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Protective and curative mechanisms of echinochrome against 7, 12-Dimethylbenz[a]anthracene -induced renal toxicity in rats

Sarah Abdelrahman Mohamed [1], Ayman Saber Mohamed [2,*], Emad El-Zayat [3] and Mohamed Refaat Shehata [4]

[1] *M.Sc., Biotechnology, Chemistry department, Faculty of Science, Cairo University, Egypt.*
[2] *Lecturer of Physiology, Zoology Department, Faculty of Science, Cairo University, 12613, Giza, Egypt.*
[3] *Prof. of Molecular Physiology and Biotechnology, Zoology Department, Faculty of Sciences, Cairo University, Egypt.*
[4] *Prof. of Inorganic Chemistry, Chemistry Department, Faculty of Sciences, Cairo University, Egypt.*

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Abstract

Echinochrome (Ech) is one of the most important bioactive substance which is found in shells, spines, and eggs of the sea urchins. Aim: the present study was carried out to evaluate the curative and protective effects of Ech pigment against DMBA -induced renal toxicity in rats. Methods: Experimental rats were assigned into two main groups; protective group (treated with Ech for 14 days then administrated DMBA) and curative group (administrated DMBA then treated with Ech for 14 days). Each group is divided into 3 sub-groups; control, DMBA (15 mg/ kg body, weight orally), and Ech (1 mg/ kg body, weight orally). Results: The oral administration of Ech decreased the concentrations of urea, creatinine, uric acid, and MDA and increased GSH and CAT levels in both protective and curative groups. Moreover, histology of kidney tissue improved after the treatment with Ech. Conclusions: The results of the present study demonstrated the potential protective and curative activities of Ech against renal toxicity induced by DMBA through inhibiting the metabolism of DMBA and restoring the balance between ROS formation and internal antioxidant enzymes by its powerful antioxidant activity.

Keywords: Echinochrome; Renal toxicity; DMBA; Antioxidant; 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 also they are included in breathing ambient so they are increased around the population through cigarette smoking and eating contaminated food with PAHs (3,4). Several studies proved that these PAHs materials could cause several cell damage and mutations leading to cancer for humans exposed to a great dosage of PAHs (5).

One of the most dangerous polycyclic aromatic hydrocarbons is 7, 12-dimethylbenz [a] anthracene (DMBA) which is an important environmental pollutant. DMBA is one of the PAHs which causes renaltoxicity, carcinogenicity in addition to changing phase I and II enzymes involved in the liver metabolic process (6). Furthermore, it is considered an immunosuppressor and tumor initiator (7)

During metabolic activation of DMBA, extravagant reactive oxygen species (ROS) are released (6). There are many studies have agreed that DMBA induces reactive oxygen species production during DMBA metabolic activation that

* Corresponding author: Ayman Saber Mohamed
Lecturer of Physiology, Zoology Department, Faculty of Science, Cairo University, 12613, Giza, Egypt.

leads to lipid peroxidation, DNA damage, and cell antioxidant defense system depletion (8).Beside, the liver is one of the first organs that can be exposed to the damaging effect of DMBA (9).

Marine natural products have become used as treatments for many diseases (10). The chemical and physical circumstance in the marine environment results in presenting a variety of molecules with unique structural characteristics by many different classes of marine organisms (11). The sea urchin is a wide distribute species in the Atlantic and the Mediterranean coasts (12). It belongs to the family of Echinidae, has spherical morphology reaches to 7 cm in diameter with soft, thick, and long spins up to 3 cm (13). Echinochrome (Ech) is considered one of the most popular and important substance which are found in shells, spines, and eggs of the sea urchins that possesses high antioxidant activity (14). It is a water-insoluble compound that possesses strong antioxidant activity and is considered to be the active gradient in the Histochrome drug (15). Due to its high antioxidant activity, Ech can act through many antioxidant mechanisms; include reduction of oxidative stress (16), interaction with lipoperoxide radicals (17), chelation of metal ions (18), inhibition of lipid peroxidation (19,20), and regulation of the cell redox potential (21). In addition, recent studies discoverwd 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 representing its mechanism against DMBA -induced kidney toxicity in rat.

