Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221−1691.270979

Impact factor: 1.59

Hepatoprotective activity of *Dypsis lutescens* against *D*−galactosamine−induced hepatotoxicity in rats and its phytoconstituents

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ARTICLE INFO

Article history:
Received 24 April 2019
Revision 3 June 2019
Accepted 30 October 2019
Available online 20 November 2019

Keywords:
Dypsis lutescens
Flavonoid glycosides
Phenolic acids
Hepatoprotective
Antioxidant
Anti-inflammatory

ABSTRACT

Objective: To isolate and identify the polyphenolic constituents of *Dypsis lutescens*, and evaluate the hepatoprotective activity of the ethanolic extract of *Dypsis lutescens* leaves.

Methods: Hepatoprotective, antioxidant and anti-inflammatory effects of two doses of *Dypsis lutescens* ethanolic leaf extract were investigated in five groups of six rats each administered with the ethanolic extract of *Dypsis lutescens* leaves. Liver function parameters were assessed, histopathological study was carried out, the anti-inflammatory mediators and the antioxidant potential in the liver tissues were evaluated. In addition, the total ethanolic extract of *Dypsis lutescens* leaves was subjected to different chromatographic separation techniques to yield ten phenolic compounds. The isolated compounds structures were spectroscopically elucidated.

Results: Hepatoprotective activity of *Dypsis lutescens* ethanolic extract was estimated for the first time and showed significant activity against histopathological changes induced by *D*-galactosamine in liver. The extract improved the liver functions. Compared to the *D*-galactosamine group, the architecture of the liver in the treated groups was improved in the histopathological examination. These results proved the hepatoprotective activity of *Dypsis lutescens* and its ability in attenuating liver oxidative damage and inflammation. Phytochemical investigations of the total extract afforded ten compounds from the genus *Dypsis*.

Conclusions: The alcoholic extract of *Dypsis lutescens* exerted potential hepatoprotective action, maintaining liver health and functions.

1. Introduction

The liver is a vital organ and takes part in metabolisms of carbohydrates, proteins and lipids as well as in detoxification[1,2]. In modern society, hepatic diseases as hepatitis A, hepatitis B, hepatitis C, hepatocellular carcinoma, liver cirrhosis and fatty liver cause serious health problems and great economic losses. In the traditional medicine, medicinal plants have been used for the treatment of hepatic diseases successfully and could promote natural healing

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Funding: The research fund for this work has been received from the National Research Centre, Egypt (project No. 11010325).

How to cite this article: El-Ghonemy MM, El-Kashak WA, Mohamed TK, Omara EA, Hussein J, Farrag ARH, et al. Hepatoprotective activity of *Dypsis lutescens* against *D*-galactosamine-induced hepatotoxicity in rats and its phytoconstituents. Asian Pac J Trop Biomed 2019; 9(11): 467-473.

process of the liver. Hence, this motivates further investigations for an efficient hepatoprotective drugs from natural sources[3].

Arecaceae family has been neglected chemically, probably because of the difficulty in collecting fresh material[4]. They are flowering plants in monotypic Arecales order[5]. *Dypsis* is a one of Arecaceae plants, and comprises 65 species that are domestic to Madagascar, Tanzania, and diverse islands in the Indian Ocean[6]. There are five *Dypsis* species grown in Egypt namely *Dypsis lutescens* (*D. lutescens*), *Dypsis decaryi*, *Dypsis leptochilios*, *Dypsis lastelliana*, and *Dypsis capada*.

D. lutescens is widely used as an ornamental plant, which possesses strong antioxidant and anticancer activities. Phytochemical screening of *D. lutescens* (Golden cane palm) revealed presence of flavonoids, tannins, lignans, triterpenes and steroids[6]. The biological and phytochemical studies on genus *Dypsis* are scarce. We selected species *D. lutescens* (Golden cane palm), isolated and identified its chemical constituents, and evaluated the hepatoprotective activity of its ethanolic extract.

2. Materials and methods

2.1. Collection of D. lutescens

D. lutescens leaves (H. Wendl) were collected from El-Zohrea Botanical Garden, Giza, Egypt in April 2014, which were identified by Consultants of Plant Taxonomy, Dr. Mohamed El-Gebaly at the Ministry of Agriculture. A voucher sample (M-146) was deposited in National Research Centre's herbarium, Egypt. The plant samples were air-dried at room temperature, powdered by electric mill, and kept in closed containers for the phytochemical and bioactivity studies.

