

IMPACT OF BEETROOT (*BETA VULGARIS RUBRA*) AND/OR SWISS CHARD (*BETA VULGARIS CICLA*) JUICES ORAL ADMINISTRATION AGAINST BARIUM CHLORIDE-INDUCED HYPOKALEMIA, ATPASE DISTURBANCE HEART AND LUNG TOXICITY IN RATS

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ABSTRACT

Objective: The research designed to explore, for the 1st time, the probable antioxidant activity and protective effect of oral administration of beetroot (*Beta vulgaris Rubra*) and Swiss chard (*B. vulgaris Cicla*) juices against barium chloride (BaCl₂)-induced toxicity in rats through investigating the changes on complete blood picture, heart and lung oxidative biomarkers, cardiac function tests, inflammatory markers as well as adenosine triphosphatase (ATPase) activity, hypokalemia, and electrolyte disturbances.

Methods: Seventy-five adult male albino rats of Sprague-Dawley strains (150±5 g) were divided into five groups (15/each) except healthy control group which contains 10 rats, and BaCl₂ control group that contains 20 rats as follows: Group I: Healthy control; Group II: BaCl₂ control, and Groups III, IV, and V: BaCl₂ intoxicated groups supplemented with 1 ml of beetroot, Swiss chard, and combination of both juices, respectively/kg body weight 3 times per week orally.

Results: Results explored that beetroot and Swiss chard juices contain significant amount of polyphenols and flavonoids as well as macro- and micronutrients that improved the complete blood picture, heart and lung oxidative stress parameters, cardiac function tests, inflammatory markers, ATPase activity, hypokalemia, and also electrolyte balance in supplemented groups compared to BaCl₂ control group.

Conclusion: This search illustrated that fresh beetroot and Swiss chard juices can improve various biochemical abnormalities resulted from BaCl₂ toxicity. BaCl₂ intoxicated rats that were supplemented with combination of juices showed the most significant improvements.

Keywords: Beetroot, Swiss chard, Barium chloride, Oxidative stress, Inflammation, Heart, Lung, adenosine triphosphatase, Hypokalemia.

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INTRODUCTION

Chemical contamination is a major global issue of food and water safety and quality. Exposure to chemicals at toxic levels, associated with nutritional imbalances, is suspected to be involved in causing several diseases. Protection of our diet from these hazards should be considered one of the essential public health issues in all countries [1].

Barium (Ba) is an alkaline earth element that oxidized easily if exposed to air. It is present as Ba²⁺ ion in surface and ground water and mainly derived from rock and minerals. World widely drinking water contaminated with Ba became an important public health problem. The amount of Ba concentration in drinking water in different countries was reached 1 µg–500 µg/l. The daily intake of Ba from drinking water daily is 2–1200 µg. Ba chloride (BaCl₂) is used in pigments industry, glass and ceramics, refining aluminum, tanning leather, photographic paper, and boiler compounds for water softening [2].

Ba is found naturally in different food stuffs. Among plant products, tea and coffee represent highest concentrations (2.7 and 1.2 mg/100 g). In cereal products, bran flakes have the highest concentration (0.39 mg/100 g). Unpeeled apples have the highest concentration of Ba in the fruit group and contain about (0.075 mg/100 g). Eggs contain within 0.76 mg/100 g while meats contain 0.04 mg/100 mg [3].

When Ba ingested, it passes rapidly through the gastrointestinal mucosa, goes into blood circulation, then distributed to soft tissues within 30 min, mainly aorta, lung, heart, brain, spleen, and kidney. The exact mechanism of Ba toxicity is a blockage of passive trans-membrane potassium (K⁺) conductance in excitable cells by the Ba²⁺ ion as well as production of reactive oxygen species (ROS) inside the body [4]. The

distinctive systemic role of Ba toxicity is "violent contraction of cardiac, smooth, and striated muscle." Clinically, this action is demonstrated as skeletal muscle contraction, colic, diarrhea, salivation, and vomiting. Later, blood pressure drops and skeletal muscles exhibit paralysis. Finally, death occurs as a result of cardiac failure and arrhythmia [5].

