Arabian Journal of Chemistry (2016) 9, 649–655

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

A new cytotoxic ceramide from *Heteroxenia ghardaqensis* and protective effect of chloroform extract against cadmium toxicity in rats

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Received 29 June 2014; accepted 24 November 2014
Available online 8 December 2014

KEYWORDS

Heteroxenia ghardaqensis;
Ceramide;
Adrenal toxicity;
Cytotoxicity

Abstract A new ceramide 2*S*,3*R*-4*E*,8*E*-2-(hexadecanoylamino)-docosa-4,8-diene-1,3-diol **(1)**, along with two known ceramides, namely, 2*S*,3*R*-4*E*,8*E*-2-(octadecanoylamino)-octadeca -4,8-diene-1,3-diol **(2)** and 2*S*,3*R*-4*E*-2-(octadecanoylamino)-octadec-4-ene-1-ol **(3)** were isolated from the chloroform extract of the Egyptian Red Sea soft coral *Heteroxenia ghardaqensis*. Additionally, cholesterol, cholesterol-3*β*-acetate, 7β-hydroxy cholesterol, cosanyl octadecanoate, and hexadecanyl octadecanoate were also isolated. The isolated compounds were identified by spectroscopic methods involving 1D, 2D-NMR and ESI-MS. The ceramides **(1)** and **(2)** showed moderate cytotoxic activity as growth inhibitors of human hept-G2 cancer cell lines. The protective action of the chloroform extract on cadmium-induced adrenal toxicity was observed by the decrease of the hydropic degeneration in the cortex and normal medulla.

1. Introduction

The Red Sea ecosystem represents one of the most promising areas as a source of medicinal and nutritional natural products (Elshamy et al., 2013). Soft corals (Octocorallia, Alcyonacea) contain not only diverse classes of secondary metabolites. The extracts of soft corals have various pharmacological activities such as antitumor, antibacterial, antiviral, antifungal, anti-inflammatory, antipyretic, hypoglycaemic and antioxidant (Edrada et al., 2000; Mohamed et al., 2012). The soft

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.11.055

corals belonging to the genus *Heteroxenia* are rich in sesquiterpenoids, diterpenes, ceramides, acylglycerols and sterols (Elshamy et al., 2013; Edrada et al., 2000; Mohamed et al., 2012; Kashman et al., 1978).

Cadmium (Cd^{2+}) is a ubiquitous industrial and environmental pollutant that accumulates in humans. The tissues that accumulate Cd^{2+} include the kidneys, lungs, reproductive organs and nervous system. Cd^{2+} causes genotoxicity and cancer in some tissues and nonmalignant chronic toxicity in others. Cadmium accumulates in the adrenal gland after entering the body. The order of important organs that contain cadmium is adrenal gland > liver > kidney > hypothalamus > cerebral cortex, with the adrenal gland ranked as No. 1 (Mithal, 1981; Leal et al., 2007; Mukhtar et al., 2002).

Continuing our work on *H. ghardaqensis* (Elshamy et al., 2013), and in our target for finding new leading bioactive materials from some Egyptian Red Sea marine organisms. The present study deals with the isolation and identification of cytotoxic ceramides and other secondary metabolites. Additionally, the protective role of $CHCl_3$ extract against adrenal glands toxicity induced by cadmium in rats was evaluated.

2. Experimental

2.1. General experimental procedures

Optical rotation was measured by Jasco P2000 polarimeter. IR spectra were carried out on a Nexus 670 FT-Ramen spectrophotometer with KBr discs. NMR spectra were measured on Mercury Bruker AV 600 MHz spectrometer with TMS as internal standard. ESI-MS spectra were measured on JEOL JMS-AX 500. Silica gel (200–300 mesh) was used for column chromatography, pre-coated silica gel 60 GF254 plates were used for PTLC, Sephadex LH-20 column chromatography was used for purification. The TLC plates were visualized by spraying with ceric (IV) sulphate, vanillin/H_2SO_4 and MeOH/H_2SO_4 (3:1) reagents followed by heating at 70 °C for ceramides detection.

