Chemical composition and therapeutic potential of three Cycas species in brain damage and pancreatitis provoked by gamma-radiation exposure in rats

Ismail, Ahmed; Hassan, Hossam M.; Moawad, Abeer S.; Abdel Fattah, Salma M.; Sherif, Noheir H.; Abdelmohsen, Usama R.; Radwan, Mohamed M.; Rateb, Mostafa E.; Hetta, Mona H.

Published in:
Journal of Radiation Research and Applied Sciences

DOI:
10.1080/16878507.2020.1722908

Published: 19/02/2020

Document Version
Publisher's PDF, also known as Version of record

Journal of Radiation Research and Applied Sciences

ISSN: (Print) 1687-8507 (Online) Journal homepage: https://www.tandfonline.com/loi/trra20

Chemical composition and therapeutic potential of three *Cycas* species in brain damage and pancreatitis provoked by γ-radiation exposure in rats

Ahmed Ismail, Hossam M. Hassan, Abeer S. Moawad, Salma M. Abdel Fattah, Noheir H. Sherif, Usama R. Abdelmohsen, Mohamed M. Radwan, Mostafa E. Rateb & Mona H. Hetta

To cite this article: Ahmed Ismail, Hossam M. Hassan, Abeer S. Moawad, Salma M. Abdel Fattah, Noheir H. Sherif, Usama R. Abdelmohsen, Mohamed M. Radwan, Mostafa E. Rateb & Mona H. Hetta (2020) Chemical composition and therapeutic potential of three *Cycas* species in brain damage and pancreatitis provoked by γ-radiation exposure in rats, Journal of Radiation Research and Applied Sciences, 13:1, 200-214, DOI: 10.1080/16878507.2020.1722908

To link to this article: https://doi.org/10.1080/16878507.2020.1722908

Published online: 19 Feb 2020.

JOURNAL OF RADIATION RESEARCH AND APPLIED SCIENCES
2020, VOL. 13, NO. 1, 200–214
https://doi.org/10.1080/16878507.2020.1722908

Chemical composition and therapeutic potential of three *Cycas* species in brain damage and pancreatitis provoked by γ-radiation exposure in rats

Ahmed Ismail[b], Hossam M. Hassan[a], Abeer S. Moawad[a], Salma M. Abdel Fattah[c], Noheir H. Sherif[c,i], Usama R. Abdelmohsen[d,e], Mohamed M. Radwan[f,g], Mostafa E. Rateb[a,h] and Mona H. Hetta[b]

[a]Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Banī Suwayf, Egypt; [b]Pharmacognosy Department, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt; [c]Drug Radiation Research Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt; [d]Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt; [e]Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, Universities Zone, New Minia City, Egypt; [f]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, USA; [g]Pharmacognosy Department, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt; [h]School of Computing, Engineering & Physical Sciences, University of the West of Scotland, Paisley, UK; [i]Pharmacognosy Department,Faculty of Pharmacy, Nahda University, Banī Suwayf, Egypt

ABSTRACT

Metabolomic profiling of the crude extracts of the three *Cycas* plants leaves; *Cycas armstrongii* Miq., *Cycas circinalis* L., and *Cycas revoluta* Thunb. showed the presence of diverse secondary metabolites. A detailed phytochemical study of *C. armstrongii* fractions led to isolation of 15 known components of different classes, but this is the first time to report them for this species. These components were chemically identified as (naringenin, dihydroamentoflavone, 2,3-dihydrohinokiflavone, amentoflavone, 2,3-dihydrobilobetin, isoginkgetin, prunin, naringin, vanillic acid, *p*-coumaric acid, *β*-sitosterol, stigmasterol, *β*-sitosterol glucoside, 3,7,9,11-tetramethyl heptadecanoic acid, and *N*-(3′-one-5′-methyl)-hexyl-alanine). Moreover, the radioprotective potential of the three *Cycas* plant species was also investigated, and the ionizing radiation was performed by the exposure of rats whole-body to 8 Gy. The extracts of the three *Cycas* species were administered at a dose of 200 mg/kg each using an intra-gastric tube. Results indicated that *Cycas* spp. extracts significantly ameliorated radiation-induced brain and pancreatic damage as well as showing protection against radiation-induced oxidative stress. The results were also proved by histopathological study.

ARTICLE HISTORY
Received 21 November 2019
Accepted 26 December 2019

KEYWORDS
Cycas; metabolomics; ionizing radiation; brain; pancreas; histopathology

1. Introduction

Cycas is known to be the only genus of family Cycadaceae and represented in Egypt, by nine species; *C. armstrongii* Miq., *C. revoluta* Thunb., *C. circinalis* L., *C. litoralis* K.D. Hill, *C. thouarsii* R.Br., *C. media* R.Br, *C. tansachana* K.D. Hill, *C. rumphii* Miq., and *C. pectinata* Griff. In a survey for benzoic and cinnamic acids and their derivatives in the Cycadaceae, the presence of caffeic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, protocatechuic, and ferulic acids in *C. revoluta* and *C. circinalis* leaves was reported (Wallace, 1972).

Previous chemical study of the constituents of *C. circinalis* L. and *C. revoluta* Thunb. led to the isolation and identification of 2 dihydrobiflavone glycosides, 15 biflavonoids, 4 flavan-3-ols, 6 flavonoid glycosides, 3 lignans, 2 nor-isoprenoids, and 1 flavanone (Moawad et al., 2010; Moawad, Hetta, Zjawiony, Hifnawy, & Ferreira, 2014). Both *C. revoluta* Thunb. and *C. circinalis* L. proved to exhibit cytotoxic, antimicrobial, and antiprotozoal activities (Moawad et al., 2010) while *C. pectinata* Griff. fruits reported to have an antidiabetic activity (Laishram et al., 2014).

Plants provide a great challenge in metabolomics due to the high chemical and physical diversity of their metabolites (Alonso & Stepanova, 2015). Therefore, no single analytical method can determine all plant metabolites simultaneously. Liquid chromatography-mass spectrometry (LC-HRESIMS) is considered as an advantageous analytical technique for metabolic profiling by detecting a broad range of chemical compounds at the same time without the tedious isolation procedures.

Radiation damage is mediated by free radicals which react with body tissues to generate lipid peroxidation, DNA lesions, and enzyme inactivation. Whole-body exposures to any form of radiation are known to alter its general physiology of the animal (Sharma, Parmar, Sharma, Verma, & Goyal, 2011). Total body irradiation (TBI) has been used in the clinical treatment of many malignancies to produce sufficient immunosuppression and prevent allograft rejections (Ravichandran et al., 2013).

The lack of chemical and biological reports on *C. armstrongii* Miq. encouraged us to perform a phytochemical study of this species to explore its active constituents and main therapeutic purposes. In this study, we performed comparative analytical and biological studies of three *Cycas* species growing in Egypt, since no chemical or biological reports

CONTACT Mona H. Hetta mhm07@fayoum.edu.eg; Mostafa E. Rateb Mostafa.Rateb@uws.ac.uk

on *Cycas armstrongii* Miq. had been reported so far. In addition, their potential as therapeutic agents in radiation-induced brain and pancreatic damage was also explored.

2. Materials and methods

2.1. Instruments and reagents

Nuclear Magnetic Resonance analysis was performed on Bruker Avance III 400 MHz for ^{1}HNMR and 100 MHz for ^{13}CNMR (Bruker AG, Switzerland) with BBFO Smart Probe.

Thin-layer chromatography was performed on pre-coated Si gel 60 F_{254} plates (Fluka-Sigma-Aldrich Chemicals-Germany), vacuum liquid chromatography (VLC) and column chromatography were done using Si gel H and Si gel 60 (E. Merck, Darmstadt, Germany), respectively. Sephadex LH20 for CC (Amersham Pharmacia Biotech B, Uppsala, Sweden).

2.2. Metabolomic analysis

The metabolomic analysis of crude ethanolic extracts of three *Cycas* species under investigation was performed using the previous published analytical technique of LC–HRESIMS (Abdelhafez et al., 2018).

2.3. Ameliorative role of Cycas species extract on γ radiation toxicity in brain and pancreas of albino rats

2.3.1. Animals. In this study, 48 adult female albino rats were used, their weight range was from (180–200 g). The animals were obtained from the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Animals were kept on a standard diet for 1 week until the experiment had been started. Ionizing radiation was performed by whole-body exposure of rats to 8 Gy. The tested extracts of *Cycas* species were administered by intragastric tube in the dose of 200 mg/kg body weight for each. Animals were divided into eight groups; Normal (group 1), *C. circinalis* extract (group 2), *C. armstrongii* extract (group 3), *C. revoluta* extract (group 4), Radiation group (Group 5), Radiation plus *C. circinalis* extract (group 6), Radiation plus *C. armstrongii* extract (group 7) Radiation plus *C. revoluta* extract (group 8).

2.3.2. Radiation process. Irradiation processing was performed at (NCRRT), using a Canadian Gamma Cell-40 (^{137}Cs). Animals were subjected to γ-radiation; 2 Gy installments every week at a dose rate of 0.5 Gy/min up to 8 Gy (total dose).

2.3.3. Samples collection. Animals were fasted overnight prior to sacrificing. Six animals from each group were randomly sacrificed by cervical dislocation 7 days post irradiation. Blood samples were collected by heart puncture from the anaesthetized rats. Serum samples were prepared by centrifugation at 3000 rpm and brain samples were collected and prepared following normal laboratory procedures, for the measurement of the biochemical parameters as well as histological examination. The abdomen was dissected to remove the pancreas. Brains and pancreases were then divided into two equal portions, the first portion was homogenized in ice-cold phosphate-buffered saline (PBS) to form 10% homogenate for biochemical assays while the other portion was kept for the histopathological examination.

2.4. Hematological parameters

Platelet count and total leucocytic count were determined using (Sysmex XE 2100 and XT 2000i operator manual, USA) a previously reported method (Bain, 2001).

2.5. Biochemical assays

Serum hepatic enzymes: alanine transaminase (ALT) and aspartate transaminase (AST) activities, glucose, urea, creatinine, amylase, and lipase were estimated. Brain and pancreas homogenates were used to estimate malondialdehyde (MDA) levels as one of the main end products of lipid peroxidation, GPx methods using available commercial kits (Biodiagnostics, Cairo, Egypt), and TAC levels by using Randox total antioxidant status kit (UK). Serum insulin, amyloid A, IL-18 assayed using a commercially available ELISA kit (MyBiosource, USA) according to the manufacturer's protocol. Also, MCP-1 in brain assayed by ELISA kit.

