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Antioxidant potential of *Salicornia arabica* lipid extract and their protective effect against cadmium induced oxidative stress in erythrocytes isolated from rats

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Abstract

The *Salicornia* species have been used for treatment and prevention of a various diseases. To the best of my knowledge, little information is regarding the antioxidant and protective activities of lipid extract from *Salicornia Arabica* (SALE). The objectives of this present work were designed to determine the in vitro antioxidant activities of SALE against cadmium-induced toxicity in erythrocytes isolated from rats. The distribution of fatty acid reflected the richness in saturated fatty acids that were predominated by palmitic acid. Our analysis showed that the major fatty acid components were palmitic acid (47.78%), linoleic acid (17.75%), followed by nanadecenoic acid (10.96%). Minor fatty acids including lignoseric acid and palmatoleic acid (C16:0). The in vitro antioxidant activity of SALE was evaluated using both test DPPH and ABTS assays. An important antioxidant potential was observed. The protective potential of SALE was evaluated by estimating the levels of stress markers like MDA concentration and SOD, CAT and GPx activities. Our results showed that cadmium significantly decreased the SOD, CAT and GPx activities in erythrocytes homogenates and enhanced lipid peroxidation. The pre-treatment with SALE ameliorated antioxidant status and inhibited MDA level in erythrocytes. In conclusion, the lipid isolated from *S. arabica* showed protective potential against Cd-induced erythrocytes damage, which could be related to active compounds present in this fraction.

Keywords Cadmium · Erythrocytes · Lipid extract · Oxidative stress · *Salicornia arabica* · Stress markers

Introduction

Heavy metals are natural constituents of the earth's crust. However their geochemical cycles and biochemical balance has been disturbed by exponential increase of their use in several industrial, agricultural, domestic and technological applications [1]. Heavy metals (HMs) are also considered as trace elements because of their presence in trace concentrations (range to less than 10 ppm) in various environmental matrices. Metal-induced diseases are significant public health concerns. These metals are systemic toxicants known to induce adverse health effects in humans, including cardiovascular diseases, neurologic disorders, diabetes, hematologic and immunologic disorders, and various types of cancer [2]. The HMs affect cellular compartments like cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei and enzymes involved in metabolism, detoxification, and damage repair [3]. Metals of particular concern include, but are not limited to: arsenic, chromium, cadmium, nickel, mercury, and uranium [4]. demonstrated that the activities of antioxidant enzymes (Superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocytes of workers exposed to cadmium are remarkably higher than that in non-exposed workers. In addition, many investigators, among them [5] have demonstrated that cadmium intoxication induces a cellular damage mediated by the formation of reactive oxygen species (ROS).

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Erythrocyte has been increasingly studied because it is the easiest available human cell type. Though many model systems are frequently used to study and to investigate oxidative damage among them erythrocytes. Thus, it has been used as a cellular model. Generally, erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the redox active protein hemoglobin (Hb), which is the potent promoter of ROS [6].

Therefore, the interest in natural antioxidants compound, especially of plant origin, has greatly increased in recent years [7]. Research conducted on the antioxidant activities of some plants generally focused on the herbs and aromatic plants [8]. So natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases [9]. Moreover, it has been shown that PUFA have a number of uses in various fields and the biomedical, pharmaceutical and nutraceutical applications of commercial PUFA-rich preparations [10, 11].

The *Salicornia* species comprise the most salt tolerant land plant and frequently occur in saline areas [12]. Belonging to the family Amaranthaceae, *Salicornia* is a halophyte (literally a plant that grows in salty soil), and its seeds yield high quality edible oil, which is highly poly-unsaturated and similar to sunflower oil in fatty acid composition [12]. It has a pleasant nut-like flavor and a texture similar to olive oil. However, to our knowledge, little information is available on the antioxidant potentials of lipid compounds. Many studies concerning ecological, biological and chemical properties of *Salicornia arabica* were studied. However, there is lack information on the phytochemical and antioxidant properties of *S. arabica* lipid extract to support its ethnomedicinal uses in the treatment of several diseases [13, 14].

The aim of the present study is to evaluate an in vitro antioxidant property and protective effect of *S. arabica* lipid extract (SALE) against cadmium-induced oxidative damage in normal rat erythrocytes.