GC–MS analysis was performed on a Varian gas chromatograph interface to SSQ 3400 coupled to mass selective detector, the columns used were a DB5, 30M, ×0.25 mm, 0.5 Mm film thickness. Injector and ion source temperature was 220 °C, the ionization energy was set at 70 eV, and the volume injected was 0.88 μl at 270 °C. The oven temperature was programmed from 50 °C for 32 min, isothermal, then heating by 10 °C/min to 150 °C, isothermal, then heating by 5 °C/min to 270 °C, and isothermally for 3 min at 270 °C.

2.2. Animal material

The soft coral *H. ghardaqensis* was collected from the Red Sea on May 2010, at a depth of 3–4 m at the front of Hurghada marine station of National Institute of Oceanography and Fisheries, Hurghada, Egypt. The soft coral was collected and identified by Dr. Hashem Madkour and Dr. Tarek Abdel-Aziz, National Institute of Oceanography and Fisheries, Hurghada, Egypt. A voucher sample was deposited at the National Research Center under the code HG-28.

2.3. Extraction and isolation

The frozen marine organism (wet weight 2.0 kg) was broken down into small pieces and extracted at room temperature with sufficient amount of chloroform three times. After filtration, the chloroform extract was concentrated under reduced pressure at 50 °C. The residue (15.0 g) was subjected to silica gel (250 g) column (50 cm $L \times 6$ cm D), eluted with petroleum ether (40–60)/EtOAc with increasing polarity (200 ml each fraction) to give 32 fractions which were finally collected to seven subfractions after examination by TLC. The fraction eluted by solvent ratio (7:3) was subjected to PTLC, developed with *n*-hexane/EtOAc (1:1, Rf 0.43) and then finally purified on Sephadex LH-20, using methanol to afford compound **1** (15 mg). Similarly, the known ceramides **2, 3** and the other compounds were isolated and purified using different chromatographic techniques.

2.4. Methanolysis of ceramides

Each ceramide compound (5 mg) was refluxed with 1.5 ml of 0.9 N HCl in 82% methanol for 16 h. The reaction mixture was cooled and extracted with 20 ml *n*-hexane. The *n*-hexane layer was concentrated and gave the fatty acid methyl ester which could be identified through GC–MS analysis (Li et al., 2007).

2.5. Spectroscopic data of ceramides

2.5.1. 2S,3R-4E,8E-2-(hexadecanoylamino)-docosa-4,8-diene 1,3-diol (1)

Yellow powder, mp 80.0–82.0 °C, $[\alpha]_D^{25}$ – 7.6° (c 0.01, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3416, 2956, 2919, 2850, 1642, 1618, 1049, 964, 720, 621. HRESI-MS $(M + Na)^+$; at m/z 615.5245 (M + Na, 100%), 593.4753 (M + 1, 20%), ESI-MS: 593 (M + 1, 100%), 522 (35%), 507 (15%), 481 (10%), 423 (20%), 395 (80%), 368 (60%), 328 (25%), 298 (20%), 255 (15%), 223 (25%), 198 (70%). ^{1}H NMR ($CDCl_3$, 600 MHz): 3.68 (dd, J = 11.7, 3.8, H-1a, 1H), 3.88 (dd, J = 11.7, 3.5, H-1b, 1H), 3.90 (m, H-2, 1H), 4.29 (m, H-3, 1H), 5.36 (dd, J = 15.8, 6.18, H-4, 1H), 5.76 (dt, J = 15.8, 6.18, H-5, 1H), 2.06 (m, H-6, 2H), 2.10 (m, H-7, 2H), 5.40 (dt, J = 15.2, 6.54, H-8, 1H), 5.52 (dt, J = 15.2, 6.5, H-9, 1H), 1.94 (m, H-10, 2H), 2.22 (t, J = 7.5, H-2′, 2H), 1.62 (m, H-3′, 2H), 1.28 (br s, n CH_2), 0.86 (t, J = 6.8, H-22 and 16′, 6H), 6.27 (d, J = 7.5, NH, 1H)), 2.93 (br s, OH-1, 3). ^{13}C NMR ($CDCl_3$, 150 MHz): 62.5 (C-1), 54.5 (C-2), 76.8 (C-3), 129.0 (C-4), 133.6 (C-5), 32.0 (C-6), 32.2 (C-7), 131.4 (C-8), 129.2 (C-9), 32.7 (C-10), 174.1 (C-1‘), 36.9 (C-2‘), 25.8 (C-3′), 32.4 (C14′, C-20), 22.7 (C-15′, C-21), 29.3–29.8 (n CH_2), 14.2 (C-22, 16′). GC–MS of fatty acid methyl ester **1B**: MS m/z: 270, 251, 227, 185, 158, 143, 130, 101, 87, 74, 69, and 55.

