manuscript, designed the study, performed the experiments, interpreted the results, wrote the manuscript. All authors revised the manuscript and confirmed it for publication

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Compliance with ethics requirements

The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and comply with the guidelines for the care and use of laboratory animals. Approval was granted by the ZU-IACUC committee with approval number ZU-IACUC/3/F/145/2019.10. Ethical approval: informed consent was not applicable to this study.

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