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Ameliorative iron-deficiency anemia levels using natural orange juice and fortified with different concentrations of mango, strawberries and beetroot juices in male albino rats

Nabila Y. Mahmoud Abdulmaguid

Associate professor || Department of Nutrition and Food Science || Faculty of Home Economics || Al Azhar University || Egypt
Associate professor || Department of Food Science and Nutrition || Faculty of Science || Taif University || Taif || KSA

ABSTRACT: Iron deficiency anemia is the most prevalent type of anemia all over the world. It occurs as a result of iron malabsorption or low supply from food. This research aimed to study the role of natural orange juice and fortified with different concentrations of mango, strawberries and beetroot juices on anemic rats. Juices chemical composition was determined. Sensory characteristics were evaluated for different concentrations of juices. Male Albino rats were used to study the nutritional effect of juices on biological evaluation, hematological and biochemical analysis. The results showed increase the ratio of macro and micro nutrients, especially minerals in fresh orange juice, which is fortified by mango, strawberry and beetroots juices. Also, sensory characteristics of juice have improved. Biological evaluations as feed intake, body weight gain, feed efficiency ratio, and internal organ weight were also increased. The levels of complete blood count, serum protein, ferritin, iron and zinc were incremented. The study confirms the importance of possible using orange juice fortified with fresh fruit and vegetables juices to treatment iron deficiency anemia as a safe way of beings.

Keywords: biological evaluation, biochemical analysis, complete blood count, chemical composition, sensory characteristics

Brief summary

Nutrition is associated with many reasons for health. The lack of iron-rich food is the main factors that inhibit its absorption and consider the main causes of iron deficiency anemia. Natural juices are one of the most important fast and delicious foods for the body. The process is strengthening the juice to increase the level of nutrients with juice and then bioavailability of iron and many minerals in the blood. Hence, the research idea is carried to sensory evaluated the fortified juices and study the impact on treatment of iron deficiency anemia.

INTRODUCTION

Iron deficiency is the most prevalent form of malnutrition in the world which has afflicted about 2 billion of people. Iron deficiency anemia is the common type of anemia. It is a result of low iron in diet or iron mal-absorption. Its daily amount required in body is very little (1 g/day), which varies among people

in accordance with their age, sex and physiological status. Vitamin C enhances iron absorption. So that, consuming vitamin C is often recommended along with rich iron foods [1].

Orange is tasty and juicy fruit belongs to the family *Rutaceae*. Orange pulp is an excellent source of vitamin C providing 64% of an individual daily requirement [2]. Citrus juices are considered a rich source of antioxidants including vitamin C, phenolic compounds (flavonoids) and carotenoids, which cannot synthesize in human body [3]. Orange juice contains many phytochemicals including carotenoids (beta-carotene, lutein and beta-cryptoxanthin), flavonoids (naringenin) [4]. Numerous volatile organic compounds are producing orange aroma, as aldehydes, esters, terpenes, alcohols, and ketones [5]. Mango (*Mangifera indica* L.) is one of the most important tropical fruits consumed worldwide, which excellent source of fiber, bioactive compounds as provitamin A carotenoids, vitamin C and phenolic compounds [6]. Strawberry (*Fragaria x ananassa Duch*.) fruits are a rich source of phytochemicals (plant chemicals) especially phenolic compounds. Many researches have shown that antioxidant as phenolic compounds have properties anticancer, anti-atherosclerotic and anti-neurodegenerative substances in both *in vitro* and *in vivo* [7]. In recent years increased attention has been focused on utilization of healthy foods. The beetroot (*Beta vulgaris*) are being an alkaline food (pH from 7.5 to 8.0) so, they have many health benefits. Its have antioxidant properties from their significant amount of vitamin C and B complex vitamins [8]. The juice of beetroot is useful remedy for sexual weakness and to expel kidney and bladder stones [9]. The therapeutic use of beetroot includes antitumor, carminative, hemostatic, renal protective properties and potential herb in cardiovascular conditions [10]. So as to, beetroot consider as a powerful antioxidant [11].

MATERIAL AND METHODS

Materials:

Orange, mango, strawberries, and beet roots were obtained from local market in Tanta, Egypt. Casein, vitamins, minerals and cellulose were purchased from EL-Gomhoryia Company, Cairo, Egypt. Tannic acid was obtained from Modern Lab for chemicals and Lab Equipment Cairo, Egypt. Thirty male albino rats of an average body weight 160±10 g of (Sprague Dawley Strain), age 8-10 weeks were obtained from the laboratory animal house of faculty of science, Cairo University. Kits used to determination the biochemical tests were obtained from Biomerieux Company France.

Methods

Preparation of juice: Orange, mango and strawberries were cleaned with tap water, peeled and then orange mango and strawberries juice was extracted using juice blender of one each. Beetroot was peeled out and sliced, crushed in a grinder with addition of subsequent water, then pulped by using hydraulic

press and the extracted juice was again filtered by using a four layer muslin cloth to remove remaining pomace.