Material and methods

Plant material and lipid extraction

Salicornia arabica were obtained from the region of Sfax in south of Tunisia; the period of collection was February 2017. The plant was authenticated by Ferjani Ben Abdallah, Professor in the Laboratory of Biology and Vegetable Ecophysiology in the Faculty of Science of Sfax. The voucher sample was created by The National Botanical Research Institute of Tunisia. Whole plant powders were prepared. Sample to solvent ratio was kept constant for the method of extraction. Briefly, 20 grams of arial part powder samples were extracted with a mixture of chloroform–methanol (2:1, v/v) according to the method of Folch et al. [15].

Fatty acid profiles

Fatty acid methyl esters (FAME) were prepared from the lipid extract by transesterification using a direct transmethylation method [16]. Then, the FAMEs were extracted with hexane and determined quantitatively by capillary gas chromatography. We used a Chromopack, CP 9001 gas chromatograph, HPS 5890 series II chromatograph, equipped with a polar 25 m capillary column CP wax 58 (Varian SA, Le Ulis, France) (0.32 mm diameter and a layer thickness of 0.52 mm) and a flame detector (FID). We used a split–splitless injection system with nitrogen as carrier gas. The oven was programmed to rise from an initial temperature of 180–250 °C at a rate of 10 °C min^{-1} (from 180 to 220), 2 °C min^{-1} (from 220 to 240) and 5 °C min^{-1} (from 240 to 250) with the FFAC column. Individual FAMEs were identified by comparing retention times with those obtained from Supelco and with laboratory standards.

Determination of antioxidant activities

The antioxidant potential of *S. arabica* lipid extract was determined by radical scavenging assays: the 2, 2 diphenylpicrylhydrazyl radical (DPPH˙) and the 2,2′-azinobis-(3 ethylbenzothiazoline-6 sulfonic acid radical (ABTS˙$^+$).

Free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH˙)

The free radical scavenging capability of each extract solution on DPPH radicals was determined as described previously [17]. Briefly, 4 ml of methanol solution of DPPH (0.1 mM) was mixed with 1 ml of lipid extract solution at different concentrations (0–1 mg ml^{-1}). The reaction mixture was incubated in a dark room for 30 min and the free radical scavenging ability was estimated by measuring the absorbance at 515 nm. The reaction was carried out in capped glass test tubes that were tightly wrapped with aluminum foil. The DPPH radical stock solution was freshly prepared every day for the reaction, and precautionary measures were taken to reduce the loss of free radical activity during the experiment. The inhibition percentage of DPPH radicals was calculated as:

$$\% \, \text{DPPH scavenging} = \left[\frac{\left(A_{\text{control}} - A_{\text{test}} \right)}{A_{\text{control}}} \right] \times 100 \qquad (1)$$

where A_{control} is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction.

Free radical scavenging ability by the use of ABTS radical cation (ABTS assay)

Antioxidant activities of *S. arabica* were also analyzed by investigating their ability to scavenge the ABTS$^{.+}$ free radical using a modified methodology previously reported by Ozgen et al. [18]. When combined with an oxidant (2.45 mM potassium persulfate), ABTS (7 mM in 20 mM sodium acetate buffer, pH 4.5) reacts to create a stable, dark blue-green radical solution following 12–16 h of incubation in the dark (4 °C). The solution was then diluted to an absorbance of 0.7 ± 0.01 at 734 nm to form the test reagent. Reaction mixtures containing 20 µl of sample and 3 ml of reagent were incubated in a water bath at 30 °C for 30 min. As unpaired electrons are sequestered by antioxidants in the sample the test solution turns color less and the absorbance at 734 nm is reduced. As unpaired electrons are sequestered by antioxidants in the sample the test solution turns color less and the absorbance at 734 nm is reduced. Radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = \left[\frac{(A_{control} - A_{test})}{A_{control}} \right] \times 100 \qquad (2)$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction.