2.6. MicroRNA216a

The TaqMan® MicroRNA Assays were performed using the using Applied Biosystems real-time PCR instrument protocols mentioned in Brattelid et al. (2011).

2.7. Real-time PCR analysis

Polytron homogenizer and TriPure isolation reagent (Roche Diagnostics, Basel, Switzerland) was used to homogenize the tissue samples at 10,000 rpm, then the total RNA was extracted and isolated according to the manufacturer's protocols (Dasgupta, Das, Izumi, Venkatesan, & Barat, 2004). Livak and Schmittgen method was applied to measure the level of mRNA (Livak & Schmittgen, 2001). GAPDH (R&D Systems Inc., USA) was used as a housekeeping gene. qPCR was performed using the specific primers (Table 1).

Table 1. qPCR was performed using the specific primers.

Gene name	Primer sequence
GAPDH	Forward 5′ GAGACCTTCAACACCCCAGC 3′ Reverse 5′ ATGTCACGCACGATTTCCC 3′
NFkB	Forward 5′ CATGAAGAGAAGACACTGACCATGGAAA3′ Reverse 5′ TGGATAGAGGCTAAGTGT AGACACG 3′
COX-2	Forward 5′ GGCACAAATATGATGTTCGCATT3′ Reverse5′ CAGGTCCTCGCTTCTGATCTGT3′
GFAP	Forward5′ GACCGCTTTGCTAGCTACATCG-3′ Reverse 5′ -GGTTTCATCTTGGAGCTTCTGC-3′ .

2.8. Histopathological examination

Liver tissue specimens were fixed and stained with hematoxylin and eosin (H&E) reagent, then examined with a light microscope (Bancroft & Stevens, 1996).

2.9. Plant material

Cycas species were purchased from Zoheria garden, Giza, Egypt in October 2014. Dr Abd-Elhaleem Mohamed (Department of Plant Taxonomy, Agricultural Research Center, Egypt) confirmed the identity of the plants. Voucher specimens of *C. armstrongii* Miq., *C. revoluta* Thunb., and *C. circinalis* L., species no. BuPD; 41–43, respectively, were deposited at the department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University.

2.10. Preparation of extracts

The aerial parts of three *Cycas* species under investigation: *C. armestrongii* (4.25 kg), *C. revoluta* (4.5 kg), and *C. circinalis* (4 kg), were air-dried, powdered and saved in tightly closed amber colored glass containers at room temperature. One kilogram of dried powder of each species was extracted by 70% ethanol at room temperature by maceration. The solvent was, in each case, removed by vacuum distillation to give residues of total alcoholic extracts used for analytical and metabolomic analysis. Aliquots of these extracts were also used for biological screening.

2.11. Phytochemical analysis

Cycas armstrongii Miq., aqueous alcoholic extract was dissolved in distilled water and successively fractionated with *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAC), *n*-butanol, and water (H_2O).

The hexane extract of the leaflets of *Cycas armstrongii* Miq. was saponified according to Ismail (2013). An aliquot of the unsaponifiable matter of the leaflets of *Cycas armstrongii* Miq. (2.5 g) was fractionated on Si gel VLC column (12.5 × 7 cm, 150 g). Hexane/EtOAc mixtures with increasing polarity were used as eluent in 5% stepwise increments till 100%. Fractions (30 ml, each) were pooled and similar fractions were combined to give two main fractions (A and B). Fraction A; (0-30% EtOAc in hexane, 610 mg). The residue was further purified by rechromatography on a Si gel column (50 × 2.2 cm, 30 g). Gradient elution was started with hexane followed by increasing amounts of ethyl acetate. Subfraction A_1 upon evaporation of the solvent yielded compound **54** (13mg) as a white powder while subfraction A_2 was subjected to further purification and recrystallization giving compound **55** (17 mg) as white needle crystals. Fraction B; 40-100% EtOAc in hexane, 420 mg), the residue obtained upon evaporation of the eluent was further purified by rechromatography on Si gel column (15 cm×1 cm, 10 g). Gradient elution was performed out using $CHCl_3$ with increasing amounts of MeOH (5% increments) yielding compound **51** (8 mg) as a white powder and mixture of two compounds **51** and **52** (25 mg).

An aliquot (3.5 g) of the DCM extract was subjected to VLC on Si gel (45 x 3 cm, 150 g) and eluted with *n*-hexane – EtOAc mixture in 5% increments. Similar fractions were combined to give three subfractions (A1-A3). Subfraction A1 (450 mg) was rechromatographed on Si gel (60 cm × 1.4 cm, 50 g) with gradient elution using DCM-MeOH in 5% increments, the fraction eluted with 20% MeOH in DCM was rechromatographed on Sephadex LH-20 eluted with (DCM: MeOH 1:1) to yield compound **41** (12 mg). The subfraction A2 (500 mg) was rechromatographed on Si gel (60 cm × 1.4 cm, 50 g) with gradient DCM – MeOH in 5% increments followed by Sephadex LH-20 eluted with MeOH afforded compound **42** (23 mg) while the subfraction A3 (413 mg) yielded A3.1 (318 mg) which then repurified on Si gel (60 cm × 1.4 cm, 30 g) with gradient DCM-MeOH (1% increment), followed by Sephadex LH-20 using DCM giving four compounds; **43** (22 mg), **44** (250 mg), **45** (9 mg) and **53** (10 mg).

The ethyl acetate extract (4.2 g) was chromatographed on Si gel column (52 x 3.5 cm, 100 g) using DCM – MeOH as an eluent in a gradient mode to yield three subfractions. The DCM-MeOH (95:5) eluate was subjected to Si gel column which gave compound **44** (45 mg), DCM-MeOH (80:20) eluate was purified using Sephadex LH-20 eluted with MeOH giving compound **46** (16 mg), The DCM _ MeOH (65:35) eluate was

Table 2. Chemical profiling of the secondary metabolites of *Cycas armstrongii* Miq., *Cycas circinalis* L. and *Cycas revoluta* Thunb. aerial parts, using LC–HRESIMS.

No.	Blank mzXML peak area	Row *m/z*	Row retention time	MR85-*C. armstrongii* mzXML peak area	MR86-*C. circinalis* mzXML peak area	MR87-*C. revoluta* mzXML peak area	*C. armstrongii* conc. Ug/g	*C. circinalis* conc. Ug/g	*C. revoluta* conc. Ug/g
1	Protocatechuic acid	155.1080	10.16695	3,582,621.0640	0.00	10,776,280.1021	352,379.1367	0.00	1,059,932.4380
2	*P*-Coumaric acid	165.0553	4.0183	685,763.3179	499,335.6607	10,820,928.1934	170,656.5205	124,262.8240	2,692,856.1300
3	Vanillic acid	169.0853	7.4822	1,971,487.593	0	4,337,500.316	263,487.1348	0	579,702.1166
4	Caffiec acid	181.0181	9.9473	123,836.1829	134,442.7314	0	12,449.23266	13,515.50738	0
5	Loliolide	197.1206	5.5833	5,301,058.845	11,125,240.6298	0	949,443.3752	1,992,580.411	0
6	*N*-(3 -One-5 -methyl)-hexylalanine	219.1076	9.8889	34,515.99844	0	0	3490.3956	0	0
7	Vomifoliol	225.1479	5.205972222	26,468,784.3153	25,872,315.8578	43,741,746.9550	5,084,311.4760	4,969,737.592	8,402,224.424
8	4-Acetoxybenzoic acid butyl ester	237.1073	7.416033333	0.00	0.00	4,493,902.9100	0.00	0.00	605,971.2394
9	Cycasin	253.2208	10.4878	351,107.7824	7,481,359.638	8,279,196.307	33,477.7343	713,339.2740	789,412.1081
10	Naringenin	273.0716	9.1997	4,257,278.8963	19,060,641.4033	22,673,732.0920	462,763.6346	2,071,880.163	2,464,620.93
11	Isopimara-7,15-diene	273.2611	18.4483	33,640.0708	79,387.1205	203,679.7531	1823.469301	4303.200728	11,040.5171
12	Phthalic acid dibutyl ester	279.1345	4.2282	879,016.52	0.00	0.00	207,894.6088	0.00	0.00
13	Isopimara-7,15-dien-19-ol	289.2441	10.5173	315,089.4588	479,733.7528	1,526,677.311	29,959.1428	45,613.7506	145,158.5963
14	5,8-Dihydroxy-9,12-octadecadienoic acid	315.2939	20.8344	0.00	0.00	1,837,152.9590	0.00	0.00	88,178.7567
15	9,12-Dihydroxy-15-nonadecenoic acid	329.2556	13.1502	0.00	183,423.9883	0.00	0.00	13,948.3270	0.00
16	5-Hydroxy-6,7,8-trimethoxy-flavanone	331.241	7.5714	0.00	0.00	5,220,326.044	0.00	0.00	689,475.0733
17	Pregnane-3,12,14,20-tetrol	353.2522	16.0800	58,880.6516	0.00	0.00	3661.7282	0.00	0.00
18	Pinoresinol	359.1478	6.0611	18,005,862.2883	18,790,342.8157	0.00	2,970,733.332	3,100,162.427	0.00
19	Lariciresinol	361.1718	6.186466667	530,391.3456	1,260,862.2	4,811,793.191	85,734.1313	203,809.7461	777,793.4401
20	Glanduloidin C	399.179	3.2751	28,672.8287	0.00	0.00	8754.5721	0.00	0.00
21	Stigmasterol	413.3635	20.9897	756,714.8115	272,379.6973	1,303,323.597	36,051.5875	12,976.7785	62,093.2537
22	Neocycasin A	415.1563	5.6752	40,057.9659	70,867.4447	106,051.4114	7058.3061	12,487.0075	18,686.5038
23	*B*-Sitosterol	415.3612	25.1644	4,167,372.403	0	3,949,813.942	165,605.7622	0	156,960.2822
24	Lanosterol	427.3784	26.9741	31,783.2059	217,615.7813	647,368.1265	1178.2831	8067.5627	23,999.5598
25	Prunin	435.1277	6.8164	4,311,779.26	8,107,127.241	18,461,352.8903	632,553.9293	1,189,345.484	2,708,348.598
26	Salvileucolide methyl ester	449.2872	19.8565	25,174,607.5938	16,026,143.0122	66,897,845.9697	1,267,827.039	807,098.0793	3,369,065.342
27	*β*-Carotene	537.4231	18.1692	193,118.8959	9,796,272.659	16,234,831.2605	10,628.8702	539,166.8716	893,532.0082
28	Hinokiflavone	539.0928	10.67962778	149,334,300.7609	94,277,185.7176	76,376,466.5120	13,983,099.77	8,827,759.514	7,151,603.792
29	Amentoflavone	539.0975	10.67962778	167,222,325.5043	105,314,323.2726	86,494,368.6881	15,658,066.83	9,861,235.379	8,099,005.92
30	Dihydrohinokiflavone	541.1124	11.0600	97,648,520.2907	103,921,702.0894	174,994,870.2444	541.1124	11.0600	541.1124
31	Dihydroamentoflavone	541.1172	11.0895	81,399,838.8453	85,040,108.1351	153,181,582.0775	541.1172	11.0895	541.1172
32	Neocycasin J	547.443	26.8426	8,706,609.459	7,496,655.942	11,988,765.4226	324,357.0979	279,281.341	446,630.9391
33	2,3-Dihydrobilobetin	555.1284	12.1156	6,297,411.604	140,960,817.0445	61,655,350.7753	519,773.3007	11,634,568.89	5,088,885.274
34	Tetrahydrobilobetin	557.1543	12.0276	2,013,683.203	21,745,353.8576	7,009,667.115	167,420.5486	1,807,940.33	582,793.9134
35	Isoginkgetin	567.1266	13.9014	3,683,668.848	48,416,583.0698	14,411,459.3292	264,985.3522	3,482,855.232	1,036,690.806
36	*β*-Sitosterol-D-glucoside	577.4579	10.5023	35,001.3207	13,038,720.1476	15,737,409.3525	3332.7114	1,241,504.354	1,498,464.727
37	2 -O rhamnosylvitexin	579.281	14.9397	6,741,181.18	10,321,102.9682	8,697,438.715	451,224.665	690,848.696	582,167.8375
38	Naringin	581.1497	5.5518	8,056,340.698	0	0	1,451,104.859	0.00	0.00
39	Kaempferol 3-O-rutinoside	594.9529	22.2445	0.00	0.00	15,669,762.4614	0.00	0.00	704,433.1166
40	Kaempferol-3-O-Rhamnosyl-(1→2)-galactoside	595.3259	22.2451	0.00	37,709.6293	0	0	1695.183	0.00