2.5.2. 2S,3R-4E-2-(octadecanoylamino)-octadec-4-ene-1-ol (3)

White amorphous solid, mp 78.0–79.0 °C. IR ν_{max} (KBr) cm^{-1}; 3445, 2922, 2853, 1642, 723. Positive ESI-MS $(M + H)^+$; at m/z 550 (M^+, 100%,), 507 (45%), 481 (35%), 381 (25%), 325 (55%), 283 (80%), 269 (75%), 225 (60%), 168 (30%). ^{1}H NMR ($CDCl_3$, 600 MHz): 3.64 (d, J = 5.5, 3.8, H-1a,

1H), 3.85 (m, H-1b, 1H), 4.02 (m, H-2, 1H), 2.25 (m, H-3, 2H), 5.30 (m, H-4, 1H), 5.35 (m, H-5, 1H), 1.98 (m, H-6, 2H), 2.28 (t, J = 7.5, H-2′, 2H), 1.58 (m, H-3′, 2H), 1.26 (brs, n CH_2), 0.86 (t, J = 7.5, H-18 and 18′, 6H), 5.39 (d, J = 7.0, NH, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz): 64.5 (C-1), 50.8 (C-2), 34.8 (C-3), 129.8 (C-4), 130.1 (C-5), 32.0 (C-6), 174.1 (C-1′), 36.6 (C-2′), 25.8 (C-3′), 26.0 (C-3′), 29.3–29.8 (n CH_2), 32.4 (C-16 and 16′), 22.7 (C-17, 17′), 14.2 (C-18, 18′).

2.6. Cytotoxic activity

2.6.1. Cell culture

Human hepatocarcinoma cell lines (Hep-G2), which were purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested compounds. Cells were routinely cultured in Eagle's Minimum Essential Medium which was supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 μg/ml amphotericin B. Cells were maintained at sub-confluence at 37 °C in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37 °C. Cells were used when confluence had reached 75%. Tested ceramides were weighed, dissolved in dimethyl sulphoxide (DMSO), and diluted thousand times in the assay to begin with the intended concentration. All cell culture materials were obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were purchased from Sigma/Aldrich, USA. All experiments were repeated three times.

2.6.2. Reagents preparation

MTT solution: 5 mg/ml of MTT in 0.9% NaCl. Acidified isopropanol: 0.04 N HCl in absolute isopropanol.

2.6.3. Calculation

Percentage of relative viability (V) was calculated using the following equation:

$$V\% = A_{\text{treated}}/A_{\text{cont}} \times 100$$

where V%: Percentage of relative viability, A_{treated}: Absorbance of treated cells, A_{cont}: Absorbance of control cells.

The half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve.

2.6.4. Anti-tumour activity

Cytotoxicity of tested ceramides (1 and 2) against HepG2 was measured using the MTT cell viability assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and forms a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue colour. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm (Hansen et al., 1989).

Cells (0.5 × 105 cells/well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 μl of serial dilutions of the tested compounds for 48 h at 37 °C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 μl MTT solution/well was added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 μl of acidified isopropanol/well and plate was shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control for the solid sample only, with cytotoxicity indicated by $<100\%$ relative viability.

2.7. Protective role of chloroform extract of the coral against cadmium-induced adrenal glands toxicity

2.7.1. Experimental animals

Thirty male rats were used and divided into 6 groups (5 rats/group). **Group I**: the control group, **group II**: rats given one dose of chloroform extract (60 mg/kg b.w.) for 10 days, **group III:** rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) (Koriem et al., 2009) for 10 days, **group IV**: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract of coral (20 mg/kg b.w.) for 10 days, **group V**: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract of coral (40 mg/kg b.w.) for 10 days, **group VI**: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract of coral (60 mg/kg b.w.) for 10 days.

