IMPACT OF INTAKE OF PLANT FOODS' IRON ON THE RECOVERY FROM IRON DEFICIENCY IN RATS

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SUMMARY: In the present research, two iron concentrates prepared from fruit and vegetable juices in addition to other plant foods products expected to be rich in iron and nutrients that enhance iron absorption have been chemically and biologically evaluated. Iron and Zn and other mineral and phytochemicals that have been claimed to reduce iron absorption (Ca, polyphenols and tannins) and micronutrients that reported to enhance iron absorption (Vitamin C, toccopherols and carotenoids) have been determined. The efficiency and safety of iron concentrates were evaluated in iron deficiency model of rats.

The iron concentrates have been shown to contain variable levels of Fe, Zn, Ca, polyphenols, tannins, vitamin C, toccopherols and carotenoids. The sum of promoters and inhibitors in mixture 1 was higher than that in mixture 2. The two iron concentrates showed improvements of iron status, however mixture 2 (3.5 strawberry: 3.5 pomegranate: 1 blackstrap: 0.5 carrot: 0.5 pumpkin:1 orange in addition to 10% lettuce juice) was more efficient than mixture 1 (4.7 strawberry: 2.7 beetroot: 1.3carrot:1.7 guava in addition of 1.3%wheat germ oil and 3.3%lettuce juice). Iron deficiency anemia induced oxidative stress which was reduced on supplementation of the iron concentrates. Both iron concentrates showed safety concerning liver and kidney functions.

Key Words: Plant foods, iron concentrates, iron deficiency, iron status.

INTRODUCTION

Iron deficiency is a well-known form of nutritional deficiency. It occurs when iron available to the erythrocyte precursors is insufficient. Its prevalence is higher particularly among young children and women of reproductive age. It can cause developmental delays and behavioral disturbances in children. In pregnant women, it increases the risk for a preterm delivery and a deliverance of a low-birth weight baby. Iron deficiency indicates iron depletion and iron-deficiency anemia in the worst case. In the human body, iron is stored in proteins such as ferritin and hemosiderin. Ferritin is water soluble and present in plasma, whereas hemosiderin is water insoluble and always found within cells. In iron-deficiency anemia, the stored iron is depleted and the iron transported by transferrin is decreased. So, iron-deficiency anemia is characterized by the reduction or absence of Fe stores, low serum concentrations of Fe and hemoglobin and decreased hematocrit. Due to its effects on development and growth, resistance to infections, and association with mortality of infants younger than 2 years, iron deficiency anemia is considered a major public health problem and is the most common nutritional deficiency in the world. Oral iron supplements, such as ferrous sulfate, are easily available for

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management of such cases of deficiency. However, iron overdose may produce symptoms of gastrointestinal malabsorption (1, 2). However there are also concerns on the pro-oxidant potential of iron in promoting ill health. It is increasingly recognized that optimal iron nutrition is a balance between risks and benefits and that the balance point is likely to differ between individuals and between environments. The challenge is to avert iron deficiency without contributing to iron overload to achieve iron absorption without risking toxicity. An important issue is that other nutrients and phytochemicals in the diet may affect non-heme iron absorption either by inhibition or enhancement. Polyphenols and tannins, have been reported to hinder iron absorption. However ascorbic acid and alpha tocopherols have been shown to promote iron absorption. Calcium, zinc and beta- carotene showed conflictions among literature (3-7).

The objective of the present research was to study the potential use of iron rich freeze dried fruit and vegetable juice mixtures (iron concentrates) in improving iron status in iron deficient rats. The aim included studying the effect of administration of such mixtures on oxidative state and liver and kidney functions to evaluate their safety. Among the aim of the current stage was to study the impact of presence of ascorbic acid, polyphenols, tannins, beta- carotene, alpha tocopherols, calcium, and zinc with iron on iron bioavailability.

MATERIALS AND METHODS

Materials

Male and female Sprague-Dawley rats of 54 g. ± 3.88 (mean ± SD) body weight were obtained from the animal house of the National Research Center and housed individually in stainless steel cages with 12-h light/12-h dark cycle. Food and mineral-free water were available ad libitum to all rats.

