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ORIGINAL ARTICLE

Anti-Eczematic and Molecular Modeling of Anthraquinones Isolated from the Seeds of *Asphodelus microcarpus* Salzm. Viv. Growing in Egypt

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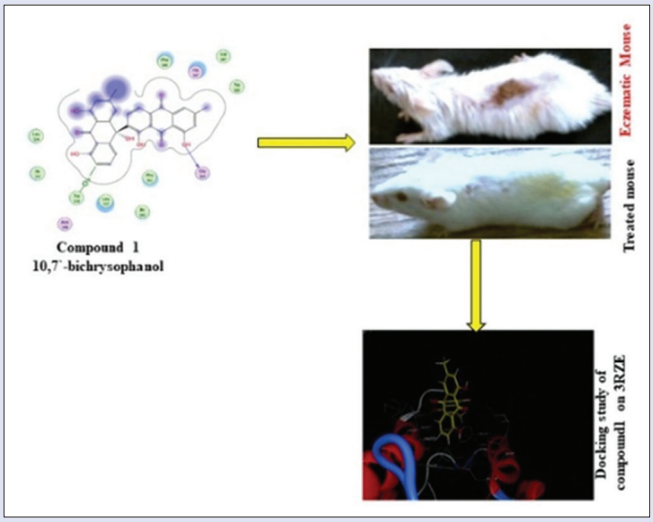
ABSTRACT

Background: Eczema or atopic dermatitis is a widely spread skin disorder; the topical application of corticosteroids is the first choice for treatment. Natural products have a great contribution in the treatment of this disease; *Asphodelus microcarpus* seeds are rich in anthraquinones and known to possess both anti-inflammatory and antidermatitis effects. **Objective:** The objective of the study is to investigate the anti-eczematic activity, acute toxicity, and molecular modeling of *A. microcarpus* seeds. **Materials and Methods:** Nuclear magnetic resonance, ultraviolet, and mass spectroscopy were applied for characterization of isolated metabolites; induction of eczema was conducted by 2% and 0.2% w/v dinitrochlorobenzene in acetone; eczema was treated with topical application of the different seed extracts in the form of ointments (1% w/w); Swiss albino mice (25–30 g) were used for the determination of LD_{50} and anti-eczematic effect. Docking studies were performed by Molecular Operating Environment software. **Results:** *A. microcarpus* seed extract exhibited promising ant-eczematic activity, six anthraquinones were isolated from chloroform portion and characterized as 10,7`-bichrysophanol (1), asphodelin (2), chrysophanol-8-O-methyl ether (3), chrysophanol (4), physcion (5), and emodin (6). Compounds 1, 3, and 5 exerted significant anti-eczematic effect. **Conclusion:** Six known anthraquinone derivatives were isolated and characterized for the first time from the seeds of *A. microcarpus*. Chloroform fraction (1% w/w) showed significant anti-eczematic effect compared to standard mometasone furoate (0.1 w/w). The docking study proved the anti-eczematic activity of anthraquinone content by their affinity to the target human histamine H_1 receptor.

Key words: Anthraquinones, *Asphodelus microcarpus,* docking, eczema, *Xanthorrhoeaceae*

SUMMARY

- Six known isolated metabolites were obtained from chloroform extract of *Asphodelus microcarpus*; the potent anti-eczematic effect of chloroform fraction on mice-induced eczema was postulated by docking study. 10,7`-bichrysophanol showed the highest affinity as antihistaminic agent toward the target human histamine H_1 receptor.



Abbreviations used: DNCB: Dinitrochlorobenzene; DMSO: Dimethyl sulfoxide; *A. microcarpus: Asphodelus microcarpus*; LD_{50}: The mean lethal dose; 3RZE: Human histamine H_1 receptor.