2.7.2. Experimental design

Thirty male rats were used and divided into 5 groups (6 rats/group). Group I: the control group, group II: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) (Koriem et al., 2009) for 10 days, group III: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract (20 mg/kg b.w.) for 10 days, group IV: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract (40 mg/kg b.w.) for 10 days, group V: rats given one dose of cadmium chloride daily equivalent to 1/10 LD_{50} (8.8 mg/kg b.w.) and chloroform extract (60 mg/kg b.w.) for 10 days.

2.7.3. Histopathological study of adrenal glands

Adrenal glands were dissected out and fixed instantaneously in 10% formal saline for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (m.p 55–60 °C). Paraffin (m.p. 55–60 °C) sections of 6 μm thicknesses were prepared and stained with haematoxylin and eosin (Koriem et al., 2009). In this method the paraffin sections were stained in haematoxylin for 5 min. Sections were washed in running water for bluing and then stained in 1% watery eosin for 2 min, washed in water, dehydrated, cleared and mounted in Canada balsam. The cytoplasm stained shades of pink and red and the nuclei gave blue colour.

3. Results and discussion

A new ceramide, 2*S*,3*R*-4*E*,8*E*-2-(hexadecanoylamino)-docosa-4,8-diene-1,3-diol **(1)**, along with two known ceramides, namely, 2*S*,3*R*-4*E*,8*E*-2-(octadecanoylamino)-octadeca-4,8-diene-1,3-diol **(2)** (Inagaki et al., 2004), 2*S*,3*R*-4*E*-2-(octadecanoylamino)-octadec-4-ene-1-ol **(3)** (Tian et al., 2001) was isolated from the chloroform extract of *H. ghardaqensis* (Fig. 1A). This is the first report of the spectroscopic data of ceramide **(3)**.

Ceramide (**1**) was obtained as white crystals (15 mg, mp 80–82 °C $[\alpha]_D^{25}$ – 7.6° (c 0.01, $CHCl_3$)), HRESI-MS and ESI-MS of compound **(1)** showed a pseudo-molecular ion peak $[M + Na]^+$ at *m/z*: 615.5245, $[M + H]^+$ at *m/z*: 593.4753, and ESI-MS: $[M + H]^+$ at *m/z*: 593, corresponding to the molecular formula $C_{38}H_{74}NO_3$. IR spectrum indicated the presence of secondary amide at ν_{max} 1642 cm^{-1}, and hydroxyl groups at ν_{max} 3316 cm^{-1}. The ^1H NMR and ^{13}C NMR spectral data of **(1)** indicated that it had a ceramide like structure. The ^1H NMR spectrum showed signals at δ 4.29 (t, H-3), δ 3.90 (m, H-2), δ 3.88 (dd, H-1b), δ 3.68 (dd, H-1a) which agree well with those reported for *N-acyl-D*-erythro-sphingosine (Huang et al., 2010; Liu et al., 2010). The spectrum showed proton signals for four olefinic protons at δ 5.36 (dd, H-9), δ 5.40 (dd, H-8), δ 5. 52 (dd, H-4) and δ 5.76 (dd, H-5). The *E* geometry was deduced from the *trans* coupling constants for H-4/H-5, H-8/H-9 and comparing these values with those reported before (Wang et al., 2000; Phillips et al., 2009). ^{13}C NMR spectrum showed 18 + n CH_2 carbon resonances. The spectrum revealed the presence of carbonyl carbon at δ 174.1, as well as the four olefinic carbon signals at δ 129.0 (C-4), 129.2 (C-8), 131.4 (C-9) and 133.6 (C-5). The signals of two methyl groups at δ 0.88 (6H, t, J = 7 Hz) and the very strong signal of polymethylene (CH_2)n at δ 1.28 (br s) in the ^1H NMR spectrum but lack of upfield methine and tertiary carbon signals in the ^{13}C NMR spectrum revealed that **1** must contain two long branchless carbon chains. It also showed signal for carbon adjacent to an amide group at δ 54.5. DEPT spectrum showed two methyl signals, 9 + n CH_2 methylene signals and 7 methine groups. The protonated carbons were assigned by HMQC experiment.