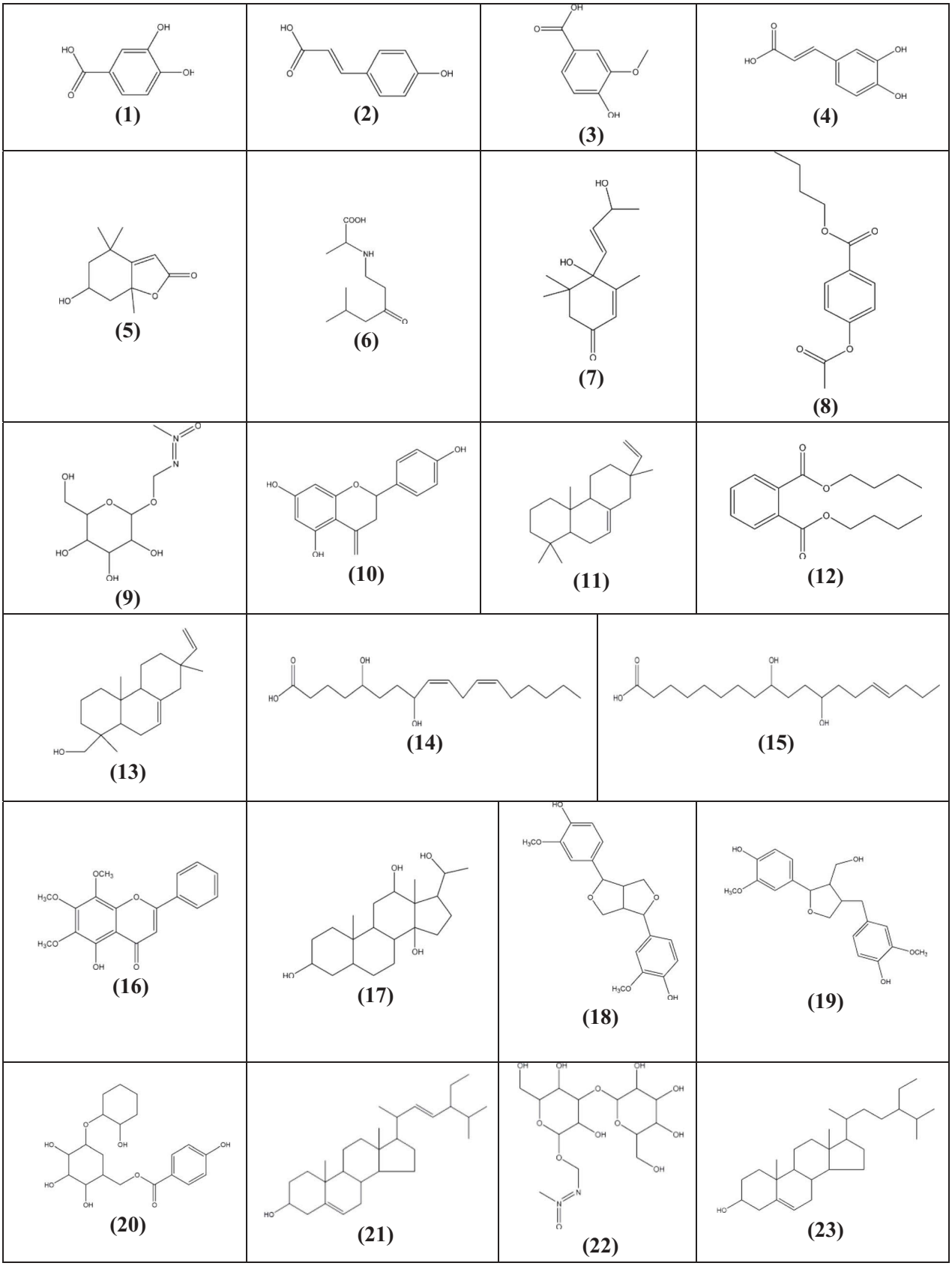

Figure 1. Structure the dereplicated secondary metabolites of *Cycas armstrongii* Miq., *Cycas circinalis* L. and *Cycas revoluta* Thunb. aerial parts identified by LC–HRESIMS.

further subjected to CC Si gel (45 x 2.5 cm, 40 g) eluted successively with gradient DCM _ MeOH and purified using sephadex LH-20 eluted with MeOH resulted in compound **47** (17 mg).

The n-BuOH (1.2 g) extract was chromatographed on polyamide eluted with H_2O – MeOH mixtures and then on an HP-resin column with H_2O – MeOH to obtain compound **48**, (18 mg).

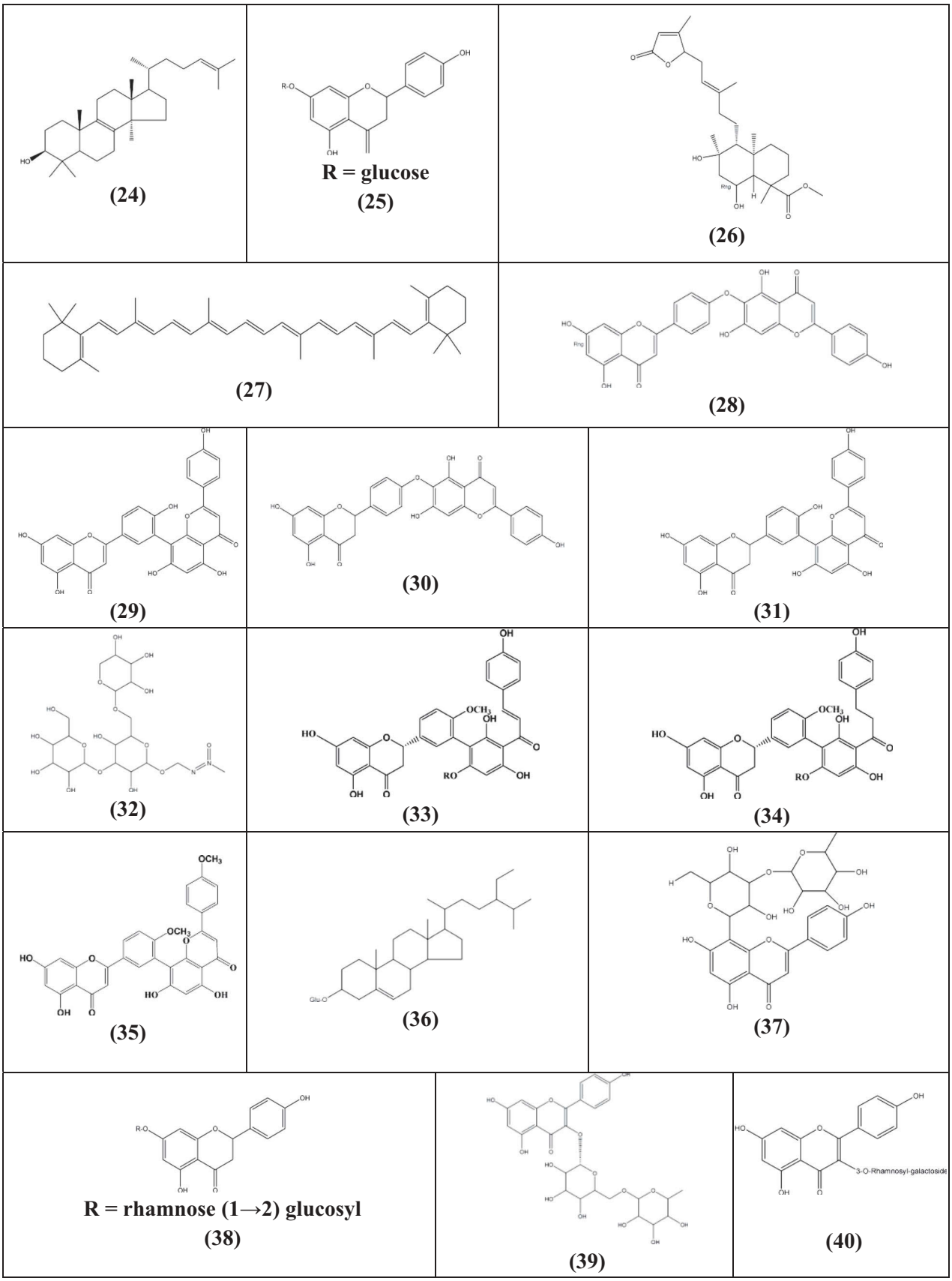

Figure 1. (Continued).