2.2. Chemicals, biochemicals and instruments

Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP) and malondialdhyde (MDA) assay kits were obtained from Biodiagnostic Company (Dokki, Giza, Egypt). Silymarin and *D*-galactosamine (*D*-GaIN) were purchased from Medical Union Pharmaceuticals Company (Abou Sultan, Ismalia, Egypt) as a gelatinous capsule under the trade name of Hepaticum; each capsule contained 140 mg of silymarin. Sephadex LH-20 (Sigma-Aldrich-Steinheim, Germany), micro crystalline cellulose (E. Merk Darmstadt, Germany), and polyamide 6(Riedel-DeHaen AG, SeelzeHaen AG, SeelzeHanver, Germany) were used for column chromatography. Whatman filter papers (1 and 3 mm) were used for paper chromatography (Whatman, Maid stone, Kent, England). Whatman paper chromatography (No. 1) was performed using two systems: butanol/ acetic acid/ water at 4: 1: 5 (BAW) and 15% HOAc. Rotatory evaporator (Heidolph, Germany) used for concentration and drying of extracts and fractions. Ultraviolet lamp: (254, 365 nm, Vl, 6LC, Marine Lavalee-Cedex, France) used for localizing spots on paper, and column chromatography. ESI-MS was recorded by a Bruker APEX Ⅱ mass spectrometer. NMR spectra were recorded on a Bruker AMX 400 MHz, at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. Proton and carbon-13 NMR chemical shifts (δ) values were reported as ppm relative to tetramethylsilane in dimethyl sulfoxide-d_6 and *J* value in Hz.

2.3. Plant extraction and compounds identification

The dry leaves of *D. lutescens* (2.5 kg) were extracted with 70% ethanol followed by evaporation of the filtrate under reduced pressure, then defatted by petroleum ether and finally extracted with *n*-butanol, yielding a brown gum (200 g). 2D paper chromatography of the extract was performed and eluted with suitable solvent which revealed the presence of phenolic compounds, giving dark purple spots under UV light which turned orange or yellow when fumed with ammonia vapor, and giving a positive color when sprayed with $FeCl_3$, then the extract was fractionated on polyamide 6 CC. The column was eluted with H_2O-MeOH step gradient. The obtained fractions were subjected to paper chromatography using BAW and 15% AcOH as developing solvents, and the similar fractions were collected together to give four major fractions (Ⅰ-Ⅳ), which were examined by 2D paper chromatography. Fraction Ⅰ was obtained from the polyamide column by 20% methanol, which was separated on Sephadex LH-20 CC using H_2O-MeOH as a solvent giving four subfractions. The first subfraction was separated on Sephadex LH-20 CC using H_2O to afford compound 1 (60 mg). The second subfraction was eluted on Sephadex LH-20 CC starting with H_2O, then methanol with decreasing polarities to give compound 2 (40 mg), while the third one was eluted on Sephadex LH-20 CC using saturated butanol to afford compound 3 (50 mg). The fourth subfraction was eluted with H_2O-MeOH step gradient to give compound 4 (40 mg). Fraction Ⅱ was eluted from the polyamide column by 40% methanol, and applied on Sephadex LH-20 CC using mixtures of H_2O and MeOH to give two subfractions; the first subfraction was separated using H_2O, then the polarity decreased by adding MeOH to give compound 5 (20 mg). The second subfraction was fractionated on cellulose CC using H_2O-EtOH, to give pure compound 6 (19 mg). Fraction Ⅲ was obtained by 60% methanol from the polyamide column, and separated on preparative paper chromatography using 15% AcOH as eluent to give two compounds 7 (33 mg) and 8 (40 mg). Fraction Ⅳ was eluted from the polyamide column by 90% methanol and purified on Sephadex LH-20 CC using 80% methanol to afford two pure compounds 9 (25 mg), and 10 (20 mg). All isolated compounds were extra purified on Sephadex columns using equal ratio of methanol and water.

