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COMPARATIVE STUDY OF THE EFFECT OF IRON ASCORBATE AND BIO-IRON RICH DIETARY COMPOUND ON BIOCHEMICAL MARKERS IN IRON DEFICIENCY ANAEMIA

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ABSTRACT

Background:

Iron deficiency is first cause of nutritional anaemia in infancy and the further growth period. It can be prevented with appropriate, adequate and timely intervention. Anaemia has been a prevalent health issue in India with over 70% children.79% of children below the age of three suffer from varying degrees of anaemia. The management of this entity is supplementing elemental iron or bio-iron through food or as medicine. Elemental iron supplements have a few drawbacks and present as unwarranted effects like constipation, vomiting, metallic taste, non-compliance, iron deposition in the tissues and sometimes iron toxicity. Bio iron i.e. iron of natural origin (plant and animal origin) bypasses these symptoms, it is readily absorbed and readily bio-converted into haemoglobin.

Methodology:

The present study compares the supplements elemental iron and bio iron i.e. iron of natural origin from Figs, Black Currants and Dates combination in the management of Iron Deficiency Anaemia in children.

Results and Conclusions:

The effect of these supplements was studied on anaemia markers and it was observed that it had positive effect on rise in haemoglobin, serum iron level and serum ferritin levels with a marked fall in total iron binding capacity, on administration of the control and trial drug for 30 days.

Keywords: Iron Deficiency Anemia (IDA), Hemoglobin, Serum Ferritin, Serum Iron, Iron Ascorbate, Figs, Dates, Black Currants

INTRODUCTION

Anaemia has been a prevalent health issue in India. According to the National Family Health Survey (NFHS) data for anaemia prevalence among children under three years of age shows 79% which is five per cent more than the previous survey conducted. Nearly 73 million children below the age of three (79%) suffer from varying degrees of anaemia. Over 50 million children suffer from moderate-to-severe anaemia. Based on studies by the National Nutrition Monitoring Bureau, anaemia prevalence among children between one to five years of age is around 66%, with a varying figures of 33 to 93% across different states of India. Anaemia in childhood can cause an irreparable damage, physical and intellectual, particularly to the development of a young child.

IDA is a blood disorder where the blood has a reduced ability to carry oxygen due to a lower than normal number of red blood cells, or a reduction in the amount of hemoglobin. Anaemia is the most common disorder of the blood, of the developing world. "Anaemia" is not a diagnosis in itself. It is an objective sign of the presence of other diseases. iron deficiency is considered to be the most common cause of anaemia, at least in children and females, though other conditions of nutrition such as vitamin B_{12} and folate deficiencies, parasitic infections, chronic gut inflammation leading to reduced absorption of iron, chronic diseases, endocrine disorder and various inherited disorder can also causes anaemia **[1, 2]**.

Table 1: W.H.O. Haemoglobin Thresholds used to define ANAEMIA [1 gm/dl:0.6206mmol/L] [3]

Age/Gender Group	Hb Threshold [gm/dl]	Hb Threshold [mmol/L]
Children (0.5–5.0 yrs)	11.0	6.8
Children (5–12 yrs)	11.5	7.1
Teens (12–15 yrs)	12.0	7.4
Women, non-pregnant (>15yrs)	12.0	7.4
Men (>15yrs)	13.0	8.1

0.5 g of iron is present in iron a full-term new-born as against of 5 g in adults. An average of 0.8 mg of iron needs to be absorbed daily during the initial 15 years of life **[4, 5]**. Small additional amount of iron is necessary to overcome the normal losses of iron caused by shedding of cells. Thus it necessitates absorption of about 1mg of iron daily to maintain positive iron balance during childhood. Less than 10% of dietary iron is usually absorbed, and thus 8-10 mg of iron is necessary daily, to maintain iron levels.

