Estimation of Vitamin C and the Fructose Levels in Some Medicinal Plants and their Effects on Iron Bioavailability in Rats

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Abstract

Iron deficiency is considered as a common problem facing the general world population. In the current research, experiments were conducted to evaluate the effects of aqueous extracts of *Acacia senegal* (Gum Arabic, GA), *Spinacia oleracea* (spinach), *Zea mays* (corn), and *Capsicum annuum* (red chili pepper) on iron and ferritin levels in rats. Vitamin C and fructose levels were first estimated in these plants by using High-Performance Liquid Chromatography (HPLC). The results showed that the GA extract contained the highest level of fructose (853 mg.L^{-1}), followed by red chili pepper (635 mg.L^{-1}), corn (521 mg.L^{-1}), and spinach (271 mg.L^{-1}) extracts. Also, the results of vitamin C estimation showed levels of 3.376, 0.645, 0.579, and 0.347 mg/ml in the extracts of spinach, red chili pepper, corn, and GA, respectively. Next, thirty male albino rats (age 8-12 weeks, weight 120-200 g) were divided randomly into six groups, each kept in a separate polypropylene rat cage. Treatments of rats with various plant extracts were conducted following a CRD complete random design, with five replicates for each treatment. The results of lipid profile test revealed that GA treatment caused the highest reduction in the serum levels of TC, TG, LDL, VLDL, and HDL in the treated rats (76.25, 55.25,23, 15.25, and 38 mg/dl , respectively), as compared to the other plant extracts. Evidence from this study suggests that the higher serum level of fructose in the group of rats treated with all plant extracts enhances the process of ferritin formation, possibly via increasing the level of bioavailable iron.

Keywords: Vitamin C estimation**,** fructose estimation, medicinal plants, non-heme Iron, Anemia, Gum Arabic.

تقدير فيتامين سي والفركتوز في بعض النباتات الطبية وتأثيره على جاهزية الحديد غير معدني في الجرذان

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الخلاصة

تعتبر الأنيميا مشكلة شائعة تواجه العالم. وقد توقع بصورة عامة ان 50% من حالات الأنيميا هي بسبب نقصان الحديد. اقيمت التجربة لترفع تأثير المستخلص المائي للنباتات الطبية (الصمغ العربي، السبانغ، الذرة والفلفل الأحمر الحار) على مستويات الحديد والفرتين في الفئران المصابة بالأنيميا. في هذه الدراسة تم تقدير نسبة الفيتامين سي والفركتوز في هذه النباتات باستخدام تقنية كروماتوغرافيا السائل عالية الدقة. ثلاثون جرذ

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ذكر البينو بعمر (8-12) اسابيع و وزن (120-200غم) قسمت عشوائيا الى ستة مجاميع: كل مجموعة حفظت في قفص بوليبروبلين للجرذان باستخدام التصميم الكامل العشوائي. اظهرت النتائج ان اعلى تركيز للفركتوز كان في الصمغ العربي اذ بلغ لقد سجل الصمغ العربي أعلى تركيز من الفركتوز (853 ملغم / لتر) و يليها الفلفل الأحمر الحار (635 ملغم / لتر) ثم الذرة (521 ملغم / لتر) و اخيرا السبانخ (271 ملغم / لتر). في حين كانت نتائج فيتامين سي   3.37ملغم/مل، 645،0ملغم/مل، 579،0ملغم/مل، و 347،0ملغم/مل السبانخ و الفلفل الاحمر الحار، الذرة، و الصمغ العربي على التوالي. كذلك لقد تم قياس الدهون. لقد بينت النتائج ان الصمغ العربي كان من أفضل المعاملات في خفض الدهون للكوليسترول، كوليسترول ثلاثيات الغليسريد، كوليسترول البروتين الدهني منخفض الكثافة، كوليسترول البروتين الدهني منخفض الكثافة للغاية، كوليسترول البروتين الدهني عالي الكثافة، اذ بلغت 76.25 ملغم/ديسيلتر ، 55.25 ملغم/ديسيلتر، 23 ملغم/ديسيلتر، 15.25 ملغم/ديسيلتر و38 ملغم/ديسيلتر على التوالي. و اخيرا، فقد اظهرت النتائج استنتجنا ان الفركتوز قد رفع من نسبة امتصاص الحديد في الفئران قيد التجربة. و نستنتج ان الفركتوز قد رفع من تكوين الفرتين و الحديد في الفئران في المجموعة التي تضمنت كل المستخلصات النباتية.