2.4. Animals

Albino female rats [(115.46 ± 5.72) g], from the Animal House of National Research Centre, were grouped and housed in polyacrylic cages (38 cm×23 cm×10 cm). Rats were kept and fed under normal conditions with dark and light cycle (12/12h) for a week before the experiment. The experimental procedure was approved by guidelines of the Institutional Animal Ethical Committee of National Research Centre (No.16-303), for project No. 11010325 on April 2016.

2.5. Acute toxicity study

This study was performed in two phases using twenty one rats according to Lorke[7]. In the first phase, rats were divided into three tested groups and one control group with three rats in each group. Rats in tested groups were administered with 10, 100 and 1 000 mg extract/kg body weight (b.w.) to determine the range of doses causing any toxic effect. All rats received a single dose of the extract after at least 5 d of adaptation. In the second phase, nine rats (three rats per dose) were further administered with extract at 1 600, 2 900 and 5 000 mg/kg b.w. to determine LD_{50} value. The extract was washed with PBS solution, dissolved in water and given orally. All rats were monitored frequently on the day of administration for 2 weeks for any sign of acute toxicity. The rats were sacrificed and then autopsied. The pathological changes of the internal organs were examined macroscopically. Weight gain is regarded as the indicator for the recovery from acute toxicity. After estimation of LD_{50}, the doses were selected for the hepatoprotective investigations using 5% and 10% of the highest dose examined which was 5 000 mg/kg b.w. So a low dose as 250 mg/kg and a high dose as 500 mg/kg of the extract were used.

2.6. Study design

Thirty rats were grouped into five groups of six rats in each group. The first group (normal control group) was administered with distilled water 5 mL/kg orally for 8 d. The second group (*D*-GaIN group as model group) was injected intraperitoneally with 200 mg/kg *D*-GaIN after 8 d of oral administration of distilled water. The third group (positive treatment group) received 100 mg/kg silymarin orally for 8 d. In the fourth group (*D*-GaIN + low dose of *D. lutescens* extract), low dose of the extract (250 mg/kg) was administered orally for 8 d, while, the fifth group (*D*-GaIN + high dose of *D. lutescens* extract) received high dose of the extract (500 mg/kg) orally for 8 d. On the eighth day (last day), the third, fourth and fifth groups received a single dose of *D*-GaIN (200 mg/kg *i.p.*) after 1 hour of extract administration.

2.7. Biochemical study

2.7.1. Liver functions tests

The activities of GOT, GPT, ALP, levels of serum total protein and total bilirubin were assessed according to previous literature[8-11], respectively.

2.7.2. Oxidant/antioxidant parameters assays

Serum nitric oxide (NO), MDA and paraoxinase 1 (PON1) activity were measured as previously described[12-14] respectively.

2.7.3. Inflammatory markers determination

Matrix metallopeptidase 1 (MMP1) levels were assessed using ELISA by Bio-Plex Pro™ Human MMP Panel kit (Bio-Rad, USA). The procedures were carried out according to the manufacturer instructions and results were recorded on Multi-Analyte Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad, USA). Tissue hyaluronic acid as the indicator of liver extracellular degradation was also estimated.

2.8. Histopathological study

Rats from different groups were sacrificed at the end of the experiment, rapidly dissected and the liver was removed and kept in 10% neutral formalin for 24 h, dehydrated in ascending grades of alcohol and embedded in paraffin. Sections at 4 μm thickness were taken, stained with hematoxylin and eosin (H&E) and examined under light microscope.

2.9. Statistical analysis

Statistical results were performed by one-way ANOVA, and then followed by the Tukey-Kramer test using Graph Pad software, InStat version 20. Results were reported as the means ± standard error of the mean (SEM). The significant level was set at P less than 0.05.

3. Results

3.1. Phenolic compounds from D. lutescens

Figure 1 illustrates ten phenolic compounds isolated from aqueous ethanolic extract of *D. lutescens* leaves including eight flavonoids: vicenin Ⅱ (1), prechafuroside B (4), violanthin (5), orientin (6), isoorientin (7), vitexin (8), apigenin (9), luteolin (10), together with two phenolic acids; gallic acid (2) and *p*-hydroxybenzoic acid (3) which were identified using different spectroscopic tools. Data of compounds 1, 4, 5 are listed in Table 1.