Table1. Formulation of different samples of juice

no.	Sample Juice %	Orange (ml)	Mango (ml)	Strawberries (ml)	Beetroot (ml)
1	OJ 100%	100	-	-	-
2	OMSBJ 5%	85	5	5	5
3	OMSBJ 10%	70	10	10	10
4	OMSBJ 15%	55	15	15	15
5	OMSBJ 20%	40	20	20	20

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroots Juice

Chemical Composition: Orange, strawberry, mango and beet juices were chemically analyzed for moisture, crude protein, fat, crude fiber and ash according to methods described in Official Methods of Analysis [12]. Total carbohydrates were calculated by difference. The energy value was calculated using the at water factors of 4, 9 and 4 for protein, fat and carbohydrate, respectively [13]. Samples were wet acid-digested, using a nitric acid and perchloric acid mixture (HNO:HClO, 5:1 w/v). The total amounts of Fe and Zn in the digested samples were determined by atomic absorption spectrophotometry (Thermo– Elmental, Model 300VA, UK) [14]. Vitamin C levels were spectrophotometrically (Model No 6300, Designed and manufactured in UK by I en way LTD) analyzed by the method in which 2, 6 - dichlorophenol endophenol dye is reduced by ascorbic acid [15]. Carotenoids were analyzed by reversed phase HPLC using water 600 system equipped with auto sampler injector, degasser, pump and water 996 UV – visible photodiode array detector operating at 450nm. The data were stored and processed by means of Millennium 4.00 software (Waters, Stockholm, Sweden). Absorption spectra were recorded between 250 and 500 nm [16].

Sensory Evaluation of juice: Juices prepared and blend to evaluate for their sensory characteristics by twenty panelists from the staff of Food Science and nutrition Dep., Faculty of Science, Taif University KSA. The scoring scheme was established as mentioned by Okayasu and Naito [17] as followed: Color (10), concentration (25), odor (20), taste (25), appearance (20) and overall score 100 degrees.

Experimental design: Animals were acclimatized for one week to laboratory condition, kept under temperature 20±5 °C and humidity control 55±5 % with a 12 h light/ dark cycle. Rats were housed in stainless steel cages (one rat per cage). Rats were fed on basal diet (casein – basal diet), composed of 12 g of casein (85 % protein); corn oil (10% fat); minerals mixture (4 % minerals); vitamins mixture (1% vitamins); cellulose (4% fiber); and corn starch (71 % starch), and tap water supply was given ad-libitum and checked daily. Composition of mineral mixture was prepared according to Hegsted et al. [18].

Composition of vitamin mixture performed according to Campbell [19]. Rats group were divided into two main groups as follow: main group (1) 6 rats were fed on basal diet only and kept as normal group. Main group (2) 24 rats were received basal diet containing 20g/kg body weight tannic acid to induce iron deficiency anemia [20] for successive 21days. First subgroup (6 rats) was left as anemic rats group; the other three subgroups were administrated orally 2 ml/juice daily on empty stomach as following: the second subgroup 6 rats were administrated orally fresh orange juice (OJ). The third subgroup 6 rats were administrated orally fresh fortified juice as mixture of orange juice 70%, strawberry juice15% and mango juice15% (OMSJ). The fourth subgroup 6 rats were administrated orally fresh fortified juice as mixture of orange juice 55%, strawberry juice15%, mango juice15% and beet roots juice 15% (OMSBJ) for successive 28 days. Principles of laboratory animal care were followed by ethical committee for animal research Cairo University, Faculty of Science [21].

Biological evaluation: During the experiment period (7 weeks), the quantities of diet which were consumed (feed intake) and/or wasted were recorded every day. In addition, rat's weight was recorded weekly. At the end of the experiment, feed intake, body weight gain (BWG %), organs weight as a percent of total body weight and feed efficiency ratio (FER) were calculated, body weight gain and feed efficiency ratio were calculated according to Chapman et al. [22]. Kidney, liver, heart and spleen were removed carefully from each rat after an abdominal laparotomy, washed with saline solution, dried with filter paper and weighted according to the method described by Drury and Wallington [23].

Biochemical analysis: At the end of experiment period, rats were scarified under diethyl ether 80% anesthetized. Blood samples were collected after 12 hours fasting using the dorsal aorta/posterior vena cava. A part of blood samples placed in EDTA tube; 0.2 ml blood sample was placed in a test tube containing 5 ml Drabkin's solution. The tube was left at room temperature for 10 min. The developed color was calorimetrically measured using spectrophotometer (Spekol11 No. 849101) at 548 nm as described by Rice [24]. Also, part of blood samples were taken with a micro capillary tube and centrifuged at 5000 rpm for 5 min to determine hematocrit value. Blood samples were taken with a micro capillary tube and centrifuged at 5000 rpm for 5 min. The volume of blood cells was measured by using a graded scale; (MCV), (MCH), (MCHC), red blood cell, white blood cells and platelets count were measured according to Fischbach [25]. The remaining part of blood sample was received in clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully removed, transferred in clean cuvette tube, and stored frozen at -20°C for analysis. Analysis was done at Medical College Laboratory, Zgazig University. Serum samples were analyzed for determination of total protein according to Sonnenwirth and Jaret [26] and serum iron and ferritin according to Douglas et al. [27]. Serum zinc concentrations were measured by flame atomic absorption technique according to Tietz [28].