Total phenols extraction from leaflets of *C. armstrongii* Miq.was performed as in assay of extraction and isolation of phenols from *Cycas beddomei* Dyer. (Alekhya, Yasodamma, & Chaithra, 2013). Two compounds were isolated; **49** (15 mg), and **50** (14 mg).

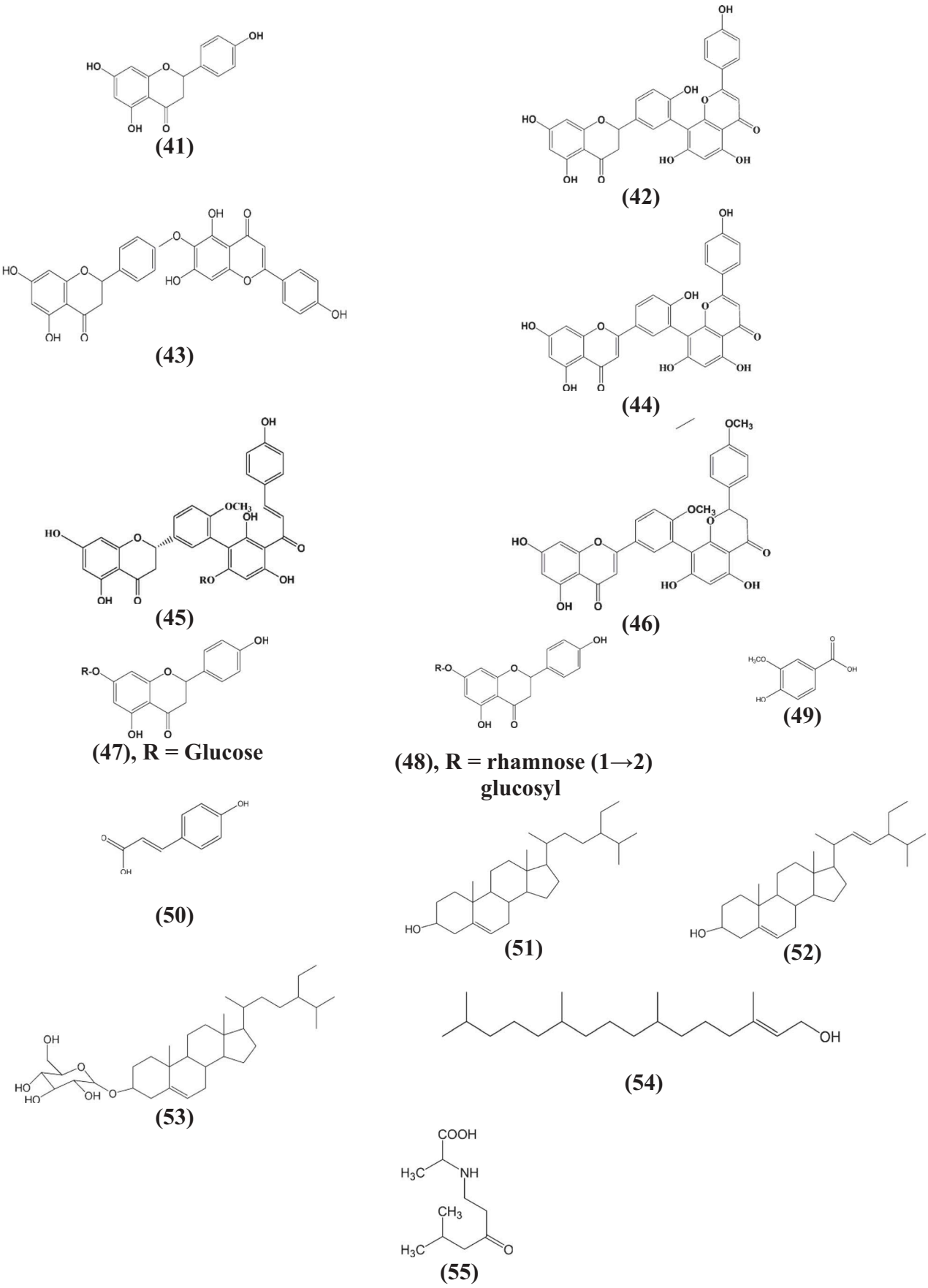

Figure 2. Structures of compounds isolated from *Cycas armstrongii* Miq.

2.12. Determination of LD_{50}

The LD_{50} of the total ethanol (70%) of each *Cycas* extract was estimated according to Spearman-Karber's method (Zalabani, Hetta, & Ismail, 2013). The median lethal dose (LD50) was calculated (Lorke, 1983).

2.13. Statistical analysis

Biochemical data were reported as means ± SE. The results were submitted to one-way *ANOVA*, and means were compared between groups by Duncan's multiple range tests and least-significant difference test. Statistically significant results were achieved when *P*-value<0.05 (Graph Pad Prism 5).

3. Results and discussion

3.1. Metabolomic analysis

Chemical profiling of the three *Cycas* species using LC–HRESIMS resulted in the identification of 40 phenolic metabolites (1–40) which belonged to different chemical classes including flavonoids and phenolic acids. The identification of these metabolites was performed by employing macros and algorithms that coupled MZmine with online and in-house databases (METLIN and DNP databases for plant natural products) (Table 2, Figure 1).

3.2. Investigation of the chemical constituents of Cycas armstrongii *Miq*

Fifteen compounds of different classes were isolated from *C. armstrongii* Miq., (Figure 2); 10 phenolics; Naringenin (**41**), dihydroamentoflavone (**42**), 2,3-dihydrohinokiflavone (**43**), amentoflavone (**44**), 2,3-dihydrobilobetin (**45**), isoginkgetin (**46**), prunin (**47**), Naringin (**48**), vanillic acid (**49**) (Chang et al., 2009) and *p*-coumaric acid (**50**) (Jerald, 1990). Three steroidal compounds; β-sitosterol (**51**), mixture of β-sitosterol and stigmasterol (**52**) (Habib, Nikkon, Rahman, Haque, & Karim, 2007), and β- sitosterol glucoside (**53**) (Ismail, 2013). One acyclic diterpene alcohol, 3,7,9,11-tetramethyl heptadecanoic acid (**54**) (Abdelgawad, Ma, Hetta, Ross, & Badria, 2015) and one non-protein amino acid, *N*-(3′-one-5′-methyl)-hexyl-alanine (**55**) (Li, Brownson, Mabry, Perera, & Bell, 1996). All the isolated compounds have been identified by comparing their chemical, spectral, and physical data with those reported in literature.

3.3. Liver and kidney functions

To show the toxicity of the extracts, liver and kidney functions were tested. Compared to the control group, γ-radiation induced a significant increase in serum activities of ALT and AST by 363% and 341%, respectively ($p < 0.001$). Blood urea and serum creatinine concentrations turned out to be markedly increased by 63% and 441%, respectively, in γ-irradiated rats as compared to control group. Three extracts, Ext 1 (*C. circinalis*), Ext 2 (*C. armstrongii*) and Ext 3 (*C. revoluta*) treatment induced a significant reduction in serum activities of ALT (56%, 61.8% and 61% respectively) and AST (56.6%, 56.3%, and 57.7%, respectively) as compared to γ-radiation group. Furthermore, a significant decrease in the concentration of blood urea returning to normal values and serum concentrations of creatinine (46%, 60%, and 51%, respectively) as compared to radiation group (Table 3).

Data were expressed as mean±SEM. Ext 1 (*C. circinalis*), Ext 2 (*C. armstrongii*) and Ext 3 (*C. revoluta*).

3.4. Glucose and insulin levels

Compared to the control group, γ-radiation induced a significant increase in serum glucose by 67.6% ($p< 0.001$) while γ-radiation caused a significant reduction in serum insulin level by 47.2%. *Cycas* treatment (3 extracts) produced a significant decrease in serum glucose (22.5%, 29%, and 29%, respectively) and elevation of insulin (53%, 49%, and 80% respectively) as compared to γ- radiation group (Figure 3).

3.5. White blood count and platelet count

γ-Radiation induced a significant decrease in WBCs by 77.5% ($p< 0.001$). Platelets count was exhibited by the

Table 3. Effect of three *Cycas* extracts on ALT, AST, blood urea, and serum creatinine after exposure to gamma radiation.

	Animal groups							
Parameters	Control	Ext1	Ext2	Ext3	IRR	IRR + Ext1	IRR + Ext2	IRR + Ext3
ALT (U/L)	15.33 ± 0.8819	17.83 ± 0.70	16.67 ± 0.71	13.50 ± 0.84	71.67 ± 1.98*	30.83± 1.25*#	27.33± 1.65*#	27.83± 0.94*#
AST (U/L)	15.83 ± 1.30	15.83 ± 0.94	17.17 ± 1.014	16.67 ± 1.11	69.83 ± 6.670*	30.33± 1.14*#	30.50± 0.88*#	29.50± 1.98*#
Urea (mg%)	49.03 ± 1.770	44.70 ± 2.17	44.83 ± 1.14	42.23 ± 2.32	80.20 ± 2.37*	53.57± 1.37*#	50.50± 3.02*#	43.43± 1.78*#
Creatinine (mg%)	0.155 ± 0.007	0.166 ± 0.014	0.14 ± 0.005	0.19 ± 0.018	0.84 ± 0.036*	0.45± 0.008*#	0.33± 0.035*#	0.41± 0.034*#

*Significantly different from control. #Significantly different from irradiation.
U/L, Unit per liter.

