



Nutritive Value of Roselle and Anti-anemic Effect of Its Beverages in Male Rats Exposed to Lead Toxicity: Comparative Study

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IN Egypt, both iron deficiency anemia (IDA) and lead pollution represent formidable health challenges. This study was conducted to identify the nutritive value of Roselle (*Hibiscus sabdariffa* L.) dried red calyces and compare the protective anti-anemic effects of cold and hot beverages of Roselle (CRB and HRB, respectively) in lead –intoxicated rats. Thirty-two adult male albino rats were divided into four equal groups, including a normal control group, while other groups were administered lead acetate (20 mg/kg/24 hr) and kept untreated (group 2), or received CRB and HRB (0.5 mL/100 g b.w./24 hr), respectively for 6 weeks. Finally, body weight gain was calculated. Serum lead and some anemia –related minerals were determined. Besides, liver antioxidant capacity and hematological indices in serum and whole blood were evaluated. Moreover, Roselle dried red calyces were chemically analyzed, while some physicochemical analyses were performed in both beverages. Results indicated the high nutritional value of dried Roselle red calyces, as they are good source of calories, protein and vitamin C. Moreover, they are rich in carbohydrates, fiber, calcium, iron and zinc. Biologically, lead exposure caused a significant increase in serum lead which in turn induced overweight, hematologic disorders, along with oxidative stress. Due to its higher anthocyanin content and total antioxidant activity, CRB was more efficient than HRB in preventing the toxic effects of lead. Accordingly, the present study confirms that dried Roselle red calyces are good source of health promoters and strongly indicates the protective effect of cold Roselle beverage against lead acetate -induced IDA.

Keywords: *Hibiscus sabdariffa* L. drinks, Anthocyanin content, Hematology.

Introduction

In Egypt, both iron deficiency anemia (IDA) and lead pollution represent formidable health challenges (Zawilla, 2013 and Moawad et al., 2016). The prevalence of IDA among pregnant women and children in Egypt was 45% and 48.5%, respectively (WHO, 2008 and El-Zanaty & Way, 2006). On the other hand, studies indicated that various concentrations of lead (Pb) were found in different types of Egyptian foods, either of animal or plant origin (Abou-Arab & Abou Donia, 2000; Abou-Arab, 2001; Abou-Arab & Abou Donia, 2002; Abou Donia, 2008) besides its presence in air and water.

Pb is considered to be a multi-target toxicant.

Oxidative stress has been proposed as a possible mechanism involved in Pb toxicity (Dewanjee et al., 2013). The Pb exposure is associated with multiple ailments and abnormalities affecting humans in different ages (Jaishankar et al., 2014). One of them is IDA. It is the most dangerous, since it is really considered the ailment of all ages and its high prevalence has substantial health and economic costs, including poor pregnancy outcome, impaired school performance, and decreased productivity (Zimmermann and Hurrell, 2007).

In general, the association between lead poisoning and iron deficiency anemia has been investigated in many human and animal studies. In the study of Turgut et al. (2007), the level of

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DOI: 10.21608/ejfs.2021.52490.1087

Received : 6/12/2020; Accepted: 12/4/2021

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lead in serum was significantly higher in children with IDA than those of controls ($p < 0.01$), while Hegazy et al. (2010) reported that Pb level $\square 10 \mu\text{g}/100 \text{ cm}^3$ was significantly associated with anemia, decreased iron absorption and hematological parameters affection.

Roselle (*Hibiscus sabdariffa* L.) is a medicinal plant belonging to the Malvaceae family, with calyces vary in color from white-yellow to deep red, and that is attributed to their anthocyanin content. Worldwide, red varieties dominate the Roselle market, while light-red and white varieties are mainly found locally. In Egypt, the red dried calyces of *H. sabdariffa* (*Hs*) are used to prepare cold beverages and infusions, widely known as Karkadeh. In general, *Hs* is a source of a large number of bioactive compounds including phenolic compounds, such as anthocyanins (such as delphinidin-3-*O*-sambubioside the most abundant), phenolic acids (chlorogenic acid) and flavonols (quercetin and kaempferol derivatives). It is also rich in α -tocopherol and organic acids (quinic acid as the major). These compounds are responsible for the various health promoting properties of *Hs* which include anti-obesity, hypolipidemic, anti-carcinogenic, hypotensive, diuretic, antioxidant and anti-microbial effects (Ali et al., 2005 and Bedi et al., 2020).

As a result of the increased prevalence of both lead pollution and IDA among Egyptians as well as their health threatening effects, dietary interventions aim to alleviate the hematological effects of lead are needed. This study was carried out to identify the nutritive value of Roselle (*Hibiscus sabdariffa* L.) red calyces and compare the protective anti-anemic effects of both cold and hot beverages of Roselle in lead-intoxicated rats.

Material and Methods

Materials

Plant material

Dried red calyces of Roselle (*Hibiscus sabdariffa* L.) were purchased from the local market for medicinal plants and herbs, Tanta city, Al-Gharbiyah governorate, Egypt. The herb was identified by the Department of Flora, Agricultural Museum, Ministry of Agriculture and the Herbarium of the Department of Botany, Faculty of Science, Cairo University.

Animals

A total of 32 healthy male albino rats (Sprague_ Dawley strain) weighing $150 \pm 5 \text{ g}$ (*Egypt. J. Food Sci.* **49**, No. 1 (2021)

were obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt.