1. Introduction

Evidence showing that simple sugars, such as fructose, affect iron absorption first appeared in the 1960s. That work showed that sugar was able to chelate inorganic iron and form soluble, stable, low molecular weight complexes. It has been proposed that fructose increases iron bioavailability by increasing ferrous iron formation. These sugar-iron complexes were shown to be readily absorbed across the intestinal mucosa of rodent models. Given that intake of fructose increased worldwide in the past 40 years, especially in the western world, while at the same time iron deficiency and iron excess remains significant public health concerns, understanding the nutritional implications of iron-sugar interactions is particularly relevant [1]. Increasing iron absorption from fortified foods by adding enhancers of iron absorption may increase the efficacy of fortification strategies, particularly in diets containing inhibitors such as tannins and phytate [1]. The importance of ascorbic acid (AA) as a means of improving iron nutrition was underlined in 1970 by the U.S. Food and Nutrition Board when it suggested the promotion of "a program featuring iron, in addition to vitamin C, as a breakfast nutrient" [2]. The use of ferritin as an indicator of iron availability was pioneered by Glahn *et al*. [3] and it is now widely used as a surrogate marker for iron uptake (for recent examples see [4]). Ferric iron is the dominant form of iron in foods, which is far less bioavailable than ferrous iron. One of the main characteristics of ascorbic acid is its ability to reduce ferric to ferrous iron. Gum Arabic is a natural branched-chain multifunctional hydrocolloid with a highly neutral, or slightly acidic, arabino-galactan-protein complex containing magnesium, calcium, and potassium [2]. GA consists of three fractions: (a) The smallest fraction having the highest protein content is a glycoprotein which differs in its amino acid composition from that of the gum arabic glycoprotein (GAGP) complex. (b) a smaller fraction is a higher molecular weight arabinogalactan-protein complex (GAGP-GA glycoprotein) in which arabinogalactan chains are covalently linked to a protein chain through serine and hydroxyproline groups. The attached arabinogalactan in the complex contains ~13% (by mole) glucuronic acid. (c) the major fraction is a highly branched polysaccharide consisting of G-(1-3) galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid (found in nature as magnesium, potassium and calcium salts) [3]. Gum Arabic is a natural vegetable product, with hypoglycemic and prebiotic effects, having a low calorific value. It is used in drinks, meal substitutes, cereal bars, and bakery products. Also, noncarcinogenic soluble fiber dairy products and confections use acacia gum for its health benefits [5, 6, 7]. Red chili pepper is (*Capsicum annuum*) used as a food additive in many regions due to its pungency, aroma, and color. It has been safely consumed in large amounts in many countries. Spinach (*Spinacia oleracea*) is a leafy green

vegetable that contains ascorbic acid and other organic acids, which are well known promoters of iron absorption from the diet. Corn (*Zea mays)* is one of the world's most important cereal grains. It is a dietary staple for more than 200 million people and provides approximately 20% of the world's calories. The aim of the present study is to evaluate the effects of aqueous extracts of *Acacia senegal, Spinacia oleracea, Zea mays* and *Capsicum annuum* on iron and ferritin levels in rats.

2. Materials and methods
2.1 Collection of the plants
   The plant samples that included the leaves of *Spinacia oleracea*, the fruits of *Capsicum annuum*, the kernels of *Zea mays*, and the powder of *Acacia senegal* were obtained from the local markets in Baghdad, Iraq. The first three samples were kept in an oven at 40 °C for one week, until they were hard and then grinded by a grinder to give small size pieces of 2 mm. Then, all the samples were stored in glass containers at room temperature in a dry dark place until use.

2.2 Preparation of plants aqueous extracts
   An aqueous extract was prepared for each plant by applying the water extraction method without boiling [8].

2.3. Estimation of fructose in the plant samples
2.3.1 Processing
   The method used for the quantitative estimation of fructose is the phenol-sulfuric acid method. In a hot acidic medium, fructose was dehydrated to 5 hydroxymethyl fufural, which forms a yellow-orange colored product with phenol and has absorption maximum at 490 nm when tested with UV spectroscopy [9].

2.3.2 Fructose level in the standardized sample
   The method used to estimate fructose level in the standardized sample was applied according to a previously published study [9].

2.3.3 Fructose levels in medicinal plant samples
   To have a standard comparison of samples of each type of plant, the procedure carried out to estimate fructose levels was repeated in a similar fashion with all plant extract samples. The absorbance was determined at concentrations of 0.01- 0.09µg/ml [9].

2.3.4 Vitamin C estimation
The determination of vitamin C was performed by using HPLC technique [10].

2.4 Laboratory animals
   Thirty male albino Sprague Dawley rats with an age range of 8-12 weeks and a weight range of 120-200 g were purchased from Al-Nahrain University Biotechnology Research Center and housed at room temperature (25 C°). The experimental protocol was designed according to OCED, 2001 [11].