Potassium is a very important mineral inside human body. Potassium disorders as hypokalemia is common electrolyte disorder caused by changes in potassium intake, transcellular shifts, or altered excretion. Metal toxicity, diuretic use, and gastrointestinal losses are common causes of hypokalemia. Severe, potassium disorders can lead to life-threatening cardiac disturbances and neuromuscular dysfunction [6].

Plants play a very important role to mankind due to their medicinal as well as nutritional properties. Plants are considered cheap and ideal source of minerals including potassium as well as phytochemicals [7]. Plants also have been used as a traditional medicine in treating different diseases [8]. Beetroot (*Beta vulgaris Rubra*) and Swiss chard (*B. vulgaris Cicla*) are vegetables belong to the *Chenopodiaceae* family, commonly used in traditional cooking, and considered cheap source of nutrients [9].

Red beetroot (*B. vulgaris Rubra*) is a naturally occurring root vegetable and a rich source of bioactive and phytochemical components as betalains (e.g., betacyanins and betaxanthins), flavonoids, saponins, as well as inorganic nitrate. It also contains minerals as potassium, iron, sodium, calcium, phosphorous, magnesium, and manganese. Beetroot is a good supply of vitamins such as A, C, and B-complex. Beetroot juice supplementation as a cost-effective strategy possesses valuable roles in the improving many clinical and pathologic conditions. Moreover,

consuming beetroot in the form of juice, pickled, pureed, boiled, jam processed, powder, bread, and oven dried across different food cultures has been increased [10].

Swiss chard (*B. vulgaris Cicla*) is a green leafy vegetable cultivated widely with low cost and many uses in cooked dishes. Leaves of chard are rich in vitamins as A, C, and B as well as folic acid. It considered as a good source of minerals such as potassium, calcium, phosphorus, and iron. It is used as a common traditional medicine for many diseases. Phytochemical screenings of chard leaves revealed the occurrence of fatty acids as (palmitic, stearic, oleic, linoleic, and linolenic acids), phospholipids, glycolipids, flavonoids, phenolic acids, pectin, polysaccharides, saponins, betalains, and also apigenin. Chard leaves can be used in cooking taro (alqilqas), cooked as spinach or in making salads [11].

In this research, we will explore for the 1st time the effect of natural freshly prepared juices of beetroot and Swiss chard on BaCl₂ toxic effects in rats.

MATERIALS AND METHODS

Materials

Chemicals

BaCl₂ was the purest grades available Sigma-Aldrich (St. Louis, MO, USA). It was dissolved in distilled water and given to rats at a dose of 200 mg/kg body weight [12].

Plants

Beetroot and Swiss chard were purchased from local market (January 2020) and authenticated by botanist (Department of Botany, Women Faculty, Ain Shams University).

Diet

Rats were fed on standard commercial diet according to the National Research Council [13]. This diet was obtained from Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt).

Animals

Seventy-five adult male albino rats of Sprague-Dawley strains weighing (150±5 g) were supplied by Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). All animal experimentations were carried out in conformity with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and were approved by the Institutional Animal Ethics Committee.

Methods

Preparation of fresh juices

One gram of beetroot or Swiss chard was mixed with 5 ml distilled water in the electric mixer and another 5 ml of water were used to wash any residue in the mixer then juice is filtered to give 10% juice concentration. Rats were supplemented with juices at a dose of 1 ml/kg body weight [14].

Measurement of total polyphenols, total flavonoids content, and total antioxidant activity of fresh juices samples

The amount of total polyphenols and total flavonoids content in each plant juice was determined by Folin-Ciocalteu reagent as described by Arnous *et al.* [15], Joyeux *et al.* [16], respectively. Total antioxidant activity of juices was determined by the 1,1-diphenyl-2-picrylhydrazyl method according to Brand-Williams *et al.* [17]. The experiments were repeated in triplicate.

Determination of some nutrients content in fresh juices samples

The samples were analyzed proximately (moisture, ash, crude protein, crude fat, and crude fiber) using method of AOAC International [18]. Carbohydrate content was determined by difference method from

other constituents. Minerals (Fe, Na, and K) were determined by atomic emission spectroscopy according to AOAC International [18].

Experimental design

All rats were housed individually with constant controlled environments in stainless steel cages and fed on the balanced diet with drinking water *ad libitum* for 7 days for acclimatization. Animals classified randomly into five groups of 15 animals (15/each), except healthy control group which contain 10 rats and BaCl₂ control group which contains 20 rats.

