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Echinochrome prevents 7,12-dimethylbenz[a]anthracene toxicity in liver of rats: docking and *in vivo* study

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ABSTRACT

7,12-dimethylbenz[a]anthracene (DMBA), an environmental pollutant, is one of the most dangerous polycyclic aromatic hydrocarbons. Echinochrome (Ech) is considered one of the most popular and important substances that is found in shells, spines, and eggs of sea urchins that possesses high antioxidant activity. The present study was carried out to evaluate the curative and protective effects of echinochrome pigment and demonstrate its mechanism against DMBA-induced liver toxicity. Docking calculations were performed on CYP1B1, CYP1A1 and mEH protein model. Experimental rats were assigned into two main groups: protective group (treated with echinochrome for 14 days and then administrated DMBA) and curative group (administrated DMBA and then treated with echinochrome for 14 days). Each group is divided into 3 sub-groups: control, DMBA (15 mg/kg body, weight orally), and DMBA/echinochrome (1 mg/kg body, weight orally) groups. According to docking results, the binding of echinochrome A to CYP1A1 domain is higher than the binding of DMBA to CYP1A1 domain. Administration of echinochrome decreased aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and malondialdehyde levels and increased glutathione reduced and catalase levels in both protective and curative groups. Histology of hepatic tissues improved after the treatment with echinochrome. The results of this study demonstrated the potential protective and curative activities of echinochrome against DMBA toxicity. Echinochrome inhibits the activities of CYP1A1, CYP1B1 and mEH enzymes preventing DMBA bioactivation and restored the balance between reactive oxygen species formation and internal antioxidant enzymes by its powerful antioxidant activity.

KEYWORDS: echinochrome, DMBA, toxicity, oxidative stress, docking, sea urchin.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and their alkylated derivatives are very harmful pollutant compounds in the environment [1]. They are formed as a product of pyrolytic processes of organic substances and the incomplete combustion of organic waste, natural gas, coke, grilled flesh, wood, and fossil fuel [2]. PAHs can be absorbed by dermal contact, inhalation, and ingestion. Their levels among the population increase through cigarette smoking and food contaminated with PAHs [3]. Several studies have proven that these PAH materials could cause severe cell damage and mutations leading to cancer in humans exposed to a high dosage of PAHs [4].

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7,12-dimethylbenz[a]anthracene (DMBA), an environmental pollutant, is one of the most dangerous polycyclic aromatic hydrocarbons. DMBA is one of the PAHs which causes hepatotoxicity and carcinogenicity in addition to changing phase I and II enzymes involved in the liver metabolic process [5]. Furthermore, it is considered an immunosuppressor and tumor initiator [6]. CYP1B1 is a member of the cytochrome P450 enzyme family 1, subfamily B and polypeptide 1. Metabolism and bioactivation of polycyclic aromatic hydrocarbons is performed in the liver by CYP1B1 [7]. Furthermore, microsomal epoxide hydrolase (mEH) is involved in the metabolism of xenobiotics like DMBA to generate dihydrodiol intermediates in the presence of the cytochrome CYP1B1 [8].

During metabolic activation of DMBA, extravagant reactive oxygen species (ROS) are released [9]. Many studies have agreed that DMBA induces reactive oxygen species production during DMBA metabolic activation that leads to lipid peroxidation, DNA damage, and cell antioxidant defense system depletion [10]. Besides, the liver is one of the first organs that can be exposed to the damaging effect of DMBA [11]. Marine natural products have been used in the treatment of many diseases [12]. The biodiversity in the marine environment results in presenting a variety of molecules with unique structural characteristics by many different classes of marine organisms [13]. The sea urchin is a species widely distributed in the Atlantic and the Mediterranean coasts [14]. It belongs to the family of Echinidae, has spherical morphology and reaches to 7 cm in diameter with soft, thick, and long spins up to 3 cm.

Echinochrome (Ech) is considered one of the most popular and important substances that is found in shells, spines, and eggs of sea urchins that possesses high antioxidant activity [15]. It is a water-insoluble compound that possesses strong antioxidant activity and is considered to be the active ingredient of the Histochrome drug [16]. The antioxidant mechanisms of Ech include reduction of oxidative stress [17], interaction with lipoperoxide radicals [18], chelation of metal ions [19], inhibition of lipid peroxidation [20], and regulation of the cell redox potential [21]. Also, recent studies have discovered the hypoglycemic [22], anticancer [23] and hypolipidemic [24] activities of Ech.

Thus, the present study was carried out to evaluate the curative and protective effects of echinochrome (Ech) pigment and demonstrate its mechanism against DMBA-induced liver toxicity in rats.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Dimethyl sulfoxide (DMSO) and 7,12-dimethylbenz[a]anthracene (DMBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The standardized echinochrome was purchased in a powder form from the G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Vladivostok. All kits were purchased from the Biodiagnostic Company (El Motor St, Dokki, Egypt).

2.2. Computational protein-ligand docking simulation

Docking calculations were performed using Docking Server (www.dockingserver.com) [25]. Gasteiger partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were performed on CYP1B1 (PDB ID: 6iq5), CYP1A1 (PDB ID: 6O5Y) and mEH (PDB ID: 1qo7) protein model.

2.3. Sea urchin collection

Sea urchins (*Paracentrotus lividus*) were collected from the Mediterranean sea of Alexandria (Egypt) in icebox and transported to the laboratory. The samples were rinsed with seawater to remove sand and other growing organisms at the collection place and placed in the ice box. Identification of the collected samples was done by using the standard literature of the taxonomic guide. The experimental use of urchins was done in agreement with the Wild Animal Welfare regulations.