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INTRODUCTION

Asphodelus microcarpus (Xanthorrhoeaceae) is a herbaceous plant with roots of several spindle-shaped tubers, widely distributed over the coastal Mediterranean region.[1] Previous studies revealed the isolation of various metabolites as sterols, lipids, anthraquinones, triterpenes, and aryl coumarins from *A. microcarpus*.[2] It has been reported that natural anthraquinone derivatives possess potent anti-eczematic activity more than that of the other secondary metabolites such as flavonoid and saponins.[3,4] It has been used in otitis and toothache and as diuretic,[5] as well as a wild food source in the Medieval Levant, skin emollient, and demulcent and conventionally for the treatment of lung diseases and several ailments.[6,7] Eczema is defined as chronic inflammation of the skin manifested by itching and red patches, which may occur due to leakage of the skin barrier or stimulation of immune response. It may be mostly acute or less frequently chronic.[8] It is characterized by appearance of redness, oozing, itching, blistering,

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scaling, weeping, and/or thickening of the skin.[9] Eczema is classified into two major types; atopic, due to excessive IgE response, and nonatopic when IgE response is not observed. Eighty percent of cases are atopic and assumed to be due to other allergic reactions such as allergic rhinitis, food allergies, and asthma, which can all show an IgE response. Atopic eczema is mainly a childhood disease affecting about 20% of children; hence, it is considered the most common skin diseases worldwide. Atopic eczema does not affect only the skin but also extend beyond the skin of children to cause sleep disorders and increased psychological problems. Atopic eczema not only affects the health of the sufferer but also has a significant economic effect as it costs around $3.8 billion per year for the treatment in the United States.[8] From the economic point of view, exploration of effective treatment is of great importance. Treatment of eczema starts with application of moisturizers (occlusive, humectants, and emollients), topical corticosteroids, topical antihistamines, topical calcineurin inhibitors, systemic immunosuppressants, and phototherapy.[10,11] The side effects of corticosteroids are fully known and should be put into consideration; it may include possible suppression of the hypothalamic–pituitary–adrenal axis, particularly in children.[11] This research is focusing on discovering a proper and effective treatment for eczema, as it is a highly prevalence disease, especially among the children. The utilization of natural products as anti-dermatitis exhibited promising outcomes in the United States. However, discovering new anti-eczematic herbal medicines of high benefits and low adverse effects is of great interest.[12] In continuation of our research to explore anti-eczematic plants and/or lead compounds of natural source, *A. microcarpus* seeds were investigated.[13] The acute toxicity and anti-eczematic effect of the active metabolites on mice were studied. The docking studies suggested and postulated the mechanism of binding affinity of the secondary metabolites on human histamine H_1 (3RZE) receptor.

MATERIALS AND METHODS

General experimental procedures

Ultraviolet (UV) spectra were determined using PyeUnicam spp. 1750 spectrophotometer. Electron impact mass spectrometry (EI-MS) was carried on 502 mass spectrometers having a direct inlet system and operating at 70 eV (samples inserted between 180°C and 240°C) or on a VG Micro mass 165 spectrometer at 18, 35 or 70 eV with inlet temperature between 180°C and 240°C. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) measurements were obtained with Joel 300 NMR spectrometer operating at 300 MHz (for ^{1}H) and 75 MHz (for ^{13}C) in DMSO-*d*6 solution; chemical shifts were expressed in δ (ppm) with reference to TMS, coupling constant (*J*) in Hertz. Si gel (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia) were used for open column chromatography. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100°C for 5 min, or spraying with ammonia solution.

Plant material

A. microcarpus Salzm. Viv.(*Xanthorrhoeaceae*) seeds were collected from Ageba coast, Marsa-Matrouh, Egypt, in July 2016 and were kindly identified by Prof. Ibrahim El-Garf, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt. The plant name is indexed in the plant list as an accepted name; a voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Animals

Healthy adult male Swiss albino mice of 25–30 g body weight were obtained from the animal house of Pharmacology Department, Pharmacy College, Al-Azhar University, Cairo, Egypt. All mice were reserved at 25°C ± 1°C and 55% relative humidity with 12:12-h light: dark cycle; they were supplied with standard rodent chow and water *ad libitum*; animals were transferred to the laboratory 12 h before the experiments and were only fed with water *ad libitum*. They were housed and cared in accordance with the protocols of Al-Azhar University, and experiments were approved by the Ethical Committee for Animal Care of the University.