Lipid peroxidation inhibition assay

The lipid peroxidation inhibition assay was estimated using the protocol described by Halliwell and Gutteridge [19]. Briefly, 10% of rat kidney homogenate in potassium chloride (0.15 M) was prepared. Then, a volume of 0.5 ml of live homogenate and 1 ml of *S. arabica* lipid extract at different concentrations were mixed. Lipid peroxidation was enhanced by adding ferrous sulfate (50 µl, 0.07 M) and incubated at room temperature for 30 min. After incubation, 50 µl of thiobarbituric acid (TBA) (0.8% in 1.1% SDS) was added. Finally, the reaction mixture was incubated in boiling water for 15 min. After cooling, 5 ml of butanol was added and centrifuged at 1000×*g* for 10 min. The percentage of inhibition was calculated according to the following formula:

$$\% \text{ Inhibition} = \left[\frac{(A_{control} - A_{test})}{A_{control}} \right] \times 100 \qquad (3)$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction.

Protein glycation

The ability of SALE to attenuate protein glycation was estimated by the method described by Vinson and Howard [20]. Briefly, 1 ml of bovine serum albumin (BSA, 7 mg ml^{-1}) in phosphate buffer (50 mM, pH 7.4) containing 0.02% (w/v) sodium azide was pre-incubated with the SALE at final concentrations 10, 20, 50 and 100 µg ml^{-1} for 30 min at room temperature. Then, 50 µl of glucose (25 mM) and 50 µl of fructose (25 mM) solutions were added to the reaction mixture. The results were determined using a fluorescence reader with an excitation wavelength of 350 nm and an emission wavelength of 450. Quercetin was used as standard (20 µg ml^{-1}). The results were expressed as arbitrary units of fluorescence from the glycated protein.

In vitro protective effects of *S. arabica* lipid extract (SALE)

Preparation of erythrocytes suspension

Blood was obtained from normal rats. The experiment was carried out in accordance with the guidelines issued by the Ethical Committee of Sfax University. Erythrocytes were separated from the plasma by centrifuging at 1200×*g* for 10 min at 4 °C. Erythrocytes were washed three times with PBS (0.1 µM NaCl, 2.7 KCl, 1.7 Kh$_2$PO$_4$, 10 mM Na$_2$HPO$_4$ pH 7.4). The 20% suspension of erythrocytes was obtained by resuspending the erythrocytes in PBS (pH 7.4) [21].

Cytoprotective effects

The cytoprotective effect of the *S. Arabica* lipid extract was evaluated against cadmium chloride (CdCl$_2$). In this study, the mean level of CdCl$_2$ was about 200 µM. Briefly, 250 µg ml^{-1} of SALE were added to a test tube containing 200 µl of 20% (v/v) suspension of erythrocyte. To each tube, 100 µl of 200 µM CdCl$_2$ (in 0.1 M PBS) was added. The tube containing cells without cadmium served as negative control. The different cultures were allocated randomly into four experimental groups:

– *Group 1* Control cells which were treated with 100 µl of PBS for 3 h;
– *Group 2* The cells were treated with 100 µl of PBS containing 250 µg ml^{-1} of SALE;
– *Group 3* The cells were treated with 200 µM of CdCl$_2$ dissolved in PBS solution;
– *Group 4* The cells were pre-treated with 250 µg ml^{-1} of SALE and cadmium (200 µM) for 3 h.

Determination of antioxidant and oxidative status of erythrocytes cell

The treated erythrocytes suspension was centrifuged at $1200 \times g$ for 10 min at 4 °C and the supernatant was discarded. Five volumes of PBS (pH 7.4) were pipetted into the residue so as to completely lyse the erythrocytes. Then the mixture was centrifuged again at $12,000 \times g$ for 5 min. The supernatant was collected for analysis.

Reduced glutathione was determined using a spectrophotometric method [22]. Superoxide dismutase activity was determined by the nitro blue tetrazolium reduction method [23]. The reaction mixture concentration 50 mM of liver tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2 μM riboflavin and 75 mM nitro blue tetrazolium (NBT). The absorbance was detected at 560 nm and the results are expressed as units (U) of SOD activity/mg protein. Catalase (CAT) activity was assayed spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi [24]. Enzymatic reaction was initiated by adding an aliquot of 20 μl of the homogenized tissue and the substrate (H_2O_2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of μmoles H_2O_2 consumed/ min/mg of protein. Glutathione peroxidase (GPx) activity was determined according to Flohé and Günzler [25]. The enzyme activity was expressed as nmoles of GSH oxidized/min/mg protein.