2.5 Experimental design
   Rats were divided into six groups, each group contained five rats, which were subjected to oral administration of different aqueous plant extracts for 30 days [11].
Group one involved the administration of distilled water (control).
Group two involved the administration of only spinach extract (0.05 g/ml).
Group three involved the administration of spinach (0.05 g/ml) and GA e (0.1 g/ml) extracts.
Group four involved the administration of spinach (0.05 g/ml) and red chili pepper (0.1 g/ml) extracts.
Group five involved the administration of spinach (0.05 g/ml) and corn (0.1 g/ml) extracts.
Group six involved the administration of all plant extracts (0.05 g/ml of spinach and 0.1 g/ml of each of the other plants).

2.6 Collection of blood samples

Five milliliters of blood were withdrawn from the heart of the animals directly by cardiac puncture using a medical syringe. Blood samples were placed in anticoagulant-free gel tubes to obtain serum. The blood was centrifuged at 3000 rpm for 15 minutes to ensure a sufficient amount of serum that is free of red blood cells. The serum was then collected in special plastic tubes and stored at -20 °C until use [12].

2.7 Determination of iron and ferritin levels in rats

After 30 days of treatment, blood samples were collected from the experimental rats and tested for iron levels by applying the iron ferrozine colorimetric method [13,14].

2.8 Measurement of lipid profile

All lipid parameters were measured by using kits obtained from BioSystems (Spain), except VLDL concentration which was estimated by using the following equation reported by Freidwold *et al*. [15]:

VLDL = TG / 5

Statistical Analysis

The program that was used to detect the effects of difference factors on the studied parameters was the Statistical Analysis System- SAS (2012), by using CRD complete random design with five replicates for each treatment. Least significant difference (LSD) test (Analysis of Variance-ANOVA) was used to compare between means and find statistically significant differences [16].

Results and discussion

3.1 Iron

3.1.1 The effects of plant extracts on iron and ferritin bioavailability

The results in Table 1 show that the highest level of vitamin C was 3.376 mg/ml, which was found in the extract of *S. oleracea*, while the lowest level was 0.347 mg/ml in *A. senegal* .

Table 1- The concentration of vitamin C in the aqueous extracts of the studied plants, as measured by the use of HPLC analysis.

Plant	Vitamin C con. (mg/ml)
Spinacia oleracea	3.376
Capsicum annuum	0.645
Zea mays	0.579
Acacia senegal	0.347

The results in Table 2 demonstrate that the highest concentration of fructose (853.54 mg/ml) was found in the extract of *A. senegal*, while the lowest (271.72 mg/ml) was in *S. oleracea*.

Table 2- The concentration of fructose in the studied plants

Plant	Fructose con. (mg/ml)
Spinacia oleracea	271.7273
Capsicum annuum	635.3636
Zea mays	521.7273
Acacia senegal	853.5455

Moreover, the results of chemical analysis revealed that iron concentration was the highest (80.10 mg/ml) in *S. oleracea* and the lowest (16.428 mg/ml) in *A. senegal* (Table 3).
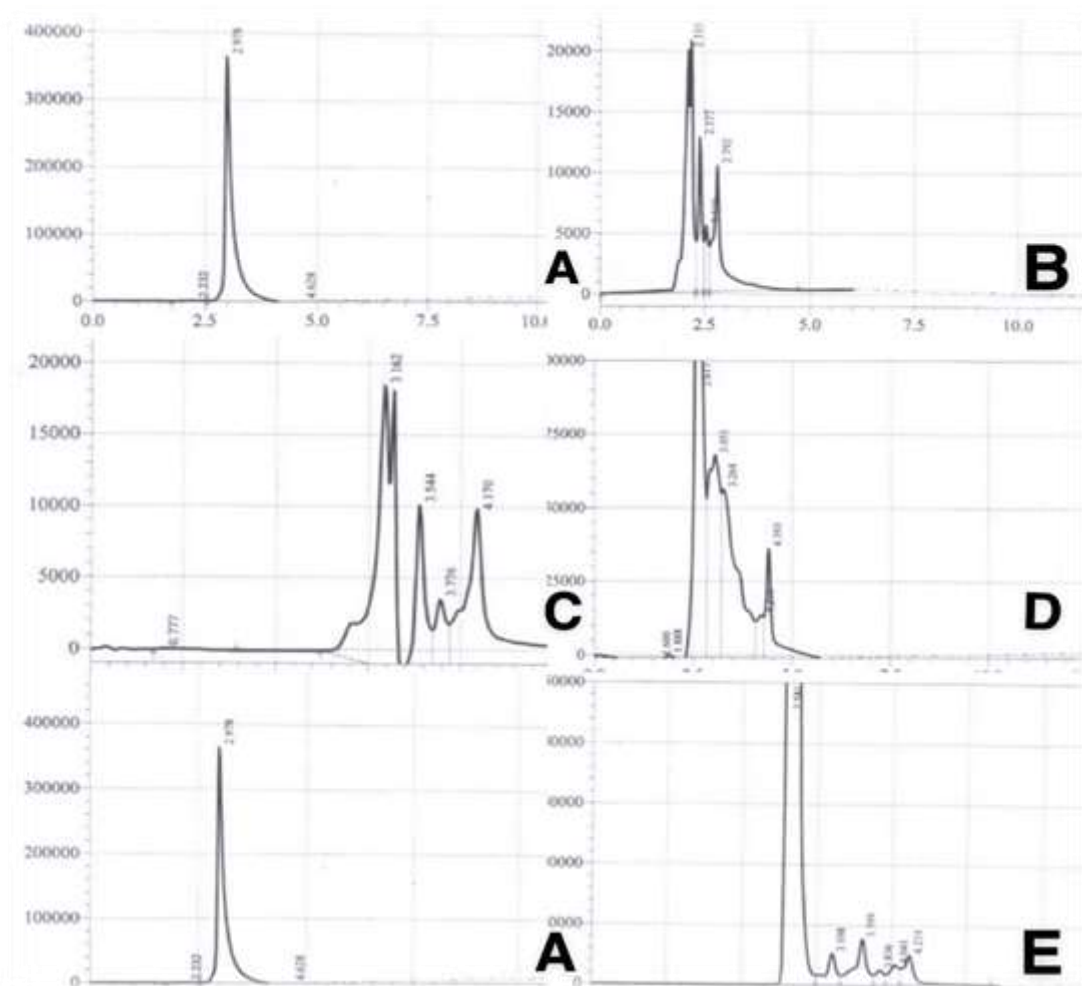
Figure 1-Vitamin C estimation by HPLC (A; standard, B; Gum Arabic, C; red chili pepper D; spinach, E; corn.