Induced eczema in mice

A concentration of 1% w/w of *A. microcarpus* seed extracts (methanol, chloroform, ethyl acetate and *n*-butanol) were prepared in the form of vaseline ointment. Animals were divided into six groups (12 animals each). Positive control: 0.1% w/w mometasone furoate ointment (Elocon® ointment, Schering-Plough Corporation/ USA). Negative control (placebo): vaseline (white soft paraffin). Allergen (sensitizer), to initiate eczematous reaction of the animals' skin, dinitrochlorobenzene (DNCB) was used as allergen and was purchased from Sigma Company. The initial sensitizing dose was prepared of 2% w/v DNCB in acetone and the second dose of 0.2% DNCB.[14] All animal groups (12 each) were sensitized by topical application of 2% DNCB.[14] Four days later, the eczema was induced in about 90% of the test animals. A repeated dose of 0.2% of DNCB solution was applied topically to those animals that showed no symptoms of eczema. Induced eczema was conducted in 2 days after the second DNCB dose. All groups were treated once daily with topical application of ointment with concentration of 1% w/w 70% methanolic extract, 1% w/w chloroform fraction, 1% w/w ethyl acetate fraction, and 1% w/w *n*-butanol fraction in Vaseline; a positive control of (0.1% w/w mometasone furoate ointment) was used. Treatment was continued until complete recovery of the induced eczema. The animals were daily examined, and the improved cases were recorded. At the end of the test period, the total number of the improved animals was recorded. The data were expressed as the percentage of healed animals out of treated ones. The nonparametric Chi-square test was used for analysis of the difference among the groups.[14]

Determination of the mean lethal dose (LD_{50})

40% w/v of methanolic extract (70%) of *A. microcarpus* seeds was suspended in distilled water. Nine groups of animals (6 mice each) were used.

Preliminary experiments were performed to determine the lowest dose that kills all mice (LD_{100}), the highest dose which kills nothing (LD_0), and that dose able to kills 50 % of the mice (LD_{50}). The doses were administrated orally by intubation to 6 mice per dose level. The mice were left 24 h after which mortalities were calculated adopting the Spearman-Karber method.[15]

Molecular docking study

Instruments used were Dell Precision™ T3600 Workstation (Intel Xeon E5-1660 3.3 GHz, 16 GB 1600 MHz DDR3 ECC RDIMM 1 TB [7200 RPM], 1 GB NVIDIA Quadro 2000, Windows 7 Professional [64 Bit]). Software, Molecular Operating Environment (MOE), package version 2016.08, was used for docking studies. The crystal structures of the human histamine H_1 (3RZE) receptor was obtained from the Protein Data Bank.[16,17] Docking of the co-crystallized ligand was carried out to study the scoring energy(s), root mean, and amino-acid interactions. Docking

was performed using London dG force, and refinement of the results was done using force field energy. The compounds for docking were achieved via their 3D structure. Certain steps were considered as 3D protonation of the structures, running conformational analysis using a systemic search, selecting the least energetic conformer, and applying the same docking protocol used with ligands. Amino acid interactions and the hydrogen bond lengths were detected.

The MOE package version 2016.08 is the software of choice for docking experiments.