Statistical analysis: Data were expressed as mean ± standard deviation. Values were statistically analyzed by one-way analysis of variance (ANOVA test) according to Snedecor and Cochran [29] by using SPSS 20.0 software package. Differences were considered significant at P values (0.05).

RESULTS

Chemical composition: Data present in table 2 illustrated the chemical composition percentage of standard orange juice (OJ). Second sample fortified juice as mixture of orange juice 70%, strawberry juice15% and mango juice15% (OMSJ). Third sample fortified juice as mixture of orange juice55%, strawberry juice15%, mango juice15% and beet roots juice 15% (OMSBJ). From the obtained data, it could be noticed that standard (OJ) contained (on dry weight basis) 0% fat, 0.6% protein, 8.3% carbohydrate, 88.3% moisture, 0.1% fiber, 0.5% ash and 35.6 kcal energy. While OMSJ contained 0% fat, 0.7% protein, 10.2 % carbohydrate, 88.8 % moisture, 0.5 % fiber, 0.4 % ash and 43.6 kcal energy. As regard OMSBJ contained 0% fat, 0.8% protein, 10.4 % carbohydrate, 88.02 % moisture, 0.6 % fiber, 0.6 % ash and 44.8 kcal energy.

Table2. Chemical composition of juices value per 100 ml

Sample	Protein %	Fat %	Moisture %	Ash %	Fiber %	Carbohydrate%	Energy kcal.
OJ	0.6	0	88.3	0.5	0.1	8.3	35.6
OMSJ	0.7	0	88.8	0.4	0.5	10.2	43.6
OMSBJ	0.8	0	88.02	1.0	0.6	10.4	44..8

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroots Juice

The results in table 3 showed the mineral contents of juices, OMSBJ had the significantly highest iron content 0.8 mg/100 g followed by OMSJ and OJ (0.6 and 0.4 mg/100 g, respectively). The increase might be due to the hydrolytic enzymes released more free Fe from its organic juice. Also OMSBJ had significantly highest Zinc content 0.2 mg/100 g. followed by OMSJ and OJ (0.1 and 0.08 mg/100 g respectively). The results in the same Table showed vitamins composition of juices. It could be noticed that, OJ contained (55mg/100g) of vitamin C that was the highest content and OMSJ decreased gradually followed by OMSBJ. The best value was for OJ (55 mg/100g) vitamin C. In addition, OMSBJ (66.5 µg/100ml) the highest value of Carotenoids followed by OMSJ and OJ (43.8 and 40.5 µg/100ml, respectively).

Table3. Minerals and vitamins composition of juices value per 100 ml

Sample	Iron (Fe) mg	Zinc (Zn) mg	Vitamin C mg	Carotenoids µg
OJ	0.4	0.08	55	40.5
OMSJ	0.6	0.1	50.8	43.8
OMSBJ	0.8	0.2	43.6	66.5

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroots Juice

Sensory Characteristics: Table 4 summarized values of sensory evaluation of orange juice fortified with mango, strawberry and beet juices. Color properties showed significant increase of fortified juice in all samples as compared to pure orange juice. Concentration degrees recorded high significant increase in orange juice 20% as compared to pure juice followed by 15, 10 and 5% fortified samples. Oder properties recorded high significant increase in orange juice 20 and 15% as compared to pure juice followed by 5 and 10% fortified samples. Taste status showed significant increase in fortified sample 20% and pure orange juice followed by 15, 5 and 10% samples. Acceptance degrees showed significant increase in pure orange juice, fortified 15 and 20% followed by 5 and 10% samples. Total percent of all sensory properties showed non-significant increase as compared to pure orange juice. So, the appreciation recorded overall good mark of all samples for sensory tests.

Table4. Sensory Characteristics of orange juice and fortified samples with mango, strawberry and beet juices at different levels

Groups	Color (10)	Concentration (25)	Oder (20)	Taste (25)	Acceptance (20)	Total%	Appreciation
OJ 100%	8.2±0.2 ^{b}	20.3±0.34 ^{c}	17.9±0.5 ^{b}	21±0.74 ^{a}	17.8±0.63 ^{a}	84±0.5 ^{a}	G
OMSBJ 5%	9.5±0.1 ^{a}	22.2±0.34 ^{b}	17.7±0.34 ^{b}	19.9±0.52 ^{b}	16.7±0.27 ^{b}	86±0.4 ^{a}	G
OMSBJ 10%	9.3±0.2 ^{a}	22.4±0.55 ^{b}	17.3±0.39 ^{b}	19.8±0.67^{b}	15.8±0.54 ^{c}	85±0.5 ^{a}	G
OMSBJ 15%	9.8±0.3 ^{a}	22.5±0.47 ^{b}	18.6±0.55 ^{ab}	20.5±0.57 ^{b}	17.3±0.64 ^{a}	88±0.7 ^{a}	G
OMSBJ 20%	9.4±0.4 ^{a}	23.7±0.7 ^{a}	19.2±0.4 ^{a}	21.3±0. 4 ^{a}	17.1±0.3 ^{a}	87±0.6 ^{a}	G