Strawberry, pomegranate, orange, guava, pumpkin, beet root, carrot, lettuce, and blackstrap (Molasses), were purchased from local market. Wheat germ was obtained from South Cairo and Giza Milling Co, Egypt.

Methods

Pomegranate, orange, pumpkin and beet root were washed by tap water, peeled and made individually into juice using fruit juicer. Strawberry, guava, carrot and lettuce were washed by tap water and also made into juice individually.

Wheat germ was placed in a continuous extraction apparatus and subjected to extraction using petroleum ether (40-60° C) for extraction of oil. The solvent was completely removed by evaporation under reduced pressure.

Two juice mixtures have been prepared to have high iron content. The ratios of different juices (V:V) in mixture (1) were 4.7 strawberry: 2.7 beetroot: 1.3 carrot:1.7 guava in addition of 1.3%wheat germ oil and 3.3%lettuce juice. Mixture (2) contains 3.5 strawberry: 3.5 pomegranate: 1 blackstrap (Molasses): 0.5 carrot: 0.5 pumpkin: 1 orange in addition of 10% lettuce juice. The two juice mixtures were freeze dried and analyzed for their content from total polyphenol (8, 9), condensed tannin (10, 11), vitamin C (12), β-carotene (13), α-tochopherol (14) and iron, calcium and zinc by atomic absorption technique as described by the AOAC (15).

A balanced diet (B) was prepared (Table 1), the salt mixture was made according to Briggs and Williams (16) but devoid of iron salts. Iron in form of ferrous ammonium sulphate (FAS) was added as 30 mg Fe/kg of diet to prepare iron adequate diet and as 2 mg Fe/kg of diet to prepare iron deficient diet according to Chang *et al.* (17). A sufficient quantity of freeze dried mixture 1 was added to diet B (on the expense of carbohydrates) so as to contain 30 mg Fe/kg of diet (diet of mixture 1). An appropriate amount of freeze dried mixture 2 was added to diet B (on the expense of carbohydrates) so as to contain 30 mg Fe/kg of diet (diet of mixture 2).

Rats were assigned to one of five groups (n=6/group). Rats of the control healthy group were fed iron adequate diet for 28days. Rats of the iron deficient group were fed iron deficient

Table1: Composition of balanced diet.

Ingredients of the diet	g/100g
Casein*	12.5
Corn oil	10
Salt mixture	3.5
Vitamin mixture**	1
Sucrose	23
Starch	46.2
Methionine	0.3
Cellulose	3.5
Total	**100**

* 12.5 g casein has been estimated to contain 10 g protein (15).
** The vitamin mixture was prepared according to Morcos (18).

diet for 28. Another group of rats was run where rats were fed iron deficient diet for 14 days followed by feeding iron adequate diet for 14 days. Two test groups were run where rats of the first and second test groups were fed iron deficient diet for 14 days followed by feeding diet of mixture 1 and 2, respectively for another 14 days. During the experiment, body weight and food intake were recorded once weekly. At the end of study total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Blood samples were collected from all rats after an overnight fast. Blood hemoglobin and hematocrit were estimated according to Vankampen and Zijlstra (19) and Strumia *et al.* (20) respectively. Plasma was separated for determination of iron (21) and zinc (22). Malondialdehyde (MDA) was determined as indicator for lipid peroxidation (23). The safety of iron concentrate (freeze dried juice mixtures) were studied through evaluation of liver and kidney function. Plasma level of creatinine (24) and urea (25) were determined as indicator of kidney function, while the activity of AST and ALT (26) were assessed as indicator of liver function. Mean corpuscular hemoglobin concentration (MCHC) was calculated according to Ney (27). Rats were killed and their livers and spleens were removed and weighed. Iron was determined in these organs by atomic absorption technique.

Statistical analysis

Data were expressed as the mean standard error. SPSS software (SPSS Inc., Chicago, IL, USA) was used for analysis. Differences between dietary groups were determined by one-way analysis of variance, and $P < 0.05$ was considered statistically significant. Analysis of variance was performed with Duncan test to determine whether dietary groups were significantly different from each other.