Extraction and isolation

The air-dried powdered seeds of *A. microcarpus* (500 g) were exhaustively extracted by percolation with 70% MeOH (3 Lx3). The combined methanolic extracts were concentrated under vacuum at 40°C to dryness (80 g). The crude extract (80 g) was suspended in distilled water (200 ml) and defatted with petroleum ether. The defatted extract (60 g) was partitioned successively with chloroform, ethyl acetate, and *n*-butanol to give 10 g, 10 g, and 15 g, respectively. The chloroform fraction (10 g) was applied on Si gel column and eluted with *n*-hexane-ethyl acetate (100:0 → 50:50) to give four fractions; A (3.7 g), B (2.8 g), C (750 mg), and D (300 mg). Fraction A (3.7 g) (eluted with *n*-hexane-ethyl acetate [80:20)] was further re-chromatographed on Si gel column with benzene to give three subfractions of A_1 (1.5 g), A_2 (750 mg), and A_3 (200 mg). Fractions A_1, A_2, and A_3 were separately re-chromatographed on Si gel column with benzene and finally purified by gel filtration using Sephadex LH-20, eluted with methanol to afford four compounds; compound 1 (128 mg) – orange needle crystals; compound 2 (50 mg) – orange yellow needles from fraction A_1; compound 3 (110 mg) – reddish-brown needle from fraction A_2; and compound 4 (60 mg) – orange red prisms from fraction A_3. Fraction B (2.8 g) (eluted with *n*-hexane-ethyl acetate [70:30]) was further re-chromatographed over Si gel column with benzene-chloroform (40:60) to give three sub fractions of B_1 (170 mg), B_2 (750 mg), and B_3 (320 mg). Fraction B_2 and B_3 were separately re-chromatographed through Si gel column with *n*-hexane-ethyl acetate (80:20) and finally purified on Sephadex LH-20, eluted with methanol to afford two compounds; compound 5 (135 mg) – orange needles from fraction B_2 and compound 6 (50 mg) – orange yellow needles from fraction B_3.

RESULTS AND DISCUSSION

Structure elucidation

The UV, IR, MS, and NMR spectra of all isolated compounds confirmed the presence of anthraquinone moiety.

Compound 1; UV: λ_{max} (MeOH): 258, 288, 392 and 429 nm; IR: (KBr) 3460 (OH), 1675 (C = O) and 1620 (C = C) cm^{-1}, EI mass spectrum m/z 508$[M]^+$, 490$[M\text{-}H_2O]^+$, 476$[M\text{-}H_2O\text{-}CH_3]^+$, 448$[M\text{-}H_2O\text{-}CH_3\text{-}CO]^+$, 254[chrysophanol unit]$^+$, 240[chrysophanol unit– $CH_3]^+$. Compound 2; UV: λ_{max} (MeOH): 256, 298, 392 and 420 nm; IR: (KBr) 3464 (OH), 1679 (C = O) and 1625 (C = C) cm^{-1}. EI mass spectrum m/z 506 $[M]^+$, 488 $[M\text{-}H_2O]^+$, 477 $[M\text{-}H\text{-}CO]^+$, 253[chrysophanol unit-H]$^+$, 240 [chrysophanol unit– $CH_3]^+$. Compound 3; UV: λ_{max} (MeOH): 225, 252, 280 and 410 nm; IR: (KBr) 3454 (OH), 1670 (C = O) and 1630 (C = C) cm^{-1}, EI mass spectrum m/z 268 $[M]^+$, 210 $[M\text{-}OCH_3\text{-}CO]^+$, 182 $[M\text{-}OCH_3\text{-}2CO]^+$. Compound 4; UV: λ_{max} (MeOH): 225, 252, 280 and 412 nm; IR: (KBr) 3450 (OH), 1674 (C = O) and 1640 (C = C) cm^{-1}. EI mass spectrum m/z 254 $[M]^+$, 255 $[M + H]^+$, 237 $[M + H\text{-}H_2O]^+$, 226 $[M\text{-}CO]^+$, 198 $[M\text{-}2CO]^+$. Compound 5; UV: λ_{max} (MeOH): 223, 252, 265, 288 and 438 nm; IR: (KBr) 3460 (OH), 1680 (C = O), 1640 (C = C) and 1570 (C = C) cm^{-1}. EI mass spectrum m/z 284 $[M]^+$, 285 $[M + H]^+$, 226 $[M\text{-}OCH_3\text{-}CO]^+$, 198 $[M\text{-}OCH_3\text{-}2CO]^+$. Compound 6; UV: λ_{max} (MeOH): 222, 225, 252, 289 and 436 nm; IR: (KBr) 3440 (OH), 1675(C = O), 1625 (C = C) and 1570 (C = C) cm^{-1}. EI mass spectrum m/z 270 [M] +, 271 [M + H] +, 197 $[M + H\text{-}H_2O\text{-}2CO]^+$. While compounds 1–4 shared the chrysophanol skeleton.[18,19] The dimeric nature of compounds 1 and 2 was proved by fragment ion peaks along with their degree of unsaturation.[18,20] The ^{1}H-NMR spectra of compounds 1, 3, 4, 5, and 6 showed a pair of meta-coupled protons, while compound 2 exhibited singlet proton with the absence of (H-4) signal, indicating the site of attachment. The ^{13}C-NMR spectra of compounds 1 and 2 are quite similar except in the absence of C-10 quinonoid carbonyl signal in compound 1 along with the appearance of another oxygenated carbon signal corresponding to a tertiary alcoholic group which is indicative for the site of attachment. The replacement of ABX spin system by a pair of ortho-coupled protons of (H-5`) and (H-6`); indicating that the point of attachment in the second monomer of compounds 1 and 2 is at C-7`.[21,22] The structure elucidation of compounds 1 and 2 was established as 10,7`-bichrysophanol and asphodelin compared to those spectra reported in literature.[21,22] The ^{13}C-NMR spectra of compounds 3, 4, 5, and 6 were almost similar; the upfield shift of C-6 and C-8 in compound 3 and C-6 in compound 5 indicates their substitution by OCH_3.[23,24] The structure elucidation of compounds 3, 4, 5, and 6 was fully characterized as chrysophanol-8-O-methyl ether,[25] chrysophanol,[26] physcion,[24] and emodin,[27] upon comparison with the literature [Figure 1 and Tables 1, 2].