Chemicals, kits and other required materials

Casein ($> 80 \text{ g protein}/100 \text{ g}$), vitamins, minerals, cellulose, choline chloride, DL-methionine, lead acetate and other required chemicals were obtained from El-Gomhouria Company for trading drugs, chemicals and medical appliances, Cairo, Egypt. Kits used for biochemical determinations were obtained from Biodiagnostics and Gama Trade Companies for chemicals, Cairo, Egypt. Sucrose, soybean oil and corn starch were obtained from the local market, Tanta city, Al-Gharbiyah governorate, Egypt.

Methods

Chemical analysis of Roselle dried red calyces

Dried red calyces of Roselle were chemically analyzed in order to determine its macronutrients including crude protein, crude fat, and crude fiber according to A.O.A.C. (2000). Total carbohydrates were calculated by difference. The energy value was calculated using the Atwater factors of 4, 9 and 4 for protein, fats and digested carbohydrates, respectively, according to Chaney (2006). Dried red calyces of Roselle were also wet acid-digested, using a nitric acid and perchloric acid mixture (HNO_3 : HClO_4 , 5:1 weight/volume "w/v"), then the total amounts of calcium (Ca), iron (Fe) and zinc (Zn) were determined by atomic absorption spectrophotometry (Thermo-Elmental, Model 300VA, UK). Vitamin C (vit C) concentration was spectrophotometrically (Model No 6300, Designed and manufactured in UK by I en way LTD) determined by the method in which 2, 6 - dichlorophenolendo phenol dye is reduced by ascorbic acid according to Anonymous (1966).

Preparation of Roselle beverages

Dry red calyces of Roselle were crushed to a fine powder using a hammer mill (Thomas Willey mills, model Ed-5, Germany). After that, they were sieved with a screen of 2 mm pore size, stored in dry closed glass jars and kept at room temperature in the dark until used. The traditional methods were followed in preparing both cold and hot Roselle beverages. In details, cold Roselle beverage (CRB) was prepared by putting the Roselle powder in a suitable jar, followed by adding distilled water ($25 \text{ }^\circ\text{C}$, 40 mL/1 g) to this powder, then the jar was covered and refrigerated overnight (12 hr). After that, the obtained

beverage was filtered. On the other hand, hot Roselle beverage (HRB) was prepared by putting the Roselle powder in a suitable jar, followed by pouring hot distilled water (95 °C, 40 mL/1 g) over this powder, then the lid was put on loosely to infuse them in the water solution for 15 min. After that, the obtained liquid was filtered. Both cold and hot Roselle beverages were prepared daily without sugar or other sweeteners. The used sun dried calyces of Roselle to water ratio (1:40 w/v) was found to be suitable for a beverage with similar color intensity as compared to commercial products according to Ramirez-Rodrigues et al. (2011).

Determination of total anthocyanin content in Roselle beverages

Anthocyanins were determined according to the pH-differential method described by Lee et al. (2005). The chemical equilibrium that exists between the red-colored flavylumcation, and the colorless hydrated hemiketal form of the various anthocyanins, which shifts towards the former at low pH values, is the base of this assay. Two aliquots of each sample were prepared, one with potassium chloride solution (250 mM, pH 1.0), and the other with sodium acetate buffer (400 mM, pH 4.5). Once mixed with each buffer, they were incubated at room temperature for 15 min, to allow the reaction to reach equilibrium. Using a UV-Vis spectrophotometer (Cary, model 50 Bio, Varian, Italy), absorbance (Abs) was then read at 510 and 700 nm in disposable cells (1 cm path length). The instrument was set to zero using distilled water. To calculate total anthocyanin content, the following equation (Eq. 1) was used:

$$\text{Eq. 1: TAC} = (\text{Abs} \times \text{MW} \times \text{DF} \times 1000) / \epsilon \times l$$

Where, Abs = (Abs 510- Abs 700) pH1.0 - (Abs510-Abs700) pH4.5, MW is the molecular weight (449.2 g/mol) of cyanidin-3-glucoside and ϵ is molar extinction coefficient (26900 M⁻¹cm⁻¹) of cyanidin-3-glucoside, too. DF is the dilution factor, 1000 is a factor to convert g to mg, and l is the cell's path length (1 cm). Results were expressed as mg of cyanidin-3-glucoside equivalents/100 mL (mg C3G/100 mL).

Determination of antioxidant activity of Roselle beverages

To quantify antioxidant activity of Roselle beverages, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used (Villa-Rodríguez et al., 2011). A stock solution was prepared by dissolving 2.5 mg of the DPPH radical in 100 mL of pure

methanol. Using a UV-VIS spectrophotometer, the absorbance of the solution was adjusted to 0.70±0.02 (time 0) at 515 nm. A 10 µL aliquot of the sample was mixed with 140 µL of the DPPH solution (absorbance adjusted previously), incubated in the dark for 30 min (time 30), and its absorbance was then read at 515 nm. Percentage of DPPH inhibition was calculated with the following equation (Eq. 2):

$$\text{Eq. 2: \% of DPPH inhibition} =$$

$$[(\text{Abs}_{\text{time0}} - \text{Abs}_{\text{time30}}) / \text{Abs}_{\text{time0}}] \times 100$$

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard for the assay, and results were presented as µmol of Trolox equivalents (TE)/100 mL.