Rats were treated as follows: Group I: Healthy control group, rats received a placebo 1 ml distilled water 3 times weekly by oral intubation; Group II: BaCl₂ control group, rats received 200 mg/kg BaCl₂ dissolved in distilled water 3 times weekly by oral intubation; Group III: Rats received 200 mg/kg BaCl₂ and supplemented with beetroot juice 3 times weekly by oral intubation; Group IV: Rats received 200 mg/kg BaCl₂ and supplemented with Swiss chard juice 3 times weekly by oral intubation; and Group V: Rats received 200 mg/kg BaCl₂ and supplemented with beetroot and Swiss chard juices (1:1) 3 times weekly by oral intubation.

Handling of blood, lung, and heart samples

At the end of the experimental period (35 days), rats were fasted overnight, sacrificed under sodium barbiturate anesthesia. Blood samples were collected from the hepatic portal vein, serum was separated for biochemical analyses. Heart and lung samples were separated, rinsed, dried on filter paper, homogenized in Tris hydrochloride buffer (pH 7.4), and centrifuged. The resulting supernatants were stored at -80°C immediately until doing the biochemical analysis.

Biochemical analysis

Complete blood picture (CBC) in whole blood was determined according to Dacie and Lewis [19]. Heart and lung malondialdehyde (MDA), advanced oxidation protein products (AOPP), and reduced glutathione (GSH) levels as well as catalase (CAT) activity were determined according to Draper and Hadley [20], Witko *et al.* [21], Beutler *et al.* [22], Aebi [23]. Serum creatine kinase isoenzyme-MB (CK-MB), lactate dehydrogenase (LDH), and also cardiac and lung Na⁺-K⁺ adenosine triphosphatase (ATPase) activities were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain) according to Reitman and Frankel [24], Dawson *et al.* [25], Kawamoto *et al.* [26], respectively, while the assessment of serum cardiac troponin I (cTnI) level was carried out by enzyme-linked immunosorbent assay (ELISA) using kit purchased from Cloud-Clone Corp., USA, according to Apple *et al.* [27]. Cardiac collagen and cardiac matrix metalloproteinase-1 (MMP-1) were determined by quantitative sandwich immunoassay technique ELISA kit (Cusabio, USA) according to Neuman and Logan [28] and enzyme-linked immunosorbent assay kits (Cloud-Clone Corp., USA) according to Zhang *et al.* [29], respectively. Serum was analyzed for tumor necrosis factor-alpha (TNF-α) level and myeloperoxidase (MPO) activity using Koma Biotech ELISA kit. Serum sodium and potassium levels were determined using the flame photometry method (410 flame photometer, Chiron Diagnostics) following the manufacturer's guidelines [30].

Statistical analysis

Results were expressed as mean ± standard deviation of the mean. Differences among means were tested for statistical significance by one-way analysis of variance using SPSS package version 16. Statistical significance was considered when p≤0.05 according to Levesque [31].

RESULTS

Total flavonoids, total polyphenols content, and total antioxidant capacity of beetroot and Swiss chard juices

Table 1 illustrates that both beetroot and Swiss chard juices have a high content of active components such as flavonoids and polyphenols and consequently have high antioxidant capacity. On comparison between beetroot and Swiss chard juice results, it was found that beetroot juice has a higher content of these active constituents.

Nutritional composition of 100 ml of beetroot and Swiss chard juices

Table 2 reveals that both beetroot and Swiss chard juices are good sources of both macro- and micronutrients compared with their prices. It was found that Swiss chard juice has a higher content of moisture, ash, protein, fiber, as well as minerals as sodium, potassium, and iron when compared with beetroot juice which has a higher content of both carbohydrate and fat.