The induced eczema in mice

The sensitized animals were treated by topical application of the ointments, freshly prepared in concentration of 1% w/w of the different seed fractions. Results revealed that: Group 1 – 9 mice out of 12 (75%) treated with 1% w/w 70% methanolic extract were improved in 5–12 days; Group 2 – all mice (100%) treated with 1% w/w chloroform fraction were improved within 4–9 days; Group 3 – 11 mice out of 12 (91.7%) treated with 1% w/w ethyl acetate fraction were improved in 4–10 days; Group 4 – 9 mice out of 12 (75%) treated with 1% w/w *n*-butanol fraction were improved 5–12 days; Group 5 – 9 mice out of 12 (75%) treated with 0.1% mometasone furoate ointment were improved during 4–12 days; Group 6 treated with vaseline showed no improvement. It is clear that

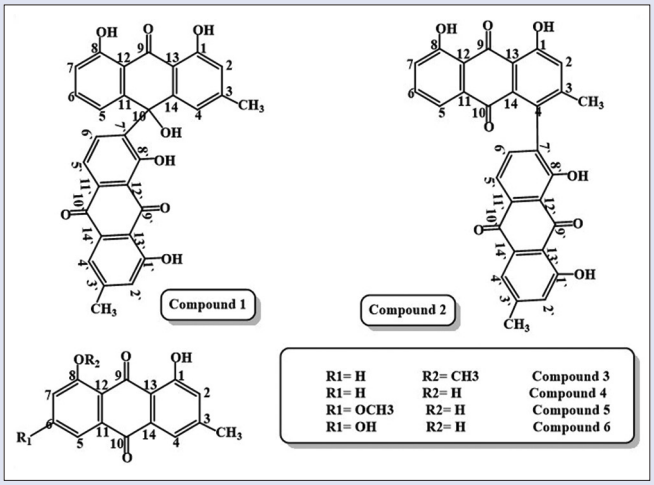

Figure 1: Secondary metabolites isolated from *Asphodelus microcarpus*

Table 1: ^{1}H NMR spectral data of compounds (1-6) (300 MHz, dimethyl sulfoxide-d_6)