Location of double bonds was established by ^1H,^1H-COSY and HMBC (Fig 2). In ^1H-^1H COSY, there were correlations for H-4/H-3, H-5/H-6, and H-7/H-8. In HMBC experiment, there were correlations for H-3/C-5 (J^3) and H-4/C-3 (J^2) which confirmed the location of double bond at C-4. Furthermore, the location of other double bond at C-8 was deduced by correlations of H-8/C-7 (J^2), H-9/C-10 (J^2) and H-9/C-7 (J^3). The relative stereochemistry of ceramide **(1)** was assigned by comparing the coupling constants values with those previously reported (Huang et al., 2010; Yue et al., 2001; Liu et al., 2010; Han et al., 2005).

The length of the long chain base (LCB) and the fatty acid moiety (FA) were determined by ESI-MS and methanolysis. Two diagnostic fragment aliphatic ions at *m/z* 394 (calcd for $C_{24}H_{44}NO_3$: 393) and at *m/z* 198 (calcd for $C_{14}H_{29}$: 197) were observed due to α-cleavage (McLafferty rearrangement). This indicated the presence of palmitic acid as FA in compound **(1)**. Furthermore, the secondary amide position was assigned on basis of typical fragment ions at *m/z* 256 and *m/z* 338 which were formed by α-cleavage and aliphatic chain fragment. Moreover, the Δ 4,5 and Δ 8,9 double bonds were confirmed on allylic cleavage fragment ions at *m/z* 368 $[C_{22}H_{42}NO_3]^+$ and 224 $[C_{16}H_{31}]^+$, which were formed by elimination of dodecene (Fig 3).

Methanolysis of compound **(1)** yielded sphingosine **(1a)** and fatty acid methyl ester **(1b)** (Fig. 1B). The fatty acid methyl ester **(1b)** was analysed using GC/MS. The GC/MS showed that the molecular ion peak was at *m/z* 270 ($C_{16}H_{32}OCH_3$, Rt 39.83) methyl hexadecanoate). Thus, the lengths of the sphingosine and the fatty chain moieties of compound **(1)** were unambiguously determined as C22 and C16 for amino alcohol (sphingosine) and fatty acid moieties, respectively. According to the above mentioned data, ceramide **(1)** was characterized as 2*S*,3*R*-4*E*,8*E*-2-(hexadecanoylamino)-docosa-4,8-diene-1,3-diol which is reported here for the first time in nature.

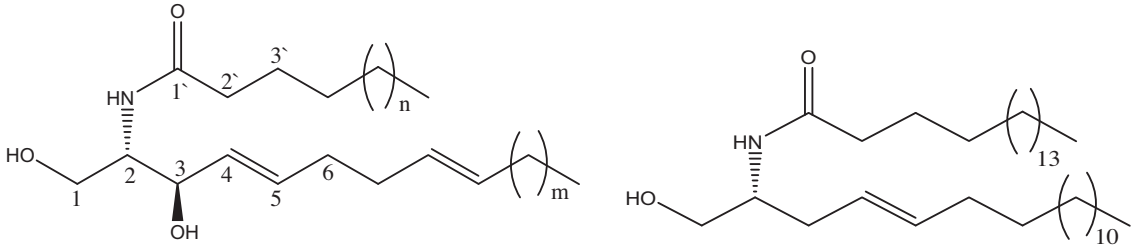
3.1. Cytotoxic activity of ceramides

Using MTT assay, the effect of the two ceramides **(1)** and **(2)** on the proliferation of Hep-G2 cells was studied after 48 h of incubation. The treatment of Hep-G2 cells with compounds **(1)** and **(2)** showed moderate cytotoxic effect against Hep-G2 as concluded from their IC_{50} values that equal to 144.3 and 147.68 μg/ml (Fig. 4A and B, respectively).