Table 3-The concentration of iron in the studied plants

Plant	Iron con. (mg/ml)
Spinacia oleracea	80.109
Capsicum annuum	23.394
Zea mays	21.654
Acacia senegal	16.428

According to Table 4, the last group of animals (G6), which was treated orally with 1 ml of each of the studied plant extracts, , had significantly higher iron and ferritin levels (by 50% and 36%, , respectively) compared with the control (G1) group.

Table 4-The levels of iron and ferritin in the studied groups

Groups	Means ± SE	
	Iron (mg/ml)	Ferritin (mg/ml)
G1	149.00 ±21.98 ab	153.68 ±38.78 ab
G2	140.00 ±17.68 c	31.50 ±22.87 c
G3	80.80 ±17.87 bc	15.93 ±6.07 c
G4	105.33 ±4.97 bc	98.33 ±23.18 bc
G5	110.60 ±16.42 bc	79.22 ±30.49 bc
G6	224.00 ±57.23 a	209.00 ±25.27 a
LSD value	92.857 *	84.728 *
Means having with the different letters in same column differed significantly * (P≤0.05).		

G1: Control (D.W), G2: Spinach, G3: Spinach + Gum Arabic, G4: Spinach + Red chili pepper, G5: Spinach + Corn, G6: Spinach + Corn + Gum Arabic + Red chili pepper.

 Non-heme iron bioavailability is influenced by many dietary factors. This study suggests that fructose increases iron bioavailability in the rats of group two (treated with only spinach), which showed slightly lower serum levels of iron and ferritin compared with the control. This may be due to the type of non-heme iron in spinach, which cannot be absorbed as easily as heme iron without certain factors, such as vitamin C or fructose [17]. It has been reported that vitamin A and β-carotene can enhance iron absorption and thereby contribute to an increase in hemoglobin levels [18]. As for group three, which involved rats treated with *S. oleracea* and *A. senegal*, the levels of iron and ferritin were significantly low, which may be due to fiber content in GA [19]. Some studies showed that fibers decrease the absorption of non-heme iron[19]. This effect of fibers is reflected on dynamics of nutrients uptake and gut microflora, as well as nutrient bioavailability, microbial makeup, and gastrointestinal function. Besides, in group four (treated with *S. oleracea* and *C. annuum*), the levels of iron and ferritin were decreased. The binding of iron to phenolic compounds that are found in chili pepper prevents the absorption of non-heme iron in humans. Apparently, the known enhancing effects of chili and capsaicin on gastric acid secretion are not relevant to iron consumption. Chili was shown to have no significant effect on the absorption of iron from acid-soluble ferric pyrophosphate compared to ferrous sulfate. Furthermore, with the use of fresh chili instead of dried chili powder, as used in our research, the inhibitory effect of chili is be predicted to be lower. As a known enhancer of iron absorption, chili lyophilization is required to reduce the ascorbic acid content [20]. The daily consumption of all kinds of pepper was reported to be approximately 24 g and that for chili pepper was 9 g. The most abundant forms of capsaicinoids found in hot red peppers are capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. About 70% of the burning sensation experienced from consumption of chili red peppers is attributed to capsaicin. Capsaicin is used extensively as a treatment for pain. Evidence showed that capsaicin has effects on regulating homeostasis and digestive tract, in addition to other deleterious effects. Capsicum and capsaicinoids improve hormone function and metabolism, stabilize blood glucose, reduce insulin and leptin resistance, regulate endothelial function, inhibit LDL-cholesterol oxidation, and prevent cancer due to antioxidant activity. Spicy food consumption showed highly consistent inverse associations with total mortality among both men and women after adjustment with potential risk factors [21].