Color density determination of Roselle beverages

Color density of the two tested beverages was determined using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan). The absorbance was measured at 420, 520 and 700 nm, for a 10 mm optical path length. The analysis was performed in duplicate and reagents used were all analytical grade. For calculation, the following equation (Eq. 3) was used according to Giusti and Wrolstad (2001).

$$\text{Eq. 3: Color density } (\mu\text{g/mL}) = [(A_{420 \text{ nm}} - A_{700 \text{ nm}}) + (A_{520 \text{ nm}} - A_{700 \text{ nm}})]$$

Preparation of experimental diet

Basal diet was formulated according to Reeves et al. (1993) with some modification. Each 100 g of the formulated basal diet consisted of 14, 4, 5, 3.5, 1, 0.25, 0.3 and 10 g of casein, soybean oil, cellulose, mineral mixture, vitamin mixture, choline chloride, DL-methionine and sucrose, respectively, while corn starch was added up to 100 g.

Animals & study design

Animals were housed in well-aerated cages under hygienic conditions in a room maintained at suitable humidity, 22 – 25 °C and a 12 hr light-dark cycle, and fed on basal diet for one week for adaptation. After that, rats were weighed and divided into four groups of 8 rats each. The first group was kept as a normal control group and fed on basal diet only, while groups from 2 to 4 received lead acetate (20 mg/kg body weight/24 h) according to Abdel Moneim et al. (2011). At the same time, group 2 was fed on basal diet only, while the 3rd and the 4th groups fed on basal diet and received CRB and HRB, respectively

(0.5 mL/100 g body weight/24 h). Rats received lead acetate, CRB and HRB orally by a stomach tube all over the experimental period (6 weeks). Meanwhile the experiment, diet and water were provided *ad-libitum* and body weight was recorded once a week.

Blood and tissue sampling

At the end of the experiment, animals were weighed, fasted overnight, then exposed to very light ether anesthesia and blood samples were withdrawn from eye plexus of veins and transferred into tubes with ethylenediamine tetraacetic acid (EDTA) in order to determine complete blood count (CBC) parameters. After sacrifice, other blood samples were collected from the aorta of each rat into dry clean centrifuge tubes. Sera were carefully separated by centrifugation of blood samples at 3000 rpm (round per min) for 10 min at room temperature, then transferred into dry clean Eppendorf tubes and kept frozen at $-20\text{ }^{\circ}\text{C}$ till analyzed. Moreover, livers and spleens were removed by careful dissection, washed in ice-cold NaCl (0.9 g/ 100 mL), dried using filter paper and weighed. After that, a specimen from each liver was stored at $-80\text{ }^{\circ}\text{C}$ until homogenate preparation.

Preparation of liver tissue homogenate

In order to prepare liver tissue homogenate, one gram of liver tissue was homogenized in ice-cold solution of potassium chloride (1.15 g/100 mL) in 50 mmol L⁻¹ potassium phosphate buffer solution (pH 7.4). Homogenization was performed and sonicated by ultrasonics homogenizer, 4710 Ultrasonics Homogenizer (Cole-Parmer Instrument Co., USA). The homogenates were centrifuged at 4000 rpm for 5 min at $4\text{ }^{\circ}\text{C}$. The supernatants were collected and stored at $-80\text{ }^{\circ}\text{C}$ for latter biochemical analysis.

Body weight gain and relative organ weight calculation

Body weight gain (BWG) was calculated by subtracting the initial weight of each rat from its final weight. As for the relative weights of liver and spleen (RLW and RSW), they were calculated according to Angervall & Carlström (1963) using the following equation (Eq. 4):

$$\text{Eq. 4: Relative organ weight (g/100 g)} = \left[\frac{\text{Organ weight (g)}}{\text{Final body weight (g)}} \right] \times 100$$

Determination of serum lead and anemia-related minerals

Serum lead concentration was determined by stable isotope dilution using a thermal ionization

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mass spectrometer (Manton et al., 2001). In the same time, zinc (Zn), iron (Fe) and copper (Cu) were determined in serum according to the methods described by Johnsen & Eliasson (1987); Ramsay (1957) and Abe et al. (1989), respectively.

Determination of total antioxidant capacity in liver tissue homogenate

In liver tissue homogenate, total antioxidant capacity (TAC) was determined according to Koracevic et al. (2001).

Determination of hematological indices in liver and serum

In liver tissue homogenate, ferritin was determined according to the method of Zuyderhoudt (1975), while total iron binding capacity (TIBC) in serum was measured according to Olson & Hamlin (1969). Concerning transferrin saturation (T_{sat}) in serum, it was calculated using the following equation (Eq. 5) used by Abd El-Azeem et al. (2016):

$$\text{Eq. 5: } T_{\text{sat}} (\%) = \left(\frac{\text{serum iron}}{\text{serum TIBC}} \right) \times 100$$

Determination of CBC parameters

CBC parameters were determined immediately by SYSMEX KX-21N Hematology Analyzer (Sysmex Cooperation, Kobe, Japan). They include hemoglobin (Hb) concentration, packed cell volume (PCV) as well as the total count of red blood cells (R.B.Cs), white blood cells (W.B.Cs) and platelets (PLTs).

Statistical analysis

Statistical analysis was carried out using the program of Statistical Package for the Social Science (SPSS), PC statistical software (Version 20; Untitled – SPSS Data Editor). The results were expressed as mean \pm standard deviation (mean \pm SD). Data was analyzed using one-way classification, analysis of variance (ANOVA) test. The differences among means were tested for significance using Duncan test at $p < 0.05$. Moreover, independent samples T test was used to analyze data of color density analysis.