RESULTS AND DISCUSSION

Table 2 showed mixture 1 to contain higher contents of iron, calcium, zinc, ascorbic acid, polyphenols, tannins, beta- carotene and alpha tocopherols than mixture 2. As can be seen from the materials and methods, an appropriate amount of mixture 1 and 2 containing 30 mg iron was added to diet, so as the final concentration of iron would be 30 mg Fe/kg of diet. This added amount was 167.5 and 214 g from mixture 1 and 2, respectively. The Fe, Ca, Zn, vitamin C and alpha tocopherol concentration in the added amount were 30, 220, 5.64, 203 and 41.9 mg respectively in mixture 1 and 30, 183.6, 2.6, 146 and 8.8, respectively in mixture 2. Polyphenol and condensed tannins were 37.8 and 7.4 g. in mixture 1 and 25.5 and 5.8 in mixture 2, respectively. ß-carotene was 19177 and 4000 μg in mixture 1 and 2 respectively. This showed that mixture 1 contains higher concentration of calcium, zinc, ascorbic acid, polyphenols, condensed tannins, beta- carotene and alpha tocopherols than mix-

Table 2 : Chemical composition of the freeze dried mixtures.

Micronutrients and phytochemicals	Mixture 1	Mixture 2
Fe (mg/100 g dry sample)	17.91	13.99
Ca (mg/100 g dry sample)	131.25	85.63
Zn (mg/100 g dry sample)	3.37	1.23
Vitamin C (mg/100 g dry sample)	121.15	68.26
Polyphenol as gallic acid equivalent (g/100g dry sample)	22.57	11.90
Condensed tannins as catechin equivalent (g/100g dry sample)	4.39	2.70
ß-carotene (μg/g dry sample)	114.49	18.69
α tocopherol (mg/g dry sample)	0.25	0.041

ture 2. Examination of the relative proportions of promoters and inhibitors of iron absorption in juice mixtures may be useful in predicting the overall iron bioavailability from mixed juices.

Nutritional advice that aims to improve iron status should emphasize not only on rich sources of iron but also factors that may enhance or inhibit absorption (28). It has been reported by Khoshnevisan *et al.* (29) that citrus fruits and fruits rich in vitamin C improved iron status in iron depleted preschool children. In an epidemiological study it has been noticed that there was low intake of green vegetables and fruits and vitamin C and E in pregnant anemic women which are considered as enhancers of iron absorption (30). Polyphenols consumption was shown to inhibit non -heme iron absorption (31, 32). Layrisse *et al.* (33) reported that beta-carotene form complex with iron keeping it soluble in the intestinal lumen and preventing the inhibitory effect of polyphenol on iron absorption. Other authors showed carotene to inhibit iron absorption (34). In a study carried out by Siegenberg *et al.* (35), it was noted that ascorbic acid prevents the dose dependent inhibitory effect of tannic acid on non -heme iron absorption in man.

Concerning minerals, it has been reported that iron absorption is slightly affected by zinc deficiency but it can be reduced by high zinc intakes (over 50 mg/day) (4). While it is usually stated that calcium impairs iron absorption, the inconsistent results across experiments suggest that calcium-iron interactions are complex. The addition of calcium phosphate reduced the absorption of nonheme iron from a semi-synthetic meal by 50 percent, whereas calcium alone did not (36). Calcium salts also lowered iron absorption by 55 percent from a typical breakfast meal with low iron availability and a high calcium content (6), and by 28 percent from a high iron availability hamburger meal with a low calcium content (6). Similarly, 165 mg calcium added as calcium chloride, milk, or cheese inhibited the absorption of nonheme iron from wheat rolls by 50 to 60 percent, while 300 to 600 mg calcium reduced the absorption of heme iron as well (37). The inhibitory effect of calcium appears to be dose related up to 300 mg calcium, after which there is little additional inhibition. It is likely that iron absorption can be protected by consuming calcium-rich foods and iron-rich foods at different meals.

Table 3 showed non significant change in body weight gain among all studied groups. Food intake was significantly high in case of groups given the freeze dried mixtures compared to normal healthy control. No significant change in food efficiency ratio was noticed between normal healthy control, iron deficient group and iron deficient group fed iron adequate diet (FAS). Food efficiency ratio of rats given mixture 2 showed non significant change compared to normal healthy control, iron deficient group and iron deficient group fed iron adequate

Table 3 : Nutritional parameters of different experimental groups.