Effect of beetroot and/or Swiss chard juices on complete blood picture in BaCl₂ intoxicated rats

Treatment of experimental rats with BaCl₂ in (Table 3) caused a significant reduction (p≤0.05) in red blood cells (RBCs), hemoglobin (Hb), and platelet (PLT) values and caused a significant increase in white blood cells (WBCs) count on comparison with healthy control group while supplementation with both beetroot and Swiss chard juices

Table 1: Total flavonoids, total polyphenols, and total antioxidant capacity of beetroot and Swiss chard juices

| Parameter | Beetroot juice | Swiss chard juice |
|---|----------------|-------------------|
| Total flavonoids (mg/1 ml fresh juice) | 0.58 | 0.31 |
| Total polyphenols (mg gallic acid equivalents/1 ml fresh juice) | 6.8 | 1.5 |
| Total antioxidant capacity (%) | 90.2 | 78.6 |

Table 2: Nutritional composition of 100 ml beetroot and Swiss chard juices

| Nutrient content | Beetroot value/100 ml | Swiss chard value/100 ml |
|-------------------------|-----------------------|--------------------------|
| Moisture (g) | 83.4 | 89.57 |
| Ash (g) | 0.80 | 2.23 |
| Protein (g) | 0.90 | 1.88 |
| Carbohydrates (g) | 13.76 | 4.13 |
| Fat (g) | 0.12 | 0.10 |
| Total dietary fiber (g) | 1.02 | 2.09 |
| Sodium (mg) | 80.32 | 179 |
| Potassium (mg) | 314.17 | 548.57 |
| Iron (mg) | 0.93 | 2.26 |

Table 3: Effect of beetroot and/or Swiss chard juices on complete blood picture in BaCl₂ intoxicated rats

| Group | Parameter | | | |
|---|----------------------------|-------------------------|----------------------------|---------------------------|
| | RBCs (10 ⁶ /μl) | Hb (g/dl) | WBCs (10 ³ /μl) | PLT (10 ³ /μl) |
| Healthy control group | 9.30±0.50 ^a | 14.22±0.77 ^a | 7.92±0.052 ^e | 963.73±3.02 ^a |
| BaCl ₂ control group | 2.02±0.064 ^e | 6.32±0.18 ^e | 16.91±0.21 ^a | 493.11±1.75 ^e |
| Ba intoxicated rats supplemented with beetroot juice | 4.63±0.10 ^d | 9.35±0.40 ^d | 11.47±0.75 ^c | 696.03±2.79 ^d |
| Ba intoxicated rats supplemented with Swiss chard juice | 5.92±0.36 ^c | 11.94±0.65 ^c | 13.17±0.30 ^b | 832.82±2.08 ^c |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 7.27±0.42 ^b | 13.12±0.13 ^b | 9.25±0.092 ^d | 900.63±1.09 ^b |

Values are expressed as means±SD, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride, SD: Standard deviation, RBCs: Red blood cells, Hb: Hemoglobin, PLT: Platelet, WBCs: White blood cells

Table 4: Impact of beetroot and/or Swiss chard juices supplementation on heart oxidative status in BaCl₂ treated rats

| Group | Parameter | | | |
|---|-------------------------|------------------------|------------------------|-------------------------|
| | MDA (μmol/g) | AOPP (nmol /mg) | GSH (mg/g) | CAT (U/g) |
| Healthy control group | 7.89±0.04 ^e | 2.59±0.26 ^e | 6.32±0.86 ^a | 30.34±0.36 ^a |
| BaCl ₂ control group | 16.51±1.2 ^a | 6.89±0.83 ^a | 2.82±0.06 ^e | 19.96±0.16 ^e |
| Ba intoxicated rats supplemented with beetroot juice | 10.59±0.41 ^c | 4.15±0.41 ^c | 4.58±0.43 ^c | 26.27±0.98 ^c |
| Ba intoxicated rats supplemented with Swiss chard juice | 12.83±0.56 ^b | 5.33±0.15 ^b | 3.90±0.20 ^d | 23.51±0.40 ^d |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 8.61±0.73 ^d | 3.43±0.57 ^d | 5.44±0.19 ^b | 28.14±0.56 ^b |

Values are expressed as means±S. D, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride, MDA: Malondialdehyde, AOPP: Advanced oxidation protein product, GSH: Glutathione, CAT: Catalase

caused a significant improvements (p≤0.05) in all parameters and the most improvements were found in the group that supplemented with both juices.

Impact of beetroot and/or Swiss chard juices supplementation on heart oxidative status in BaCl₂ treated rats

In Table 4, there were a significant increase (p≤0.05) in oxidative stress parameters and, on the other hand, a significant decrease in antioxidant parameters in the heart of BaCl₂ control group as compared to healthy control group. While Barium intoxicated rats supplemented with beetroot and swiss chard juices showed significant improvement in heart antioxidant status (p≤0.05) in comparison with Barium chloride control group.