Results and Discussion

Chemical composition of dried red calyces of Roselle

In Table 1, the concentrations of macronutrients, some minerals and vit C in dried red calyces of Roselle were presented. It was found that 100 g of the used sample contain 8.30, 1.15 and 73.18 g of crude protein, crude

fat and total carbohydrates, respectively. Crude fiber content also was 18.02 g/100 g. Thus, the total energy provided is 264.19 kilocalorie. As for mineral content, Ca, Fe and Zn contents were found to be 450, 20 and 11 mg, respectively, while total ash was 11.25 g/100 g. Besides, vit C content was found to be 15 mg/ 100 g of the used sample.

Roselle as a herb is known to have a high nutritional value. Herein, chemical analysis indicated that 100 g of the used sample of Roselle as dried red calyces provide 264.19 kilocalorie, i.e. 13.21% of the caloric intake for the human adult (2000 kilocalorie/ 24 hr). According to Food and Drug Administration (FDA) (2016), crude protein, crude fat, total carbohydrates and crude fiber contents in 100 g of the used sample of dried Roselle red calyces represent 16.6, 1.47, 26.61 and 64.36% of the new daily values (DVs) based on a caloric intake of 2000 kilocalorie for adults, while calcium and Fe contents represent 34.62 and 111.11%. The new DVs of Zn and vit C are not equal in males and females. They were found to be 11 and 90 mg for males, and 8 and 75 mg for females, respectively. Accordingly, Zn content in 100 g of the used sample represents 100 % and 137.5 %, whereas vit. C content represents 16.67% and 20% of the new DVs for males and females (19-30 years), respectively. So, dried Roselle red calyces can be considered good source of calories and protein generally as well as vit C for adult males. On the other hand, they are rich in carbohydrates and calcium and excellent source of fiber, Fe, Zn, in general, and vit C for females.

Total anthocyanin content, total antioxidant activity and color density of Roselle beverages

Results presented in Table 2 indicated that CRB was higher in both anthocyanin content and antioxidant activity than HRB. On the other hand, the high anthocyanin content of CRB resulted in a significant elevation in its color density compared to HRB. Many studies discussed the effect of both temperature and period of extraction on anthocyanin content. For example, Ramirez-Rodrigues et al. (2011) found that both cold (25 °C for 240 min) and hot (90 °C for 16 min) aqueous extracts of *Hibiscus sabdariffa* L. calyces had the two major anthocyanins: delphinidin-3-sambubioside and cyanidin-3-sambubioside. Both extracts also were found to yield similar phytochemical properties.

Recently, Salmerón-Ruiz et al. (2019) used response surface methodology to identify temperature (70–100 °C), calyces-to-water ratio (1–20 g/100 mL), and time (1–30 min) that would produce a *Hibiscus* infusion with the highest total anthocyanin content and antioxidant activity. They stated that the best infusion was that prepared using 10 g dry calyces /100 mL at 88.7 °C for 15.5 min. In the present study, the two beverages were prepared using the same calyces-to-water ratio (1:40 w/v), however, the cold beverage was prepared at 25 °C for 12 hr, while the hot beverage was prepared at 95 °C for 15 min. Thus, it could be understood that calyces-to-water ratio had no effect on this difference in total anthocyanin content and total antioxidant activity between the two studied beverages. Although low temperature, long period of extraction looks like to be useful in extracting more anthocyanin, and hence obtaining a beverage with higher antioxidant activity, since anthocyanins are the major antioxidant compounds in *Hibiscus* calyces. In the same time, and according to Salmerón-Ruiz et al. (2019), temperatures above 88.7 °C decreased total anthocyanin content in the resulted infusions, which means that anthocyanins degraded at higher temperature.

Body weight gain & absolute and relative liver and spleen weights

At the beginning of the experiment, there were no significant differences in the body weight of all experimental groups, while at the end, body weight gain of untreated lead-exposed group was found to be significantly ($P < 0.05$) higher than that of normal control group (Table 3). This finding was in line with the results of several human and animal studies. For example, Wang et al. (2016) reported that there is a positive association between blood lead level and BMI in Chinese women, but not in men. In an animal study, the metabolic disorders associated with chronic exposure of adult rats to lead toxicity, including insulin insensitivity and weight gain, may be induced through altered methylation of metabolism-related genes (Sun et al., 2017).

Both cold and hot beverages of Roselle red calyces (CRB and HRB, respectively), as noticed in Table 3, induced significant decreases compared with untreated lead-exposed group, however, CRB was so efficient that could return BWG toward its normal value. The weight loss promoting effect of *Hibiscus sabdariffa* L. calyx beverages (CRB and HRB) is in harmony with Omar et al. (2018) who revealed that oral

administration of *Hs* aqueous extracts (150, 200, 250, and 300 mg/kg) for 10 weeks caused a dose-dependent reduction in BWG and abdominal fat in obese rats. The mechanisms by which *Hs* can induce weight loss were investigated in many studies. They included the ability of *Hs* to suppress appetite and adipogenesis and inhibit the activities of carbohydrate digestive enzymes including α - β -glucosidase and α -amylase (Omar et al., 2018; Ojulari et al., 2019; Gondokesumo et al., 2017).

On the other hand, both absolute and relative weights of liver and spleen were not affected

significantly by lead exposure or *Roselle* beverages administration (Table 3).

Serum anemia –related minerals & total liver antioxidant capacity

Table 4 showed the effect of *Roselle* red calyx beverages on serum anemia – related minerals and total liver antioxidant capacity in lead-intoxicated versus normal rats. It could be noticed that regular exposure to lead acetate induced a significant ($P < 0.05$) rise in serum Pb level, while serum level of Zn as well as total antioxidant capacity in liver tissue homogenate showed significant decrease. In contrast, serum level of free iron decreased insignificantly, while serum copper recorded an insignificant increase.