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroots Juice. 90-100 Very Good (VG), 80-89 Good (G), 70-79 satisfactory (S), Less than 70 Questionable (Q)

Biological evaluation: As regard to table 5, it is showed significant decrease of feed intake (FI) for anemic rats as compared to normal rats (17.15±0.42 and 18.68±0.30 g/day, respectively). OMSBJ group showed the highest value of FI in treated group (19.33±0.27 g/day), followed by OMSJ and OJ. On the other hand, there were non-significant differences between juice groups. Body weight gain (BWG %) cleared a significant increase in anemic rats group as compared to normal rats group (-6.21±0.86 and 22.84±1.66%, respectively). All treated groups showed significant increase in (BWG %) as compared to anemic rats

group (14.95±1.41, 14.88±0.17 and 13.71±1.79 %, respectively) for OMSJ, OMSBJ and OJ. The best result recorded in OMSJ group. Regarding to, feed efficiency ratio (FER) anemic rats group showed significant decrease as compared to normal rats group (-0.02±0.005 and 0.07±0.004). There was a significant increase between all treated groups (0.04±0.17, 0.04±0.003 and 0.03±0.004, respectively) as compared to anemic rats group. OMSJ and OMSBJ showed the highest increase in FER which closet to normal rats group.

Table5. Feed intake, body weight gain% & feed efficiency ratio of anemic rats treated juice groups

Groups	FI (g)/day	BWG (%)	FER
Normal rats	0.30[a] 18.68±	22.84±1.66[a]	0.07±0.004[a]
Anemic rats	17.15±0.42[b]	-6.21±0.86[c]	- 0.02±0.005[c]
OJ	19.12±0.35[a]	13.71±1.79[b]	0.03±0.004[b]
OMSJ	19.16±0.17[a]	14.95±1.41[b]	0.04±0.003[b]
OMSBJ	19.33±0.27[a]	14.88±0.17[b]	0.04±0.17[b]

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroot Juice. Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

The mean values in table 6 illustrated significant increase of relative organs weight (liver, heart and spleen) for anemic rats group (3.10±0.08, 0.56±0.06 and 0.71±0.03 g/100g, respectively) as compared to normal rats group (2.58±0.1, 0.51±0.06 and 0.60±0.04 g/100g, respectively). On the other hand, relative kidneys weight was showed significant decrease for anemic rats group compared to normal rats group. For relative liver weight, there was a significant decrease in all treated groups (2.68±0.03, 2.73±0.06 and 2.80±0.06g/100g, respectively) as compared to anemic rats group. The best result found in OMSJ group. As regard to relative heart weight, only the groups fed on OMSJ group showed non-significant differences (0.62±0.09 g/100g) comparing with anemic rats. Values of relative kidneys weight showed significant increase in all treated groups (0.98±0.03, 0.99±0.05 and 1.00±0.04 g/100g, respectively) as compared to anemic rats group. For relative spleen weight, there was a significant decrease in all treated groups (0.69±0.05, 0.68±0.05 and 0.65±0.04 g/100g, respectively) for OMSBJ, OJ and OMSJ as compared to anemic rats group.

Table6. Relative organs weight of anemic rats and treated juice groups

Groups	Liver	Heart	Kidneys	Spleen
Normal rats	2.58±0.10[b]	0.51±0.06[c]	0.89±0.05[b]	0.60±0.04[c]
Anemic rats	3.10±0.08[a]	0.56±0.06[b]	0.83±0.04[c]	0.71±0.03[a]
OJ	2.80±0.06[b]	0.68±0.04[a]	0.98±0.03[a]	0.68±0.05[b]
OMSJ	2.68±0.03[b]	0.62±0.09[ab]	1.00±0.04[a]	0.65±0.04[b]
OMSBJ	2.73±0.06[b]	0.65±0.06[a]	0.99±0.05[a]	0.69±0.05[b]