3.2. Role of chloroform extract of the coral against cadmium-induced adrenal toxicity

3.2.1. Histopathological results

Microscopic examination of the adrenal glands of control and rats that administered with chloroform extract of coral



1 n = 11, m = 12

2 n = 13, m = 8

3

Figure 1A Structures of isolated ceramides.

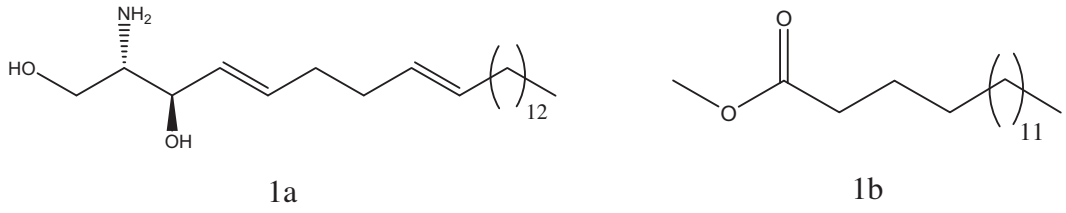


Figure 1B Structures of methanolysis products of a new ceramide **1**.

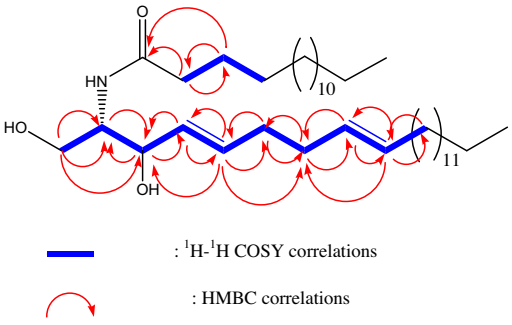


Figure 2 ^{1}H-^{1}H COSY and key HMBC correlations of ceramide 1.

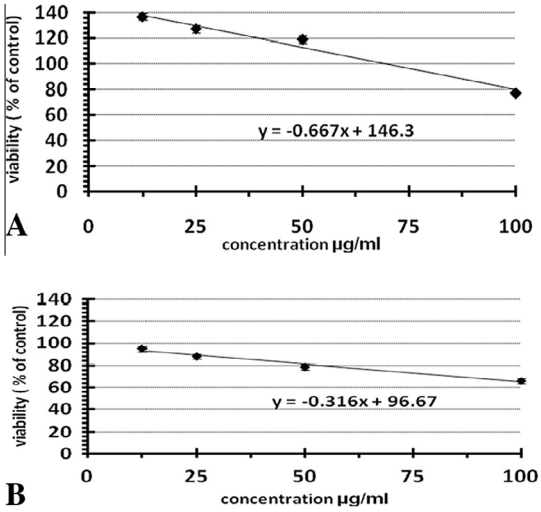


Figure 4 Cytotoxic effect of ceramides **1** and **2** against Hept-G2 cells using MTT assay (n = 4), data expressed as the mean value of cell viability (% of control) ± S.D.

(60 mg/kg/bw) showed the normal structure, outer capsule, zona glomerulosa, sudanophobe layer, zona fasciculate, zona reticularis of the cortex and medulla (Fig. 5A and B). Examination of sections of adrenal glands of rats treated with $CdCl_2$ (8.8 mg/kg b.w.) showed hydropic degeneration, focal necrosis in the adrenal cortex and disturbance of the medulla (Fig. 5C), congestion and haemorrhagic area in the medulla (Fig. 5D). Sections of adrenal glands of rats treated with of $CdCl_2$ and chloroform extract of coral (20 and 40 mg/kg/b.w.) showed mild hydropic degeneration in the cortex (Fig. 5E and F, respectively). Sections of adrenal gland of rats treated with of $CdCl_2$ and chloroform extract of coral (60 mg/kg/bw) showed mild hydropic degeneration in the cortex and normal medulla (Fig. 5G).