Parameters	Normal healthy control	Iron deficient group	Iron deficient group fed iron adequate diet (FAS)	Group fed diet of mix 1	Group fed diet of mix 2
Initial BW(g)	53.7 ± 1.476^{a}	54 ± 1.390^{a}	53.8 ± 1.579^{a}	54.0 ± 1.483^{a}	54.0 ± 1.461^{a}
Final BW (g)	150.7 ± 6.015^{a}	144.5 ± 4.334^{a}	147.2 ± 2.040^{a}	143 ± 3.652^{a}	152.5 ± 5.284^{a}
Body weight gain (g)	96.7 ± 4.870^{a}	90.5 ± 3.191^{a}	93.3 ± 1.054^{a}	89.0 ± 2.338^{a}	98.5 ± 4.624^{a}
Total food intake (g)	324.5 ± 2.605^{a}	330.8 ± 7.115^{ab}	329.8 ± 3.655^{ab}	357.5 ± 14.839^{c}	349.8 ± 2.358^{bc}
Food intake (g/day)	11.6 ± 0.095^{a}	11.8 ± 0.246^{ab}	11.8 ± 0.120^{ab}	12.8 ± 0.522^{c}	12.5 ± 0.079^{bc}
Food effeciency ratio	0.298 ± 0.016^{b}	0.274 ± 0.008^{ab}	0.284 ± 0.005^{ab}	0.251 ± 0.011^{a}	0.282 ± 0.014^{ab}

Values are mean $\pm$ SE (n=6). Values in the same row with different superscript letters are significantly different at $p < 0.05$.

Table 4 : Weight and iron content of liver and spleen of rats of different experimental groups.

Parameters	Normal healthy control	Iron deficient group	Iron deficient group fed iron adequate diet (FAS)	Freeze dried mix 1	Freeze dried mix 2
Liver weight (g)	5.09 ± 0.193[a]	5.29 ± 0.148[a]	5.27 ± 0.288[a]	5.06 ± 0.430[a]	4.79 ± 0.368[a]
Liver weight/ body weight (%)	3.50 ± 0.148[a]	3.60 ± 0.114[a]	3.66 ± 0.198[a]	3.53 ± 0.257[a]	3.13 ± 0.184[a]
Fe mg/liver weight	0.211 ± 0.052[a]	0.015 ± 0.003[b]	0.017 ± 0.002[b]	0.031 ± 0.011[b]	0.068 ± 0.037[b]
Fe mg/g liver tissue	0.040 ± 0.009[a]	0.003 ± 0.0002[b]	0.003 ± 0.0002[b]	0.007 ± 0.003[b]	0.016 ± 0.010[b]
Spleen weight (g)	0.79 ± 0.126[a]	0.78 ± 0.126[a]	0.82 ± 0.119[a]	0.64 ± 0.093[a]	0.55 ± 0.128[a]
Spleen weight/ body weight (%)	0.554 ± 0.110[a]	0.530 ± 0.081[a]	0.575 ± 0.090[a]	0.445 ± 0.056[a]	0.368 ± 0.096[a]
Fe mg/spleen weight	0.106 ± 0.035[a]	0.101 ± 0.017[a]	0.113 ± 0.20[a]	0.073 ± 0.019[a]	0.123 ± 0.056[a]
Fe mg/g spleen tissue	0.130 ± 0.037[a]	0.129 ± 0.014[a]	0.133 ± 0.010[a]	0.112 ± 0.014[a]	0.225 ± 0.113[a]

Values are mean ± SE (n=6). Values in the same row with different superscript letters are significantly different at $p<0.05$.