Effect of beetroot and/or Swiss chard juices on lung oxidative status in BaCl₂ intoxicated rats

Table 5 shows a significant elevation (p≤0.05) of oxidative stress parameters resulting in accumulation of MDA and AOPP in lung tissues and suppressed antioxidant power of the lung that was represented in significant decrease (p≤0.05) in CAT activity and GSH level in Barium chloride control group. While, on the other hand, Barium intoxicated rats supplemented with beetroot and swiss chard juices acquired a strong antioxidant capacity that reduced the bad effect of BaCl₂ on the lung oxidative status.

Effect of beetroot and/or Swiss chard juices supplementation on cardiac enzyme activities in BaCl₂ intoxicated rats

Significant alterations (Table 6) in serum LDH, CK-MB, and cTnl activities were seen in rats treated with BaCl₂ as compared to control group indicating tissue degeneration. Administration of fresh juices to rats treated with BaCl₂ showed significant (p≤0.05) reduction in serum activities when compared to BaCl₂ treated group indicating tissue improvement.

Impact of beet root and/or Swiss chard juices on serum TNF-α level and MPO activity, heart collagen, and MMP-1 levels, in BaCl₂ intoxicated rats

Treatment of rats with BaCl₂ led to marked (p≤0.05) elevation in serum TNF-α level and MPO activity and also cardiac collagen and MMP-1 levels (Table 7) indicating inflammatory response and cardiac injury. Moreover, beetroot and/or Swiss chard juices supplementation to rats treated with BaCl₂ showed significant (p≤0.05) decrease of TNF-α level, MPO activity, cardiac collagen, and MMP-1levels compared to the BaCl₂ treated group.

Effect of beetroot and/or Swiss chard juices on heart and lung Na⁺-K⁺ ATPase activity and serum Na⁺ and K⁺ levels in BaCl₂ intoxicated rats

Results tabulated in Table 8, BaCl₂ treated rats showed a significant disturbance (p≤0.05) in electrolyte balance appeared by the decrement of both heart and lung Na⁺-K⁺ ATPase activity and serum K⁺ level associated with a significant increase in serum Na⁺ level. Fresh juices contain significant amount of antioxidants and potassium that improved the electrolyte balance in BaCl₂ treated groups.

DISCUSSION

Ba is a mineral that known to be environmental pollutants cause harmful effects on human health. The current study aims to examine

the probable ability of beetroot and/or Swiss chard juices, used as nutritional supplements, to reveal toxic effects caused by BaCl₂ in heart and lung of adult rats as they contain significant amounts of active components such as flavonoids and polyphenols as well as macro- and micronutrients that may compete with Ba toxic effects.

The results of the analysis of fresh juices samples revealed that beetroot and chard leaves may provide a natural source of antioxidants and electrolytes, especially potassium that is important for general health and can help in defending against toxic heavy metals like Ba. These results agreed with Zein [9], Zein *et al.* [10], Ninfali and Angelino [32], Ninfali and Antonini [33] who stated that beetroot and chard leaves and leave juices are of high biological value and contain antioxidants

Table 5: Effect of beetroot and/or Swiss chard juices on lung oxidative status in BaCl₂ intoxicated rats

| Group | Parameter | | | |
|---|------------------------|-------------------------|------------------------|-------------------------|
| | MDA (μmol/g) | AOPP (nmol/mg) | GSH (mg/g) | CAT (U/g) |
| Healthy control group | 1.29±0.03 ^e | 0.96±0.015 ^e | 3.65±0.49 ^a | 88.91±1.5 ^a |
| BaCl ₂ control group | 9.18±0.76 ^a | 3.44±0.18 ^a | 0.75±0.15 ^e | 54.16±0.93 ^e |
| Ba intoxicated rats supplemented with beetroot juice | 5.63±0.17 ^c | 2.05±0.31 ^c | 1.86±0.03 ^c | 68.77±0.45 ^c |
| Ba intoxicated rats supplemented with Swiss chard juice | 7.12±0.25 ^b | 2.85±0.04 ^b | 1.36±0.04 ^d | 59.83±0.64 ^d |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 3.91±0.09 ^d | 1.35±0.26 ^d | 2.9±0.21 ^b | 77.10±0.18 ^b |