2.4. Extraction of echinochrome (Ech)

Once the internal organs were removed, the shells and spins were washed with a stream of cold water, air-dried at 4 °C for 2 days in the dark and then were ground. The powders (5 g) were dissolved by gradually adding 10 ml of 6 M HCl.

The pigments of the solution were isolated 3 times with the same volume of diethyl ether. The ether layer collected was washed using 5% NaCl to remove any remaining acid. The ether solution containing the pigments was dried over the anhydrous sodium sulfate and the solvent was evaporated under reduced pressure [15, 26]. The extract containing the Ech pigment was stored at -30 °C in the dark. Each 10 gram of shells and spins powders gives 4.12 mg Ech.

2.5. High performance liquid chromatography (HPLC) analysis

Chromatographic imprint analysis of Ech was performed using the liquid chromatographic apparatus Shimadzu, which comprised of two LC20AD pumps, a DGU-20 A3 degasser, and an SPD M20 A diode array detector. The separation was carried out on a 4.6 mm i. d. × 150 mm, 5 µm particle, Luna C18 column (Phenomenex) with a Security Guard pre-column (2 mm) containing the same adsorbent, operating at room temperature and with a flow rate of 1.0 mL/min. The binary mobile phase comprised of 0.1% formic acid (A) and (B) acetonitrile-methanol (5:9, v/v). The elution form was as following: 0-25 min 30-80% B in A (linear gradient). The injection volume was 20 µL. Detection was executed within wavelengths between 200 to 800 nm. The data analysis system included the LC Solution (Shimadzu). The extract was dissolved in ethanol at a concentration of 3 mg/mL and filtered (0.45 µm).

2.6. Experimental animals

The experimental animals used in this study were male Wistar albino (*Rattus norvegicus*) rats (130-150 g). Rats were purchased from the National Research Center (NRC, Dokki, Giza). Animals were housed in polycarbonate boxes with steel-wire tops and bedded with wood shavings (6 rats per box), in the well-ventilated animal house of the Zoology Department, Faculty of Science, Cairo University. They were supplied with a standard laboratory diet and water *ad libitum*. The animals were kept under fixed suitable conditions of housing and handling comprising of a 12 hr/12 hr light-dark cycle at (22-25 °C) room temperature. Animals were kept in the laboratory for 7 days to get adapted to laboratory conditions prior to the beginning of the experiments.

2.7. Ethical consideration

Experimental protocols and procedures in this study were approved by the Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CU/I/F/55/18). All the experimental procedures were performed according to international guidelines for the care and use of laboratory animals.

2.8. Experimental design

Thirty-six male Wistar albino rats were assigned into two main groups, the Pre-treated group (18 rats), and the Post-treated group (18 rats).

A. The Pre-treated (protective) group was divided into 3 subgroups, each subgroup containing 6 rats:

- **Subgroup 1:** served as control and received 1 ml of 2% DMSO daily before a single dosage of corn oil by oral gavage.
- **Subgroup 2:** received 1 ml of 2% DMSO for 14 days before a single dosage of DMBA (15 mg/kg body, weight orally) [27].
- **Subgroup 3:** received 1 ml Ech (1 mg/kg body weight, in 2% DMSO, orally) [28] for 14 days before a single dosage of DMBA (15 mg/kg body weight, orally).

The animals were then euthanized 4 days after DMBA administration.

B. The Post-treated (curative) group was also divided into 3 subgroups, each subgroup containing 6 rats:

- **Subgroup 1:** served as a control, administrated a single dosage of corn oil by oral gavage, and then, after 4 days, received 1 ml of 2% DMSO for 14 days.
- **Subgroup 2:** administrated a single dosage of DMBA (15 mg/kg body weight, orally), and then, after 4 days, received 1 ml 2% DMSO for 14 days.
- **Subgroup 3:** administrated a single dosage of DMBA (15 mg/kg body weight, orally), and then, after 4 days, received 1 ml of Ech (1 mg/kg body weight, in 2% DMSO, orally) [23] for 14 days.

2.9. Animal handling and collection of the samples

At the end of the experiment, the animals were euthanized with sodium pentobarbital (150 mg/kg, i.p) [29]. The blood samples of the animals were immediately collected in sterile centrifuge tubes. The liver was enucleated and transferred to a filter paper for removing blood traces. Pieces of the liver tissues were stored at -80 °C for biochemical analyses. One of the pieces of the liver tissues was suspended in 10% formal saline for histopathological studies.

2.10. Serum preparation

Blood samples were centrifuged for 20 min. at 3000 rpm. The collected serum was stored at -20 °C until use for biochemical tests.

2.11. Liver homogenate preparation

Liver tissue was homogenized in ice-cold 0.1 M Tris-HCl buffers (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min. at 4 °C. The resultant supernatant was kept in -20 °C for use in the biochemical analyses.

2.12. Biochemical assessment

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Reitman and Frankel [30]. Serum alkaline phosphatase (ALP) [31], malondialdehyde (MDA) [32], glutathione reduced (GSH) [33], and catalase [34] were determined according to the manufactures' instructions using Biodiagnostic kits (Giza, Egypt).