Lipid peroxidation was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) as described by Niehaus and Samuelsson [26]. An aliquot of 0.5 ml of liver extract supernatant was mixed with 1 ml of trichloroacetic acid solution and centrifuged at $2500 \times g$ for 10 min. One milliliter of a solution containing 0.67% thiobarbituric acid (TBA) and 0.5 ml of supernatant were incubated for 15 min at 90 °C and cooled. Absorbance of TBA–MDA complex was determined at 532 nm. Lipid peroxidation was expressed as nmoles of thiobarbituric acid reactive substances (TBARS)/ mg of tissue.

Statistical analysis

Statistics were performed on SPSS software (version 20). Data were expressed as the mean ± standard deviation and analyzed by one way ANOVA. Significance level was determined ($p < 0.05$) and significant difference was determined using Duncan's Multiple Range Test (DMRT).

Results and discussion

Fatty acid profile

The yield of SALE is about 3.74%. Consumption of these fatty acids as a dietary supplement or as a food ingredient has the potential to provide health benefits. This may include benefits such as reducing pain related to inflammation by acting as strong antioxidants [27].The percent compositions of the 9 FAs from *Salicorniaarabica* are are shown in Table 1 and Fig. 1. The major FA components were palmitic acid (C16:0) (47.78%), linoleic acid (C18:2) (17.75%), followed by nanadecenoic acid (C19:1) (10.96%). Minor FAs including lignoseric acid (C24:0), stearic acid (C18:0) and palmatoleic acid (C16:0). The margaric acid (C17:0), arachidic acid (C20:0), and myristic acid (C14:0) were also observed in this species.

Antioxidant potential of *S. arabica* lipid extract

Various saturated and unsaturated fatty acids were evaluated for antioxidant activities and are present in numerous plant-derived food products and showed substantial antioxidant activities compared to positive controls tested. Saturated fatty acids such as palmitic acid showed high antioxidant activity [27]. Besides, olive oil rich in palmitic acid has high antioxidant properties [28]. Antioxidant activity evaluated by DPPH and ABTS assays for the lipid extract results were presented in Fig. 2 a, b. The values of DPPH scavenging activity ranged from 72.10% in the SALE to 93.41% in BHT (Synthetic antioxidants).

Table 1 Fatty acid composition (%) of *S. arabica*

CX:Y	Fatty acid	Retention time	FA composition (%)
C14:0	Myristic acid	2.66	2.94 ± 0.24
C16:0	Palmitic acid	3.55	47.78 ± 1.04
C17:0	Margaric acid	3.83	1.69 ± 0.35
C18:0	Stearic acid	4.70	6.21 ± 0.61
C20:0	Arachidic acid	6.01	2.62 ± 0.41
C24:0	Lignoseric acid	8.14	6.72 ± 0.64
∑SFA			67.96 ± 2.04
C16:1	Palmatoleic acid	3.72	3.30 ± 0.17
C19:1	Nanadecenoic acid	5.64	10.96 ± 0.42
∑MUFA			14.26 ± 1.07
C18:2	Linoleic acid	5.19	17.75 ± 0.76
∑PUFA			17.75 ± 0.76