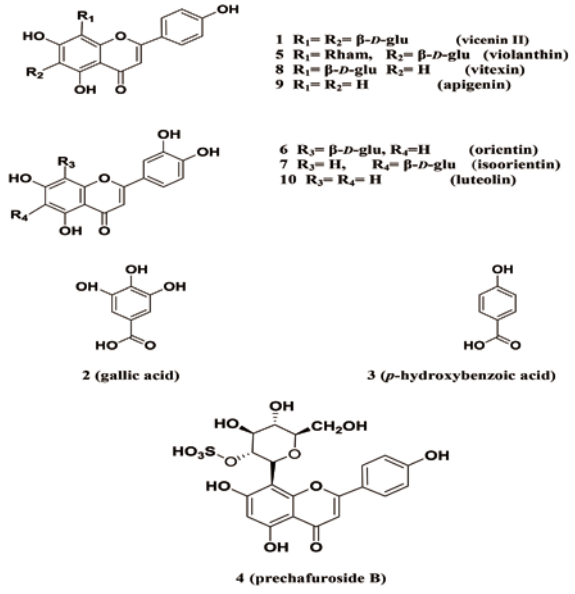

Figure 1. Chemical structures of isolated compounds.

3.2. Acute toxicity study

The extract did not produce any obvious toxic symptoms or mortality up to 5 000 mg/kg b.w. in all groups after 14 d suggesting that LD_{50} of the extract were higher than 5 000 mg/kg b.w.

3.3. Biochemical examination

D-GaIN significantly increased GOT, GPT, ALP, total protein, total bilirubin, NO, MDA, hyaluronic acid and MMP1 levels, and reduced PON1 enzyme levels as compared to control group (P<0.05) (Table 2-4). *D. lutescens* effectively restored these changes in dose dependence, which was similar to silymarin.

3.4. Histopathological examination

The light microscopy examination revealed normal hepatocytes radiating from the central vein and blood sinusoids with prominent nucleus in control group (Figure 2A). *D*-GaIN group showed disordered hepatic structure associated with centrilobular infiltration of mononuclear cells which led to severe centrilobular necrosis. Hepatocytes in these areas showed vacuolation. In addition, dilation, hemorrhage of sinusoids, activated Kupffer cells, pyknotic and apoptotic nuclei were also observed (Figure 2B). Silymarin group showed attenuation of hepatic damage and reduction of hepatocellular necrosis and inflammatory cell infiltration. Few activated Kupffer cells and dilated blood sinusoid were also observed (Figure 2C). Low dose of *D. lutescens* treated group moderately improved these changes (Figure 2D), while high dose of *D. lutescens*

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data for compounds (1, 4, 5) in DMSO-d_6.

Atom no.	Vicenin][(1)		Prechafuroside B (4)		Violanthin (5)	
	δ C	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)	δ C	δ H (*J* in Hz)
2	164.61		162.80		165.94	
3	100.92	6.82 (s)	102.20	6.67 (s)	102.08	6.77 (s)
4	180.36		182.01		182.27	
5	155.55		155.38		155.70	
6	107.96		98.40	6.13 (s)	109.82	
7	162.45		164.68		160.68	
8	105.44		104.59		105.64	
9	158.48		159.80		161.48	
10	103.02		104.20		102.80	
1′	121.90		121.01			
2′,6′	129.51	8.03 (d, 8.60)	129.19	8.02 (d, 8.71)		7.88 (d, 8.30)
3′,5′	116.34	6.92 (d, 8.60)	116.00	6.89 (d, 8.71)		6.84 (d, 8.30)
4′	161.76		161.50			
1″	74.21	4.88 (d, 9.94)	71.46	4.80 (d, 9.60)	73.91	4.62 (d, 9.30)
1‴	73.69	4.76 (d, 10.00)			77.33	4.91 (s)
2″	71.02		76.80	4.64 (dd, 9.90 & 10.40)	70.50	
3″	78.95		78.40		79.40	
4″	70.23	3.32-3.83 (m, 10 sugar protons)	71.00	3.33-3.80 (m, 4 sugar protons)	70.10	
5″	82.61		81.71		82.00	3.31-3.90 (m, sugar protons)
6″	61.65		61.80		61.52	
2‴	70.98				75.65	
3‴	77.91				74.81	
4‴	69.48				72.62	
5‴	82.32				72.18	
6‴	60.62				19.42	0.96 (d, 6.40)

d: doublet. s: singlet. m: multiplet. dd: doublet of doublet. *J*: coupling constant. DMSO: dimethyl sulfoxide.