2.13. Histopathological examination

The liver was fixed in 10% neutral-buffered formalin. The fixed specimens were washed, dehydrated, and embedded in paraffin wax. The tissues were sectioned at a thickness of 4-5 µm and stained with hematoxylin and eosin (H&E) according to Bancroft and Stevens [35], as routine procedures for histopathological examination.

2.14. Statistical analysis

Values were expressed as means ± SE. The comparisons within groups were evaluated utilizing one-way analysis of variance (ANOVA) while Duncan post hoc test was used to compare the group means and $p < 0.05$ was considered statistically significant. SPSS for Windows (version 15.0) was used for the statistical analysis. Percentage of Ech improvement in all measured parameters was calculated from the following equation:

$$\text{percentage of change} = \left| \frac{mean\ of\ Ech - Mean\ of\ DMBA}{mean\ of\ DMBA} \right| \times 100$$

3. RESULTS

3.1. Computational protein-ligand docking

Computational protein-ligand docking simulation results suggested that the affinity of CYP1B1 to bind with Ech-A was more than that of DMBA (Figure 1; Table 1).

The possible binding modes of echinochrome A at mEH active sites are shown in Table 1 and Figure 2.

According to docking results, the binding of echinochrome A to CYP1A1 domain is higher than the binding of DMBA with CYP1A1domain (Figure 3; Table 1).

3.2. Characterization of the extracted Ech

The HPLC studies showed a major peak at retention time 3.75 min that was identical to the standard Ech. The purity of the extracted Ech was 96.22% (Figure 4).

3.3. Liver function enzymes

Table 2 shows a significant increase ($P < 0.05$) in serum ALP, ALT, and AST activities in DMBA groups, as compared to the corresponding control groups, while a significant decrease ($P < 0.05$) was observed in serum ALP, ALT, and AST activities after oral administration of Ech (1 mg/Kg body weight), as compared to the corresponding DMBA groups. The percentage of change of Ech in the protective group was higher than the curative group.

3.4. Oxidative stress markers

A significant increase ($P < 0.05$) was observed in the MDA concentration of DMBA groups while GSH and CAT levels decreased, as compared to the corresponding control groups. Meanwhile oral administration of Ech (1 mg/Kg body weight) caused a significant decrease ($P < 0.05$) in MDA

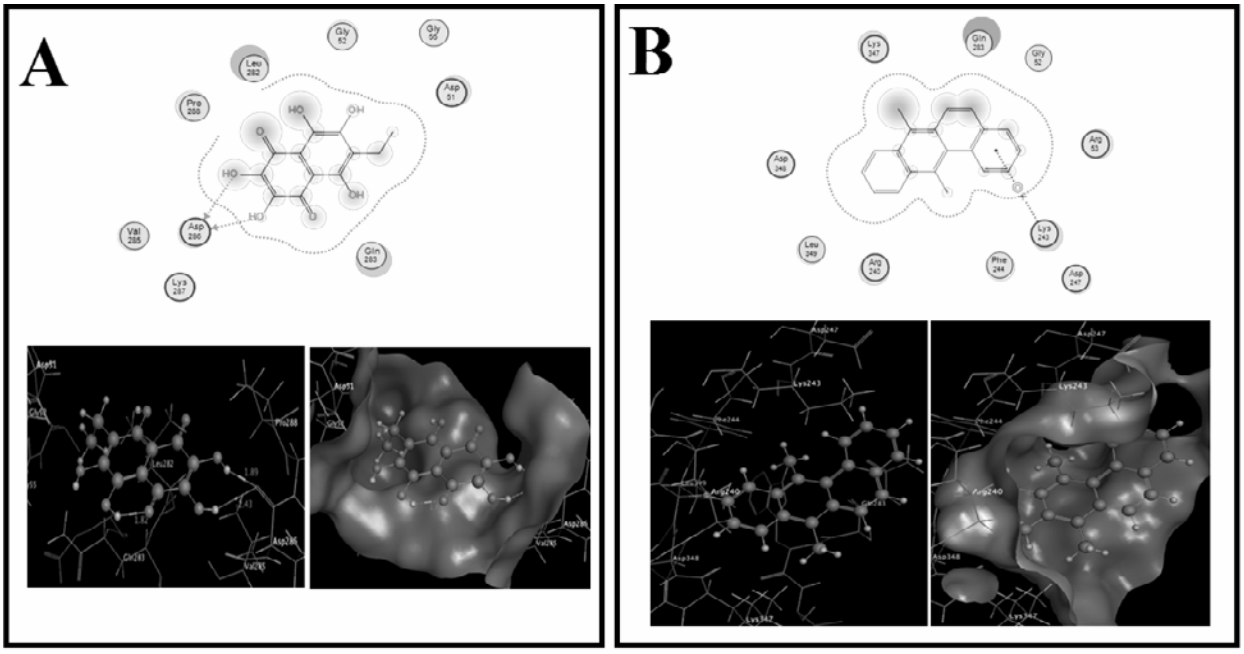

Figure 1. Molecular docking simulation studies of the interaction between Ech (A) and DMBA (B) with the active site of the CYP1B1 (PDB ID: 6iq5). Hydrophobic interactions with amino acid residues are shown using dotted curves. The docked conformation of the compound is shown using ball and stick representation.

Table 1. The docking data calculations of the interaction of Ech and DMBA with the active site of CYP1B1 (PDB ID: 6iq5), CYP1A1 (PDB ID: 6O5Y) and mEH (PDB ID: 1qo7).