TABLE 1. Chemical composition of dried red calyces of Roselle per 100 g .

Nutrient	Concentration
Macronutrient:	
Moisture	
Crude protein	6.12 g
Crude fat	8.30 g
Ash	1.15 g
Crude fiber	11.25 g
Total carbohydrates	18.02 g
Energy	73.18 g
	264.19 kilocalorie
Micronutrients:	
Calcium	450 mg
Iron	20 mg
Zinc	11 mg
Vit. C	15 mg

TABLE 2. Total anthocyanin content, total antioxidant activity and color density of Roselle beverages.

Parameters	Beverages	
	CRB	HRB
Total anthocyanin content (mg C3G/100 mL)	167.32	100.83
Total antioxidant activity (μ mol TE/100 mL)	1270.11	780.20
Color density (μ g/mL)	23.00 \pm 2.00 ^b	15.88 \pm 1.05 ^a

• CRB= Cold Roselle beverage, HRB= Hot Roselle beverage, C3G= Cyanidin-3-glucoside equivalents, TE= Trolox equivalents.

TABLE 3. Effect of *Roselle* red calyx beverages on body weight gain, absolute and relative liver and spleen weights in lead-intoxicated versus normal rats.

Groups	Normal control	Lead-intoxicated	Lead intoxicated +CRB	Lead intoxicated + HRB
Parameters				
Initial weight (g)	158.33±15.28	161.67±20.21	151.67±10.41	166.33±12.66
Final weight (g)	221.60±23.33 ^{ab}	249.30±16.43 ^b	216.07±7.18 ^a	239.86±3.77 ^{ab}
BWG (g)	63.27±8.15 ^a	87.63±8.33 ^c	64.40±6.10 ^a	73.53±9.32 ^b
LW (g)	7.11±0.98	7.02±0.76	6.59±0.83	7.01±0.75
RLW (g/100 g)	3.26±0.78	2.82±0.30	3.05±0.40	2.93±0.35
SW (g)	1.27±0.08	1.18±0.17	1.23±0.16	1.20±0.18
RSW (g/100 g)	0.58±0.10	0.47±0.07	0.57±0.07	0.50±0.07

● Results are expressed as mean ± SD.

● Values that have different letters in each row differ significantly ($P \leq 0.05$), while the difference among those with similar letters completely or partially is not significant.

● CRB= Cold Roselle beverage, HRB= Hot Roselle beverage, BWG= Body weight gain, LW= Liver weight, RLW= Relative liver weight, SW= Spleen weight, RSW= Relative spleen weight.

The elevating effect of lead acetate on serum Pb level was in agreement with the results of Amah et al. (2014). On the other hand, reduction of total antioxidant capacity in liver tissue homogenate through exposure to lead acetate is in line with El-Tantawy (2016) who reported that Pb exposure dysregulated antioxidant/oxidant balance in liver tissue homogenate. Pb intoxication was reported to induce lipid peroxidation indirectly via damaging of the protective antioxidant barrier which in turn happens through binding to thiol groups of antioxidant enzymes (Flora et al., 2008).

The inverse association between serum levels of Pb and Zn, in the present study, was in accordance with many animal and human studies. For example, Taha et al. (2013) found that serum Zn was significantly decreased in lead acetate trihydrate –injected rats. Similarly, Dioka et al. (2004) noticed that Zn level in the blood reduced by 34 % in artisans who were occupationally exposed to lead. The negative correlation between the serum levels of Pb on a hand and Zn on the other hand may be attributed to 1) decreasing the absorption rate and biologic availability of Zn in the body, mainly because of their competition for binding to the sulfhydryl (-SH) group site in various enzymes, other proteins (especially metallothionein) and tissues (Telisman, 1995; Ahamed et al., 2007), 2) hypoalbuminemia as most of plasma Zn is protein bound (Victery et al., 1981), and 3) stimulation of urinary excretion of Zn and interfering with its reabsorption in kidney (Morawiec, 1991).

On the other hand, the insignificant lowering effect of lead exposure on serum free iron concentration were in accordance with Kasperczyk et al. (2012) who found that iron level decreased insignificantly in the low Pb -exposed male employees compared with the control group. However, Kim et al. (2003) reported a decrease in the serum Fe level in lead-exposed workers, but a significantly lower dietary Fe intake was observed concurrently. The present results may be attributed to the fact that although high blood Pb level was associated with decreased iron absorption (Hegazy et al., 2010) resulted in decreased serum iron, Pb in the same time reduces the activity of ferrochelatase, the terminal enzyme responsible for catalyzing the insertion of ferrous iron into protoporphyrin IX, yielding heme (Jin et al., 2008). Labbé et al. (2000) explained that Zn protoporphyrin (ZPP) is a compound found in red blood cells when heme production is inhibited by lead and/or by lack of iron. Instead of incorporating a ferrous ion, to form heme, protoporphyrin IX, the immediate precursor of heme, incorporates a zinc ion, forming ZPP. This explanation not only added another mechanism by which lead exposure decreases serum Zn concentration, but also counted one of the mechanisms by which Pb exposure decreased hemoglobin level as discussed latter.