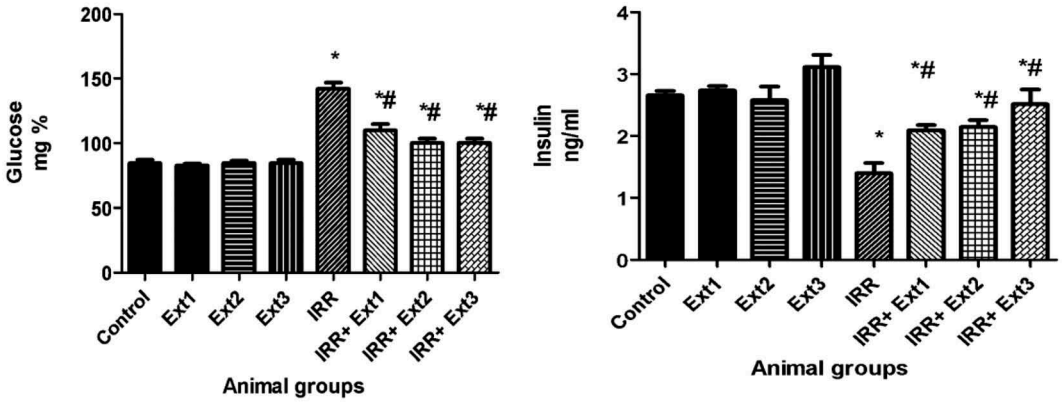

Figure 3. Effect of the *Cycas* extracts on glucose and insulin levels. Ext 1 (*Cycas circinalis*), Ext 2 (*Cycas armstrongii*) and Ext 3 (*Cycas revoluta*). *Irradiation only, #Significantly different from irradiation.

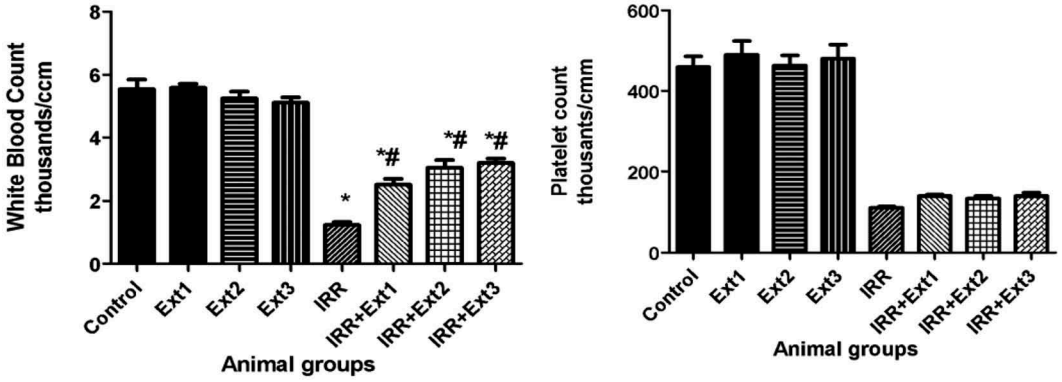

Figure 4. Effect of the *Cycas* extracts on WBCs and platelets count. Ext 1 (*Cycas circinalis*), Ext 2 (*Cycas armstrongii*) and Ext 3 (*Cycas revoluta*). *Irradiation only, #Significantly different from irradiation.

same trend where γ-radiation caused a significant reduction in platelet count by 77%. *Cycas* treatment (3 extracts) induced a significant increase in WBCs by (101%, 142%, and 157%, respectively) and non-significant elevation of platelet count (31%, 26.8%, and 31%, respectively) as compared to γ-radiation group (Figure 4).

3.6. IL-18, amyloid A

γ-Radiation induced a significant increase in serum IL-18 and amyloid A by 153% and 67.7%, respectively ($p < 0.001$). On the other hand, *Cycas* treatment (3 extracts) induced a significant reduction in serum IL-18 and amyloid A (25.7%, 38.7%, and 50.4%, respectively in case of IL-18) and in respect of amyloid A (65%, 68%, and 68.5%, respectively) as compared to γ-radiation group (Figure 5).

3.7. Oxidative stress parameters

Compared to the control group, γ-radiation showed a significant elevation in MDA in tissues of brain and pancreas by 58.2% and 67.26%, respectively ($p < 0.001$) while γ-radiation induced a significant

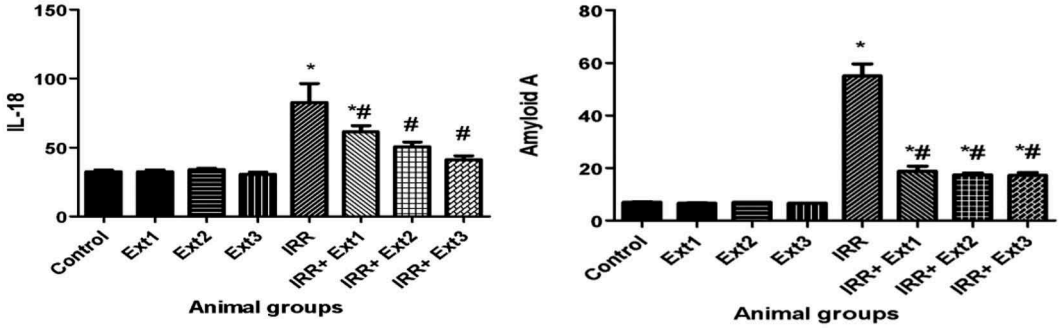

Figure 5. Effect of the *Cycas* extracts on IL-18 and Amyloid A. Ext 1 (*Cycas circinalis*).
Ext 2 (*Cycas armstrongii*) and Ext 3 (*Cycas revoluta*). *Irradiation only, #Significantly different from irradiation.

Table 4. Effect of three *Cycas* extracts administration on oxidative stress parameters in brain and pancreas against irradiation.

Tissue and parameter		Animal groups							
		Control	Ext1	Ext2	Ext3	IRR	IRR + Ext1	IRR + Ext2	IRR + Ext3
Brain	TAC	50.95 ± 2.494	57.03 ± 2.82	65.57 ± 0.84*	59.20 ± 1.803*	17.98 ± 0.62*	42.62± 1.11*#	47.72± 0.47*#	48.45± 1.40*#
	Px (µg/g)	124.1± 1.17	131.0 ± 1.77	131.0 ± 1.340	132.3 ± 1.99*	51.05 ± 0.75*	120.9± 2.25*#	121.7± 2.28*#	118.2± 2.00*#
	MDA (nmole/g)	8.53 ± 0.52	5.917± 0.258	5.450± 0.2	5.93± 0.30	58.20± 4.58*	21.73± 1.47*#	31.00± 2.18*#	25.20± 1.81*#
Pancreas	TAC	51.0 ± 2.53	59.35 ± 2.23*	64.22 ± 0.65*	69.58 ± 1.05*	19.35 ± 1.145*	48.13± 3.9*#	40.20± 4.87*#	59.87± 2.5*#
	GPx (µg/g)	138.0 ± 3.13	149.4 ± 1.62	146.3 ± 4.0	154.8 ± 0.86*	70.25 ± 3.18*	100.6± 2.86*#	111.6± 3.05*#	112.4± 1.98*#
	MDA (nmole/g)	5.27 ± 0.46	4.400± 0.11	3.98± 0.044	3.900± 0.057	40.65± 2.057*	21.63± 1.80*#	20.20± 0.94*#	16.90± 0.94*#

*Significantly different from control. #Significantly different from irradiation

decrease in Gpx and TAC in tissues of brain and pancreas by 58.5% and 65.5% for brain, respectively ($p < 0.001$) and 49% and62% for pancreas, respectively. *Cycas* treatment (3 extracts) induced a significant reduction in MDA of brain by 62.6%, 46.7%, 56.7%, respectively and MDA for pancreas 49%, 50%, 58%, respectively as compared to the γ-radiation group. Furthermore, a significant increase in GPx and TAC were shown. The increase of GPx in brain was 137%, 138%, 131%, respectively. The increase of TAC in brain was 137%, 165%, 169%, respectively, as compared to the γ-radiation group. In addition, the increase of GPx in pancreas was 43%, 58.8%, 58.8%, respectively and the increase of TAC in pancreas was 148%, 107%, 209%, respectively, as compared by γ-radiation group (Table 4). Data were expressed as mean ± SEM. Ext 1 (*Cycas circinalis*), Ext 2 (*Cycas armstrongii*) and Ext 3 (*Cycas revoluta*).

3.8. Detection of brain injury (NFkb, Cox2, MCP-1, GFAP)

γ-Radiation elevated the expression of NFkb, Cox2, and GFAP (by 5.4-, 6-, 4.75-fold, respectively) and caused a significant increase in MCP-1by 60% compared to control group. Treatment with the three extracts exhibited relief of the parameters in the brain by significantly downregulating NFkb, Cox2, GFAP expression compared to the γ-radiation group. The decrease in NFkb was 53%, 62%, 69%, respectively. The decrease in Cox2

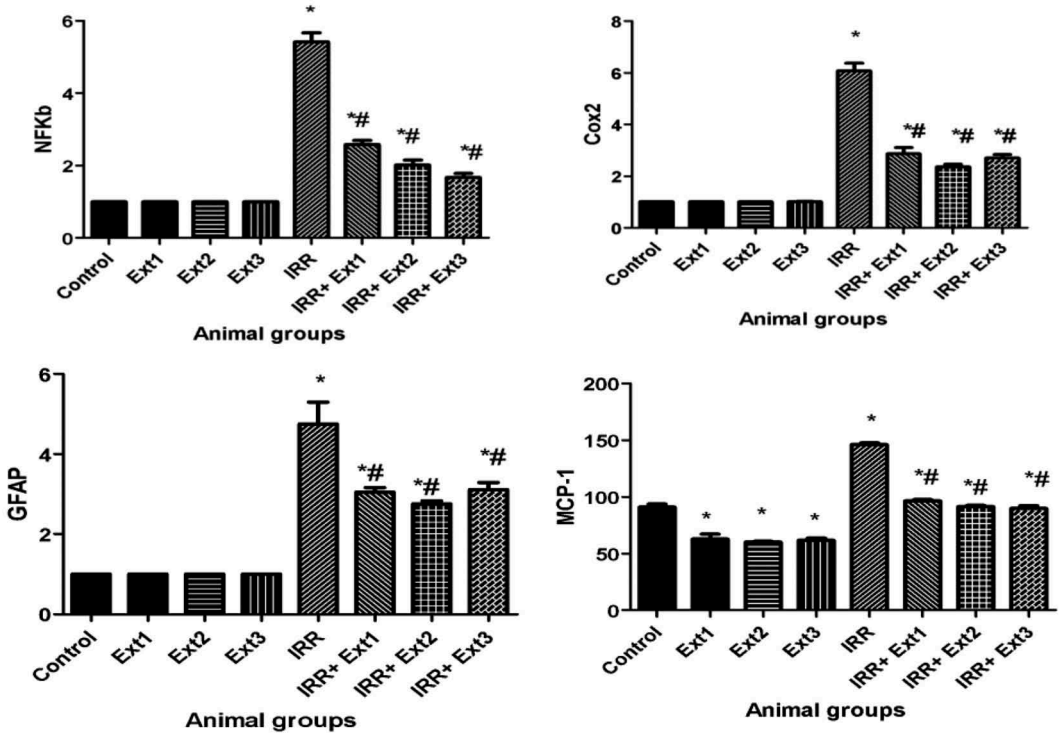

Figure 6. Effect of the *Cycas* extracts on NF-kB, Cox2, GFAP, MCP-1. Ext 1(*Cycas circinalis*), Ext 2 (*Cycas armstrongii*) and Ext 3(*Cycas revoluta*). *Irradiation only, #Significantly different from irradiation.