Position	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
2	6.78, brs	7.43, s	7.06, d, 1.4	7.04, brs	6.85, brs	6.30, brs
4	6.66, brs	-	7.37, d, 1.4	7.37, brs	7.70, brs	7.50, brs
5	6.80, d, 7.8	7.34, d, 7.5	7.55, d, 8.3	7.25, d, 8.4	7.60, d, 2.2	7.17, s
6	7.48, dd, 8.1, 7.8	7.72, t, 7.5	7.70, dd, 8.3, 8.1	7.68, dd, 8.4, 7.2	-	-
7	6.92, d, 8.1	7.52, d, 7.5	7.76, d, 8.1	7.56, d, 7.2	7.15, d, 2.2	6.85, s
CH_3	2.21, s	2.46, s	2.35, s	2.35, s	2.39 s	2.25, s
OCH_3	-	-	3.92, s	-	3.80 s	-
2`	6.90, brs	7.22, brs	-	-	-	-
3`	7.19, brs	-	-	-	-	-
4`	-	7.60, brs	-	-	-	-
5`	7.76, d, 8.1	7.54, d, 7.2	-	-	–	-
6`	8.63, d, 8.1	7.81, d, 7.2	-	-	-	-
CH_3	2.07, s	2.07, s	-	-	-	-

Table 2: ^{13}C NMR spectral data of compounds (1-6) (75 MHz, dimethyl sulfoxide -d_6)

Position	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
1	161.39	161.84	161.57	161.52	164.31	164.42
2	116.36	124.95	119.21	120.61	123.75	123.76
3	147.85	149.30	147.22	149.06	147.23	147.82
4	120.86	127.80	123.90	124.22	120.45	120.14
5	119.33	124.08	119.20	123.9	108.51	108.26
6	136.74	137.38	136.03	137.17	165.42	166.13
7	116.65	123.90	119.68	119.18	107.63	107.69
8	161.11	161.62	160.53	161.30	161.24	161.26
9	192.60	192.60	187.42	191.35	189.23	189.06
10	69.52	181.00	181.97	181.00	180.69	180.77
11	148.30	130.84	134.78	133.10	134.57	134.58
12	115.25	115.08	119.10	115.60	108.56	109.05
13	113.25	113.77	114.45	113.50	112.85	112.92
14	149.00	131.90	131.90	132.75	132.29	132.35
CH_3	22.10	21.66	21.41	21.55	21.30	21.44
OCH_3	-	-	56.46	-	56.00	-
1`	161.00	160.78	-	-	-	-
2`	118.85	120.61	-	-	-	-
3`	147.75	148.72	-	-	-	-
4`	123.29	119.63	-	-	-	-
5`	120.43	119.48	-	-	-	-
6`	132.79	137.02	-	-	-	-
7`	132.62	136.46	-	-	-	-
8`	157.50	158.92	-	-	-	-
9`	192.60	192.90	-	-	-	-
10`	180.65	181.48	-	-	-	-
11`	132.13	133.18	-	-	-	-
12`	114.21	114.97	-	-	-	-
13`	112.14	113.58	-	-	-	
14`	141.62	133.72	-	-	-	-
CH_3	22.10	20.69	-	-	-	-

Table 3: One-way variance analysis of the effect of different fractions of *Asphodelus microcarpus* seeds

	Group 2	Group 3	Group 4	Group 5
Group 1 (*P*)	0.016	0.067 (NS)	0.645 (NS)	1.0 (NS)
Group 2 (*P*)		0.101 (NS)	0.003	0.001
Group 3 (*P*)			0.013	0.007
Group 4 (*P*)				0.0374

NS: Not significant

1% w/w concentration of 70% total extract, chloroform fraction, ethyl acetate fraction, *n*-butanol fraction, and 0.1% mometasone furoate ointment are statistically comparable. ANOVA (one-way analysis) was performed using the MINITAB program (version 12.21). Analysis by ANOVA revealed that the effect of chloroform and ethyl acetate fractions significantly differed from the effect of mometasone furoate ($P = 0.001$ and 0.007, respectively). However, there was no significant difference observed between the effect of mometasone furoate and the other extracts ($P < 0.05$) [Table 3 and Figure 2].

The mean lethal dose

The mean lethal dose (LD_{50}) of any drug is the amount of the drug administered to the animals, causing lethal effects in 50% of them. Spearman-Karber method assumes that the toxicity of an orally administered drug is directly proportional to its absorption rate from gastrointestinal tract. The experiment revealed that the LD_{100} is 9.91 g/kg (mice) while the LD_0 is 3.28 g/kg (mice) and LD_{50} is 5.08 g/kg (mice) with Fiducial limit of 4.45 and 5.81 g/kg (mice) for 70% methanolic extract of *A. microcarpus* seeds [Figure 3].