Regarding serum copper, its level was found to increase insignificantly in untreated lead –exposed group. It was found that lead competes

with copper during absorption analogically to zinc (Cerklewski & Forbes, 1977), i.e. Pb exposure can decrease serum Cu level. However, some studies showed that lead exposure is associated with an elevated activity of superoxide dismutase isoenzyme that contains Cu and Zn (CuZn-SOD) in both serum and erythrocytes. CuZn-SOD is part of the antioxidant defense system and its activity may be elevated because of lead-induced oxidative stress (Kasperczyk et al., 2004; Kasperczyk et al., 2005). The increase in serum Cu level in untreated lead-exposed group may also be caused by competitive displacement of copper from tissues by lead ions (Qian et al., 2005). Increased bioavailability of displaced Cu may induce reactive oxygen species generation via the Fenton reaction and contribute to oxidative stress enhancement.

According to the present results illustrated in Table 4, CRB and HRB decreased serum lead and increased total liver antioxidant capacity compared with untreated lead-intoxicated group, however the changes induced by HRB were not significant. These findings were in harmony with a large number of *in vitro* and *in vivo* studies. According to Olusola (2011), both the whole aqueous and anthocyanin-rich extracts of Roselle induced significant antioxidant effects. Huang et al. (2015) explained that the high antioxidant activities of Roselle calyces can be attributed to its polyphenolic compounds such as protocatechuic acid, catechins, caffeic acid and epigallocatechin-gallate. Moreover, Omar et al. (2018) revealed that delphinidin-3-O-sambubioside and delphinidin-3-O-sambubioside, the main anthocyanins detected in Roselle extract, were found to be responsible to a large extent for its antioxidant properties. The antioxidant activity of Roselle calyces is due to their strong scavenging effect on reactive oxygen and free radicals (Farombi & Fakoya, 2005; Sayago-Ayerdi et al., 2007), inhibition of xanthine oxidase activity, protective action against tert-butyl hydroperoxide (t-BHP)-induced oxidative damage (Tseng et al., 1997), protection of cell from damage by lipid peroxidation (Farombi & Fakoya, 2005), inhibition in Cu²⁺-mediated oxidation of low density lipoprotein and the formation of thiobarbituric acid reactive substances (TBARS) (Hirunpanich et al., 2005; Ochani & D'Mello, 2009), reduction of glutathione depletion, decrease of blood activities of superoxide dismutase and catalase (Usuh et al., 2005), while in the liver it increased their activities as well as decreased

malondialdehyde content (Famurewa et al., 2019; Hoseini et al., 2021).

Although CRB and HRB also increased serum Zn compared with untreated lead-intoxicated group, the differences were not significant. However, serum Zn level of CRB was the nearest from that of normal control group. As for serum Fe and Cu, CRB and HRB did not affect them significantly compared to both control and untreated lead-intoxicated group, however their levels in CRB-administered group were close to those of normal control group (Table 4).

As an alternative source of iron, *Hs* decoctions were used by Falade et al. (2005) for the treatment of anemia and some other mineral deficiency diseases. Results showed that dry fermented calyces of *Hibiscus* exhibited a very low pH value which enhanced mineral availability. The authors also attributed the enhancing effects of *Hs* decoctions on iron, zinc, calcium and magnesium bioavailability to the high concentration of ascorbic acid. Regarding iron, vit. C enhances its absorption by increasing the reduction of iron ferri (Fe³⁺) to ferro (Fe²⁺) in the small intestine.

Hematological indices in liver and serum & CBC parameters

Table 5 shows that regular exposure to lead acetate caused a significant ($P < 0.05$) elevation in total iron binding capacity (TIBC), while both liver ferritin and serum transferrin saturation (T_{sat}) were significantly lowered. On the other hand, subchronic exposure to lead acetate, as presented in Table 6, led to a significant ($P < 0.05$) decrease in hemoglobin (Hb), packed cell volume (PCV), red blood cell (R.B.Cs) count and platelet (PLTs) count, while total white blood cell (W.B.Cs) count was significantly increased.

The lowering effect of lead acetate on ferritin concentration was supported by Hegazy et al. (2010) who found that high blood lead levels were associated with low serum levels of ferritin in Egyptian children. In infants with iron deficiency anemia, Willows (2000) found a significant negative correlation between blood lead and hemoglobin and blood lead and ferritin concentrations. Like ferritin, transferrin saturation was found to be lower in untreated lead exposed group than in normal control group. This effect along with the elevating effect of Pb exposure on total iron binding capacity, observed in the present study, are in line with Kim et al.

TABLE 4. Effect of *Roselle* red calyx beverages on serum anemia – related minerals and total liver antioxidant capacity in lead- intoxicated versus normal rats.

Groups Parameters	Normal control	Lead-intoxicated	Lead intoxicated + CRB	Lead intoxicated + HRB
Pb (µg/100 mL)	83.17±14.18 ^a	105.67±10.00 ^b	83.67±8.00 ^a	102.00±11.53 ^{ab}
Zn (mg/100 mL)	134.40±16.01 ^b	108.00±14.65 ^a	127.60±13.96 ^{ab}	112.00±18.26 ^a
Fe (mg/mL)	1.39±0.20	1.14±0.17	1.32±0.20	1.16±0.17
Cu (mg/100 mL)	141.00±16.99	156.20±21.36	146.00±16.58	151.00±17.87
Liv. TAC (ng/mL)	0.31±0.04 ^b	0.15±0.03 ^a	0.28±0.04 ^b	0.16±0.03 ^a

• Results are expressed as mean ± SD.

• Values that have different letters in each row differ significantly ($P \leq 0.05$), while the difference among those with similar letters completely or partially is not significant.

• CRB= Cold Roselle beverage, HRB= Hot Roselle beverage, Pb= Lead, Fe= Iron, Zn= Zinc, Cu= Copper.