Data are shown as the mean ± SD values for three measurements

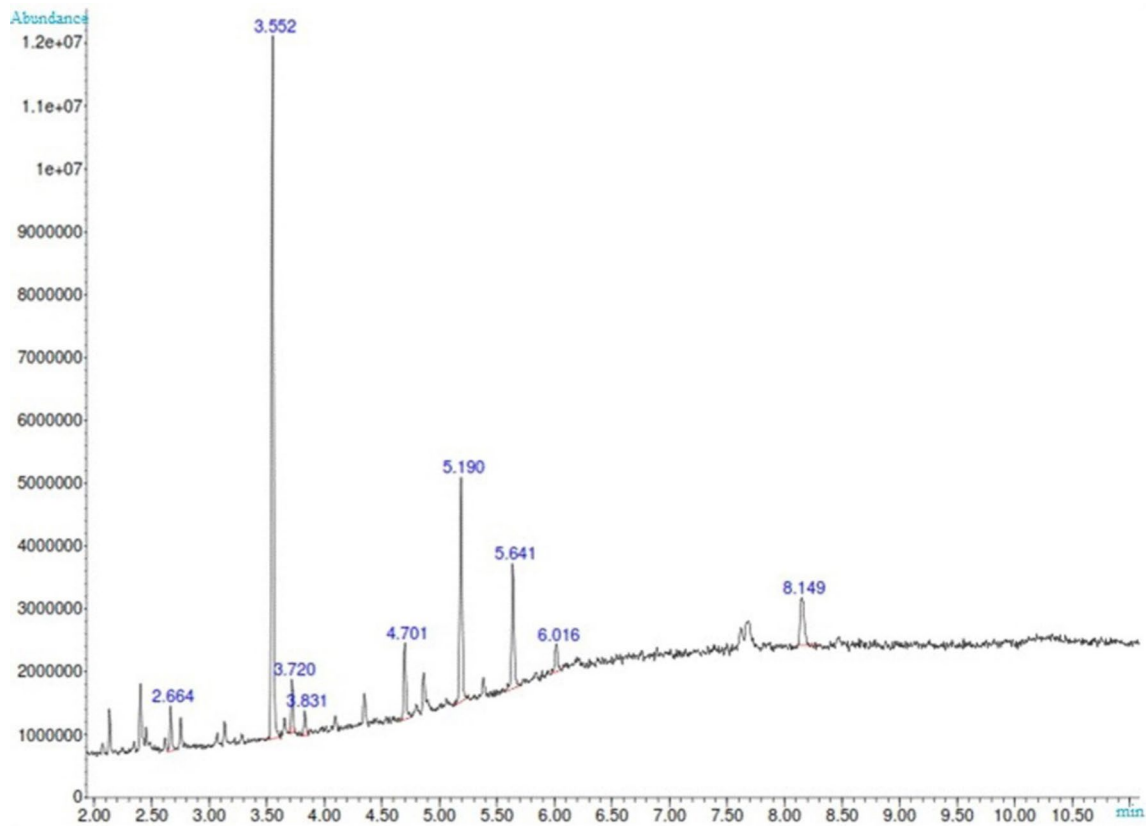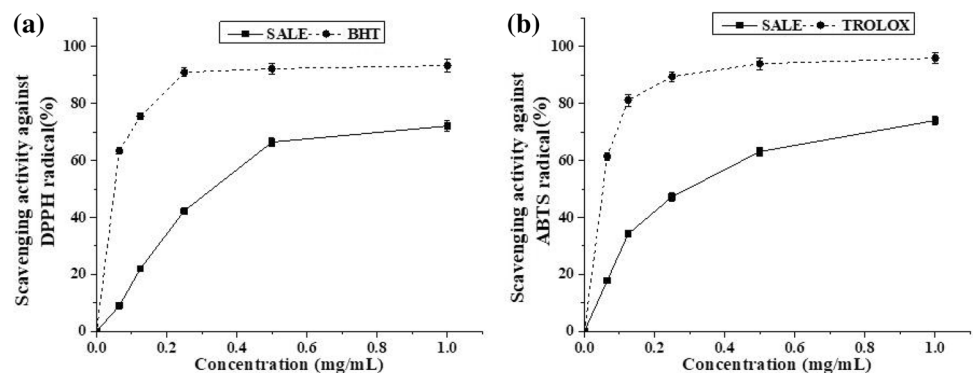*SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Fig. 1 GC–MS analysis of *S. arabica* lipid extract

Fig. 2 Antiradical effects of *S. arabica* lipid extract (SALE) for DPPH (**a**) and ABTS (**b**) compared to respective standards (BHT and TROLOX)

Concerning, the ABTS scavenging capacity of *S. arabica* lipid extract was observed an activity (74.05%) at a level of 1 mg ml^{-1} in the reaction mixture.

Protective effect of SALE against ferrous sulfate induced lipid peroxidation in vitro

The ability of the *S. arabica* lipid extract (SALE) to inhibit the lipid peroxidation was determined and compared with that of positive control (BHT at 100 μg ml^{-1}). The mixture prepared without antioxidant component considered as negative control (total). As shown in Fig. 3, the inhibition of lipid peroxidation of SALE increased in a concentration-dependent manner from 0 to 1 mg ml^{-1}. SALE exhibited the highest lipid inhibition of 77.07% at 1 mg ml^{-1}, Our results proved that SALE could effectively inhibit membrane lipid peroxidation.