Values are expressed as means±S. D, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride, MDA: Malondialdehyde, AOPP: Advanced oxidation protein product, GSH: Glutathione, CAT: Catalase

Table 6: Effect of beetroot and/or Swiss chard juices supplementation on cardiac enzyme activities in BaCl₂ intoxicated rats

| Group | Parameter | | |
|---|------------------------|--------------------------|--------------------------|
| | LDH (U/L) | CK-MB (U/L) | cTnI (Pg/ml) |
| Healthy control group | 1872±2.03 ^e | 1166±7.31 ^e | 400.76±1.49 ^e |
| BaCl ₂ control group | 4056±3.76 ^a | 2569±4.59 ^a | 906.82±6.95 ^a |
| Ba intoxicated rats supplemented with beetroot juice | 3083±1.10 ^c | 1802±6.44 ^c | 736.02±0.82 ^c |
| Ba intoxicated rats supplemented with Swiss chard juice | 3642±4.25 ^b | 2031±2.0915 ^b | 812.49±3.22 ^b |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 2650±0.79 ^d | 1684±3.07 ^d | 619.31±0.96 ^d |

Values are expressed as means±S. D, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride, CK-MB: Creatine kinase isoenzyme-MB, LDH: Lactate dehydrogenase, cTnI: Cardiac troponin I

Table 7: Impact of beetroot and/or Swiss chard juices on heart collagen and MMP-1 levels, serum TNF-α level, and MPO activity in BaCl₂ intoxicated rats

| Group | Parameter | | | |
|---|-------------------------|-------------------------|--------------------------|-------------------------|
| | TNF-α (pg/ml) | MPO (ng/ml) | Collagen (pg/100 mg) | MMP-1 (ng/100 mg) |
| Healthy control group | 37.31±0.45 ^e | 1.26±0.03 ^e | 294.92±1.03 ^e | 0.266±0.02 ^e |
| BaCl ₂ control group | 83.71±0.93 ^a | 11.57±0.88 ^a | 984.17±0.99 ^a | 5.84±0.45 ^a |
| Ba intoxicated rats supplemented with beetroot juice | 60.97±0.13 ^c | 6.17±0.25 ^c | 466.15±2.26 ^c | 3.22±0.36 ^c |
| Ba intoxicated rats supplemented with Swiss chard juice | 70.83±0.74 ^b | 9.57±0.41 ^b | 642.85±2.89 ^b | 4.70±0.14 ^b |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 44.68±0.47 ^d | 4.80±0.19 ^d | 372.42±1.38 ^d | 2.66±0.08 ^d |

Values are expressed as means±S. D, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride

Table 8: Effect of beetroot and/or Swiss chard juices on heart and lung Na⁺-K⁺ ATPase activity and serum Na⁺ and K⁺ levels in BaCl₂ intoxicated rats

| Group | Parameter | | | |
|---|---|--|--------------------------|------------------------|
| | Heart Na ⁺ -K ⁺ ATPase (μmol/pi/h/ml) | Lung Na ⁺ -K ⁺ ATPase (μmol/pi/h/ml) | Na ⁺ (mEq/1) | K ⁺ (mEq/1) |
| Healthy control group | 1.93±0.03 ^a | 14.68±0.83 ^a | 135.66±2.81 ^e | 6.05±1.31 ^a |
| BaCl ₂ control group | 0.58±0.15 ^e | 6.15±0.15 ^e | 203.94±1.79 ^a | 1.92±0.68 ^e |
| Ba intoxicated rats supplemented with beetroot juice | 0.87±0.06 ^d | 7.87±0.41 ^d | 183.81±1.34 ^b | 3.07±0.51 ^d |
| Ba intoxicated rats supplemented with Swiss chard juice | 1.07±0.31 ^c | 9.33±0.68 ^c | 167.21±2.19 ^c | 4.68±0.46 ^c |
| Ba intoxicated rats supplemented with beetroot and Swiss chard juices | 1.17±0.43 ^b | 11.94±0.90 ^b | 143.70±0.93 ^d | 5.33±1.03 ^b |

Values are expressed as means±S. D, n=10. There was no significant difference between means that have the same alphabetical superscripts letter in the same column (p≤0.05). BaCl₂: Barium chloride, ATP: Adenosine triphosphatase

that can compete oxidative stress and inflammation related to different diseases.