2. Material 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). All kits were purchased from the Biodiagnostic Company (El Motor St, Dokki, Egypt).

2.2. Sea urchin Collection

Sea urchin (*Paracentrotus lividus*) were collected from the Mediterranean sea of Alexandria (Egypt) and placed in the laboratory and kept in ice (Fig. 1). The samples were rinsed with seawater to remove sands and other growing organisms at the collection place and placed in the laboratory (Figure 1). Identification of the collected samples had done by using the standard literature of the taxonomic guide (Clark and Rowe, 1971).

Figure 1 Sea urchin (*Paracentrotus lividus*) was collected from the Mediterranean sea of Alexandria (Egypt).

2.3. Extraction of Echinochrome (Ech)

Pigments in the shell and spins were separated using the Amarowicz method with little modifications (25,26). 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 (5g) 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 until the acid to be mostly removed. The ether solution containing the pigments was dried over the anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The extract containing the echinochrome pigment was stored at -30°C in the dark.

2.4. Experimental animals

The experimental animals to be used in this study were male Wistar albino (*Rattus norvegicus*) rats (130-150 g) were used in all experiments. The rat to be used for anyone experiment was selected from rats of similar age (± 1 week) and weight (± 5 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 of a 12 hr/ 12 hr light-dark cycle at (22 - 25 ^{0}C) room temperature. Animals were kept in the laboratory for 7 days to be adapted to laboratory conditions before the experiment beginning.

2.5. 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.

2.6. 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).

2.6.1. A-The Pre-treated (protective) group divided into 3 subgroups, each subgroup contains 6 rats

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

The animals were then euthanized 4 days after DMBA administration.

2.6.2. B-The Post-treated (curative) group which divided also into 3 subgroups, each subgroup contains 6 rats

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

2.7. Animal handling and collection of the samples

At the end of the experiment, by using 3% sodium pentobarbital, all the animals were fully anesthetized, then, the animals were slaughtered from the neck using a sterile scalpel. The blood samples of the animals were immediately collected in sterile centrifuge tubes. The kidney was enucleated and transferred to a filter paper for removing blood traces. A piece of the kidney tissue was stored at -80 ^{o}C for biochemical analyses. In 10% formal saline, another piece of the kidney was suspended for preparative fixation for Histopathological fixation.

2.8. Samples preparation

2.8.1. Serum preparation

Blood samples were centrifuged for 20 min. at 3000 rpm. The collected serum, stored at -20 ^{o}C until using for biochemical tests.

2.8.2. Kidney homogenate preparation

In ice-cold 0.1 M Tris-HCl buffers (pH7.4), kidney tissues were homogenized (10% w/v). The homogenate was centrifuged at 3000 rpm for 15 min. at 4^{o}C. The resultant supernatant was kept in -20 ^{o}C for use in the biochemical analyses.

2.9. Biochemical assessments

The serum creatinine was estimated by the method of Schirmeister [27] urea [28] and uric acid [29] according to the manufacturer's instructions using Bio-diagnostic kits (Giza, Egypt).

MDA level is an index of lipid peroxidation and it was estimated by Ohkawa [30], glutathione reduced (GSH) [31], and catalase [35] were determined in the liver homogenate supernatant according to the manufactures instructions using Bio-diagnostic kits (Giza, Egypt).

2.10. Histopathological *examination*

Kidney 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 stainedwith hematoxylin and eosin (H&E) according to Bancrof and Stevens (32), as routine procedures for histopathological examination.

2.11. Statistical analysis

Values were expressed as means ±SE. The comparisons within groups were evaluated utilizing one-way analysis of variance (ANOVA) with 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 improvement used to compare the treatment efficacy of Ech in both protective and curative groups. It takes only the positive value and calculated from the following equation:

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

3. Results

3.1. Kidney function

Serum urea, uric acid, and creatinine concentrations of DMBA groups increase significantly (P <0.05), as compared to the corresponding control groups. While a significant decrease (P <0.05) in serum urea, uric acid, and creatinine concentrations after treatment with Ech (1 mg/Kg body weight, orally), as compared to the corresponding DMBA groups (Table 1). The percentage of change of Ech in protective was higher than the curative experiment.