In group five, which involved rats treated with *S. oleracea* and *Z. mays*, the levels of iron and ferritin were slightly higher in comparison with the control. This might be because *Z. mays* contains phytate and polyphenols. Phytate is known to be a possible inhibitor which chelates micronutrients (such as iron and calcium) and prevents their absorption in monogastric animals, including humans, since their digestive tract lacks the enzyme phytase [22]. Maize products, including essential fatty acids, especially linoleic acid in maize oil, play important roles in the diet by maintaining blood pressure, regulating blood cholesterol level, and preventing cardiovascular disorders. Vitamin E in maize oil is known as a key chain breaking antioxidant. Maize has comparable energy density to other cereal crops and is a relatively good source of vitamin A, but it is also rich in phytate, a compound that potently inhibits iron availability for absorption [23]. According to the results of the current study, the administration of *S. oleracea*, *C. annuum*, *A. senegal*, and *Z. mays* together into rats revealed a high increase in iron and ferritin levels at a dose of 1 ml of the extract. These results agree with those reported by other research groups [24-27]. This may be due to the high amount of vitamin C in   *S. oleracea* and *C. annuum* [28]. Besides, there are many different mechanisms that suggested and explained the interaction between vitamin C and iron. One of these mechanisms is the reduction of Fe^{+3} to Fe^{+2} in the gastrointestinal tract, which will promote

the non-heme iron absorption. The improved iron solubility of Fe^{+2} and Fe^{+3} is owed to complex formation and followed by enterocyte endorsement [29]. However, the effect of *A. senegal* and *Z. mays* in increasing iron level may be due to the high amount of fructose in these plants, which is considered as an effective agent in rising iron *in vivo* through ferritin formation, which affects the oxidative state of iron and alters it to the bioavailable ferrous form [30]. In addition, *Z. mays* contains folic acid (hematopoietic factor) [31] and tannin [32], which are considered as phytochelatores and metal binding ligands that can impact iron absorption by the assessment of ascorbic acid. Such components that were mentioned above, and others, can encourage redox activities that influence iron absorption by the help of ascorbic acid [33].

3.2 Lipid profile

The data listed in Table 5 demonstrate the outcomes of the lipid profile assay. The results of the oral administration of *A. senegal* in group three indicate that the levels of CHO, TG, LDL, and VLDL were decreased (76.25, 55.25 ,23, 15.25 mg/dl, respectively), while HDL was increased (38mg/dl). However, group five showed raised levels of CHO, TG, LDL, and VLDL (149, 153.60, 93.00, 29.80 mg/dl, respectively) , whereas the level of HDL (26.20mg/dl) was low.

Table 5- The levels of lipid profile parameters in the studied groups.

Groups	Means ± SE				
	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)	Serum VLDL (mg/dl)
G1	84.00 ±17.44 c	90.20 ±17.08 bc	38.60 ±3.66 a	32.16 ±15.93 bc	16.80 ±3.48 c
G2	88.80 ±9.81 bc	39.40 ±7.10 d	36.00 ±1.64 ab	34.96 ±9.40 bc	17.76 ±1.96 bc
G3	76.25 ±2.89 c	55.25 ±15.65 cd	38.00 ±1.08 a	23.00 ±3.32 c	15.25 ±0.58 c
G4	139.67 ±13.64 ab	118.33 ±24.25 ab	30.33 ±1.45 ab	81.40 ±12.34 ab	27.93 ±2.72 ab
G5	149.00 ±18.79 a	153.60 ±16.81 a	26.20 ±3.42 b	93.00 ±18.24 a	29.80 ±3.75 a
G6	116.80 ±24.01 abc	64.20 ±16.55 cd	31.20 ±4.87 ab	62.04 ±23.89 abc	23.36 ±4.80 abc
LSD Value	51.40 *	48.18 *	10.03 *	49.66 *	10.282*
Means having different letters in the same column differed significantly * ($p \leq 0.05$).					