Enzyme	Ligand	Atom	Receptor	Interaction	Distance (Å)*	E (kcal/mol)
CYP1B1	Ech	O 6	O ASP 286	H-donor	2.80 (1.87)	-3.0
		O 7	O ASP 286	H-donor	3.28 (2.43)	-1.1
	DMBA	6-ring	NZ LYS 243	pi-cation	4.27	-3.2
CYP1A1	Ech	O 2	OD1 ASP 313	H-donor	3.09 (2.28)	-0.9
		O 3	OD1 ASP 313	H-donor	3.23 (2.18)	-1.5
		O 6	OD2 ASP 320	H-donor	2.95 (2.06)	-3.7
	DMBA	6-ring	NZ LYS 456	pi-cation	3.87	-2.1
MEH	Ech	O 6	O ASP 286	H-donor	2.80 (1.87)	-3.0
		O 7	O ASP 286	H-donor	3.28 (2.43)	-1.1
	DMBA-epoxide	6-ring	NZ LYS 7	pi-cation	3.71	-1.2
		6-ring	NZ LYS 7	pi-cation	3.90	-1.0

*The lengths of H-bonds are in brackets. Stronger interaction between the enzyme and molecule produces more negative energy and stable complex.

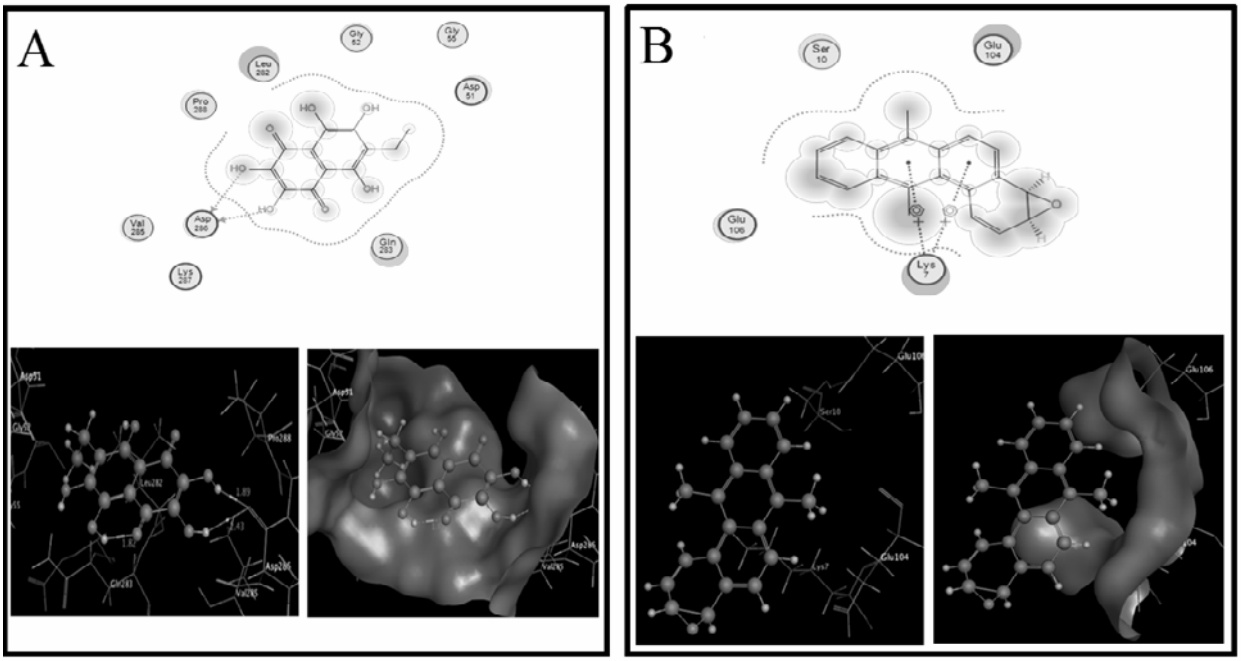

Figure 2. Molecular docking simulation studies of the interaction between Ech (A) and DMBA-epoxide (B) with the active site of mEH (PDB ID: 1qo7). Hydrophobic interactions with amino acid residues are shown using dotted curves. The docked conformation of the compound is shown using ball and stick representation.