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroot Juice. Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Complete blood count: Data listed in table 7 recorded mean values of hemoglobin concentration, hematocrit, MCV, Rbcs, Wbcs and platelets for anemic rats group were recorded significant decreases (9.4±0.2 g/dL, 28.3±1.3 %, 70.4±3.1 μm^3, 3.7±0.2 106/µL, 4. 6±0.8 103/µL and 332.2±4.2 µm3) as compared to normal rats group (13.7±0.41 g/dL, 42.2±0.84%, 92.4±4.5 µm3, 6.1±0.57 106/µL, 9. 8±1.9 103/µL and 440.6±4.6 μm^3). For hemoglobin concentration, there was a significant increase in all treated groups (12.38±0.39, 11.36±0.57 and 11.12±0.48 g/dL, respectively) for OMSBJ, OMSJ and OJ as compared to anemic rats group. The best result found in OMSBJ group. For hematocrit presentation, there was a significant increase in all treated groups (40.2±1.9, 37.1±2.3 and 36.4±1.1 %, respectively) for OMSBJ, OMSJ and OJ as compared to anemic rats group, the best result found in OMSBJ group. As regard to MCV concentration there was a significant increase in OMSBJ group (84.2±2.5 µm3) followed by OMSJ and OJ groups (78.0±2.6 and 78.8±1.9 μm^3) as compared to anemic rats group. Values of Rbcs concentration was a significant increase in OMSBJ and OMSJ groups (4.8±0.21 and 4.5±0.23 106/µL) while, in OJ group showed non- significant deference (4.1±0.29 106/µL) as compared to anemic rats group. For Wbcs concentration, there was a significant increase in OJ and OMSBJ groups (8.92±1.6 and 7.94±1.4 103/µL, respectively) and non- significant deference (5.04±1.6 103/µL) in OMSJ group as compared to anemic rats group. Concerning Platelets concentration there was a significant increase in OMSBJ group (431.0±7.17 μm^3) while, there was non- significant deference in OJ and OMSJ groups (294.4±4.63 and 286.4±6.4 μm^3) as compared to anemic rats group.

Table7. Complete blood count of anemic rats and treated juice groups

Groups	Hb g/dL	Hct %	MCV(µm3)	Rbcs 10^6/µL	Wbcs 10^3/µL	Platelets (μm^3)
Normal rats	**13.7±0.41^a**	**42.2±0.84^a**	**92.4±4.5^a**	**6.1±0.57^a**	**9. 8±1.9^a**	**440.6±4.6^a**
Anemic rats	**9.4±0.33^d**	**28.3±1.3^c**	**70.4±3.1^c**	**3.7±0.2^d**	**4. 6±0.8^b**	**332.2±4.2^b**
OJ	**11.12±0.48^c**	**36.4±1.1^b**	**78.8±1.9^c**	**4.1±0.29^{bc}**	**8.92±1.6^a**	**294.4±4.63^b**
OMSJ	**11.36±0.57^c**	**37.1±2.3^b**	**78.0±2.6^c**	**4.5±0.23^b**	**5.04±1.6^b**	**286.4±6.4^b**
OMSBJ	**12.38±0.39^b**	**40.2±1.9^a**	**84.2±2.5^b**	**4.8±0.21^b**	**7.94±1.4^a**	**431.0±7.17^a**

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroot Juice. Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Biochemical analysis: Results in table 8 revealed that a significant decrease in mineral status as total iron, zinc and ferritin for anemic rats group were significant decreases (1.52±0.39, 108.2±5.6 and 128.2±4.69

µg/dL) compared to normal rats group (3.77±0.35, 163.8±4.3 and 217.8±4. µg/dL). For total iron, all treated groups showed significant increase for OMSBJ, OMSJ and OJ as compared to anemic rats group (2.97±0.3, 2.77±0.55 and 2.47±0.5 µg/dL, respectively), the best result found in OMSBJ group. In the same table, it could be noticed a significant increase of zinc in all treated groups (145.2±2.5, 142.2±3.4 and 141.4±4.4 µg/dL, respectively) OMSBJ, OMSJ and OJ as compared to anemic rats group. Concerning to ferritin there was a significant increase in OMSBJ, OMSJ and OJ groups as compared to anemic rats group (181.2±3.57, 180.2±4.44 and 169.4±3.40µg/dL, respectively), the best result found in OMSBJ group. Serum total protein showed significant decreases of anemic rats compared to normal rats (3.44±0.29 and 5.82±0.37 g/dL). All treated groups had significant increase in level of serum total protein as compared anemic rats group. There were no-significant changes between treated groups, the best result found in OMSBJ, OMSJ and OJ groups (5.69±0.51, 5.66±0.24 and 5.16±0.9 g/dL) which nearly to normal rats group.

Table8. Minerals, ferritin and total protein of anemic rats and treated juice groups

Groups	Total Iron µg/dL	Zinc µg/dL	Ferritin µg/L	T. protein g/dL
Normal rats	3.77±0.35^{a}	163.8±4.3^{a}	217.8±4.9^{a}	5.82±0.37^{a}
Anemic rats	1.52±0.39^{c}	108.2±5.6^{c}	128.2±4.69^{d}	3.44±0.29^{b}
OJ	2.47±0.5^{b}	141.4±4.4^{b}	169.4±3.40^{c}	5.16±0.9^{a}
OMSJ	2.77±0.55^{b}	142.2±3.4^{b}	180.2±4.44^{b}	5.66±0.24^{a}
OMSBJ	2.97±0.3^{ab}	145.2±2.5^{b}	181.2±3.57^{b}	5.69±0.51^{a}

*where OJ= Orange Juice OMSJ= Orange Mango Strawberries Juice OMSBJ= Orange Mango Strawberries Beetroot Juice. Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