The histopathological study showed that distraction of cadmium chloride for ten days caused hydropic degeneration, focal necrosis in the adrenal cortex as well as congestion and haemorrhagic area in the medulla. These results were in accordance with (Mithal, 1981; Abdel-Aziem et al., 2001; Jin et al., 2013). Cadmium intoxication caused congested blood vessels, haemorrhage just adjacent to the medulla and circumscribed haemorrhage in cortex. Also it was reported that cadmium has degenerative effect on the endothelium of blood vessels. On the other hand, the same author suggested that haemorrhage has occurred due to the effect of cadmium (Mithal, 1981).

The histopathological investigation showed that the toxic effects of cadmium in rats treated with chloroform extract of coral (20, 40, and 60 mg/kg/bw) were found to be reduced as presented by decrease the hydropic degeneration in the cortex and normal medulla. The decreasing of the cadmium chloride toxicity can be attributed to the presence of gorgostane sterols (Elshamy et al., 2013), cholestane sterols, ceramides, and fatty acid esters (Inagaki et al., 2004; Djeridane et al., 2010; Mohamed et al., 2012).

The sterol content plays a basic role in the biological activity of the chloroform extract because of its high antioxidant activity

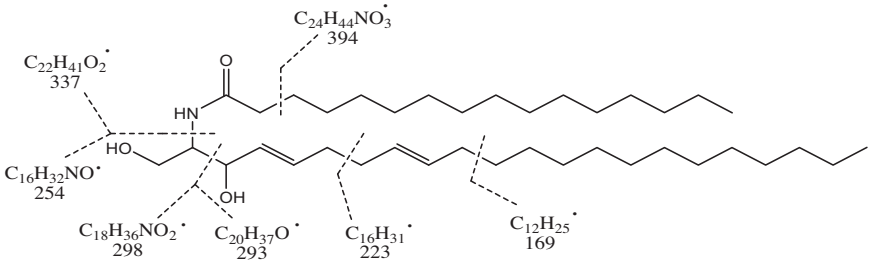


Figure 3 ESI-MS fragmentation of ceramide 1.

