

## Hepatoprotective activity of aerial parts of *Erythrina crista-galli*

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### ABSTRACT

Hepatoprotective activity was measured for *Erythrina crista-galli* extract as well as fractions. Fractions II and III have shown a remarkable protective effect against CCl<sub>4</sub>-induced hepatocyte injury. This was evidenced by their ability to significantly ameliorate CCl<sub>4</sub>-induced elevation in ALT and AST levels. This is supported by the notion that pretreatment of hepatocytes with either *Erythrina crista-galli* extracts or fractions significantly alleviated CCl<sub>4</sub>-induced GSH and SOD depletion and replenished CCL<sub>4</sub> reduction of TAC. Hepatoprotective activity mechanism is attributed at least in part, to the free radical scavenging and antioxidant activity of the phenolic compounds present in the extract proved by the phytochemical screening of the fractions.

**Keywords:** *Erythrina-crista-galli*; hepatoprotection.

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## 1. INTRODUCTION

Phenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects. Family Leguminosae which include genera that embrace phenolic-rich species is capable of synthesizing and accumulating the high percentage of phenolics particularly flavonoids [1].

The genus *Erythrina* (Leguminosae) contains more than 110 species with a broad distribution [2]. These species have been widely used in indigenous traditional medicine [3] *Erythrina* has been used in folk medicine for treatment of insomnia malaria fever, venereal disease, asthma, and toothache. The alkaloid Erythroidine was used as a muscle relaxant. Haemoerythrina

alkaloids were investigated for their anti-cancer activity [4].

Studies on phytochemical of *Erythrina* species have demonstrated alkaloids [5-8] and phenolics including flavonoids [9], isoflavonoids [10, 11], pterocarpan [12, 13], flavanones [14, 15], isoflavones [16], chalcones [17, 18] and cinnamylphenols [19, 20] as major constituents of this genus.

*Erythrina* had several pharmacological activities anti-bacterial [10, 21, 22], anti-oxidant [6, 23], anti-inflammatory [24], cytotoxic [25], protein tyrosin phosphatase inhibition [26], anti-plasmodial [27], estrogenic [28], anti-osteoporotic [29] and central nervous system activities [30].

*Erythrina crista-galli* is widely distributed in subtropical and tropical regions. It is known as cockspur coral tree. It is also commonly called "Corticeira" in Brazil, and its bark is used for rheumatism, hepatitis, sedation, and hypnogenesis. Phytochemical studies on this plant showed the presence of *Erythrina* alkaloids, benzyloquinoline alkaloids, isoflavonoids, and pterocarpan [31].

Since only a few reports are available in the current literature about the hepatoprotection activity of the leaves of *Erythrina crista-galli*, it was, therefore, found interesting to subject the extracts and fractions of the leaves as a continuation to our previous study where we isolated polyphenols with a phytoestrogen activity, here we aim to assess the hepatoprotective activity for the extract and fractions.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

Fresh leaves of *Erythrina crista-galli* (Fabaceae) were collected from plants grown in El-Giza Zoo garden, Giza, on April (2011). They were kindly authenticated by Mrs. Tereize Labib, agricultural engineer, El-Orman botanical garden, Giza, Egypt. Voucher specimens of the authenticated plant were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

### 2.2. Instruments and material for chemical investigation

For column chromatographic analysis the following adsorbents were used: Cellulose powder, E-meric, Germany. Paper chromatographic analysis was carried out on Whatman No. 1 paper (Whatman, Kent, UK) using solvent systems: 1) H<sub>2</sub>O; 2) 6% AcOH; and 3) BAW (n-BuOH/AcOH/H<sub>2</sub>O, 4:1:5, v/v, upper layer). Solvents 2 and 3 were also used for preparative paper chromatography (PPC).