diet (FAS). However, food efficiency ratio of rats given mixture 1 showed non significant change compared to rats given mixture 2, iron deficient group and iron deficient group fed iron adequate diet (FAS). Chang *et al.* (17) showed similar results concerning the non significant change in body weight gain and total food intake between iron deficient and control rats. In Table 4, it can be noticed that there was no significant change in liver and spleen weights and their percentage rations to the body weights among different experimental groups. In disagreement with the present results, Díaz-Castro *et al.* (38) reported that the body weights of the anemic rats were significantly lower, whereas the liver weight was slightly decreased in anemic rats compared with controls with consequent significant higher liver weight/body weight ratio in the anemic group. This may be due to that the period of feeding of Fe deficient diet was longer (40 days) than in the present experiment (28 days) so that it may reduce thyroid hormone concentration as previously reported (39). Iron content of liver in the present study was significantly reduced in anemic rats compared to control healthy group indicating significant depletion of liver iron stores, this result agreed with that of Chang *et al.* (17). Repletion by either FAS or the freeze dried juice mixture in the current study only produced slight increase in iron liver content which was higher in case of juice mixtures (Table 4). Iron level in spleen in the present study sowed non significant change among all the studied groups. However it showed slight decrease in anemic group compared to healthy control and on repletion, slight increase was noticed which was more remarkable on supplementation with mixture 2. The effect of supplementation of juice mixtures on iron contents of both liver and spleen reflects somehow iron bioavailability.

The bioavailability of iron from the two freeze dried juice mixtures was examined in rats as animal models by checking for recovery from iron deficiency. After Fe deprivation (2 mg/kg of diet), hematologic parameters in the anaemic group were dramatically different from those of the control healthy, with a significant low mean blood hemoglobin concentration, hematocrit and serum Fe, as noticed from Table 5. These parameters were significantly elevated in rats given the freeze dried mixtures and the rats given FAS diet compared to iron deficient group. Plasma iron levels in the three iron-repleted

Table 5: Blood and plasma parameters of different experimental groups.

Parameters	Normal healthy control	Iron deficient group	Iron deficient group fed iron adequate diet (FAS)	Freeze dried mix 1	Freeze dried mix 2
Createnine mg/dl	0.597 ± 0.010^{a}	0.592 ± 0.009^{a}	0.588 ± 0.008^{a}	0.585 ± 0.0089^{a}	0.590 ± 0.010^{a}
Urea	26.16 ± 0.850^{a}	25.54 ± 0.488^{a}	25.70 ± 0.478^{a}	26.20 ± 0.729^{a}	25.40 ± 0.690^{a}
ALT (IU/l)	58.0 ± 1.125^{a}	56.50 ± 0.619^{a}	56.67 ± 0.666^{a}	56.83 ± 0.307^{a}	57.17 ± 1.973^{a}
AST (IU/l)	136.5 ± 1.945^{a}	137.17 ± 1.013^{a}	137.33 ± 0.715^{a}	137.0 ± 0.730^{a}	136.0 ± 1.390^{a}
Hemoglobin (g/dl)	14.17 ± 0.111^{a}	12.62 ± 0.108^{c}	13.78 ± 0.114^{ab}	13.62 ± 0.212^{b}	13.88 ± 0.164^{ab}
Hematocrite %	43.83 ± 0.477^{a}	36.33 ± 0.558^{b}	42.67 ± 1.054^{a}	43.50 ± 0.847^{a}	44.17 ± 0.601^{a}
MCHC (%)	32.33 ± 0.214^{b}	34.75 ± 0.475^{a}	32.41 ± 0.867^{b}	31.39 ± 0.966^{b}	31.47 ± 0.600^{b}
Zn (ug/dl)	77.23 ± 1.452^{a}	71.22 ± 1.656^{b}	74.07 ± 1.530^{ab}	76.98 ± 1.117^{a}	77.08 ± 0.749^{a}
Fe (ug/dl)	171.57 ± 2.15^{a}	139.52 ± 2.111^{c}	154.68 ± 1.658^{b}	156.28 ± 1.731^{b}	157.72 ± 1.557^{b}
MDA (nmol/l)	4.72 ± 0.203^{d}	8.16 ± 0.144^{a}	6.70 ± 0.171^{b}	6.13 ± 0.115^{c}	6.02 ± 0.158^{c}

Values are mean ± SE (n=6). Values in the same row with different superscript letters are significantly different at $p < 0.05$.