Table 2. Liver functions tests.

Groups	Glutamate oxaloacetate transaminase (U/L)	Glutamate pyruvate transaminase (U/L)	Total protein (g/dL)	Alkaline phosphatase (U/L)	Total bilirubin (mg/dL)
Control	87.95±6.17	30.04±1.91	5.50±0.27	114.15±4.73	0.74±0.03
D-galactosamine	215.65±12.21*	81.64±3.66*	8.16±0.21*	217.05±8.88*	1.31±0.08*
Silymarin	84.39±18.81•	31.08±2.54•	6.05±0.18•	111.16±4.79•	0.81±0.03•
Dypsis lutescens (low dose)	170.46±15.90•	70.85±1.45•	6.33±0.26•	182.58±2.54•	1.03±0.03•
Dypsis lutescens (high dose)	143.26±9.08•	61.07±5.55•	4.88±0.33•	151.16±3.96•	0.79±0.03•

Data presented as mean ± SEM, *Significant at P< 0.05 compared to control group, •Significant at P< 0.05 compared to *D*-galactosamine group.

restored histological structure and the cytoplasmic vacuoles disappeared in most hepatocytes with minimized activated Kupffer cells and dilated blood sinusoid (Figure 2E).

Table 3. Oxidant/antioxidant parameters in different studied groups.

Groups	Nitric oxide (μmol/L)	Malondialdhyde (nanomol/mL)	Paraoxonase 1 (mg/dL)
Control	16.72±0.36	97.39±3.53	91.61±3.89
D-galactosamine	41.39±1.06*	241.30±5.83*	50.16±4.29*
Silymarin	17.25±0.49•	110.81±2.05•	92.20±4.37•
Dypsis lutescens (low dose)	30.19±2.61•	193.17±3.39•	66.18±3.35•
Dypsis lutescens (high dose)	26.56±2.29•	164.78±5.03•	55.91±3.56

Data presented as mean ± SEM, *Significant at $P < 0.05$ compared to control group, •Significant at $P < 0.05$ compared to *D*-galactosamine group.

Table 4. Inflammatory markers in different studied groups.

Groups	Hyaluronic acid (U/L)	Matrix metallopeptidase 1 (ng/mL)
Control	22.69±0.85	430.03±8.41
D-galactosamine	40.88±2.12*	874.11±53.93*
Silymarin	21.90±0.58•	461.81±17.23•
Dypsis lutescens (low dose)	37.78±1.26	814.51±20.25
Dypsis lutescens (high dose)	29.79±1.27•	629.18±18.64•

Data presented as mean ± SEM, *Significant at $P < 0.05$ compared to control group, •Significant at $P < 0.05$ compared to *D*-galactosamine group.

4. Discussion

Structural elucidation of compound 1 exhibited characteristic chromatographic behavior and UV spectral data of an apigenin di-*C*-glycoside[15]. ^{1}H NMR spectrum showed two *o*-doublets at δ 7.93 and 6.91 ppm, assignable to H-2′/6′ and H-3′/5′, respectively, of ring B. The observation of a singlet at 6.59 ppm of H-3 alongside the absence of H-8 and H-6 resonances has led us to expect an apigenin 6, 8-di-*C*-glycoside structure. Two anomeric proton doublets at 4.76 and 4.88 were confirmative documents for two β-*C*-glycoside residues. Another evidence for the 6, 8-di-*C*-glycosidation was deduced from the typical downfield location of ^{13}C-resonances of C-6 and C-8, and the intrinsic upfield location of C-1″ and C-1‴. Moreover, the total identity of 1 was proved by the positive HR-ESI-MS that showed a molecular ion peak at *m/z* 595.375 7 $[M+H]^+$, corresponding to a MF of $C_{27}H_{30}O_{15}$. According to the above discussed documents and comparison with the corresponding previous published data[16], the structure of 1 was finally confirmed as apigenin 6,8-di-*C*-β-D-$^4C_1$-glucopyranoside (vicenin Ⅱ), which was isolated for the first time from the genus *Dypsis*[4]. Compound 4 has shown some chromatographic and UV spectral properties of compound 1, referring to a tentative structure as an apigenin *C*-glycoside. In the aromatic region, its ^{1}H NMR spectrum gave the same splitting pattern as in case of compound 1 except for the appearance of a singlet at δ 6.13 interpretable to H-6 to conclude a tentative structure as an apigenin 8-*C*-glycoside for compound 4. Furthermore, the characteristic downfield location of H-2″ in