Under natural conditions, internal enzymatic and non-enzymatic antioxidants eliminate ROS, protecting cells against ROS. Oxidative stress resulted from the unbalance between free radicals generation and antioxidants defense causing permanent cell damage [34]. Erythrocytes are highly affected by the peroxidation process [35], their membranes exposed to covalent damage, including cross-linking and aggregation induced by ROS such as superoxides resulting from Hb auto-oxidation.

In this current search, an abnormality was observed in different blood cell parameters in BaCl₂-toxicated rats. A noticeable decrement in RBCs count, Hb level, and PLT count was detected in the Ba intoxicated group which resulted from DNA strand breakage in these cells due to oxidative stress associated with BaCl₂ administration. Moreover, in our research, Ba administration elevated the total WBCs count, which could be due to the compensatory increment in the production of blood cellular components by the bone marrow or due to inflammatory response, according to Rawat *et al.* [36]. Supplementation with beetroot and/or Swiss chard juices improved these result as a consequence of their content of antioxidants that control oxidative stress and inflammatory processes as well as the presence of essential components for newer cell generation such as protein, carbohydrate, and iron [37,38].

Metals may stimulate ROS generation including hydroxyl radical, superoxide anion, and hydrogen peroxide. When ROS generated at high concentrations, oxidative stress occurs resulting in damage to lipids, proteins, and DNA, leading to cellular dysfunction [39]. This study revealed that Ba intoxication amplified the levels of both MDA and AOPP in the heart and lung tissues, causing their impairment. MDA is the breakdown product generating from the main chain reactions, leading to polyunsaturated fatty acids oxidation. MDA considered as a dependable parameter of oxidative stress-mediated lipoprotein oxidation in heart and lung tissues. Furthermore, the increased levels of AOPP marker reflected an excess of free radical generation and protein oxidative damages, shown in Ba intoxicated heart and lung tissues as well. The increment of AOPP was probably associated with free radical-mediated protein and membrane damages.

On the other hand, antioxidant defense system prevents lipid peroxidation in mammalian cells through ROS disruption. Enzymatic and non-enzymatic compounds act to eliminate and scavenge ROS. Results of this study revealed a variation in the antioxidant defense system homeostasis, displayed as a reduction in CAT and GSH levels. Ideal level of GSH is very important factor in maintenance of the structures and functions of membranes. In addition, results displayed that Ba administration stimulates the production of ROS that disturb heart and lung functions. The diminishing of the enzymatic and non-enzymatic antioxidant defense system was considered a main event following BaCl₂-induced cardiac and lung toxicity. The study results agreed with Elwej *et al.* [40], Elwej *et al.* [41], Elwej *et al.* [42]. Supplementation of beetroot and Swiss chard juices improved these results while coadministration of both beetroot and Swiss chard juices to Ba-treated rats was more effective than beetroot juice or Swiss chard juice used separately against oxidative stress. Their synergistic properties were approved in the current work by elevating levels of non-enzymatic antioxidants, lowering lipid peroxidation, scavenging free radicals, and sustaining CAT activity and GSH levels.

Five weeks of BaCl₂ oral administration induced an increase in the serum activities of CK-MB and LDH as well as the level of cTnI due to their leakage from damaged myocytes as a consequence of peroxidation and degeneration of heart tissues [43]. ROS generation is the main promoter of cellular dysfunction that increases serum biomarkers which are sensitive indicators of cardiac injury in cardiovascular diseases [44]. Serum CK-MB activity is measured as a sensitive index at an early stage of myocardial ischemia, while higher levels of LDH

are relative to the degree of damage to myocardial tissues [45]. cTn (T, I, and C) are considered to be standard blood biomarkers with high sensitivity and specificity for myocardial degeneration [46]. These contractile proteins usually released from myocardium proportional to the degree of tissue injury and myocyte membranes distribution [47]. As a consequence of cardiac membrane damage, cTnI is leaked as a result of rapid myofibrillar breakdown or may reflect the presence of free cTnI within the sarcoplasm [48,49]. Beetroot and Swiss chard juices prohibited the leakage of LDH, CK, and cTnI from the heart into extracellular environment. This may be due to their strong antioxidant power confirming stability of membrane and lowering cardiac tissue damage.