Table 1 Curative and protective potency of Ech on kidney function markers of DMBA intoxicated rat.

Treatment	Groups	Urea (mg/dl)	Creatinine(mg/dl)	uric acid (mg/dl)
Curative	Control	26.27±0.33^{a}	0.89±0.02^{a}	5.48± 0.03^{a}
	DMBA	39.68±0.77^{c}	1.31±0.01^{c}	6.95±0.03^{c}
	Ech	30.25±0.71^{b}	1.07±0.03^{b}	6.21±0.06^{b}
	% of change	-23.76	-18.60	-10.54
Protective	Control	24.17±0.30^{a}	0.86±0.01^{a}	5.26±0.02^{a}
	DMBA	36.49±0.49^{c}	1.21±0.01^{c}	6.68±0.06^{c}
	Ech	27.01±0.29^{b}	0.94±0.02^{b}	5.76±0.13^{b}
	% of change	-25.99	-22.48	-13.82

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

3.2. Oxidative stress

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. Whereas oral administration of Ech (1 mg/Kg body weight) caused a significant decrease (P <0.05) in MDA concentration while GSH and CAT levels increased, as compared to the corresponding DMBA groups. The percentage of change of Ech in protective was higher than the curative experiment. (Table 2).

Table 2 Curative and protective potency of Ech on oxidative stress markers of DMBA intoxicated rat

Treatment	Groups	MDA (nmol/g.tissue)	GSH (mg/ g. tissue)	CAT (U/g. tissue)
		Kidney	Kidney	Kidney
Curative	Control	2.25±0.11[a]	13.04±0.07[c]	3.45±0.05[c]
	DMBA	3.06±0.07[b]	10.93±0.09[a]	1.96±0.15[a]
	Ech	2.60±0.20[a]	11.81±0.13[b]	2.53±0.12[b]
	% of change	-15.13	8.03	29.47
Protective	Control	2.47±0.12[a]	12.83±0.06[c]	3.09 ±0.14[c]
	DMBA	3.45±0.08[c]	10.89±0.13[a]	1.67±0.02[a]
	Ech	2.92±0.09[b]	12.02±0.33[b]	2.50±0.05[b]
	% of change	-15.19	10.31	49.65

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

3.3. Histopathological examination of the kidney

The control groups show the normal appearance of the tissue where glomeruli (G) appears as dense tufts of capillaries enclosed in the outer layer of Bowman capsules, urinary space (U), and renal tubules (R) (Figure 2a). DMBA groups show severe shrinking and degeneration of glomeruli, degenerated cytoplasm of some cells of the renal tubules, and deformed renal tissue architecture (Figure 2b). The groups treated with Ech show improvement in the architecture of renal tissue (Figure 2c)