During infancy, when growth is rapid, iron from cow's and breast milk makes it difficult to maintain body iron levels at the optimum. Breastfed infants have are at an advantage, because they can absorb iron 2-3 times more efficiently than infants that are fed cow's milk. Breastfed infants are at risk to land in to iron deficiency if regular intake of iron-fortified foods by six months of age is not started **[6]**. The usual dietary pattern observed in infants and toddlers with nutritional iron-deficiency anemia, is excessive consumption of cow's milk **[7, 8, 9]** (low iron content, blood loss from milk protein colitis). Under nutrition is also generally responsible for iron deficiency.

Iron deficiency and iron-deficiency anaemia:

Iron deficiency is a condition where serum iron levels fall below the threshold levels i.e. 50 mg/dL but the pathophysiology of anaemia has not set off. Iron deficiency has non hematologic systemic effects. Both iron deficiency and iron-deficiency anaemia are associated with impaired neurocognitive function in infancy **[9]**. Iron-deficiency anaemia may also be associated with, possibly irreversible, cognitive defects **[10]**. Some studies suggest an increased risk of seizures, strokes, breath-holding spells in children, and exacerbations of restless legs syndrome **[11]**. Due to the potential of adverse neurodevelopmental outcomes, reducing the frequency of iron deficiency has to be an important goal, overall. Other non-hematologic consequences of iron deficiency include pica and pagophagia, the desire to ingest ice.

Diagnosis of IDA [12]:

A thorough medical history is important to the diagnosis of iron-deficiency anemia. A travel history to areas in which hookworms and whipworms are endemic may also be helpful in guiding certain stool tests for parasites or their eggs **[13]**.

In progressive iron deficiency, a sequential biochemical and hematologic events occur.

Tissue iron stores deplete. The reduced serum ferritin reflects this depletion, Ferritin is an iron-storage protein, which can provide an estimate of body iron stores when inflammatory diseases are absent **[14]**. Later serum iron levels decrease. The iron-binding capacity of the serum (serum transferrin) increases, and the transferrin saturation falls below normal. As iron stores decrease, iron becomes unavailable to form a complex with protoporphyrin to form haem. At this point, iron deficiency progresses to iron-deficiency anemia **[15]**.

With lesser hemoglobin, the red blood cells (RBCs) become smaller called microcytes, and varied in size. The changes are associated with a decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin **[16]**. The RBC count decreases. The reticulocyte count (percentage) is normal or moderately elevated. The peripheral blood smear reveals hypochromic, microcytic RBCs with substantial variation in cell size. A presumption of iron-deficiency anemia is most of the times done by a complete blood count (CBC) showing a microcytic anemia picture with high RDW, decreased RBC count (Hematocrit), normal WBC count, and a normal or elevated platelet count. Other laboratory investigations like the reduced serum ferritin, reduced serum iron, and increased total iron-binding capacity, generally, are not necessary unless severe anemia requires a faster diagnosis, other complicating clinical signs are present, or the anemia does not respond to oral iron therapy. A diagnosis of iron deficiency in the absence of anemia is challenging. Serum ferritin is a useful tool, whose value is increased by also measuring C-reactive protein to help identify false-negative results because of concomitant inflammation.

Table 2: Laboratory studies differentiating diagnosis of microcytic anaemia [16, 17]

Study	Iron Deficiency Anaemia	A or β Thalassemia	Anaemia of Chronic Disease
Haemoglobin	Decreased	Decreased	Decreased
Mean Corpuscular Volume	Decreased	Decreased	Normal / Decreased
RDW	Increased	Normal / Minimally Increased	Normal / Increased
RBC	Decreased	Normal / Increased	Normal / Decreased
Serum Ferritin	Decreased	Normal	Increased
TIBC	Increased	Normal	Decreased
Serum Transferrin Saturation	Decreased	Normal	Decreased
FEP	Increased	Normal	Increased
Soluble Transferrin Receptor	Increased	Normal	Normal
Reticulocyte Haemoglobin Concentration	Decreased	Normal	Normal / Decreased