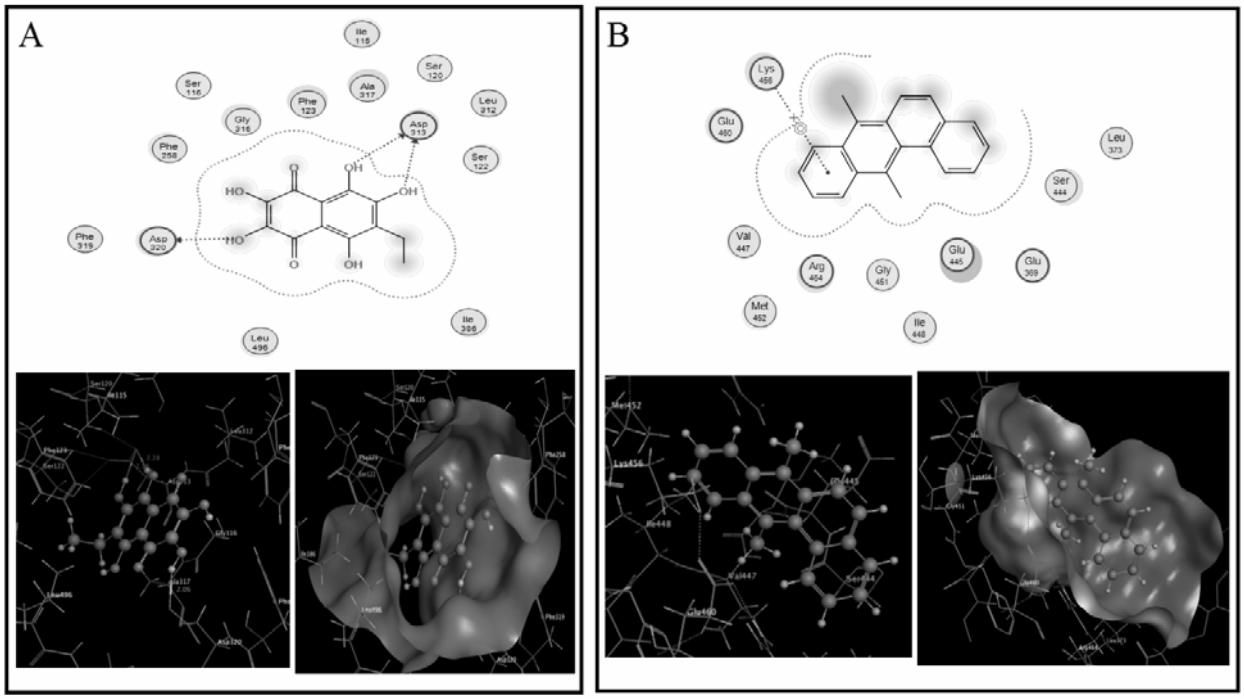

Figure 3. Molecular docking simulation studies of the interaction between Ech (A) and DMBA (B) with the active site of the CYP1A1 (PDB ID: 6O5Y). Hydrophobic interactions with amino acid residues are shown using dotted curves. The docked conformation of the compound is shown using ball and stick representation.

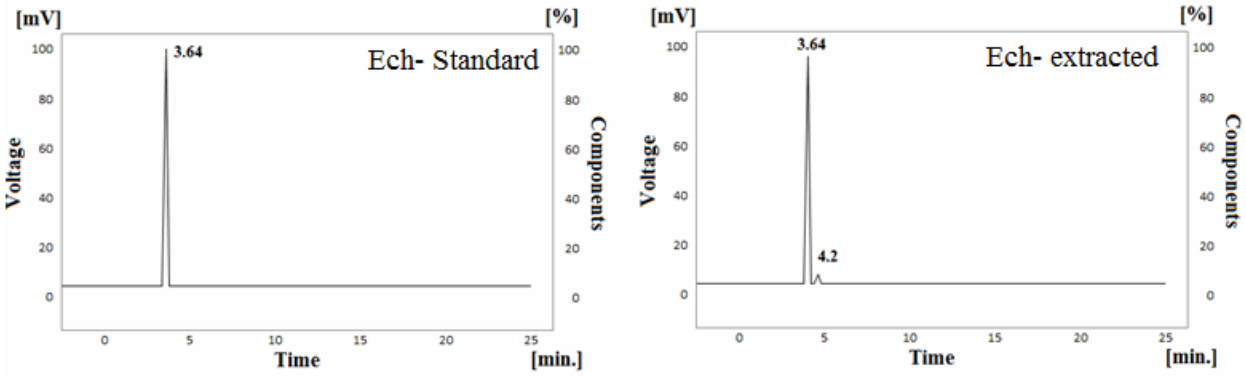

Figure 4. HPLC analysis of the standard and extracted echinochrome.

Table 2. Curative and protective potency of Ech on liver function markers of DMBA-intoxicated rats.

Treatment	Groups	ALP (U/L)	(AST) (U/L)	ALT (U/L)
Curative	Control	154.22 ± 5.43^{a}	83.08 ± 0.26^{a}	27.13 ± 0.42^{a}
	DMBA	255.43 ± 9.17^{b}	140.21 ± 0.68^{c}	48.54 ± 1.05^{c}
	Ech	187.15 ± 5.82^{a}	108.47 ± 0.41^{b}	31.59 ± 1.2^{b}
	% of change	-16.74	-22.69	-34.92
Protective	Control	143.48 ± 5.04^{a}	75.65 ± 0.24^{a}	20.79 ± 0.33^{a}
	DMBA	238.62 ± 1.4^{3b}	127.83 ± 0.11^{b}	43.5 ± 1.14^{b}
	Ech	163.10 ± 2.08^{a}	86.38 ± 1.08^{a}	12.03 ± 1.62^{a}
	% of change	-31.63	-32.49	-51.66

Values are given as means ± standard error (n = 6 per group). Each value not sharing a common superscripted letter is significantly different ($P < 0.05$).

concentration while GSH and CAT levels increased, as compared to the corresponding DMBA groups. The percentage of change of Ech in the protective group was higher than the curative group (Table 3).

3.5. Histopathological examination of the liver

The liver of control groups is formed of the classic hepatic lobules including hepatocyte (H), sinusoids (S), and Von Kupffer cells (K). Hepatocytes appeared polygonal in shape with rounded vesicular nuclei. Blood sinusoids were seen separating the cords of the liver cells and lined by flattened endothelial cells and Von Kupffer cells (Figure 5a). The DMBA groups showed enlargement of hepatocytes (H), necrosis (N), apoptosis (A), and sever destructive changes in hepatocytes (Figure 5b). The groups treated with Ech showed normal hepatocyte and improved liver architecture (Figure 5c).