(2003). Since hepatocytes are the major site for transferrin and ferritin synthesis (Ponka et al., 1998) and extensive *in vivo* and *in vitro* studies markedly exhibited the hepatotoxic effects of lead (Mudipalli, 2007; Verheij et al., 2009), which was also evidenced in the present study by decreasing total antioxidant capacity in liver tissue homogenate significantly as discussed above, it can be proposed that Pb promotes oxidative stress in liver tissue, thereby it dysregulates/ suppresses its functions including ferritin and transferrin synthesis and affinity for iron.

On the other hand, the effects of lead on R.B.Cs count, hemoglobin and hematocrit noticed in the present study were in agreement with many human (Lilis et al., 1978; Willows, 2000; Kim et al., 2003; Yilmaz et al., 2012) and animal studies (Mugahi et al., 2003 and Abd EL Rahiem et al., 2007). A shortening of erythrocyte survival time was observed in the rats exposed to lead (Terayama, 1993), and this was attributed to that lead increases the fragility of erythrocyte membranes and decreases their mobility (Terayama et al., 1986). Verheij et al. (2009) added that lead exposure results in oxidative stress of the red blood cells and increased variability in shape and size of the erythrocytes. In parallel to the decrease in R.B.Cs count in the present study, lead decreases hemoglobin level significantly. According to the current results, that iron deficiency is a cause for low hemoglobin levels is an excluded suggestion. Low hemoglobin level in untreated lead-exposed group, however, may be due to that

lead interferes with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting aminolevulinic acid dehydratase and ferrochelatase activity (Masci et al., 1998 and Baranowska- Bosiacka et al., 2000). In addition, Sivaprasad et al. (2003) explained that free radicals produced as a result of lead exposure contribute to hemoglobin denaturation and precipitation, leading to anemia. Moreover, lead was reported to induce a partial loss of hemoglobin molecule stability (Moussa and Bashandy, 2008). As for hematocrit level, the significant decrease in its value in rats exposed to lead than the control groups can be attributed to dilution of the blood associated with a decrease in the count of R.B.Cs.

According to the present work, exposure to lead acetate induced a significant increase in W.B.Cs count. This result was supported by the results of various animal studies (Berny et al., 1994; Mugahi et al., 2003; Alwaleedi, 2016). The possible causes might include increased inflammation as well as the toxic effects of lead on the hemopoietic and lymphatic organs of the body (Yagminas et al., 1990 and Berny et al., 1994). In contrast, the reducing effect of lead on platelet count observed in the present study was in accordance with Barman et al. (2014) who concluded that lead exposure may impair coagulation function through endothelial tissue injury and reduction of nitric oxide.

Both CRB and HRB decreased TIBC and W.B.Cs, while they increased T_{sat} liver ferritin,

Hb, PCV, R.B.Cs and PLTs compared with untreated lead-intoxicated group, however the changes induced by HRB were not significant, except for serum TIBC which was significantly decreased. In general, CRB was more efficient than HRB as it could return most indices toward their normal values recorded by normal control group (Tables 5 and 6).

The hematological effects of Roselle beverages in the present study were in line with Ejere et al. (2013) who noticed significant ($p < 0.05$) increase in R.B.Cs, Hb and PCV values as a result of consuming aqueous extract of *H. sabdariffa* L. calyces by normal male albino rats. In male volunteers given Roselle beverage for 2 weeks, Tazoho et al. (2016) observed significant ($p < 0.05$) elevation in the levels of R.B.Cs, Hb and PCV, which clearly indicates that the beverage contains phytochemical compounds, mainly anthocyanins, that stimulate the formation of erythropoietin, a glycoprotein which stimulates stem cells in bone marrow to produce red blood cells, in the stem cells (erythropoiesis) as well as the decreasing in the destruction of matured R.B.Cs (Kaur & Kapoor, 2005 and Ohlsson & Aher, 2010).

Besides, consumption of cold *Hibiscus* beverage induced a significant decrease ($p < 0.05$) in total W.B.Cs count compared with untreated lead-exposed group. This result was in harmony with Tazoho et al. (2016). The W.B.Cs count is an important indicator for the strength of the immune system, as they protect body cells against pathogens. However, in spite of the significant decrease of W.B.Cs in cold beverage-received group, the value of this parameter was

in the normal range recorded by normal control group, which means that the rats received cold *Hibiscus* beverage enjoyed good health because decrease in number of W.B.Cs below the normal range is an indication of allergic conditions and certain parasitism or presence of foreign body in circulating system (Ahamefule et al., 2008), but none of this was observed during this study. Moreover, increased levels of hemoglobin and transferrin saturation in *Hibiscus* beverages – received groups can be attributed to the high content of vitamin C in Roselle calyces (15 mg/100 g dry weight basis). As a support, Jalalzadeh et al. (2012) carried out a crossover randomized clinical trial in which hemodialysis patients received ascorbic acid intravenously for 6 months. Results showed that low amount of intravenous ascorbic acid could enhance Hb and T_{sat} , suggesting improved iron utilization.

In general, CRB was more efficient than HRB in preventing the hematologic disorders associated with lead exposure. This can be attributed to its higher anthocyanin content and total antioxidant activity. The higher content of vitamin C in CRB, as it is prepared at room temperature and does not expose to high temperature, may also a cause for its high efficiency compared to HRB.

Conclusion

The present study confirms that dried Roselle red calyces and Roselle red calyx beverages are good source of health promoters. It also strongly indicates the protective effect of cold Roselle beverage against lead acetate-induced IDA, thereby scientifically support its traditional use.