The response of iron-deficiency anaemia to adequate administration of iron is a critical diagnostic and therapeutic feature. Oral administration ferrous salts, **[18, 19]** most often ferrous sulphate is inexpensive and effective. No evidence is available regarding the addition of any trace metal, vitamin, or other hematinic substance leading to significant increase in the response to simple ferrous salts. Calcium and dietary fibre decrease the absorption of iron, but this can be overcome by co-administration of ascorbic acid. Tea is a significant inhibitor of iron absorption. The therapeutic dose should be calculated in terms of elemental iron. Iron in a total daily dose of 3-6 mg/kg of elemental iron in 1 or 2 doses is enough, while higher doses are used in severe cases. Parenteral iron is considered in malabsorption, where compliance is inadequate, as oral therapy is otherwise effective, less expensive and safe. Iron therapy increases the virulence of malaria and certain gram-negative bacteria. Iron overdose is mostly associated with Yersinia infection. Dietary counselling is usually necessary. Intake of cow's milk, needs be limited. Dietary iron should be increased. Iron from haem sources is more bioavailable than from non-haem sources. Iron should be continued for 2-3 months after values normalize so as to re-establish iron stores.

The dried fruits of *Fig* are reported to be an important source of different vitamins, minerals, carbohydrates, sugars, organic compounds, phenolic etc. **[20, 21]**. The fresh as well as dried fruits of figs are good source of fibre and polyphenols **[22, 23, 24]**. Figs are quoted in the traditional systems of medicine like as *Āyurveda*, *Unānī*, and *Siddha* and are used to treat diseases of the endocrine system (diabetes), respiratory system, digestive tract (indigestion and vomiting), menstrual disorders and menstrual pain etc. As per data of USFDA, dried figs are rich in fibre, copper, manganese, magnesium, potassium, calcium, iron and vitamin K. Fruits also have other nutrients in smaller proportion. Fruits of *Ficus carica (Figs)* are consumed fresh or dried, which are an excellent source of minerals, vitamins, carbohydrates, and dietary fibre, having a low fat and cholesterol content and ample amount of amino acids **[23-27]**.

Fruits of Phoenix dactylifera Linn, Dates fruits, are rich in carbohydrates in the form of sucrose, glucose and fructose (65% – 80%), and is a good source of fibres and certain essential minerals. The content of fat and protein is low but have high sugar concentration and do not have starch. They

are rich source of niacin, B6, riboflavin, thiamine, pantothenic acid, manganese, magnesium, iron and phosphorus and also contain a good amount of zinc, copper, potassium selenium and folic acid.

The basic and most important content of dried fruits of Grape Vine (*Vitis vinifera*) i.e Black Currant is manganese. *Vitis Vinifera* or grape vine also contains vitamin B6, thiamine, riboflavin, vitamin C and potassium. Various studies concluded that it also contains a chemical substance like resveratrol, a polyphenol having antioxidant properties **[28]**.

Table 3: Nutritional analysis

	Aṅjīra	*Drākśā*	*Kharjūra*
Edible Portion (Percentage)	**99.0**	**98.0**	**94.0**
Moisture (Grams)	**38.0**	**20.2**	**15.3**
Protein (Grams)	**1.3**	**0.3**	**2.5**
Total Fat (Grams)	**0.2**	**0.5**	**0.4**
Total Minerals (Grams)	**0.6**	**1.2**	**3.15**
Total Edible Fibre (Grams)	**2.2**	**1.7**	**3.9**
Carbohydrates (Grams)	**57.6**	**75.2**	**75.8**
Total Calories (Kcal)	**373**	**316**	**317**
Calcium (Milligrams)	**80.0**	**130.0**	**120.0**
Phosphorus (Milligrams)	**30.0**	**110.0**	**50.0**
Iron Compounds (Grams)	**0.87**	**0.69**	**1.54**
Elemental Iron (Milligrams)	**102**	**7.7**	**173**
Carotene (Micrograms)	**162.0**	**21.0**	**26.0**
Thiamine (Milligrams)	**0.06**	**0.03**	**0.01**
Riboflavin (Milligrams)	**0.05**	**0.14**	**0.02**
Niacin (Milligrams)	**0.6**	**0.4**	**0.9**
Ascorbic Acid (Milligrams)	**5.0**	**1.0**	**3.0**
Essential Amino Acids (Total Nitrogen Gram Percent)	**2.8**	**2.3**	**0.4**