Inhibition of protein glycation by SALE

The effect of SALE on protein glycation was determined using a glycation-inducing reaction system with bovine
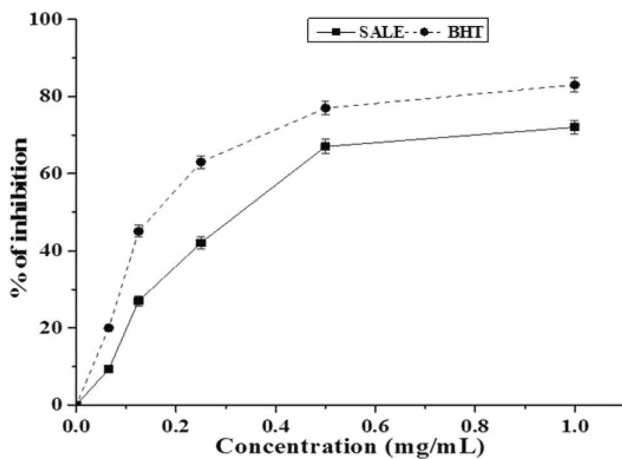
Fig. 3 The inhibition effect of *S. arabica* lipid extract (SALE) on lipid peroxidation. BHT used as standard. Values are mean $\pm$ SD (n $=$ 3)
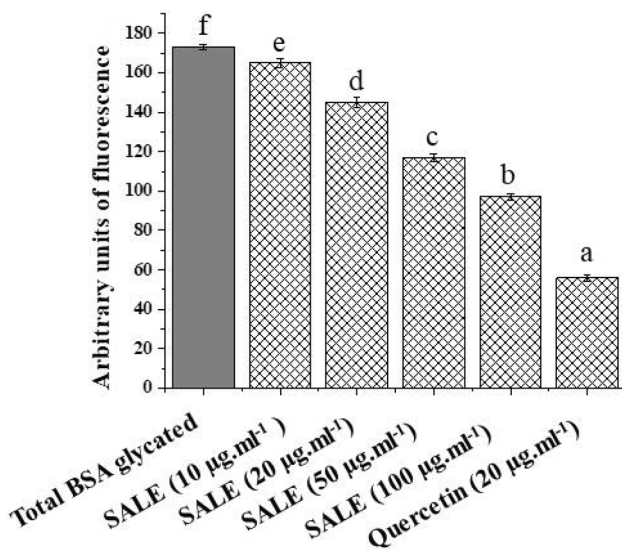

Fig. 4 Inhibition of protein glycation by *S. arabica* lipid extract (SALE). Results are expressed as arbitrary units of fluorescence. Values having different letters on a same column showed significant difference (p $<$ 0.05)

albumin, fructose and glucose. As displayed in Fig. 4, the SALE showed a strong inhibition of protein glycation at 10, 20, 50 and 100 µg ml^{-1}. The flavonoids compound quercetin was also determined and it exhibited a significant inhibition of protein glycation at 20 µg ml^{-1}.

Effects of SALE on oxidative markers of Cd-exposed erythrocytes cells

In the last decades, discussion regarding the relationship on development of diseases induced by environmental

pollutants is increasing in the scientific literature. Among the several toxic substances to which, human and other mammalian species are exposed is cadmium, a heavy metal whose half-life ranged between 10 and 30 years. It promotes wide damage to numerous tissues such liver and blood [29]. Natural compounds have remained a very successful inventorization of new therapeutic agents. The objective of this study was to determine if *S. arabica* lipid extract (SALE) is able to protect erythrocytecells against the diseases induced by cadmium. To our best knowledge, no studies were cited in literature evaluating the protective potential of *S. arabica* lipid extract. Our study is the first comprehensive ex vivo study revealing the protective effect of the lipid extract of the *S. arabica* against Cd-induced toxicity. Honda et al. [30] found that cadmium has toxicological effect on various cells and organs in both humans and animals.