### 2.3. Plant extraction and fractionation

Fresh leaves (1 kg) were exhaustively extracted with 10 L of aqueous methanol (75%). Leaves were boiled in double distilled water for exactly 2 h. The aqueous extract was dried by lyophilization. The dry lyophilized extract was then further extracted with methanol (HPLC grade) for 30 min at 40 °C to ensure complete extraction of phenolic components. This method helps to avoid extraction of other plant metabolites as carbohydrates, water-soluble protein, inorganics, lipids, or alkaloids. Finally, the extract was completely evaporated in vacuo at a low temperature until dryness.

Fractionation of the extract (40 g) on cellulose column, using water followed by water-methanol mixtures of decreasing polarities, yielded three main fractions (I, II and III).

Two-dimensional PC of the extract and fractions II and III proved the presence of a high percentage of phenolic constituents (blue color reaction with ferric chloride TS and ammonia).

### 2.4. Cell culture for hepatoprotective activity

HepG2 cell line was purchased from VACSERA, Giza, Egypt. Cells were grown in Dulbecco's modified Eagle's medium supplemented with heat-inactivated fetal bovine serum (10%), penicillin G (100 IU/mL), and streptomycin (100 µg/mL). Cells were maintained at 37 °C in a 5% CO<sub>2</sub> atmosphere with 95% humidity. Cell culture reagents were obtained from Gibco Invitrogen (Carlsbad, CA, USA).

### 2.5. Assay for hepatoprotective activity

HepG2 monolayer culture after attachment was pretreated with the aqueous extract, aqueous methanol extract, fraction I, fraction II, and silymarin (100 µg/mL in phosphate-buffered saline) for one hour. An aliquot of 40 mM CCl<sub>4</sub> in 0.05% dimethyl sulfoxide (DMSO) was added and incubation was continued for another two hours. The supernatant medium and cell lysate

were then collected and stored at  $-20\text{ }^{\circ}\text{C}$  until analysis. Silymarin was used as a standard. The positive control was a set of cells maintained in culture medium and treated only with  $\text{CCl}_4$  (40 mM); while the negative control was a set of cells maintained in phosphate-buffered saline.

## 2.6. AST and ALT measurement

Activities of the marker enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of Reitman and Frankel (1957) [32].

## 2.7. Superoxide dismutase (SOD)

Superoxide dismutase activity was determined in the cell lysate through inhibition of pyrogallol autoxidation (Marklund and Marklund, 1974). Cytosolic fraction (20  $\mu\text{L}$ ) was added to a microcuvette containing 10  $\mu\text{L}$  pyrogallol solution (10 mM dissolved in 10 mM HCl) and 1 mL Tris-HCl buffer (50 mM, pH 8.2) containing 1 mM diethylenetriaminopentaacetic acid. The change in absorbance per minute at 420 nm was recorded for 2 min [33].

## 2.8. Glutathione reduced (GSH)

The level of GSH in cell culture supernatant was determined as protein-free sulfhydryl content using 5,5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) that is reduced by the SH-group in GSH to form 5-thio-2-nitrobenzoic acid, which has a stable yellow color measured colorimetrically at 412 nm as described by Ellman [34]. In detail, protein precipitation was attained by mixing equal volumes of cell culture supernatant and 10% trichloroacetic acid-0.005 M EDTA solution followed by centrifugation at 600g for 15 min. To 0.5 mL of the resulting supernatant, 0.85 mL phosphate buffer (0.1 M, pH 8) and 0.05 mL Ellman's reagent (10 mM) was added in a microcuvette and the optical density was measured at 412 nm.

## 2.9. Total antioxidant capacity (TAC)

The determination of the antioxidant capacity is performed by the reaction of the antioxidants in the sample with a defined amount of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The antioxidants in the sample eliminate a certain amount of the provided  $\text{H}_2\text{O}_2$ . The residual  $\text{H}_2\text{O}_2$  is determined colorimetrically by the enzymatic reaction which involves the conversion of 3,5-dichloro-2-hydroxybenzenesulphonate to a colored product.