Molecular docking study

The suggested mechanism of anti-eczematic action of the chloroformic extract was confirmed by docking; the activity is mainly attributed to hydrogen-bond formation and hydrophobic or dipole–dipole interaction between the active metabolites (1, 3, and 5) and the target receptor 3RZE [Table 4 and Figures 4-6].[28,29] The study suggested that the anthraquinone derivatives (1, 3, and 5) may be used as anti-histaminic and anti-eczematic agents.

Compound 1, as a ligand, had score energy (S) of −5.9040 kcal/mol. The interactions between compound 1 and 3RZE included hydrogen-bonding (Gly 164 with–OH) and hydrophobic [Trp 158 with H; Figure 4].

Compound 3 as a ligand had score energy (S) of –4.5872 kcal/mol. The Lys 179, Lys 191, and Tyr 431 amino acids were responsible for dipole–dipole interaction between phosphate group of the amino acids and the oxygen of phenolic group of the compound as well as the hydrogen bonding between Tyr 431 and C = O group [Figure 5].

Compound 5 is a ligand with score energy (S) of –4.2840 kcal/mol. It showed two hydrogen bonds at Asp 107 with OH and Tyr 431 with C = O, as well as dipole–dipole interaction between phosphate group of Lys 179, Lys 191, and Tyr 431 amino acids and the *p*-carbon of phenol [Figure 6].

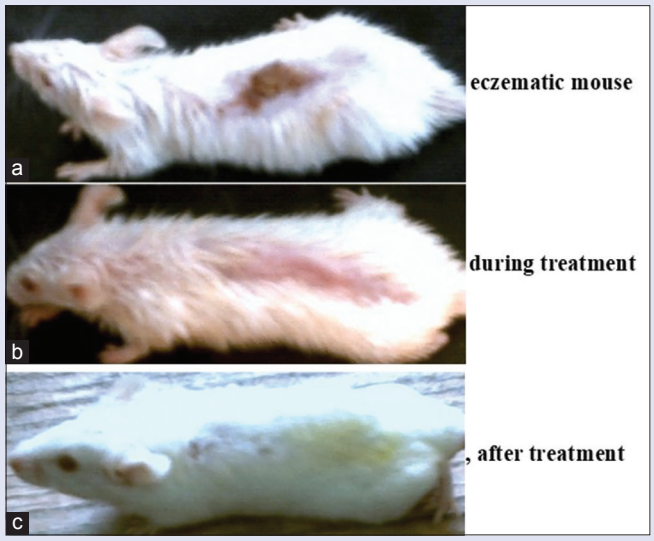

Figure 2: The phase treatment of induced eczema illustrated in photos (a-c)

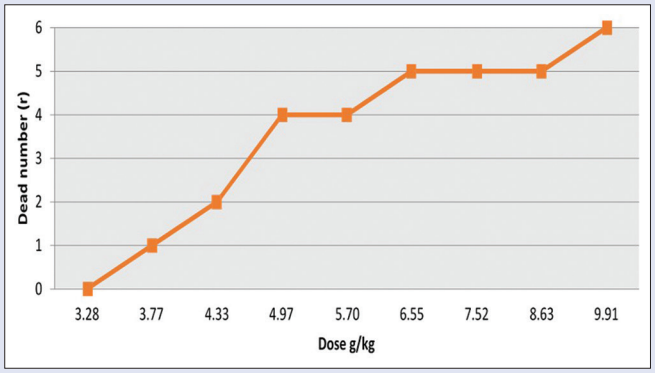

Figure 3: Determination of LD_{50} of 70% methanolic extract of *Asphodelus microcarpus* seeds