G1: Control (D.W), G2: Spinach, G3: Spinach + Gum Arabic, G4: Spinach + Red chili pepper, G5: Spinach + Corn, G6: Spinach + Corn + Gum Arabic + Red chili pepper.

Lipids are defined as biological matters which are normally hydrophobic in nature and in many circumstances soluble in organic solvents [34]. The main biological purposes of lipids include energy storage, signaling, and performing as structural components of cell membranes [35]. The administration of *S. oleracea* in group two did not have a significant effect on lipid profile. Ko *et al*. [36] revealed that the administration of plant extracts of spinach is not effective at reducing blood cholesterol. The composition of the extract used in the experiments, as well as the dosages and the experimental period, can be to the reasons of these findings.

Group three showed the best influence on lipid profile. *A. senegal* is a soluble dietary fiber that has probiotic features [37], assisting oligosaccharides or polysaccharides to be fermented by colonic bacteria to build short chain fatty acids [38]. Other studies suggested that, due to GA viscosity [39], it can have lowering effects on lipid profile parameters in humans and animals [40]. Ali *et al*. [41] suggested that GA, due to its antioxidant activity, has a lowering effect on lipid peroxidation. another study reported that GA inhibits glucose absorption in the

intestine through its interaction with sodium-glucose transporter 1 (SGLT1) which is abundant in cell membrane in mice [42].

The administration of *C. annuum* revealed a negative effect on lipid profile in rats. It increased TC, TG, LDL, VLDL, and decreased HDL serum levels. Many studies suggested that capsaicin in the red chili pepper has a beneficial effect on lipid parameters. Al-Jumayi *et al*. [43] proved that the ethanol extract of red chili has positive effects on lipid metabolism. The difference between our result and those of other studies might be due to the plant extracting method. In another 35-day trial on rabbits, intubation with 8 mg capsaicin / rabbit did not have a beneficial impact on plasma cholesterol, TG and HDL-cholesterol in regular diets. These variations were due to the effects on intestinal absorption of cholesterol by capsaicin [44].

Group five, which involved *Z. mays* oral administration, had undesirable effects on lipid profile. Although Oyeyemi *et al*. [45] showed that the consumption of corn had beneficial effects on lipid metabolism, the results obtained revealed that *Zea mays* bran may possess a laxative activity. The different results obtained by this study might be due to the usage of the kernel instead of bran of the corn, in addition to the different method of extraction. Dapčević-Hadnađev *et al*. [46] showed that the corn bran has high percentage of fibers and viscosity that have a positive effect on lipid metabolism, while the usage of kernel and the water extraction method in our study had negative effects on lipid profile parameters.

The results of the last group showed negative impacts on lipid parameters. This may be due to the synergetic effects of all the components of the plant extracts, and that the positive effect of GA (as shown in group three) was reversed by the other components, which showed negative effects on lipid metabolism in other treated groups.

In conclusion, these studies suggest that plant extracts enhance iron bioavailability, possibly through the influence of either vitamin C or fructose, as measured by ferritin formation; however, the results are inconclusive. Also, the results indicated that using these plants in the modern diet has effects on iron bioavailability, without any notable pathophysiological consequences. Thus, further studies are required to test other plant extracts, or their active components, to enhance iron bioavailability.

References

[1] T. Christides, P. Sharp, "Sugars increase non-heme iron bioavailability in human epithelial intestinal and liver cells," *PloS one*, 8(12), e83031, 2013.

[2] D. Renard, L. Lavenant-Gourgeon, M. C. Ralet, C. Sanchez, *"Acacia senegal* gum: continuum of molecular species differing by their protein to sugar ratio, molecular weight, and charges," *Biomacromolecules*, 7(9), 2637-2649, 2006.

[3] RP. Glahn, OA. Lee, A. Yeung, MI. Goldman, DD. Miller, "Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in *vitro* digestion/Caco-2 cell culture model," *J Nutr.*, 128: 1555–1561, 1998.

[4] MG. Lung'aho, AM. Mwaniki, SJ. Szalma, JJ. Hart, MA. Rutzke, "Genetic and physiological analysis of iron biofortification in maize kernels," *PLoSOne*6:e20429,2011.

[5] S. Chandraju, R. Mythily and C. S. Chidan Kumar, "Isolation of simple sugar from a hydro colloid: gum Arabic," *Recent Research in Science and Technology*, 4(11): 34-36,2012.

[6] Y. Gaspar, K. L. Johnson, J. A. McKenna, A. Bacic, C. J. Schultz, "The complex structures of arabinogalactan-proteins and the journey towards understanding function," *Plant molecular biology*, 47(1), 161-176, 2001.