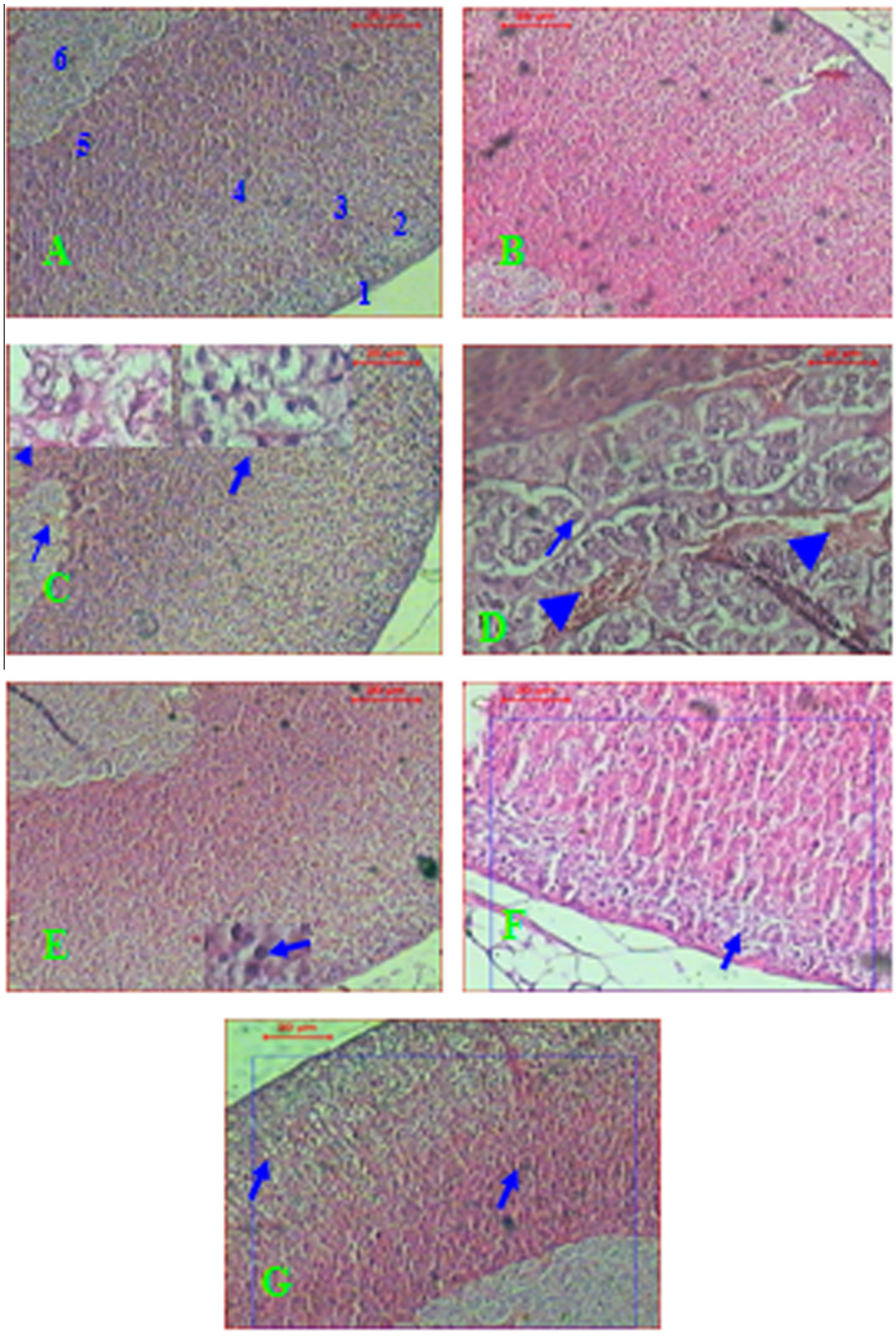


Figure 5 Sections of adrenal glands of (A) control rat shows normal structure. Notice 1 – outer capsule of the adrenal gland, 2 – zona glomerulosa of the cortex, 3 – sudanophobe layer of the cortex, 4 – zona fasciculata of the cortex, 5 – zona reticularis of the cortex and 6 – medulla, (B) rat treated with chloroform extract of coral (60 mg/kg/bw) shows normal of the cortex and normal medulla, (C) rat treated with $CdCl_2$ shows hydropic degeneration (arrow) and focal necrosis (arrowhead) in the adrenal cortex and disturbance of the medulla (thin arrow). (D) Rat treated with $CdCl_2$ shows congestion (arrow) and haemorrhagic area (arrowhead) in the medulla. (E) Rat treated with of $CdCl_2$ and chloroform extract of coral (20 mg/kg/bw) shows mild hydropic degeneration (arrow) in the cortex and normal structure of medulla. (F) Rat treated with of $CdCl_2$ and chloroform extract of coral (40 mg/kg/bw) shows mild hydropic degeneration (arrow) in the cortex. (G) Rat treated with of $CdCl_2$ and chloroform extract of coral (60 mg/kg/bw) shows mild hydropic degeneration in the cortex and normal medulla (H & E stain, bar: 20 μm).

(Djeridane et al., 2010). It was reported that the alcoholic extract of *H. fuscescens* has moderate antioxidant activity that related to the isolated sterols, especially gorgostane derivatives, ceramides and long chains (Elshamy et al., 2013; Mohamed et al., 2012).

Huang et al. (2010) stated that the high ceramide content increases antioxidant activity. Also, significant attenuation against the Cd-induced hepatotoxicity was found due to its ceramides content (Prabu et al., 2012; Smalinskiene et al.,

2009). The presence of saturated fatty acid esters plays an important role as antioxidant agents because they are rich in fatty acids and their esters (De La Cruz et al., 1999).

4. Conclusion

A new ceramide, 2*S*,3*R*-4*E*,8*E*-2-(hexadecanoylamino)-docosa-4,8-diene-1,3-diol **(1)**, along with seven known compounds was isolated from the $CHCl_3$ extract of soft coral *H. ghardaqensis*. The two ceramides **(1)** and **(2)** showed moderate cytotoxic activity as growth inhibitors of human hep-G2 cancer cell lines. The protective action of the $CHCl_3$ extract on cadmium-induced adrenal toxicity was observed by the decrease of the hydropic degeneration in the cortex and normal medulla. The protective role of *H. ghardaqensis* could be useful in the adrenal gland protection against cadmium.

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