McCauley and Washington [50] have explained that the concentration of Ba in the heart was 3 times more than other tissues or blood following oral dose of BaCl₂, causing Ba accumulation in the heart. Due to the high amount of ROS generated from Ba intoxication, cardiac structure integrity was badly affected. This was cleared from the increased level of cardiac collagen associated with increased activity of MMP-1 in the Ba intoxicated rats. Collagen is the main extracellular matrix (ECM) protein in the heart and represents a vital goal for anti-remodeling and cardioprotective treatment. The quantity and quality of collagen was controlled under numerous physiological and pathologic circumstances. Extreme collagen deposition, leading to cardiac fibrosis, is a main factor of cardiac dysfunction and arrhythmogenicity related to death. ROS affected collagen metabolism in cardiac fibroblasts by influencing the activity of both synthesizing and degradative enzymes. ROS decreased collagenase-sensitive [3H] proline incorporation as well as the presence of mRNA for procollagenase $\alpha 1(I)$, $\alpha 2(I)$, and $\alpha 1(III)$. ROS generation and inflammation also increased the total activity of extracellular MMP-1 through the transcriptional and posttranscriptional levels. MMP-1 is a proteolytic enzyme that are regulated by inflammatory signals to mediate changes in ECM [51,52]. Heavy metals toxicity induced cardiac ECM expansion and remodeling as a result of the increased fibroblasts activity leading to fibrosis. Furthermore, fibroblasts produce a number of cytokines, peptides, and enzymes among which MMP and their inhibitors directly impact of ECM turnover and homeostasis. On the other hand, these alterations were arrested in the beetroot and Swiss chard juices treated rats [53].

The TNF- α is one of the inflammatory cytokines, that is, increased following Ba-induced ROS. Oxidative stress activates p-38 mitogen which activates protein kinase and nuclear factor kappa β and thus plays a part in the sequence of signaling events involved in the production of myocardial TNF- α [54]. As a result of oxidative and inflammatory process induced by Ba toxicity, the activity of MPO increased. MPO is found mainly in the azurophilic granules of neutrophils, MPO activity measurement is used as indicator for neutrophil infiltration (inflammation) and cardiac damage in different experimental studies [55]. Moreover, excessive formation of MPO causes oxidative damage [56]. The elevation in MPO levels in Ba intoxicated rats was due to increase in the infiltration of the cardiac tissue with inflammatory cells and as a result leads to myocardial damage. However, the results of the rats supplemented with beetroot and Swiss chard juices showed marked decrease in the level of TNF- α and MPO activity as a consequence of the presence of anti-inflammatory flavonoids and polyphenols.

Reyes *et al.* [57] reported that Na⁺-K⁺ ATPase is a membrane bound enzyme responsible for the maintenance of Na⁺ and K⁺ gradients over cell membranes. It is inhibited by ROS in soft tissues. Free radicals generation as a result of BaCl₂ leads to oxidative stress that motivated functional changes such as ATP depletion. The study showed that the significant inhibition in the activity of both heart and lung Na⁺-K⁺ ATPase was due to membrane depolarization causing hypokalemia associated with hypernatremia as Na⁺-K⁺ ATPase acted to maintain the ionic gradient for cardiac and lung cells excitability. Moreover, Na⁺-K⁺ ATPase activity depletion might be due to the decreased level of its substrate (ATP) and the elevation of membrane lipid peroxidation alters membranes by free radicals. On the other hand, beetroot and

Swiss chard juices supplementation to intoxicated rats corrected the electrolyte balance and improved Na⁺-K⁺ ATPase activity due to their antioxidant power as well as nutritive value.

CONCLUSION

Beetroot and Swiss chard are super foods that contain significant amount of macro- and micronutrients as well as active components as polyphenols and flavonoids which increase their antioxidant power. Both plant juices showed a significant improvement and ability to compete against toxicity with metals as Ba. Moreover, the synergistic effect of supplementation with both juices together was more powerful in competing toxicity when compared with each one alone. This approves that combination of different colorful foods in our diet could improve our health and protect our bodies.

AUTHORS' CONTRIBUTIONS

Dr. Alyae M. S. Gabal and Dr. Gehan M. Morsy designed the study, making of protocol, managed the work done, performed the literature searches, and completed the manuscript writing.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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