[7] Y. K. Murali, R. Chandra, P. S. Murthy, "Antihyperglycemic effect of water extract of dry fruits of Terminalia chebula in experimental diabetes mellitus," *Indian Journal of Clinical Biochemistry*, 19(2), 202-204, 2004.

[8] V. M. Jain, G. N. Karibasappa, A. S. Dodamani, G. V. Mali, "Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method," *Journal of education and health promotion*, 6, 2017.

[9] Z. Gazdik, O. Zitka, J. Petrlova, V. Adam, J. Zehnalek, A. Horna, R. Kizek, "Determination of vitamin C (ascorbic acid) using high performance liquid chromatography coupled with electrochemical detection," *Sensors*, 8(11), 7097-7112. 2008.

[10] Oecd, O. E. C. D., "Guidelines for the Testing of Chemicals," Section 1: 1-16,2004.

[11] Z. Cheng, "Angiotensin II induced inflammation and vascular dysfunction: Role of oxidative stress and cyclooxygenase," Academic dissertation, University of Helsinki, 2002.

[12] A. R. Lobo, E. H. Gaievski, E. De Carli, E. P. Alvares, C. Colli, "Fructo-oligosaccharides and iron bioavailability in anemic rats: the effects on iron species distribution, ferroportin-1 expression, crypt bifurcation and crypt cell proliferation in the caecum," *British journal of nutrition*, 112(8), 1286-1295, 2014.

[13] P. Carter, "Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine)," *Analytical biochemistry*, 40(2),450-458,1971.

[14] WT. Friedwold, RI. Levy, And DS. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clin Chem*, 18(499.1972), 411, 1972.

[15] J. M. McCord, J. D. Crapo, I. Fridovich, "Superoxide dismutase assays: a review of methodolog," *Superoxide and superoxide dismutase* 1, 11-17, 1977.

[16] A. P. Perfecto, "Mineral composition of boiled, green leafy vegetables found in Hawaiʻi and iron bioavailability using the in-vitro digestion/Caco-2 cell method," (Doctoral dissertation, [Honolulu]:[University of Hawaii at Manoa],[August2012]).

[17] Y. W. Luo, W. H. Xie, "Effects of vegetables on iron and zinc availability in cereals and legumes," *International Food Research Journal*, 19(2), 455, 2012.

[18] S. Adams, C. T. Sello, G. X. Qin, D. Che, R. Han, "Does dietary fiber affect the levels of nutritional components after feed formulation?," *Fibers*, 6(2), 29, 2018.

[19] S. Tuntipopipat, K. Judprasong, C. Zeder, E. Wasantwisut, P. Winichagoon, S. Charoenkiatkul, T. Walczyk, "Chili, but not turmeric, inhibits iron absorption in young women from an iron-fortified composite meal," *The Journal of nutrition*, 136(12), 2970-2974, 2006.

[20] J. Deshpande, S. Jeyakodi, V. Juturu, "Tolerability of capsaicinoids from capsicum extract in a beadlet form: a pilot study," *Journal of toxicology*, 2016.

[21] K. Raes, D. Knockaert, K. Struijs, J. Van Camp, "Role of processing on bio accessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant," *Trends in Food Science & Technology*, 37(1), 32-41, 2014.

[22] T. R. Shah, K. Prasad, P. Kumar, "Maize—A potential source of human nutrition and health: A review," *Cogent Food & Agriculture*, 2(1), 1166995,2016.

[23] N. Khan, M. J. Ahmed, S. Z. A. Shah, " Comparative analysis of mineral content and proximate composition from chilli pepper (*Capsicum annuum* L.) germplasm," *Pure and Applied Biology* (PAB), 8(2), 1338-1347, 2019.

[24] T. Christides, "Effects of carbohydrates on iron metabolism in intestinal and liver cells" (Doctoral dissertation, University of Greenwich), 2016.

[25] L. Kochian, R. Glahn, E. Tako, "High bioavailablilty iron maize (*Zea mays* L.) developed through molecular breeding provides more absorbable iron in *vitro* (Caco-2 model) and in *vivo* (Gallus gallus)," *Nutrition journal,* 12(1), 1-11, 2013.

[26] M. M. Zangeneh, "Green synthesis and formulation a modern chemotherapeutic drug of *Spinacia oleracea* L. leaf aqueous extract conjugated silver nanoparticles; Chemical characterization and analysis of their cytotoxicity, antioxidant, and anti-acute myeloid leukemia properties in comparison to doxorubicin in a leukemic mouse model," *Applied Organometallic Chemistry*, *34*(1), e5295,2020.

[27] A. O. Patrick, U. A. Fabian, I. C. Peace, O. O. Fred, "Determination of variation of vitamin 'C' content of some fruits and vegetables consumed in Ugbokolo after prolonged storage," *Journal of Environmental Science, Toxicology and Food Technology*, 10(7), 17-19, 2016.