DISCUSSION

Iron is essential ion for oxygen transport. On the other hand, its excessive accumulation in organs can cause oxidative damage. In cases of iron deficiency, iron stored in liver withdrawn it to compensate for deficiency. This compensation is particularly important for animals during growth. Longer periods of iron deficiency caused low iron storage, decreased hemoglobin biosynthesis and giving rise to anemia [30]. Microencapsulated ferric saccharate or ferrous sulphate induced high significant of final body weight, feed efficiency, mean corpuscular volume of reticulocytes and average haemoglobin content in reticulocytes for anaemic rats supplemented with either compared to anaemic controls [31]. Fruits are considered healthy foods because; contain a large amount of antioxidants such as, vitamins, dietary fiber and minerals. There are many types of a fruit juice already preferred from consumers due to its taste, nutrients and available in the market. Also, fruit juices are wanted for its higher health benefits [32]. So that, fortification of fruit and vegetables had an effect on the color, taste, concentration and acceptance

characteristics of the products by increasing a good number of consumers accepted orange, mango, strawberries and beetroots juice.

Citric acid and ascorbic acid or vitamin C that exists in mango and orange juice increases the absorption of iron in body. Malic acid and tartaric acid existing in beetroot also lead to an increased absorption of iron [1]. Consuming healthy foods and levels of hemoglobin have an important role in human and experimental animal status. The mass of red blood cells are effect on the levels of hemoglobin. Orange juice contains an effective potent of antioxidants including flavonoids (hesperetin and naringenin most of them, glycosides), carotenoids (xanthophylls, cryptoxanthins, carotenes), vitamin C and beneficial phytochemicals, such as folate. All of these components are believed to be significant contributors to the preventive effects of fruits against many diseases [33]. Mango is common fruits and considers an important source of micronutrients such as vitamins and other phytochemicals. Mango fruits provide energy, carbohydrates, proteins, fats, dietary fiber, phenolic compounds [34] and omega-3 and -6 polyunsaturated fatty acids [35], which are vital to human health, growth and development [36]. Up to 25 different carotenoids have been found in mango pulp, mostly was beta-carotene [37]. Mangos are used in vitiated conditions, hemoptysis, hemorrhages emaciation, and anemia [38]. Strawberries contain a variety of nutrients that are necessary in the formation of red blood cells, white blood cells, iron absorption and prevents anemia due to the rich content of folic acid, vitamin C content and minerals (Iron, magnesium, calcium and potassium). The effect of iron is the main component the synthesis of hemoglobin. If iron deposits enough, formation of red blood cell in bone narrow will always be met [39]. The cost of the beetroot is low when comparing with other iron rich vegetables; also it can be stored easily. Many studies proved that beet root can be contributed to improve the haemoglobin level in the blood. Beetroot juice after administration to adolescence girls proved a significant improvement in hemoglobin level [40]. Hence, the idea of mixing and mixing fresh orange juice with mango, strawberry and beet juice was come. This mix can provide the best vitality for the active ingredients in juices, which have a significant role in improving the level of complete blood count in beings. Total serum protein range about 7.0-9.5 g/dl. It makes up about 7% of total blood volume. Serum proteins play numerous roles in the life continuity; they act as transport system for hormones, vitamins, minerals, lipids, drugs and balance the osmotic pressure of blood and tissue [41]. Serum proteins can be help in regulating the cellular activities of the cell, functioning of the immune system and as a source of energy for cells when carbohydrates have been depleted in the body [42]. The transferrin family of plasma proteins is involved in the regulation of iron, which is essential for the proper oxygenation of blood [43]. Flavonoids presenting in orange juice have antioxidant properties and suppress destructive oxygen free radicals. More existence of free radicals can damage all components of the cell, including proteins as hepatic (xanthine oxidase XO), xanthine dehydrogenase (XDH), fats and DNA activity in oxonate-induced hyperuricemic rats [44]. Citrus sinensis extract have increase total protein in the body. Its active component has responsible for the elevation of

serum proteins as d-limonene; this could in turn lead to stimulation of protein receptors synthetic pathways [45]. Supplementation with multiple nutrients (zinc, vitamin A, iron, copper, and vitamin C) at long time was more effective in improving growth than vitamin A supplementation alone or combined only with zinc. Multiple nutrients is more effective than single nutrient supplementation [46].

CONCLUSION

Nowadays, the modern trend of fresh and natural foods is of interest, despite the long treatment period, but it is safe. Hence the idea of using mixed fresh juices to increase interest and improve sensory properties. The results showed the therapeutic role of orange juice supported with mango, strawberry and beetroot juices to raise the level complete blood count, serum proteins and minerals. So, this study recommends the possibility using of orange juice, fortified with other fruits and vegetables for treatment iron deficiency anemia.