groups were still significantly lower than that of normal healthy group. Hemoglobin concentration was comparable to normal healthy group in case of mixture 2 and FAS diet. Percentage hematocrit and MCHC in rats given the freeze dried mixtures and the rats given FAS diet showed non significant change compared to normal healthy group. These results indicated that iron sources from FAS and the two freeze dried juice mixtures were bioavailable and allowed recovery from iron deficiency. It could be noticed that iron concentrates prepared during this study showed improvements of iron status, however mixture 2 was more efficient. It was also noted that the sum of inhibitors and promoters of iron absorption in mixture 2 was lower than that in mixture 1. The efficiency of mixture 2 might also be attributed to the sources of juices, which may lead to the suggestion that iron absorption is not only affected by the aforementioned promoters and inhibitors but also the sources of such components.

It was noticed that plasma zinc was reduced significantly in iron deficient group compared to normal healthy (Table 5). Plasma zinc was significantly elevated only in the groups given both freeze dried juice mixtures but not in case of the rats fed FAS diet which may be due to presence of zinc in the mixtures. The elevation of plasma zinc in case of rats given the mixtures was comparable to healthy control group.

In the present study it can be seen from Table 5, that MDA, a parameter reflecting oxidative stress, was significantly higher in iron deficient group than normal healthy control. Supplementation of iron in form of FAS or freeze dried juice mixtures to iron deficient rats produced significant reduction of MDA. It can be postulated that iron may induce oxidative stress only if it is given in an overdose or if it is given to non iron deficient subjects or if it was in ferric form. Rats given both freeze dried juice mixtures showed significant lowers levels of MDA compared to those fed FAS diet which might be due to presence of the antioxidant components; ascorbic acid, ß-carotene, alpha-tocopherol, polyphenol, condensed tannins in the mixtures which agreed with the study of Ronca *et al.* (40). There is controversy about the susceptibility of cells to lipid peroxidation in iron deficiency anemia: some investigators have claimed there is no difference in lipid peroxidation among patients with iron deficiency anemia compared with controls (41,42) but others have reported

that among patients with iron deficiency anemia oxidants are increased and antioxidants decreased, so the oxidative/antioxidative balance is shifted toward the oxidative side (43-45). It has been reported by Díaz-Castro *et al.* (38) that iron deficiency anemia does not affect lipid peroxidation in rats, suggesting that there is enough compensatory capacity to keep antioxidant defenses high. Current evidence suggests that transition metals, in particular Fe, react with hydrogen peroxide in cell nuclei, leading to oxygen radical generation (46). It has been reported that glutathione peroxidase activity in anemia is similar to that of normal cells (38, 41, 42) which was in contrast to those of many other authors (43-45). No correlations were found between Fe concentration and glutathione peroxidase. By the study of Díaz-Castro *et al.* (38). In this sense, glutathione peroxidase is involved in the reduction of the peroxides that can damage polyunsaturated fatty acids, thus preventing lipid peroxidation and the degradation of membrane phospholipids and the subsequent formation of TBARS (47). It has been postulated that an insufficient amount of Fe available, would exert a protective effect in the animal, avoiding the Fe-catalyzed generation of oxygen. radicals via Fenton and Haber-Weiss chemistries, which was in disagreement of our findings regarding lipid peroxidation. Oxidative status in iron deficiency anemia has been widely investigated (although the results obtained have been ambiguous). Iron deficiency induces changes in the cellular Fe homeostasis system. It has been suggested by Díaz-Castro et al (38) that Fe deficiency could exert a protective effect, preventing, at least in part, the generation of free radicals and lipid peroxidation which was not the case in the present study.

Liver and kidney function tests (Table 5) showed non significant change among all studied groups indicating the safety of freeze dried juices and that iron deficiency did not affect either liver or kidney function.

CONCLUSION

Iron concentrates prepared during this study showed improvements of iron status, however mixture 2 was more efficient. The sum of inhibitors and promoters of iron absorption in mixture 2 was lower than that in mixture 1. It can also be concluded that iron deficiency anemia induced oxidative stress which was reduced on supplementation of the freeze dried juice mixtures. The safety of the freeze dried mixtures towards both liver and kidney functions was confirmed during the present study.

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