4. DISCUSSION

Atmospheric pollutants such as polycyclic aromatic hydrocarbons (PAHs) spread abundantly in the environment and reach humans through air, water, and food [36]. 7,12-dimethylbenz[a]anthracene (DMBA) an environmental pollutant, is a PAH that exhibits numerous carcinogenic and toxic effects [37]. Cytotoxicity, carcinogenicity, mutagenicity, and immunosuppression are well-known activities of DMBA [38].

Table 3. Curative and protective potency of Ech on oxidative stress markers of DMBA-intoxicated rats.

Treatment	Groups	MDA (nmol/g. tissue)	GSH (mg/g. tissue)	CAT (U/g. tissue)
		Liver	Liver	Liver
Curative	Control	1.13 ± 0.03^{a}	11.63 ± 0.14^{c}	10.80 ± 0.22^{c}
	DMBA	1.62 ± 0.04^{c}	9.30 ± 0.10^{a}	7.21 ± 0.41^{a}
	Ech	1.35 ± 0.04^{b}	10.89 ± 0.11^{b}	8.37 ± 0.15^{b}
	% of change	-17.15	17.06	16.06
Protective	Control	1.06 ± 0.03^{a}	12.50 ± 0.36^{b}	9.07 ± 0.16^{c}
	DMBA	1.51 ± 0.14^{b}	10.09 ± 0.14^{a}	4.87 ± 0.53^{a}
	Ech	1.18 ± 0.01^{a}	11.96 ± 0.19^{a}	7.79 ± 0.48^{b}
	% of change	-21.63	18.53	59.78

Values are given as means ± standard error (n = 6 per group). Each value not sharing a common superscripted letter is significantly different ($P < 0.05$).

The liver is the main organ of many chemical detoxifications, which may result in liver toxicity [5]. Metabolism of DMBA in liver mediated by cytochrome CYP1B1 in liver microsomes produces toxic substances such as diol epoxides and other reactive oxygen species (ROS) [39]. Besides, these toxic substances may bind to DNA causing chromosomal mutation [40]. The present study showed a significant increase in the serum AST, ALT, and ALP levels in the DMBA groups. The elevation in ALT, AST, and ALP levels is a marker for liver injury and hepatocellular damage caused by many toxic substances [28]. This elevation in liver enzymes has been associated with the increased membrane permeability of hepatocytes leading to enzyme leakage into the blood circulation [41]. Furthermore, histopathological examination of liver tissue in the present studyrevealed damage to liver cells that were confirmed by biochemical analyses. On the other hand, Ech administration restores serum liver enzymes to near normal and improves histology of liver tissue. Docking results showed the ability of Ech to inhibit DMBA bioactivation by binding to both CYP1B1 and mEH enzymes (enzymes responsible for DMBA bioactivation).

DMBA and their metabolism products can stimulate ROS formation causing oxidative stress [42]. In the present study, DMBA groups showed an increase in MDA concentration and a decrease in GSH, and CAT levels. MDA elevation is a marker for lipid peroxidation during tissue injury and the inability of antioxidant defense systems to consume ROS [43]. DMBA inactivates mitochondrial enzymes and stimulates lipid peroxidation by its ability to generate ROS [44]. GSH is the main non-protein thiol antioxidant compound involved in the detoxification pathways [45]. Also, catalase is an important enzyme in the internal antioxidant system which catalyzes the breakdown of hydrogen peroxide, thereby protecting the cells from oxidative damage [46]. The decrease in CAT and GSH levels recorded in DMBA groups may be due to the enhanced ROS production resulting from the oxidative stress conditions [47]. However, the treatment with Ech decreases MDA concentration and induces GSH nad CAT production. These results demonstrated that Ech restored the balance between ROS formation and internal antioxidant enzymes and consequently enhanced hepatic protection [48].

5. CONCLUSION

The results of this study demonstrated the potential protective and curative activities of echinochrome (Ech) against DMBA toxicity.