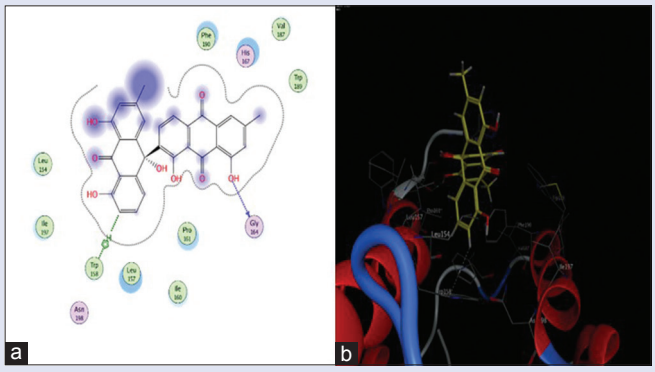

Figure 4: (a) Two-dimensional and (b) three-dimensional interactions of compound 1 with the human histamine H_1 receptor

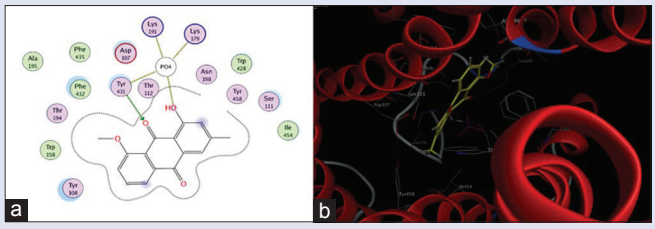

Figure 5: (a) Two-dimensional and (b) three-dimensional interactions of compound 3 with the human histamine H_1 receptor

Table 4: Molecular modeling data for compounds 1, 3, and 5 with human histamine H_1 receptor

Compound number	Affinity (Kcal/mol)	Type of interaction	Receptor amino acids' names and numbers	Functional groups
1	−5.9040	Hydrogen bond	Gly 164	OH
		Hydrophobic	Trp 158	aromatic
3	−4.5872	Dipole-dipole interaction	Lys 179	
		Hydrogen bond	Lys 191	$O\text{-}C^{+\delta}$
			Tyr 431	
			Tyr 431	C=O
5	−4.2840	Hydrogen bond	Asp 107	OH
		dipole-dipole interaction	Tyr 431	C=O
			Lys 179	$O\text{-}C^{+\delta}$ (*p*-C)
			Lys 191	
			Tyr 431	

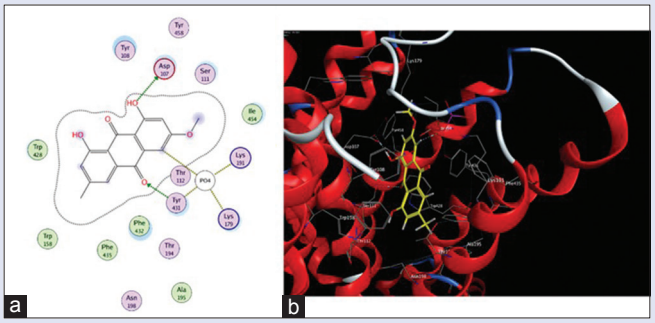

Figure 6: (a) Two-dimensional and (b) three-dimensional interactions of compound 5 with the human histamine H_1 receptor

CONCLUSION

The present study led to isolation and characterization of six known anthraquinone derivatives for the first time from the seeds of *A. microcarpus*. The1% w/w chloroform fraction exhibited significant and potent anti-eczematic effect compared to 0.1 w/w standard mometasone furoate. The docking study proved the mechanism of anti-eczematic action of chloroform fraction; anthraquinone derivatives 1, 3, and 5 exerted good affinity to the target receptor, human histamine H_1 (3RZE). The promising activity of isolated natural anthraquinones was confirmed by docking, but further studies are required for investigation of the action mechanism. The natural anthraquinone derivatives exhibited potent anti-eczematic activity more than the other secondary metabolites such as flavonoid and saponins. All parts of *A. microcarpus* are rich in anthraquinone derivatives which are concentrated in seed. To the best of our knowledge, it is the first study for phytochemical and biological evaluation *A. microcarpus* seeds.

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Conflicts of interest

There are no conflicts of interest.

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