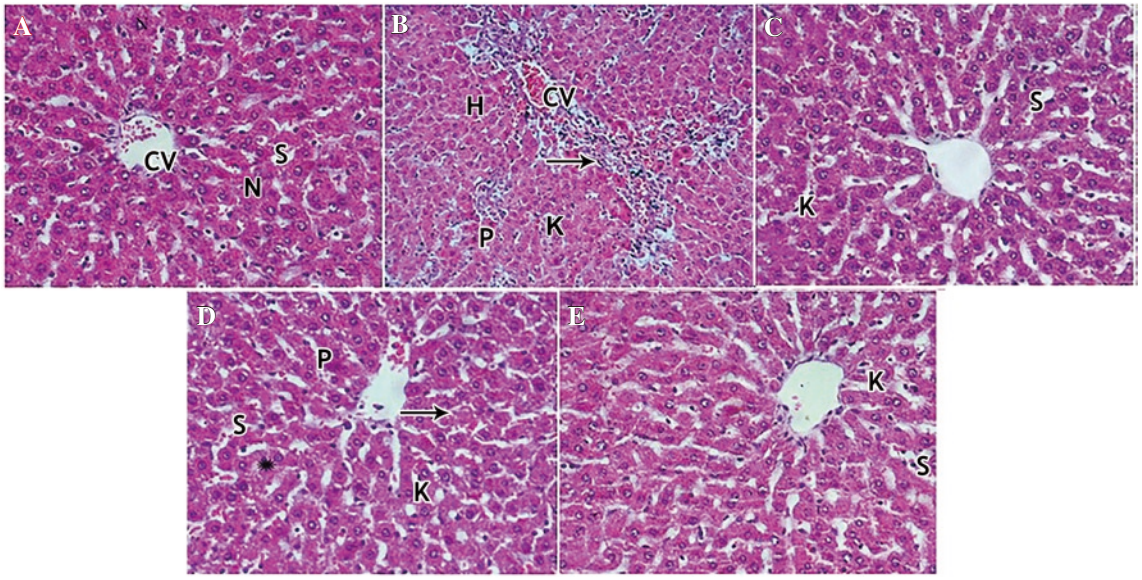

Figure 2. Photomicrographs of liver sections stained with haematoxylin and eosin. A: normal control group showing normal hepatic lobule, central vein (CV), hepatocytes with prominent nuclei (N) and hepatic sinusoids (S); B: *D*-galactosamine group showing distorted liver architecture and intense inflammatory cells infiltrating around central vein, hepatocellular necrosis (arrowhead) with hemorrhage between hepatocytes (H), activated kupffer cells (K) and pyknotic nuclei (P); C: *D*-galactosamine and silymarin group showing that hepatocytes mostly had normal appearance with minimal dilated of blood sinusoids (S) and activated kupffer cells (K) (H & E ×400), D: *D*-galactosamine + low dose of *Dypsis lutescens* extract group showing mild degeneration cells (star), dilated blood sinusoids (S) with activated kupffer cells (K) and pyknotic nuclei (P); E: *D*-galactosamine + high dose of *Dypsis lutescens* extract group showing restored hepatocytes architecture, with minimal dilated blood sinusoids (S) with activated kupffer cells (K) (H & E ×400).

the aliphatic region at 4.64 ppm was indicative of the probability for acylation of OH-2″. The presence of a sulphate group on C-2″ was established by negative ESI-MS analysis that showed a molecular ion peak at *m/z* 511.1 $[M-H]^-$ together with a fragment ion at 431.1 $[M-H-80]^-$, corresponding to the loss of sulphate group. Further confirmation for the structure of compound 4 was achieved from ^{13}C NMR, which showed a remarkable downfield shift of C-2″ to 76.80 due to the effect of the presence of sulphate group at C-2″. Depending on the mentioned data and its comparison with the corresponding literature[17], compound 4 was established as vitexin 2″-*O*-sulfate (prechafuroside B). ^1H and ^{13}C NMR spectral data of compound 5 were similar to the corresponding ones of compound 1, except for the observation of a broad singlet at 4.91 along with a doublet at 0.96 that were intrinsic signals for the anomeric (H-1‴) and methyl protons (CH_3-6‴), respectively, of an α-rhamnopyranosyl moiety. An additional evidence for the presence of a *C*-rhamnosyl moiety was deduced from ^{13}C-resonance of CH_3 at 19.40 ppm. Compound 5 was proved to be apigenin 6-*C*-β-D-$^4C_1$-glucopyranosyl-8-*C*-β-L-$^1C_4$-rhamnopyranoside (violanthin)[18]. The remaining compounds were identified using various spectroscopic tools of analysis and comparison of their data with the corresponding literature[19–25].