[28] G. J. Kontoghiorghes, A. Kolnagou, C. N. Kontoghiorghe, L. Mourouzidis, V. A. Timoshnikov, N. E. Polyakov, "Trying to Solve the Puzzle of the Interaction of Ascorbic Acid and Iron: Redox, Chelation and Therapeutic Implications," *Medicines*, 7(8), 45, 2020.

[29] M. Wiciński, G. Liczner, K. Cadelski, T. Kołnierzak, M. Nowaczewska, B. Malinowski, "Anemia of Chronic Diseases: Wider Diagnostics—Better Treatment?," *Nutrients*, 12(6), 1784, 2020.

[30] T. R. Shah, K. Prasad, P. Kumar, "Maize—A potential source of human nutrition and health: A review," *Cogent Food & Agriculture*, 2(1), 1166995,2016.

[31] S. I. Hameed, A. W. Al-Shahwany, S. J. Salih, "Evaluation of the Activity of Some Plants Extracts on Thyroid Gland Regulation in Female Albino Rats," *Iraqi Journal of Science*, 61(2), 254-265, 2020.

[32] S. H. Ko, J. H. Park, S. Y. Kim, S. W. Lee, S. S. Chun, E. Park, "Antioxidant effects of spinach (*Spinacia oleracea* L.) supplementation in hyperlipidemic rats," *Preventive nutrition and food science*, 19(1), 19, 2014.

[33] G. T. Macfarlane, S. Macfarlane, "Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics," *Journal of clinical gastroenterology*, 45, S120-S127, 2011.

[34] W. Calame, A. R. Weseler, C. Viebke, C. Flynn, A. D. Siemensma, "Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner," *British Journal of Nutrition*, 100(6), 1269-1275, 2008.

[35] D. D. Gallaher, C. A. Hassel, K. J. Lee, "Relationships between viscosity of hydroxypropyl methylcellulose and plasma cholesterol in hamsters," *The Journal of nutrition*, 123(10), 1732-1738, 1993.

[36] M. A. Levrat, M. L. Favier, C. Moundras, C. Rémésy, C. Demigné, C. Morand, "Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats," *The Journal of nutrition*, 124(4), 531-538, 1994.

[37] O. Nasir, F. Artunc, K. Wang, R. Rexhepaj, M. Föller, A. Ebrahim, N. Mohebbi, "Down regulation of mouse intestinal Na+-coupled glucose transporter SGLT1 by Gum Arabic (*Acacia senegal*)," *Cellular Physiology and Biochemistry*, 25(2-3), 203-210, 2010.

[38] H. A. Al-Jumayi, H. A. Elhendy, A. M. Darwish, "Biological Effects of Red Chili Pepper (*Capsicum annuum*) Consumption on High Fat Diet Female Albino Rats," *Pakistan Journal of Biological Sciences*: PJBS, 23(2), 150-158, 2020.

[39] J. A. Negulesco, S. A. Noel, H. A. I. Newman, E. C. Naber, H. B. Bhat, D. T. Witiak, "Effects of pure capsaicinoids (capsaicin and dihydrocapsaicin) on plasma lipid and lipoprotein concentrations of turkey poults," *Atherosclerosis*, 64(2-3), 85-90, 1987.

[40] A. W. Oyeyemi, P. C. Ugwuezumba, O. O. O. Daramola, O. Onwelu, "Comparative effects of *Zea mays* bran, Telfairia occidentalis and Citrus sinensis feeds on bowel transit rate, postprandial blood glucose and lipids profile in male wistar rats," *Nigerian Journal of Basic and Applied Sciences*, 26(1), 80-87, 2018.

[41] T. Dapčević-Hadnađev, M. Hadnađev, M. Pojić, "The healthy .components of cereal by-products and their functional properties. In Sustainable recovery and reutilization of cereal processing by-products," *Woodhead Publishing*, pp. 27-61,2018.

[42] L. Zhu, X. Luo, Z. Jin, "Effect of resveratrol on serum and liver lipid profile and antioxidant activity in hyperlipidemia rats," *Asian-Australasian Journal of Animal Sciences*, 21(6), 890-895, 2008.

[43] S. I. Hameed, A. W. Al-Shahwany, S. J. Salih, "Investigation the potential role of some medicinal plants extracts in regulating serum lipid profile in female albino rats," *Iraqi Journal of Science*, 60(12), 2561-2571, 2019.

[44] I. N ZekiI, H S. Al-Warid, "The prevalence of anemia among children infected with Entamoeba histolytica in Baghdad," *Iraqi Journal of Science*, 60(12), 2590-2599, 2019.