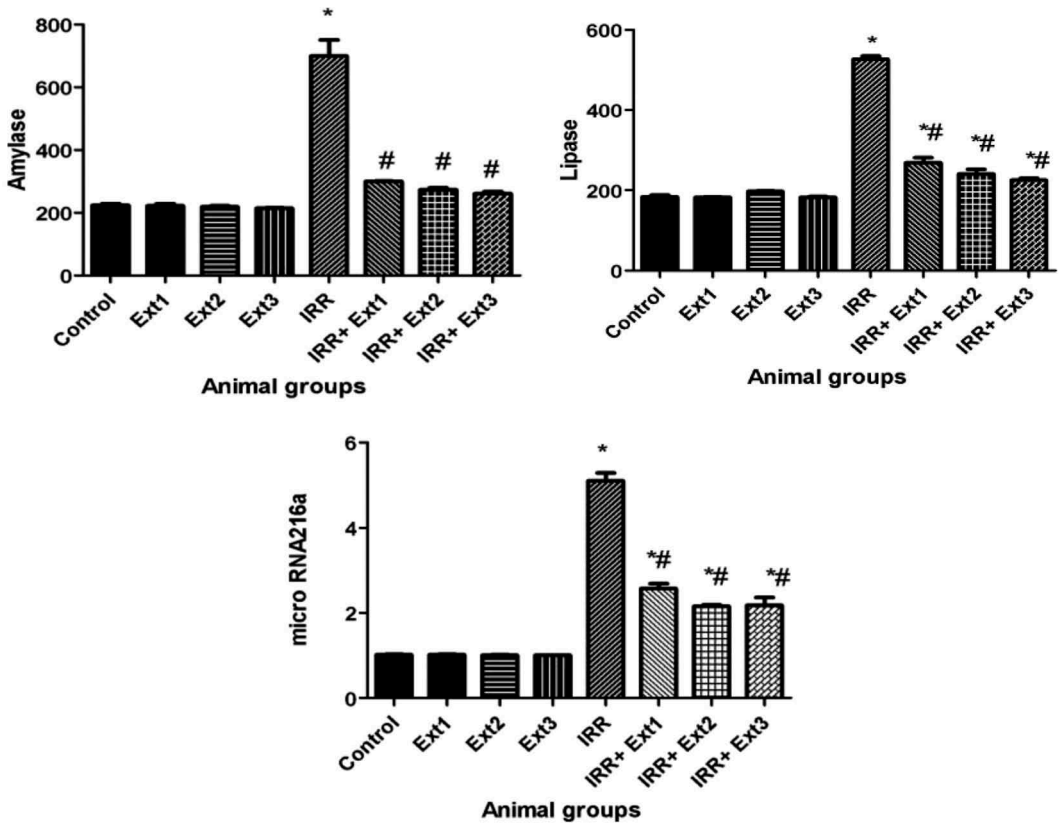

Figure 7. Effect of the *Cycas* extracts on Amylase, Lipase, microRNA216a. Ext 1 (*Cycas circinalis*), Ext 2 (*Cycas armstrongii*) and Ext 3 (*Cycas revoluta*). *Irradiation only, #Significantly different from irradiation.

was 52%, 60%, 55%, respectively. Moreover, the decrease in GFAP was 36%, 42%, 34% as compared to γ-radiation. The extracts reduced MCP-1 by 34%, 37%, 38.5%, respectively, as compared to the γ-radiation group (Figure 6).

3.9. Detection of pancreatic injury (amylase, lipase, microRNA216a)

γ-Radiation elevated the expression of microRNA216a (by 5.1-fold) and caused a significant increase in amylase and lipase by 213% and 187%, respectively. Treatment with the 3 extracts exhibited anti-inflammatory effects in pancreas by significantly downregulating microRNA216a expression compared to the γ-radiation group (by 50%, 58%, 59%, respectively). Moreover, the current study demonstrated anti-pancreatic injury of extracts by reducing serum amylase and lipase levels. They reduced amylase by 57%, 60.9%, 62%, respectively, as compared to the γ-radiation group. Further, they reduced lipase by 49%, 54%, and 57%, respectively, as compared to the γ-radiation group (Figure 7).

3.10. Histopathological examination

To further characterize the brain and pancreatic damage induced by γ-radiation, histopathological examination of the two tissues was performed. No histopathological alteration was observed on the structure of the neurons in the cerebral cortex, subiculum and fascia dentate, striatum and cerebellum both normal and three extract groups (Figure 8(a–e)) and (Figure 9(a–c)). In contrast, histological examination of brain tissue from γ-irradiated animals revealed nuclear pyknosis and degeneration in the neuronal cells of the cerebral cortex (Figure 8(f)), subiculum of the hippocampus (Figure 8(g)) and striatum (Figure 8(h)). Treatment by Ext1 showed that the neurons in the subiculum of the hippocampus were intact (Figure 9(d)) while the neurons of fascia dentate exhibited nuclear pyknosis and degeneration (Figure 9(e)). Furthermore, in the R+ Ex2 group, nuclear pyknosis and degeneration were observed in fewer neurons in the subiculum of the hippocampus (Figure 9(f)), while the fascia dentate showed more (Figure 9(g)). The examination of the R+ Ex2 group revealed that few neurons in subiculum of the hippocampus appeared nuclear pyknosis and degeneration (Figure 9(h)). On the other hand, the histology of pancreas of control and extracts alone groups, there was no histopathological alteration of the islands of Langerhans cells as endocrine portion and the acini with the ducts system as exocrine one was recorded in (Figure10(a,d,e,f)). In the radiation group, there was an atrophy in the islands of Langerhans cells (Figure10(b)) associated with congestion in the stromal blood vessels (Figure10(c)). In the R+ Ext1 group, there was atrophy in some of the islands of Langerhans while others showed moderate size (Figure10(g)). In the R+ Ext 2 group, no

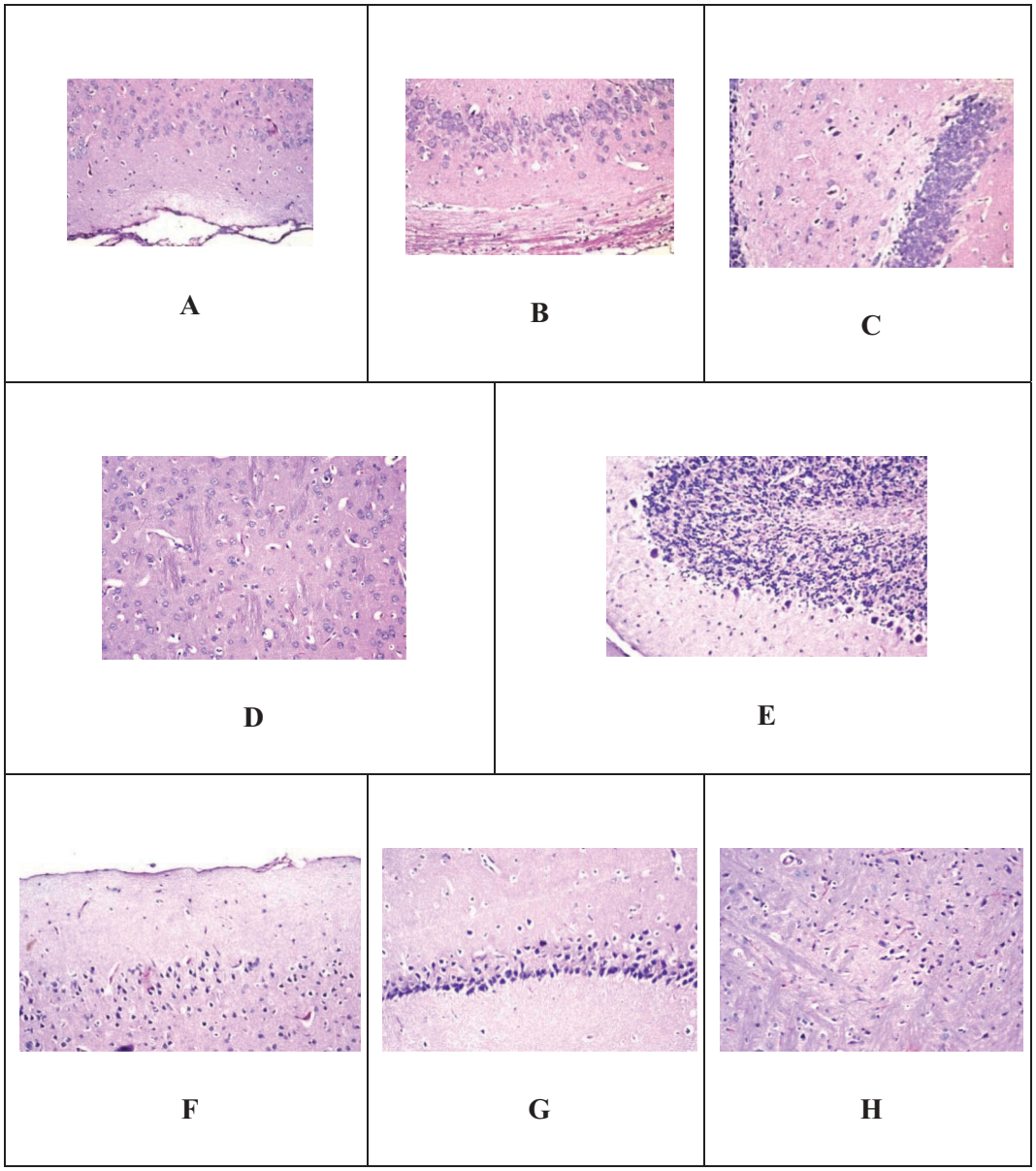

Figure 8. Histopathology of brain tissue sections (× 400).