In this study, *D*-GaIN caused significant damage to liver functions. Elevated levels of liver enzymes are considered as indicators of liver damage. It may be due to that *D*-GaIN injection affects plasma membrane permeability, and then causes release of liver enzymes inside the cell, which leads to the increase in serum enzymes levels[26–28]. Liver injury induced by *D*-GaIN administration is also due to the extreme formation of reactive hydroxyl radical (OH^-), which is responsible for acute oxidative damage to the hepatic cells contents as lipids, proteins and DNA[29], and to the increased secretion of proinflammatory cytokines such as tumor necrosis factor-α and interkulin-1α[26]. Our results showed significantly increased NO and MDA levels and significantly reduced PON1 level, in *D*-GaIN group compared to control group. In addition, *D*-GaIN group showed a significant increase in hyaluronic acid level, which indicated liver injury[30]. Remarkable relations between plasma hyaluronic acid levels and degree of hepatic damage have been found in cirrhosis, alcoholic liver disease as well as chronic hepatitis C[31], and hyaluronic acid levels can be used to estimate liver function status. The significant increase of MMP1 in *D*-GaIN group was also observed. MMPs are important markers for diagnosis of inflammatory process and elevated MMP1 level indicates liver injury and inflammation. MMP1 is secreted by stromal and inflammatory cells, such as TNF-α, transforming growth factor β and IL-1α[32]. Infection followed by immoderate inflammation may cause tissue damage, and MMPs are implicated in immunopathological changes[33].

The decrease of MMP1 and hyaluronic acid levels in the treatment group indicated the ability of the high dose of *D. lutescens* to attenuate liver inflammation and injury. In the same line, the antioxidant properties of the *Dypsis* extract were observed in this study; thus, MDA and NO levels were significantly decreased in the treatment groups compared to *D*-GaIN group. Surprisingly, the antioxidant enzyme (PON1) was significantly increased only in the low dose of *D. lutescens*.

The antioxidant and anti-inflammatory properties of *D. lutescens* help in maintaining liver health and functions as observed in our results. From the light of these results, we concluded that the hepatoprotective actions of *D. lutescens* may be attributed to a combination of different phytochemicals and bioactive constituents such as flavonoids, coumarins, sterols, terpenoids, quinones and alkaloids. These bioactive constituents are synergistic in their action, and such diversity of these compounds magnifies their biological properties. It is known that *D*-GaIN promoted the oxidative stress through induction of ROS generation[34,35]. Besides, it consumed antioxidant compounds in the liver[36], and produced free radicals that induced LPO[37], which led to liver injury and elevation of all hepatic markers levels[38]. Various studies suggested that antioxidant effects of magniferin, flavonoids and lycopene reduced acute liver injury induced by *D*-GaIN[39,40].

Our findings also showed that administration of *D. lutescens* suppressed the histopathological hepatic damage in the *D*-GalN group. These results were confirmed with the biochemical results. Therefore, it can be concluded that *D. lutescens* supplements can inhibit *D*-GaIN stimulated liver damage.

Conflict of interest statement

There are no conflicts of interest associated with this publication.

Funding

The research fund for this work has been received from the National Research Centre, Egypt (project No. 11010325).

Authors' contributions

MME and WAE were responsible for collection and extraction of the plant, and isolation of the compounds. Both MIN and TKM contributed in isolation of the compounds and interpretation of the spectroscopic data. EAO and AHF performed the histopathological studies. JH carried out the hepatoprotective, antioxidant and anti-inflammatory assays and wrote the biological part of the manuscript. MYE suggested the plan of work and contributed in writing the manuscript.

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