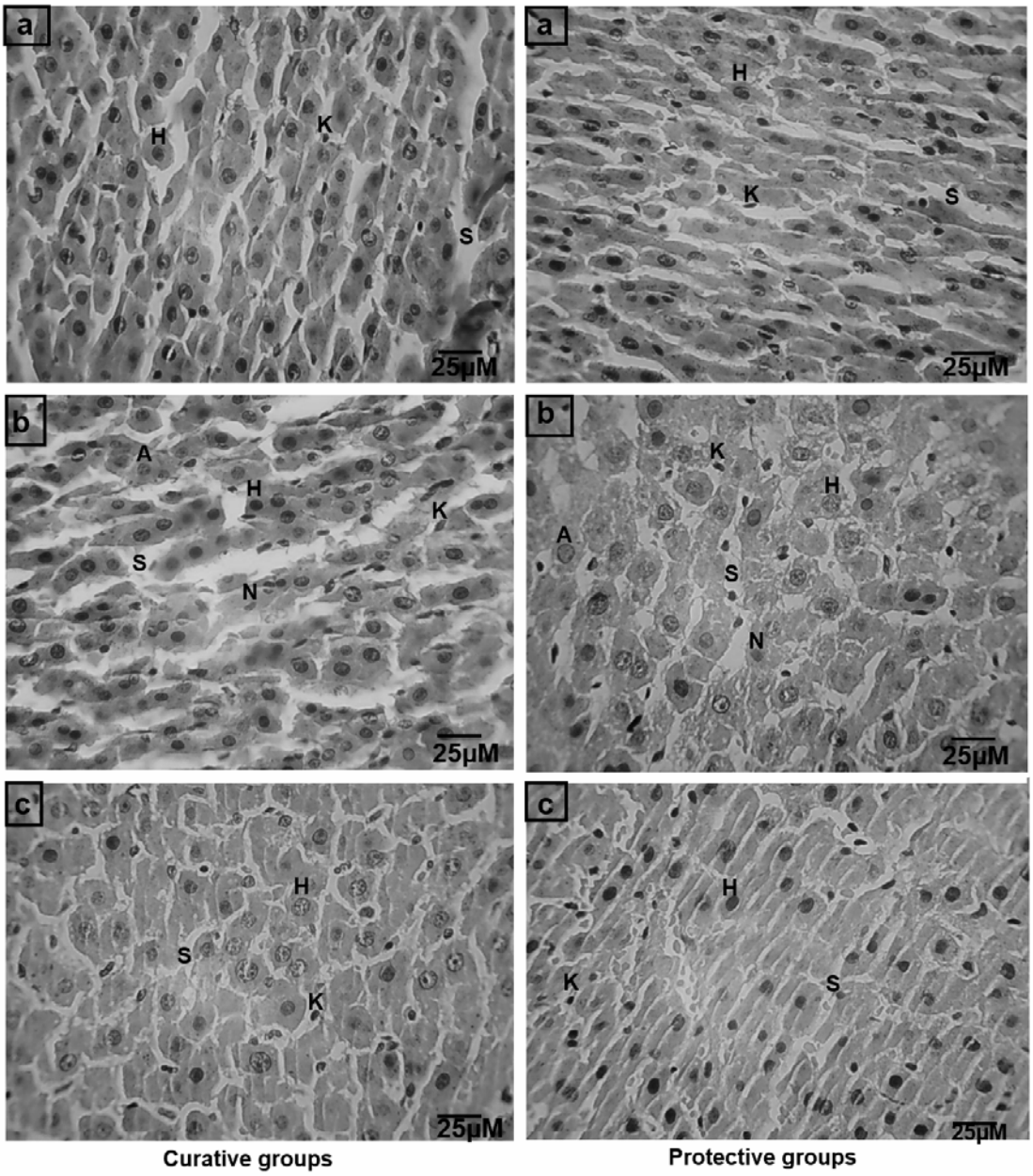

Figure 5. Histopathological examination (H & E) of liver sections: A: Control group, B: DMBA group, and C: Ech group.