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REFERENCES

1. Kazemi TA. Review of iron deficiency anemia. Journal of current research in science 2014; 2(6): 882-886.
2. Ohwesiri M. Akusu, David B. Kiin-Kabari, Caroline O. Ebere. Quality Characteristics of Orange/Pineapple Fruit Juice Blends. American Journal of Food Science and Technology 2016; 4(2): 43-47.
3. Peterson JJ, Dwyer JT, Beecher GR, Seema AB, Gebhardt SE, Haytowitz DB Holden JM. Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature. Journal of Food Composition and Analysis 2006; 19: 66-73.
4. Aschoff JK, Kaufmann S, Kalkan O, Neidhart S, Carle R, Schweiggert RM. In Vitro Bioaccessibility of Carotenoids, Flavonoids, and Vitamin C from Differently Processed Oranges and Orange Juices [Citrus sinensis (L.) Osbeck]. Journal Agric Food Chem. 2015; (8):578−587.
5. Perez-Cacho PR, RL Rouseff. Fresh squeezed orange juice odor: a review. Crit Rev Food Sci Nutr. 2008; 48(7):681−695.
6. Isabel G. Nutritional value and quality of processed mango fruits. Ph.D. thesis, Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers Reproservice, Chalmers University of Technology Göteborg, Sweden; 2016.

7. Navindra P. Seeram. Strawberry Phytochemicals and Human Health: A Review. Article January; 2007. https://www.researchgate.net/publication/228983423.
8. Singh B, Singh B Hatan. Optimization of osmotically dehydrated beetroot candy using response surface methodology," International Journal of Food and Nutritional Sciences 2013; 2 (1): 15-21.
9. Sharma N, Tanwer BS, Vijayvergia R. Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. International Journal of Pharm Tech Research 2011; 3 (1): 110–113.
10. Vali L, Tefanovits-Banyai E, Szentmihalyi K. Liver protecting effects of table beet (Beta vulgaris var.rubra) during is chemia-reperfusion. Nutrition 2007; 23 (2):172–178.
11. Christiana Winkler BW, Schroecksnadel K, Schennach H, Fuchs D. In vitro effects of beet root juice on stimulated and unstimulated peripheral blood mononuclear cells. The American Journal of Biochemistry and Biotechnology 2005; 1: 180–185.
12. A.O.A.C. Official Methods of Analysis, 17thEd Association of Official Analytic Chemists International. Arlington, Virginia 2000; USA.
13. Chaney SG. Principles of nutrition I: Macronutrients. In: Devlin, T.M. (ed.), Textbook of Biochemistry, with Clinical Correlation, 6th ed. John Wiley and sons, New York; 2006. pp.: 1071-1090.
14. Lindsey WL, Norwell MA. A new DPTATEA soil test for zinc and iron. Agronomy Abstracts 1969; 61: 84.
15. Methods of Vitamin Assay. Prepared and Edited by the Association of Vitamin Chemists, 2nd Ed. New York, USA Inc. Interscience Publishers; 1951. pp. 18:301.
16. Rodriguez-Amaya DB. Carotenoids and Food preparation: The retention of Provitamin A Carotenoids in Prepared, Processed and Stored Foods. /OMNI Project, John Snow, Inc. 1997.
17. Okayasu H, Naito S. Sensory Characteristics of Apple Juice Evaluated by Consumer and Trained Panels. Institute of Food Technologists. 2001; 66(7): 1025-29.
18. Hegested A. Salt mixture. J. Biol. Chem. 1941; 138:459.
19. Campbell, JA. Methodology of protein evaluation. Document R. 101 Led. 37Meeting, New York: PAG Nutr; 1963.
20. Kaosar A, Kazuki S, Satoshi I, Hiroshi H. Effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. Biosci. Biotechnol. Biochem. 2004; 3: 584-592.
21. Gaafar K, Soliman AM, Bashter A, Hamdi H, Badr AM, El-Shinawi M, Mohamed MM. Towards Establishing Ethical Committee for Animal Research: Challenges and Opportunities, IACUC Conference; 2013.
22. Chapman DG, Castilla R. and Campbell JA. Evaluation of protein in food. I-A. Method for the determination of protein efficiency ratio. Can. J. Biochem. Physiol. 1959; 37: 679-686.