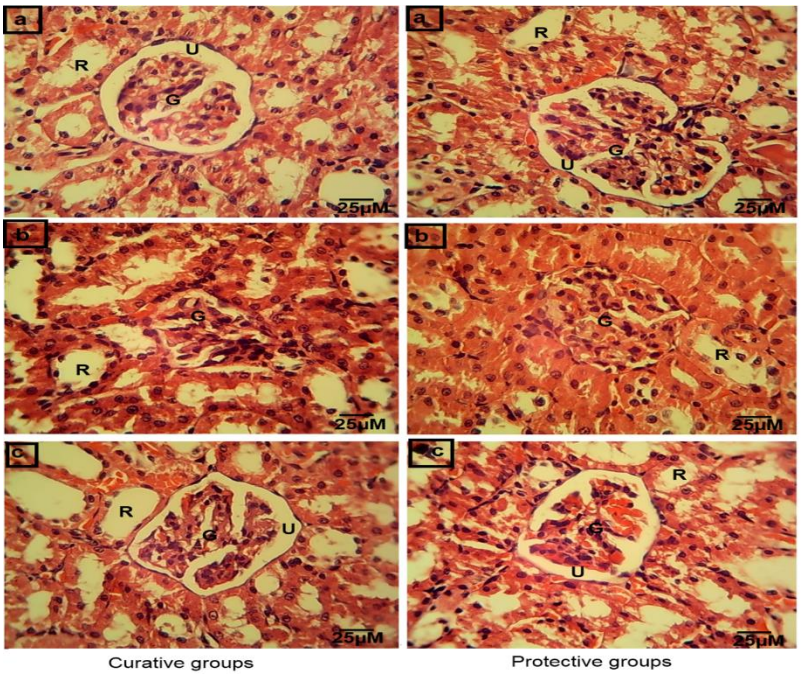

Figure 2 Histopathological examination (H & E) of kidey sections: A: Control group, B: DMBA group, and C: Ech group**.**

4. Discussion

Atmospheric pollutants such as polycyclic aromatic hydrocarbons (PAHs) spread abundantly in the environment and reach humans through air, water, and food (33). 7, 12-dimethylbenz[a]anthracene (DMBA) is environmental pollutants from PAHs that exhibit numerous carcinogenic and toxic effects (34) The cytotoxicity, carcinogenicity, mutagenicity, and immunosuppression are well-known activities of DMBA (35) (36).

Kidney is an important organ responsible for the excretion of various toxic metabolic waste products. It cannot escape the detrimental effect of the toxic metabolic products of DMBA (37).

Toxic manifestation of DMBA induced oxidative stress is associated with a wide range of macromolecular damages such as lipids, proteins and nucleic acids, thereby producing interrelated derangements of cellular metabolism including peroxidation of lipids (38). The present study showed a significant increase in the concentration of urea, creatinine and uric acid in the serum of the DMBA groups. The elevation in the concentration of urea and creatinine in the serum after DMBA treatment may be due to protein catabolism upregulation (39). Meanwhile, urea represents the nitrogenous waste produced by protein degradation (40). Therefore, the increased concentration of urea and creatinine in the serum is related and indicating to kidney dysfunction (41).+ (42). While the elevated concentration of serum uric acid reveals nephrotoxicity (39) which can be an indication of kidney impairment (40). Furthermore, histopathological examination of kidney tissue revealed damage to kidney cells that were confirmed by biochemical analyses. On the other hand, the oral administration of Ech restores the serum concentrations of urea, creatinine, and uric acid near normal and improves the histology of kidney tissue.

DMBA and their metabolism products can stimulate ROS formation such as peroxides and superoxide anion radicals, which reasons for oxidative stress formation in tissues (43). In the present study, DMBA groups showed an increase in MDA concentration and a decrease in the levels of GSH and CAT. The elevated MDA concentrations resulted from high lipid peroxidation, which leads to tissue damage and defected antioxidant defense system for blocking the generation of more free radicals (39). Similarly, Lu (44) proved that the enhanced MDA concentration is associated with the tissue injury present in kidney cells owing to the generation of ROS by DMBA. GSH is the main non-protein thiol antioxidant compound involved in the detoxification pathways (45). While; catalase is an important enzyme in the internal antioxidant system which catalyzes the breakdown of hydrogen peroxide, thereby protect the cells from oxidative damage (46). The reduction of GSH and CAT concentrations is caused by the increased radical generation during DMBA metabolism (47). Moreover, the treatment with Ech resulted in a decrease in MDA concentrations and induced the increase in GSH and CAT levels. Ech can act through many antioxidant mechanisms; include reduction of oxidative stress (48), chelation of metal ions (49), and regulation of the cell redox potential (50).

5. Conclusion

The results of this study demonstrated the potential protective and curative activities of echinochrome (Ech) against DMBA toxicity. Thanks to the antioxidant effect of Ech, it restored the balance between reactive oxygen species (ROS) formation and internal antioxidant enzymes.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors disclose that there are no conflicts of interest

Statement of ethical approval

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.

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