Extract 1 is *Cycas circinalis* L. Extract 2 is *Cycas armstrongii* Miq. Extract 3 *is Cycas revoluta* Thunb. A: Neuronal cells of cerebral cortex, B: Subiculum, C: fascia dentate, D: striatum, E: Cerebellum F: Neuronal cells of the cerebral cortex, G: Subiculum of the hippocampus, H: Striatum.

histopathological alteration was observed (Figure10(h)). In the R+ Ext 3 group, there was no histopathological alteration as recorded in (Figure 10(i)).

The variability in chemical components observed in *Cycas* species is correlated to the variability in response upon evaluation of their biological activities. The correlation between *Cycas* species and their biological activities might be attributed to a combinatorial synergistic effect of these components. *Cycas armstrongii* Miq. was selected for phytochemical study based on its prevalence effects in most of the assessed biological activities. The alcoholic extract of *C. circinalis* L. showed the best antioxidant activity followed by *C. armstrongii* Miq., then *C. revoluta* Thunb. as a result of the ease of donation of hydrogen radicals, which is related to the radical scavenging ability of antioxidants as a reason of flavonoids and phenolic contents that could be responsible for the antioxidant capacity of medicinal plants (Zalabani et al., 2012).

Phenolic compounds were reported to exert various physiological activities as antioxidant effect (Shahwar et al., 2012) and improve a variety of conditions such as microbial, protozoa, and malaria infection; vanillic acid reported to have a moderate inhibition effect against *Pseudomonas aeruginosa* (Chatterjee et al., 2015). Naringin was recently considered as antimalarial agent (Rudrapal & Chetia, 2017), while amentoflavone and its derivatives have previously been assessed as antileishmanial agents (Rizk et al., 2014). Regarding the antifungal activity, the isolated sterols and diterpene alcohol were reported as antifungal agents (Minhas, Rehaman, Yasin, Awan, & Hussain, 2013; Omoruyi, Afolayan, & Bradley, 2014).

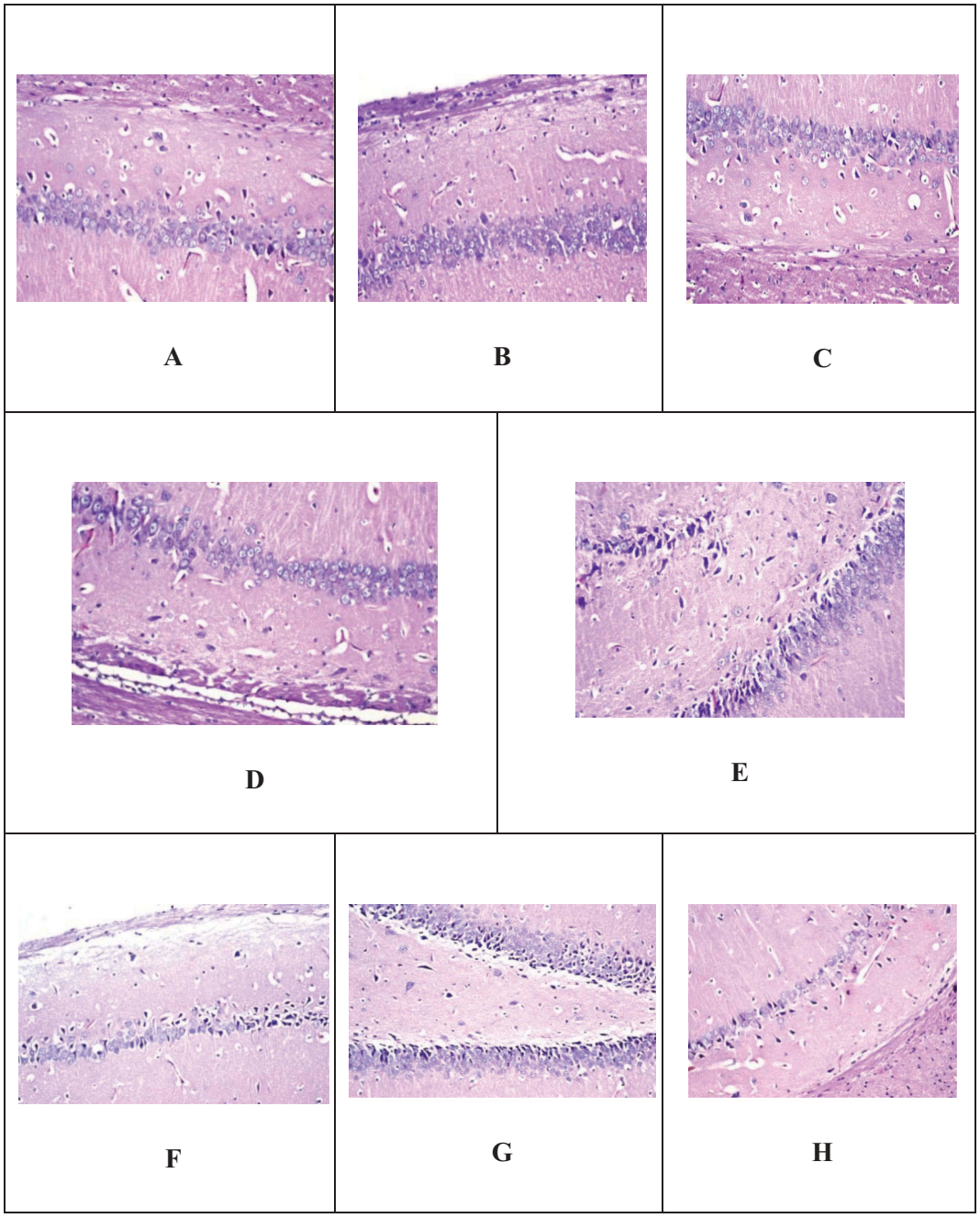

Figure 9. Histopathology of brain tissue sections (× 400).

Extract 1 is *Cycas circinalis* L. Extract 2 is *Cycas armstrongii* Miq. Extract 3 *is Cycas revoluta* Thunb. A, B, C: Three extracts alone; EXT1, EXT2, and EXT3. D: Neurons in the subiculum of the hippocampus + Treatment by Ext1. E: Neurons of fascia dentate + Treatment by Ext1. F: Neurons in subiculum of the hippocampus + Treatment by Ext2. G: Neurons in Fascia dentate + Treatment by Ext2. H: Neurons in subiculum of the hippocampus + Treatment by Ext2.

Few reports were recorded on the phytochemical studies of different *Cycas* species (Laishram et al., 2014) and no records on radiation injury. The improvement of the studied parameters in treating rats could be attributed to their active constituents and their antioxidative and anti–inflammatory effects. The extracts attenuated the oxidative stress in brain and pancreas. Serum SAA and IL-18 declined significantly than radiation exposure alone. Kumar and kumar (2015) observed that *C. circinalis* does not produce any toxic effect in male albino rats with no adverse histopathological presentations when different organs including brain, liver, and kidney were examined. Their findings of the sub-acute toxicity tests suggest that *C. circinalis* with oral administration is considered nontoxic at therapeutic doses for an extended period showing potency for enhancing the immune system and liver protection, kidney, and cardiovascular system. The sub-acute toxicity study proved that when the ethanolic extract of *C. circinalis* given orally until 1000 mg/kg body weight proved its safety and exhibited no toxicity.

Histopathological examination confirmed the biochemical evaluation and showed ameliorating effects in brain tissue. The histology of pancreas