23. Drury RA, Wallington EA. Carton's histological technique. 5thEd.Oxford University Press, New York; 1980.
24. Rice EW. Rapid determination of total hemoglobin as hemoglobin cyanide in blood containing carboxyhemoglobin. Clin. Chim. Acta; 1967. p.18: 89.
25. Fischbach FT. A Manual of laboratory and diagnostic tests. 5th Ed. New York: Lippincott Philadelphia; 1996. pp. 31-120.
26. Sonnenwirth A, Jaret L. Grad-Wholes clinical laboratory methods and diagnosis. 18th Ed London: Mosby; 1980. p.: 258-259.
27. Douglas A. Skoog, Donald M. West, F. James Holler and Stanley R. Crouch. Fundamentals of Analytical Chemistry, 9th Ed., Chapter 29. Cengage Learning 2013.
28. Tietz NW. In clinical guide to laboratory tests. W.B Saunders Company. Philadelphia; 1983. p. 384.
29. Snedecor GW, Cochran WC. Statistical Methods. 6th Ed. Press Ames USA, the Lowa State Univ.; 1967.
30. Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 2000; 28: 289–309.
31. La´zaro E, Santas J, Rafecas M. Recovery from dietary iron deficiency anaemia in rats by the intake of microencapsulated ferric saccharate. J Food Sci Technol. 2017; 54(9):2913–2918.
32. Wunwisa K, Kamolnate K. Sensory characteristics and consumer acceptance of fruit juice containing probioitcs beads in Thailand. AU J.T. 2010; 14(1): 33-38.
33. Adrian A. Franke, Robert V. Cooney, Susanne M. Henning, Laurie J. Custer. Bioavailability and antioxidant effects of orange juice components in humans. J Agric Food Chem. 2005; 53(13): 5170–5178.
34. Tharanathan RN, Yashoda HM, Prabha TN. Mango (Mangifera indica L.), the king of fruits – A review. Food Reviews International 2006; 22:95-123.
35. United States Department of Agriculture (USDA). USDA National Nutrient Database for Standard Reference. Fruit Reports, raw Mango 2010; 9(23).
36. Jahurul MHA, Zaidul ISM, Ghafoor K, Al-Juhaimi FA, Nyam KL, Norulaini NAN, Sahena F, Omar AKM. Mango (Mangifera indica L.) by-products and their valuable components: A review. Food Chemistry 2015; 183:173-180.
37. Chen JP, Tai CY, Chen BH. Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (Mangifera indica L.) Journal of Chromatography Archives. 2004; 1054:261-268.
38. Masud Parvez GM. Pharma ological Activities of Mango (Mangifera Indica) Journal of Pharmacognosy and Phytochemistry 2016; 5(3): 01-07.
39. Wulandari S, Ajunnisa. The effect of consumption strawberry juice against level of haemoglobin in third trimester of pregnant woman. International Journal of Medical Research and Pharmaceutical Sciences 2016; 3 (12): 76-82.

40. Gayathri Priya N, Malarvizhi M, Jega Jothi A. Beet root juice on haemoglobin among adolescent girls. Journal of Nursing and Health Science 2013; 2 (1): 09-13.
41. Anderson NI, Anderson NG. High resolution two dimensional electrophesis of Human plasma proteins. Proceeding of the National Academy of Sciences 1977; 74(12); 5421-5425.
42. Jeremy E. Serum proteins. Ann intern. Med. 2009; 10:457-460.
43. Geyer, PE; Kulak, NA; Pichler, G; Holdt, LM; Teupser, D; Mann, M. Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst. 2016; 2 (3): 185–95.
44. Haidari F, Keshavarz SA, Rashidi MR, Shahi MM. Orange juice and hesperetin supplementation to hyperuricemic rats alter oxidative stress markers and xanthine oxidoreductase activity. J. Clin. Biochem. Nutr. 2009; 45: 285–291.
45. Akpan OU, Effiong OO, Helen OT, Daniel AH. Citrus sinensis separately increases serum plasma proteins while its combination treatment with carbimazole reduces-the thyroid hormones connection. Frontiers in Science 2013; 3(3): 102-106.
46. Castro LCV, Costa NMB, Sant'Anna HMP, Ferreira Célia LLF, Franceschini SCC. Improvement the nutritional status of pre-school children following intervention with a supplement containing iron, zinc, copper, vitamin A, vitamin C and prebiotic. Ciência & Saúde Coletiva 2017; 22(2):359-68.

تحسين مستويات فقر الدم الناجم عن نقص الحديد باستخدام عصير البرتقال الطبيعي والمدعم بتركيزات مختلفة من عصائر المانجو والفراولة والشمندر في ذكور الفئران البيضاء

الملخص: فقر الدم الناجم عن نقص الحديد هو أكثر أنواع فقر الدم انتشارا في جميع أنحاء العالم. ويحدث نتيجة سوء امتصاص الحديد أو انخفاض المتناول من الطعام. يهدف هذا البحث إلى دراسة دور عصير البرتقال الطبيعي والمدعم بتركيزات مختلفة من عصائر المانجو والفراولة والشمندر على الفئران المصابة بفقر الدم. تم تقدير التركيب الكيميائي للعصائر. كما تم تقييم الخصائص الحسية للتركيزات المختلفة من العصائر. واستخدمت ذكور الفئران البيضاء لدراسة التأثير الغذائي للعصائر على التقييم البيولوجي وتحاليل الدم والتحاليل البيوكيميائية. وأظهرت النتائج زيادة نسبة المغذيات الكبرى والصغرى خاصة المعادن في عصير البرتقال الطازج، الذي تم تدعيمه بعصائر المانجو والفراولة والشمندر. كما تحسنت الخصائص الحسية للعصير أيضاً. وزادت معدلات التقييم البيولوجي كالمأخوذ من الغذاء ووزن الجسم المكتسب ونسبة كفاءة الغذاء ووزن الأعضاء الداخلية. وتزايدت مستويات صورة الدم الكاملة ومصل الدم للبروتين والفريتين والحديد والزنك. وأكدت الدراسة على أهمية استخدام عصير البرتقال المدعم بعصائر الفواكه والخضروات الطازجة لعلاج فقر الدم الناجم عن نقص الحديد كطريقة آمنة للكائنات الحية.

الكلمات المفتاحية: التقييم البيولوجي، التحليل البيوكيميائي ، صورة الدم الكاملة، التركيب الكيميائي، الخصائص الحسية.