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Research Article

IMPACT OF BEETROOT (*BETA VULGARIS RUBRA*) AND/OR SWISS CHARD (*BETA VULGARIS CICLA*) JUICES ORAL ADMINISTRATION AGAINST BARIUM CHLORIDE-INDUCED HYPOKALEMIA, ATPASE DISTURBANCE, AND 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 ($BaCl_2$)-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 $BaCl_2$ control group that contains 20 rats as follows: Group I: Healthy control; Group II: $BaCl_2$ control, and Groups III, IV, and V: $BaCl_2$ 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 $BaCl_2$ control group.

Conclusion: This search illustrated that fresh beetroot and Swiss chard juices can improve various biochemical abnormalities resulted from $BaCl_2$ toxicity. $BaCl_2$ 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 Ba^{2+} 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 ($BaCl_2$) 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 Ba^{2+} 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 $BaCl_2$ toxic effects in rats.

MATERIALS AND METHODS

Materials

Chemicals

$Bacl_2$ 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 $BaCl_2$ 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: $BaCl_2$ control group, rats received 200 mg/kg $BaCl_2$ dissolved in distilled water 3 times weekly by oral intubation; Group III: Rats received 200 mg/kg $BaCl_2$ and supplemented with beetroot juice 3 times weekly by oral intubation; Group IV: Rats received 200 mg/kg $BaCl_2$ and supplemented with Swiss chard juice 3 times weekly by oral intubation; and Group V: Rats received 200 mg/kg $BaCl_2$ 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 $BaCl_2$ intoxicated rats

Treatment of experimental rats with $Bacl_2$ in (Table 3) caused a significant reduction ($p \leq 0.05$) 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 caused a significant improvements ($p \leq 0.05$) in all parameters and the most improvements were found in the group that supplemented with both 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

Impact of beetroot and/or Swiss chard juices supplementation on heart oxidative status in $BaCl_2$ treated rats

In Table 4, there were a significant increase ($p \leq 0.05$) in oxidative stress parameters and, on the other hand, a significant decrease in antioxidant parameters in the heart of $BaCl_2$ 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 ($p \leq 0.05$) in comparison with Barium chloride control group.

Effect of beetroot and/or Swiss chard juices on lung oxidative status in $BaCl_2$ intoxicated rats

Table 5 shows a significant elevation ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 $Bacl_2$ on the lung oxidative status.

Effect of beetroot and/or Swiss chard juices supplementation on cardiac enzyme activities in $BaCl_2$ intoxicated rats

Significant alterations (Table 6) in serum LDH, CK-MB, and cTnI activities were seen in rats treated with $Bacl_2$ as compared to control group indicating tissue degeneration. Administration of fresh juices to rats treated with $BaCl_2$ showed significant ($p \leq 0.05$) reduction in serum activities when compared to $Bacl_2$ 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 $BaCl_2$ intoxicated rats

Treatment of rats with $Bacl_2$ led to marked ($p \leq 0.05$) 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 $BaCl_2$ showed significant ($p \leq 0.05$) decrease of TNF-α level, MPO activity, cardiac collagen, and MMP-1levels compared to the $BaCl_2$ treated group.

Table 3: Effect of beetroot and/or Swiss chard juices on complete blood picture in $BaCl_2$ 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 ($p \leq 0.05$). $BaCl_2$: 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 $BaCl_2$ 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 ($p \leq 0.05$). $BaCl_2$: Barium chloride, MDA: Malondialdehyde, AOPP: Advanced oxidation protein product, GSH: Glutathione, CAT: Catalase