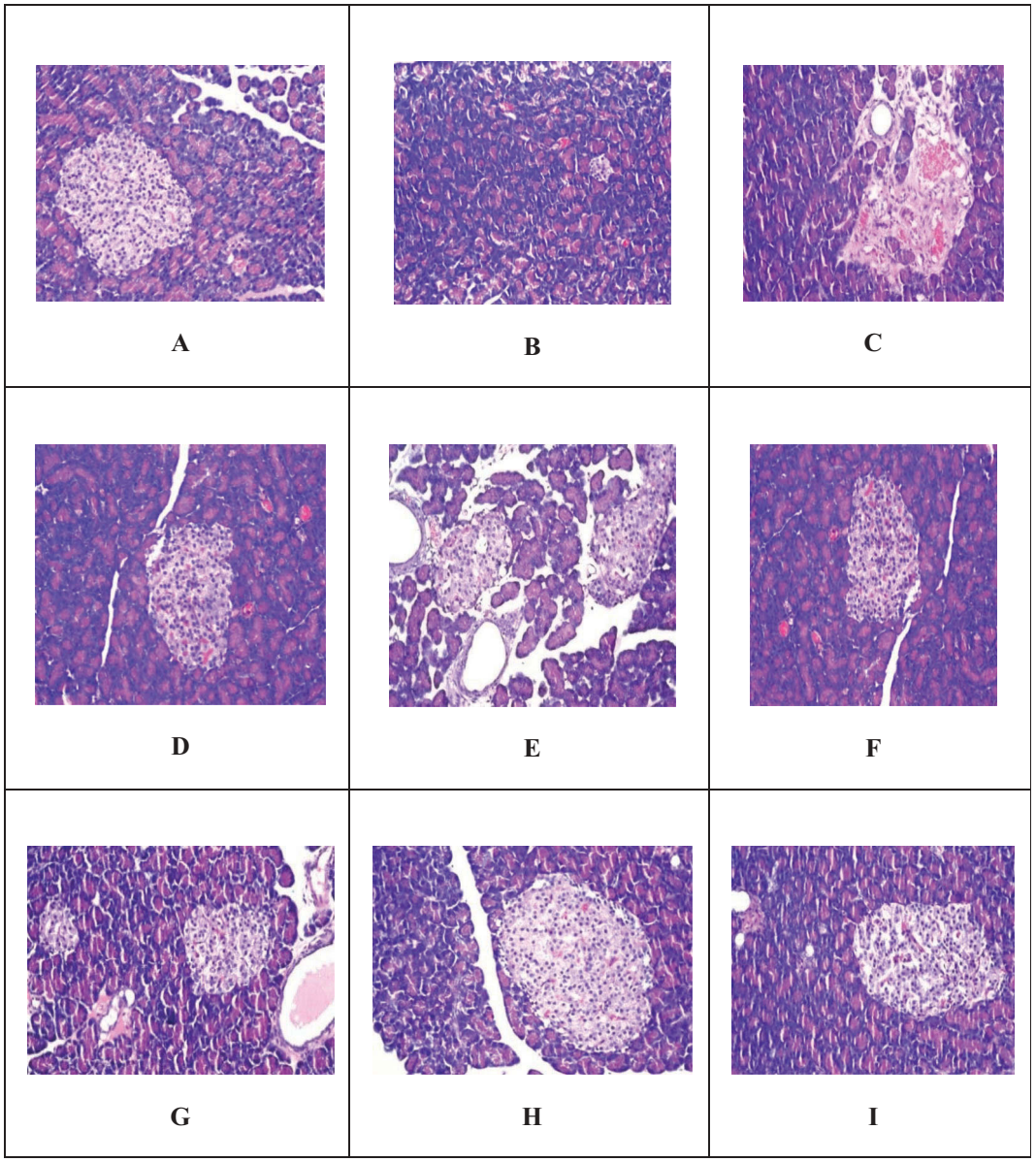

Figure 10. Histopathology of pancreas tissue sections (× 400).

Extract 1 is *Cycas circinalis* L. Extract 2 is *Cycas armstrongii* Miq. Extract 3 *is Cycas revoluta* Thunb. A: Control. B and C: Radiation alone; EXT1, EXT2, and EXT3. D, E and F: Three extracts alone. G: Radiation and extract 1. H: Radiation and extract 2. I: Radiation plus extract 3.

showed better histopathological findings than brain in which extracts render pancreatic tissues as normal control.

4. Conclusion

Although *Cycas* plants were used for decades as edible food and drug, the reported secondary metabolites of these plants are few and the phytochemical and biological profiles have been scarcely studied. Accordingly, this work was designed to explore the role of *Cycas* species in ameliorating the deleterious effects of ionizing radiation via attenuation of oxidative stress, improving blood count, alleviating inflammatory mediators in serum, brain, and pancreatic tissues after exposure of high dose of ionizing radiation. The obtained data proved the potential role of these extracts in radiation treatment and provided a proposal for *Cycas* therapeutic applications.

Acknowledgments

This work was dedicated to the soul of our late collaborator Dr Salma M. Abdel Fattah, who recently passed out to her God. This was her last piece of work, and we wish God add the benefit of this work to her good deeds.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Abeer S. Moawad http://orcid.org/0000-0002-0228-6757

References

Abdelgawad, S., Ma, G., Hetta, M., Ross, S., & Badria, F. (2015). Chemical and biological study of *Withania somnifera* (L.) dunal leaves growing in Upper Egypt: Beni-Suef region. *Journal of Natural Products*, *8*, 64–74.

Abdelhafez, O. H., Fawzy, M. A., Fahim, J. R., Desoukey, S. Y., Krischke, M., Mueller, M. J., & Abdelmohsen, U. R. (2018). Hepatoprotective potential of Malvaviscus arboreus against carbon tetrachloride-induced liver injury in rats. *PloS One*, *13*, e0202362.

Alekhya, C., Yasodamma, N., & Chaithra, D. (2013). Qualitative analysis of phenols, flavonoids and anthocyanidins of *Cycas beddomei* Dyer. An endemic gymnosperm of Seshachalam Hills ranges. *Indo American Journal of Pharmaceutical Research*, *3*, 1632–1641.

Alonso, J. M., & Stepanova, A. N. (2015). *Plant functional genomics: Methods and protocols* (pp. 221). New York: Springer Science & Business Media.

Bain, B. J. (2001). *Blood cell (A practical guide) performing a blood count* (3rd ed., pp. 23). London: Blackwell Science Ltd.

Bancroft, J. D., & Stevens, A. (1996). *Theory and practice of histological techniques* (4th ed.). New York: Churchill Livingstone.

Brattelid, T., Aarnes, E. K., Helgeland, E., Guvag, S., Eichele, H., & Jonassen, A. K. (2011). Normalization strategy is critical for the outcome of miRNA expression analyses in the rat hear. *MicroRNA and tissue injury*. *Physiological Genomics*, *43*, 604–610.

Chang, S. W., Kim, K. H., Lee, H. K., Choi, S. U., Ryu, S. Y., & Lee, K. R. (2009). Phytochemical constituents of *Bistorta manshuriensis*. *Natural Product Sciences*, *15*, 234–240.

Chatterjee, N. S., Panda, S. K., Navitha, M., Asha, K. K., Anandan, R., & Mathew, S. (2015). Vanillic acid and coumaric acid grafted chitosan derivatives: Improved grafting ratio and potential application in functional food. *Journal of Food & Nutritional Disorders*, *52*, 7153–7162.

Dasgupta, A., Das, S., Izumi, R., Venkatesan, A., & Barat, B. (2004). Targeting internal ribosome entry site (IRES) mediated translation to block hepatitis C and other RNA viruses. *FEMS Microbiology Letters*, *234*, 189–199.

Habib, M. R., Nikkon, F., Rahman, M., Haque, M. E., & Karim, M. R. (2007). Isolation of Stigmasterol and beta-sitosterolfrom methanolic extract of root bark of *Calotropis gigantea* (Linn). *Pakistan Journal of Biological Sciences*, *10*, 4174–4176.

Ismail, A. S. (2013) Phytochemical and biological study of certain *Conyza* species growing in Egypt. Master Thesis, Faculty of Pharmacy, Beni-Suef University, Egypt.

Jerald, J. N. (1990) Toxic compounds in *Cycads* (Master Thesis). University of Natal, Durban, South Africa. doi:10.1099/00221287-136-2-327

Kumar, B. S., & kumar, J. V. (2015). Sub-acute toxicity study of *Cycas circinalis* L. and *Ionidium suffruticosum* Ging in Wistar Albino Rats. *International Journal of Pharmaceutical Sciences Review and Research*, *33*, 87–92.

Laishram, S., Sheikh, Y., Moirangthem, D. S., Deb, L., Pal, B. C., Talukdar, N. C., & Borah, J. C. (2014). Anti-diabetic molecules from *Cycas pectinata* Griff. Traditionally used by the Maiba-Maibi. *Phytomedicine*, *22*, 2223–2226.

Li, C., Brownson, D. M., Mabry, T. J., Perera, C., & Bell, E. A. (1996). Nonprotein amino acids from seeds of *Cycas circinalis* and *Phaseolus vulgaris*. *Phytochemistry*, *42*, 443–445.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCt method. *Methods*, *25*, 402–440.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology Dec*, *54*, 275–287.

Minhas, F. A., Rehaman, H. U., Yasin, A., Awan, Z. I., & Hussain, M. A. (2013). Antimicrobial activities of the leaves and roots of *Elaeagnus umbellate* Thunb. *African Journal of Biotechnology*, *12*, 6754–6760.

Moawad, A., Hetta, M., Zjawiony, J. K., Hifnawy, M., & Ferreira, D. (2014). Two new dihydroamentoflavone glycosides from *Cycas revoluta*. *Natural Product Research*, *28*, 41–47.

Moawad, A., Hetta, M., Zjawiony, J. K., Jacob, M. R., Hifnawy, M., & Ferreira, D. (2010). Phytochemical Investigation of *Cycas circinalis* and *Cycas revoluta* Leaflets: Moderately active antibacterial biflavonoids. *Planta Medica*, *76*, 796–802.

Omoruyi, B. E., Afolayan, A. J., & Bradley, G. (2014). Chemical composition profiling and antifungal activity of the essential oil and plant extracts of *Mesembryanthemum edule* (L.) bolus leaves. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*, 19–30.

Ravichandran, R., Binukumar, J. P., Davis, C. A., Al Rahbi, Z., Balakrishnan, R., & Al Mandhar, Z. (2013). Total body irradiation (TBI): Preliminary experience on clinical implementation. *Journal Of Medical Physics / Association of Medical Physicists of India*, *38*, 210–211.

Rizk, Y. S., Fischer, A., Cunha, M. C., Rodrigues, P. O., Marques, M. C., Matos, M. F., … Arruda, C. C. (2014). In-vitro activity of the hydroethanolic extract and biflavonoids isolated from *Selaginella sellowii* on *Leishmania amazonensis*. *MemInst Oswaldo Cruz*, *109*, 1050–1056.

Rudrapal, M., & Chetia, D. (2017). Plant flavonoids as potential source of future antimalarial leads. *Systematic Reviews in Pharmacy*, *8*, 13–18.

Shahwar, D., Raza, M. A., Saeed, A., Riasat, M., Chattha, F. I., Javaid, M., & Ullah, S. (2012). Antioxidant potential of the extracts of *Putranjiva roxburghii, Conyza bonariensis, Woodfordia fruiticosa* and *Senecio chrysanthemoids*. *African Journal of Biotechnology*, *11*, 288–4295.

Sharma, P., Parmar, J., Sharma, P., Verma, P., & Goyal, P. K. (2011). Radiation-induced testicular injury and its amelioration by *Tinospora cordifolia* (An Indian medicinal plant) extract. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 1–9.

Wallace, J. W. (1972). A survey for benzoic and cinnamic acid of the Cycadaceae. *American Journal of Botany*, *59*, 1–4.

Zalabani, S. M., Hetta, M. H., & Ismail, A. S. (2013). Anti-inflammatory and antimicrobial activity of the different *Conyza dioscoridis* L. Desf. Organs. *Biosafety*, *2*, 106.

Zalabani, S. M., Hetta, M. H., Ross, S. A., Abo Youssef, A. M., Zaki, M. A., & Ismail, A. S. (2012). Antihyperglycemic and antioxidant activities and chemical composition of *Conyza dioscoridis* (L.) Desf. DC. growing in Egypt. *International Journal of Basic & Applied Sciences*, *6*, 257–265.