Total antioxidant capacity =  $(\text{AB}-\text{AS}) \times 3.33$  mM  $\text{H}_2\text{O}_2$

Where:

AB = the absorbance of the blank

AS = the absorbance of the sample [35]

## 2.10. Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are represented as mean  $\pm$  SEM the results were considered significant if  $P < 0.05$ .

## 3. RESULTS AND DISCUSSION

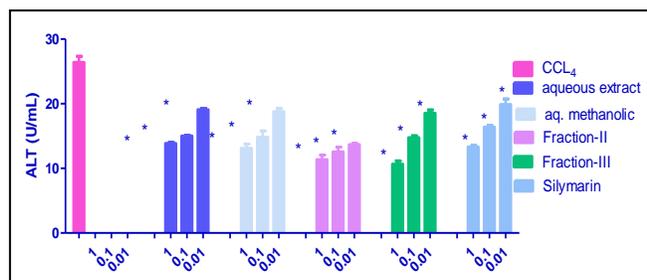
### 3.1. Phytochemical screening

Phytochemical screening of powdered dried samples of the aerial parts of *Erythrina crista-galli* cultivated in Egypt showed that the plant is rich in flavonoids, coumarins, sterols and/or triterpenes, carbohydrates and/or glycosides, alkaloids, and anthraquinones.

### 3.2. Assay for hepatoprotective activity

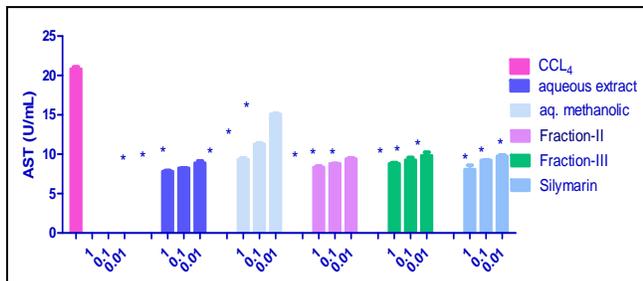
Hepatoprotective activity against  $\text{CCl}_4$ -induced damage in HepG2 cells was tested using three different concentrations (1000  $\mu\text{g}/\text{mL}$ , 100  $\mu\text{g}/\text{mL}$  and 10  $\mu\text{g}/\text{mL}$ ) of *Erythrina crista-galli* aqueous and aqueous methanol extracts and Fractions I and II. Pretreatment of hepatocytes with three concentrations (1000  $\mu\text{g}/\text{mL}$ , 100  $\mu\text{g}/\text{mL}$  and 10  $\mu\text{g}/\text{mL}$ ) of *Erythrina crista-galli* aqueous extract reduced the  $\text{CCl}_4$ -induced ALT elevation by 47%, 43% and 28%, respectively. While aqueous methanol extract reduced the  $\text{CCl}_4$ -induced ALT elevation by 39%, 44% and

29%, respectively. Fraction II showed reduction for the CCl<sub>4</sub>-induced ALT elevation by 60%, 44% and 30% respectively. While fraction III reduced CCl<sub>4</sub>-induced ALT elevation by 60%, 44% and 30% respectively. **Fig. 1 and 2** show the pretreatment of hepatocytes with three concentrations (1000 µg/mL, 100 µg/mL and 10 µg/mL) of *Erythrina crista-galli* aqueous extract reduced the CCl<sub>4</sub>-induced AST elevation by 62%, 69% and 57%, respectively.



**Fig. 1** The effect of the aqueous, aqueous methanol extracts and fractions of the aerial parts of the *Erythrina crista-galli* on ALT level. Data are mean  $\pm$  SEM, (n=3).

\* Significantly different from CCl<sub>4</sub> group at  $p < 0.05$



**Fig. 2** The effect of the aqueous, aqueous methanol extracts and fractions of the aerial parts of the *Erythrina crista-galli* on AST level. Data are mean  $\pm$  SEM, (n=3).