TABLE 5. Effect of *Roselle* red calyx beverages on hematological indices in liver and serum of lead-intoxicated versus normal rats.

Parameters	Normal control	Lead-intoxicated	Lead intoxicated + CRB	Lead intoxicated + HRB
Liv. ferritin (ng/mL)	1.75±0.31 ^c	0.52±0.07 ^a	0.95±0.20 ^b	0.77±0.12 ^{ab}
TIBC (mg/mL)	2.85±0.27 ^a	4.37±0.48 ^c	3.00±0.32 ^a	3.65±0.50 ^b
T_{sat} (%)	49.07±6.76 ^b	25.96±1.22 ^a	44.22±6.46 ^b	31.86±1.24 ^a

• Results are expressed as mean ± SD.

• Values that have different letters in each row differ significantly ($P \leq 0.05$), while the difference among those with similar letters completely or partially is not significant.

• CRB= Cold Roselle beverage, HRB= Hot Roselle beverage, TIBC= Total iron binding capacity, T_{sat} = Transferrin saturation.

TABLE 6. Effect of *Roselle* red calyx beverages on complete blood count in lead-intoxicated versus normal rats.

Groups Parameters	Normal control	Lead-intoxicated	Lead intoxicated + CRB	Lead intoxicated + HRB
Hb (g/100 mL)	11.55±1.45 ^b	8.67±1.13 ^a	11.10±0.50 ^b	10.77±1.57 ^{ab}
PCV (%)	34.70±5.25 ^c	20.00±2.70 ^a	28.33±3.51 ^{bc}	22.13±4.59 ^{ab}
R.B.Cs (×10 ⁶ /mm ³)	6.51±0.89 ^b	3.14±0.60 ^a	5.47±0.73 ^b	3.93±0.41 ^a
W.B.Cs (×10 ³ /mm ³)	12.07±1.86 ^a	20.13±3.68 ^c	13.10±1.30 ^{ab}	17.77±2.93 ^{bc}
PLTs (10 ³ /μL)	453.00±60.32 ^b	178.12±23.11 ^a	477.33±60.01 ^b	225.33±38.28 ^a

• Results are expressed as mean ±SD.

• Values that have different letters in each row differ significantly (P<0.05), while the difference among those with similar letters completely or partially is not significant.

• CRB= Cold Roselle beverage, HRB= Hot Roselle beverage, Hb= Hemoglobin, PCV= The packed cell volume, R.B.Cs= Red blood cells, W.B.Cs= White blood cells, PLTs= Platelets.

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القيمة الغذائية للكرديه والتأثير المضاد للأنيميا لمشروباته في ذكور الجرذان المعرضة لسمية الرصاص: دراسة مقارنة

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يعتبر كلا من أنيميا نقص الحديد والتلوث بالرصاص تحديات صحية جسيمة في مصر. أجريت هذه الدراسة بهدف تحديد القيمة الغذائية لكؤوس زهور الكركديه الحمراء الجافة ومقارنة التأثير الوقائي المضاد للأنيميا لمشروبي الكركديه البارد والساخن في الجرذان المعرضة لسمية الرصاص. تم تقسيم الجرذان (٣٢ جرذ أبيض ذكر بالغ) إلى أربع مجموعات متساوية تشمل المجموعة الضابطة السليمة والمجموعات الأخرى تم تجريعها بمادة خلات الرصاص (٢٠ ملجم/كجم/٢٤ ساعة)، حيث تركت إحداهن (المجموعة ٢) دون معالجة، في حين تم تجريع المجموعتين الباقيتين بمشروبي الكركديه البارد والساخن (٠,٥ مل/ ١٠٠ جم من وزن الجسم/٢٤ ساعة) على التوالي ولمدة ستة أسابيع. في نهاية التجربة تم حساب الزيادة المكتسبة في الوزن، كما تم تقدير الرصاص وبعض المعادن وثيقة الصلة بالأنيميا في السيرم. بالإضافة إلى ذلك، تم تقدير كفاءة مضادات الأكسدة بالكبد وكذلك مقاييس الدم بالسيرم والدم الكامل. علاوة على ذلك، تم تحليل كؤوس الزهور الحمراء الجافة للكرديه كيميائياً، في حين أجريت بعض التحاليل الفيزيوكيميائية لكلا المشروبين. أثبتت نتائج التحليل الكيميائي القيمة الغذائية العالية لكؤوس الزهور الحمراء الجافة للكرديه؛ حيث يمكن اعتبارها مصدر جيد للسرعات والبروتين وفيتامين ج، كما أنها غنية بالكربوهيدرات والألياف وعناصر الكالسيوم والحديد والزنك. بيولوجياً، نتج عن التعرض للرصاص زيادة معنوية في مستوى الرصاص بالسيرم والذي أدى بدوره إلى زيادة وزن الجسم واضطراب مقاييس الدم مصحوباً بالتلف التأكسدي بالكبد. أظهر المشروب البارد كفاءة أعلى في منع التأثيرات السامة للرصاص مقارنة بالساخن؛ ويرجع ذلك إلى المحتوى الأعلى من الأنثوسيانين والنشاط الأكبر لمضادات الأكسدة ككل. وبناءً عليه، فإن الدراسة الحالية تؤكد أن كؤوس زهور الكركديه الحمراء الجافة تعتبر مصدر جيد لعدد من دعائم الصحة، وتوضح بشكل قوي تأثير مشروب الكركديه البارد في الوقاية من أنيميا نقص الحديد المحدثة بواسطة خلات الرصاص.