Lipid peroxidation (LPO) is another most commonly used stress biomarker determined in terms of malondialdehyde (MDA) contents in cells. Table 2 shows the effects of Cd exposure on MDA level in erythrocytes cell. The estimated levels of these stress biomarkers of CdCl$_2$ model group significantly (p $<$ 0.01) increased compared to the control rats. Increased lipid peroxidation in erythrocytes cell may be explained by the elevation of ROS production in these cells. Kamiya et al. [31] reported that cadmium stress significantly increased ROS production in kidney tubular epithelial cells line (Cos7). Additionally, Wang et al. [32] also reported that Cd enhanced ROS production in a proximal tubular cell line, WKPT-0293Cl.2. Similar to the present study, Messaoudi et al. [33] found that cadmium treatment group stimulated MDA levels in kidney tissues. Whereas, the administration of SALE extract protected the erythrocytes cells against toxicity induced by CdCl$_2$. This result could be explained by the highest ability of SALE to inhibit lipid peroxidation in vitro

Table 2 Mean effect ($\pm$ SD) of SALE on cadmium induced damage in the concentration enzymatic antioxidant status and the levels of lipid peroxidation in erythrocytes cells of control and treatment rats

Parameters and treatments	Group 1	Group 2	Group 3	Group 4
SOD$^{\alpha}$	15.23 ± 0.45^c	15.25 ± 0.56^c	5.56 ± 0.37^a	11.23 ± 0.47^b
CAT$^{\beta}$	14.23 ± 0.37^c	14.24 ± 0.67^c	6.45 ± 0.32^a	10.45 ± 0.42^b
GPx$^{\gamma}$	24.13 ± 1.45^c	24.14 ± 1.76^c	10.41 ± 1.75^a	18.65 ± 1.45^b
MDA$^{\delta}$	0.43 ± 0.07^a	0.42 ± 0.08^a	1.05 ± 0.11^c	0.68 ± 0.12^b

Values having different letters on a same column showed significant difference (p $<$ 0.05)

α U mg^{-1} protein, β µmoles/H$_2$O$_2$ consumed min^{-1} mg^{-1} of protein, γ nmoles GSH min^{-1} mg^{-1} of protein; δ nmoles of MDA g^{-1} of tissue, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *MDA* malondialdehyde

system. The results of this present study are consistent with the observation of Ben [34] who found that the lipid extract isolated from *Dunaliella* sp. significantly inhibited lipid peroxidation in renal cells after nickel treatment.

Accumulating evidence suggests that Cd induces oxidative damage by distributing the antioxidant defense systems, and the enhancement of ROS production is responsible to suppress free-radical scavenger enzymes, such us SOD, CAT and GPx. Antioxidant enzyme activities of SOD, CAT and GPx of normal rats and Cd-treated group are given in Table 2. Accordingly, our results showed that the SOD, CAT and GPx activities were significantly ($p < 0.01$) decreased with Cd-exposure by 63.49%, 54.67% and 56.85%, respectively. SALE pre-treatment led to a significant increase in SOD, CAT and GPx activities by 50.48%, 38.27% and 44.18% comparing to Cd treated group. Our finding suggested that Cd-exposure induces oxidative injuries in erythrocytes cell by decreasing the antioxidant defense systems (SOD, CAT and GPx). Apart from macromolecules such as lipids and proteins, antioxidant enzymes systems are also affected by Cd exposure. It has been shown by Zhang et al. [21] that Cd-exposure inhibited antioxidant status in erythrocytes cell. Looking at the antioxidative defense system, it was reported that Cd exposure resulted in GSH depletion in rat kidney tissue. Our results showed that the SALE treatment ameliorated the antioxidant status in erythrocytes cell when compared with the normal group. Fatty acid compounds could be potentially employed as antioxidant agents [35]. In addition, Attia and Nasr [36] found that fatty acid could maintain normal levels of enzymatic status such as SOD and CAT activities. The antioxidant and anti-inflammatory effects of fatty acid through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously [37]. Fatty acids were found to play protective roles in the liver and kidney and they have been widely used in clinical preoperative total parenteral nutrition [38].

Conclusion

Salicornia arabica lipid extract (SALE), which is rich in fatty acid molecules can be considered to be a powerful antioxidant source even in vitro and ex vivo study. These findings show that SALE presents the highest antioxidant potential. Lipid extract isolated from *S. arabica* protected erythrocyte cells against Cd-induced oxidative damage. The prevention action offered by the investigated plant extract may involve the scavenging of free radicals generated during Cd metabolism ex vivo and/ or the stimulation of antioxidant status. The SALE can be used as potent natural antioxidant and to protect against cadmium toxicity in blood cells. Moreover, studies are required to explain detailed molecular mechanism of achievement of this plant extract against Cd-induced toxicity.

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