\* Significantly different from CCl<sub>4</sub> group at  $p < 0.05$

While aqueous alcoholic extract reduced CCl<sub>4</sub>-induced AST elevation by 55%, 46% and 28% respectively. Fraction II showed reduction for the CCl<sub>4</sub>-induced AST elevation by 60%, 58% and 55% respectively. While fraction III reduced CCl<sub>4</sub>-induced AST elevation by 58%, 56% and 53% respectively. From the aforementioned results we can conclude that *Erythrina crista-galli* aqueous and aqueous methanol extracts as well as Fractions II and III protected against CCl<sub>4</sub>-induced hepatocyte injury

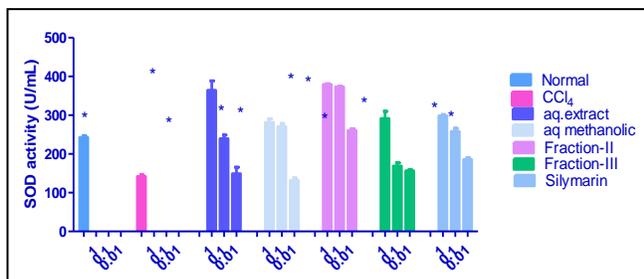
as evidenced by their ability to reduce CCl<sub>4</sub>-induced ALT and AST elevation. The hepatoprotective activity study was further substantiated by testing the influence of *Erythrina crista-galli* extracts and its fractions on CCl<sub>4</sub>-induced alteration in SOD activity and GSH level.

In vitro pretreatment of HepG2 hepatocytes with the aqueous extract concentrations significantly replenished CCl<sub>4</sub>-induced SOD reduction by 157% (2.5 folds), 69% and 4% respectively and replenished CCl<sub>4</sub>-induced GSH reduction by 120% (2.2 folds), 83% and 79% respectively. While aqueous methanol extract replenished CCl<sub>4</sub>-induced SOD reduction by 98%, 69% and 7.5% respectively and replenished CCl<sub>4</sub>-induced GSH reduction by 125%, 99% and 77% respectively. Fraction II replenished CCl<sub>4</sub>-induced SOD reduction by 167% (2 folds), 163% and 83% respectively and replenished CCl<sub>4</sub>-induced GSH reduction by 143% (2.4 folds), 112% (2.1 folds) and 112% (2.1 folds), respectively. Fraction III replenished CCl<sub>4</sub>-induced SOD reduction by 106% (2 folds), 19% and 10% respectively and replenished CCl<sub>4</sub>-induced GSH reduction by 114% (2.1 folds), 24% and 81%, respectively (**Fig. 3, 4**).

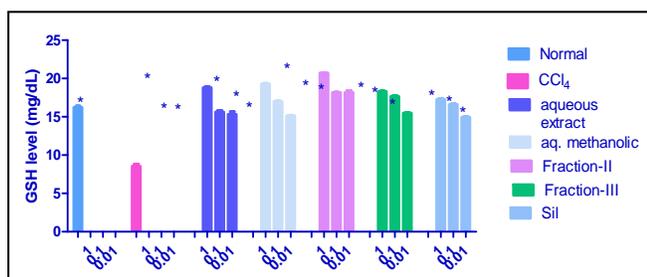
In vitro pretreatment of HepG2 hepatocytes with the aqueous extract concentrations significantly replenished CCl<sub>4</sub>-induced TAC reduction by 3.6 folds, 2.12 folds and 1.7 fold respectively. While aqueous methanol extract replenished CCl<sub>4</sub>-induced TAC reduction by 3.5 folds, 2.19 folds and 2 folds, respectively. Fraction II replenished CCl<sub>4</sub>-induced TAC reduction by 7 folds, 5.5 folds and 3.7 folds, respectively. Fraction III replenished CCl<sub>4</sub>-induced TAC reduction by 16 folds, 11 folds and 4.7 folds respectively (**Fig. 5**).