The underlying mechanisms of Ech action include inhibition of DMBA bioactivation and restoration of the balance between ROS formation and internal antioxidant enzymes by its powerful antioxidant activity.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Wei, C., Bandowe, B. A. M., Han, Y., Cao, J., Zhan, C. and Wilcke, W. 2015, Chemosphere, 134, 512-520.
2. Abbas, I., Badran, G., Verdin, A., Ledoux, F., Roumié, M., Courcot, D. and Garçon, G. 2018, Environ. Chem. Lett., 16, 439-475.
3. Wang, W., Huang, M. J., Wu, F. Y., Kang, Y., Wang, H. S., Cheung, K. C. and Wong, M. H. 2013a, Atmos. Environ., 77, 525-533.
4. Kim, K. H., Jahan, S. A., Kabir, E. and Brown, R. J. 2013, Environ. Int., 60, 71-80.
5. Arora, R., Bhushan, S., Kumar, R., Mannan, R., Kaur, P., Singh, A. P., Vig, A. P., Sharma, D. and Arora, S. 2014, PLoS One, 9(11), e112614.
6. Manoharan, S. and Selvan, M. V. 2012, J. Environ. Biol., 33(2), 255-260.
7. Shah, B. R., Xu, W. and Mraza, J. 2019, RSC Advances, 9, 21050-21062.
8. Gao, J., Lauer, F. T., Mitchell, L. A. and Burchie, S. W. 2007, Toxicol. Sci., 98(1), 137-144.
9. Kumar, R., Kaur, R., Singh, A. P. and Arora, S. 2014, PloS One, 9(2), e90083.
10. Choi, E. J. 2008, Life Sci., 82, 1059-1064.
11. Sharma, V. and Paliwal, R. 2012, Int. J. Drug Dev. Res., 4, 215-228.
12. Jimeno, J. M. 2002, Anti-Cancer Drugs, 15-19.
13. Thakur, N. L., Jain, R., Natalio, F., Hamer, B., Thakur, A. N. and Müller, W. E. 2008, Biotechnol. Adv., 26, 233-245.
14. Arafa, S., Chouaibi, M., Sadok, S. and El Abed, A. 2012, Sci. World J., 2012, 815935.
15. Kuwahara, R., Hatate, H., Chikami, A., Murata, H., Kijidani, Y. 2010, LWT Food Sci. Technol., 43(8), 1185-1190.
16. Jeong, S. H., Kim, H. K., Song, I. S., Lee, S. J., Ko, K. S., Rhee, B. D., Kim, N., Mishchenko, N. P., Fedoryev, S. A., Stonik, V. A. and Han, J. 2014, Marine Drugs, 12(5), 2922-2936.
17. Lebedev, A. V., Ivanova, M. V. and Levitsky, D. O. 2005, Life Sci., 76(8), 863-875.
18. Boguslavskaya, L. V., Khrapova, N. G. and Maksimov, O. B. 1985, Bull. Acad. Sci. USSR, Div. Chem. Sci., 34, 1345-1350.
19. Lebedev, A. V., Ivanova, M. V. and Levitsky, D. O. 2008, Hemoglobin, 32, 165-179.
20. Jeong, S. H., Kim, H. K., Song, I. S., Noh, S. J., Marquez, J., Ko, K. S., Rhee, B. D., Kim, N., Mishchenko, N. P., Fedoreyev, S. A. and Stonik, V. A. 2014, Marine Drugs, 12(8), 4602-4615.
21. Mischenko, N. P., Fedoreev, S. A., Zapara, T. A. and Ratushnyak, A. S. 2009, Bull. Exp. Biol. Med. B Exp. Biol. Med., 147, 196-200.
22. Mohamed, A. S., Soliman, A. M. and Marie, M.-A. S. 2016, Life Sci., 151, 41-49.
23. Mohamed, A. S. 2021, Nutr. Cancer, 73(1), 124-132.
24. Fahmy, S. R., Zaki, N. I., Eid, S. Z., Mohamed, A. S. and Hassanein, S. S. 2019, Nat. Prod. Bioprospect., 9(5), 337-344.
25. Bikadi, Z. and Hazai, E. 2009, J. Cheminform., 1, 15.
26. Farag, N. A., Mohamed, A. S., El Sayed, H. F., Salah EL-Din, E. Y. and Tawfik, A. A. 2022, J. Nat. Prod., 12(3), 80-86.
27. Dakrory, A. I., Al Harbi, M. S. and Mohamed, A. S. 2015, Int. J. Adv. Res., 3, 275-287.
28. Mohamed, A. S. 2021, Nutrition and Cancer, 73(1), 124-132.
29. Mohamed, A. S., Hosney, M., Bassiony, H., Hassanein, S. S., Soliman, A. M., Fahmy, S. R. and Gaafar, K. 2020, Sci. Rep., 10, 378.
30. Reitman, S. S. and Frankel, A. 1957, Am. J. Clin. Pathol., 28, 56-63.
31. Belfield, A. and Goldberg, D. M. 1971, Arch. Dis. Childhood, 46, 842-486.
32. Ohkawa, H., Ohishi, N. and Yagi, K. 1979, Anal. Biochem., 95, 351-358.
33. Beutler, E., Duron, O. and Kelly, M. B. 1963, J. Lab. Clin. Med., 61, 882-888.
34. Aebi, H. 1984, Methods Enzymol., 105, 121-126.
35. Bancrof, J. D. and Stevens, A. 1996, Theory and Practice of Histological Techniques, Churchill Levingstone, New York, USA.
36. Abdel-Shafy, H. I. and Mansour, M. S. M. 2016, Egypt. J. Pet., 25(1), 107-123.
37. Katz, I. S., Albuquerque, L. L., Suppa, A. P., de Siqueira, D. M., Rossato, C., da Silva, G. B.,

Jensen, J. R., Starobinas, N., Cabrera, W. H., De Franco, M., Borelli, P., Ibañez, O. M. and Ribeiro, O. G. 2014, Int. J. Toxicol., 33(2), 130-142.
38. Burchiel, S. W., Davis, D., Gomez, M., Montano, R. M., Barton, S. L. and Seamer, L. C. 1990, Toxicol. App., 105(3), 434-442.
39. Bhuvaneswari, B., Velmurugan, S., Abraham, K. and Nagini, S. 2004, Braz. J. Med. Biol. Res., 37(7), 1029-1034.
40. Guerin, M. R. 1978, Energy sources of polycyclic aromatic hydrocarbons. H. V. Gelboin and Ts'o POP (Editors), Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment, Academic Press, New York, 1-42.
41. Bin Dajem, S., Ali, S. B., Abdelrady, O. G., Salahaldin, N. M., Soliman, A. M., Kamal, Y. M., Abdelazim, A. Y., Mohamed, A. F., Morsy, K., Mohamed, A. S. and Fahmy, S. R. 2020, Asian Pac. J. Trop. Biomed., 10(9), 411-416.
42. Batcioglu, K., Karagözler, A. A., Ozturk, I. C., Genc, M., Ozturk, F. and Aydogdu, N. 2005, Cancer Detect. Prev., 29(1), 54-58.
43. Mohamed, A. S., Bin Dajem, S., Al Kahtani, M., Ali, S. B., Alshehri, M., Shati, A., Morsy, K. and Fahmy, S. R. 2020, Bull. Environ. Contam. Toxicol., 105(6), 827-834.
44. Arulkumaran, S., Ramprasath, V. R., Shanthi, P. and Sachdanandam, P. 2007, Chem. Biol. Interact., 167(2), 99-106.
45. Morsy, K., Fahmy, S., Mohamed, A., Ali, S., El-Garhy, M. and Shazly, M. 2019, Acta Parasitologica, 64(4), 873-886.
46. Mohamed, A. S., Fahmy, S. R., Soliman, A. M. and Gaafar, K. M. J. 2018, Am. Assoc. Lab. Anim. Sci., 57(5), 443-446.
47. Mohamed, A. S., Mahmoud, S. A., Soliman, A. M. and Fahmy, S. R. 2019, Nat. Prod. Res., 2019, 1-5.
48. Mohamed, A. S., Sadek, S. A., Hassanein, S. S. and Soliman, A. M. 2019, J. Surg. Res., 234, 317-324.