Liver injuries induced by CCl<sub>4</sub> are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for

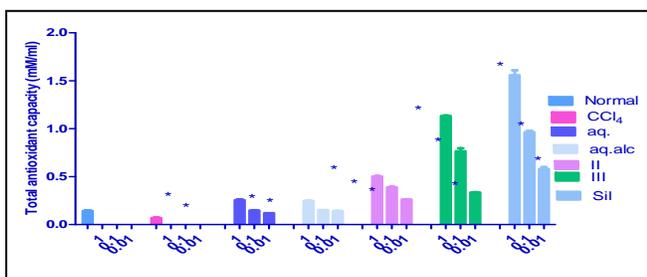
the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs [36].



**Fig. 3** The effect of the aqueous, aqueous methanol extracts and isolated fractions of the aerial parts of the *Erythrina crista-galli* on SOD activity. Data are mean  $\pm$  SEM, (n=3). \* Significantly different from CCl<sub>4</sub> group at  $p < 0.05$



**Fig. 4** The effect of the aqueous, aqueous methanol extracts and isolated fractions of the aerial parts of the *Erythrina crista-galli* on GSH activity. Data are mean  $\pm$  SEM, (n=3). \* Significantly different from CCl<sub>4</sub> group at  $p < 0.05$



**Fig. 5** The effect of the aqueous, aqueous methanol extracts and isolated fractions of the aerial parts of the *Erythrina crista-galli* on TAC activity. Data are mean  $\pm$  SEM, (n=3). \* Significantly different from CCl<sub>4</sub> group at  $p < 0.05$

Since the changes associated with CCl<sub>4</sub> induced liver damage are similar to that of acute viral hepatitis [37], CCl<sub>4</sub> mediated hepatotoxicity was chosen as the experimental model. It has been established that CCl<sub>4</sub> is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P450 dependent

monooxygenases to form a trichloromethyl radical (CCl<sub>3</sub>). The CCl<sub>3</sub> radical alkylates cellular proteins and other macromolecules with simultaneous attack on polyunsaturated fatty acids, in presence of oxygen, to produce lipid peroxides, leading to liver damage [36]. Thus, antioxidant or free radical generation inhibition is important in protection against CCl<sub>4</sub> induced liver lesions [38].

The GSH system includes reduced glutathione, GPx and glutathione-S-transferase. GSH is involved in several defense processes against oxidative damage. It protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury [39].

SOD is a scavenger of peroxide anion radicals, which could inhibit the initiation of lipid peroxidation by free radicals [40].

Taking these together, the hepatoprotective effect of *Erythrina crista-galli* extracts and its fractions is at least partly due to antioxidant activity.

#### 4. CONCLUSION

Phytochemical screening of the aqueous methanol extract of *Erythrina crista-galli* leaves indicated the presence of phenolic metabolites. The aim of the present study was to evaluate the potential hepatoprotective activity of *Erythrina crista-galli* extracts.

*Erythrina crista-galli* aqueous and aqueous methanol extracts as well as fraction II and III have shown a remarkable protective effect against CCl<sub>4</sub>-induced hepatocyte injury. This was evidenced by their ability to significantly ameliorate CCl<sub>4</sub>-induced elevation in ALT and AST levels. The observed hepatoprotective activity of the tested extracts and fractions can be, at least partly, attributed to their antioxidant

activity. This is supported by the notion that pretreatment of hepatocytes with either *Erythrina crista-galli* extracts or fractions significantly alleviated CCL<sub>4</sub>-induced GSH and SOD depletion and replenished CCL<sub>4</sub> reduction of TAC. Hepatoprotective activity mechanism is attributed at least in part, to the free radical scavenging and antioxidant activity of the phenolic compounds present in the extract.

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