MATERIALS AND METHODS:

A comparative interventional study was conducted on 160 children in an urban and semi urban area at two centers simultaneously, all diagnosed as IDA on a baseline anaemia profile including a CBC, Serum Iron, Serum Ferritin and Total Iron Binding Capacity were included between the age group of 5 years to 9 years. Ethical clearances were obtained from both the centers. The trial was registered with CTRI (Central Trial Registry of India) and a trial number was obtained on 22.10.2019, bearing no. CTRI/2019/10/021749. They were randomly divided into two groups of 80 each, by simple randomization method. The trial group which received iron ascorbate solution in the dose of 6 mg/kg/day, rounded off to the nearest multiple of 10 mg, daily once for 30 days. The patients from the trial group were asked to consume a freshly prepared paste containing Dried Figs, Black Currants and Dates, 10 grams each, soaked overnight in water, for the span of 30 days. A biochemical examination for assessment of a complete blood count, serum iron, serum ferritin and total iron binding capacity was done before the commencement and after the

end of the intervention. The post-trial were done to evaluate the effect of intervention.

RESULTS AND DISCUSSION:

The data collected in the present study in respect of biochemical parameters are detailed below and were studied for improvement in individual factors and the results were subjected to statistical analysis.

Table 4: Effect of Interventional Combination on Anaemia Profile Parameters

Parameter	Haemoglobin	Serum Iron	Serum Ferritin	TIBC
Mean BT	7.9475	35.063	17.375	535.88
Mean AT	10.056	70.8	39.713	339.25
X	2.1088	35.738	22.338	196.63
% of improvement	20.97%	50.48%	56.25%	57.96%
't' value	-29.57	-42.25	-19.37	20.337

Table 5: Effect of Iron Ascorbate on Anaemia Profile Parameters

Parameter	Haemoglobin	Serum Iron	Serum Ferritin	TIBC
Mean BT	8.009	34.75	18.9	527.588
Mean AT	10.034	69.125	47.65	328.5
X	2.025	34.375	28.75	199.088
% of improvement	20.18%	49.73%	60.34%	60.61%
't' value	-35.547	-46.425	32.667	-79.248

For the trial compound the mean grade of Haemoglobin on 1st day was 7.94 g/dL which increased to 10.056 g/dL at 30th day. The mean increment in score was 20.97% as compared to 20.18% with the use of iron ascorbate, which is significant as observed by paired t test (as p value <0.05) thus it can be said that there is significant increment on Haemoglobin. With iron ascorbate which is a form of elemental iron, the increment in haemoglobin was by 2.025 g/dL as compared to 2.1088 g/dL by the use of bio-iron. Increase in haemoglobin is not only dependent on supply of iron; though iron has a major role to play in rise in haemoglobin, but also on supply on other nutrients as the molecule is made up of two contents; the haem and the globin. Dietary iron also has an influence on the rise in haemoglobin **[29]**.

The mean grade of Serum Iron on 1st day was 35.063 mg/dL which increased to 70.8 mg/dL at 30th day with the use of bio iron, whereas with elemental iron it increased from 34.75 mg/dL to 69.125 mg/dL with a mean difference of 34.375 mg/dL in comparison to 35.738 mg/dL with use of bio iron. The mean improvement in score was 50.48% which is significant as observed by paired t test (as p value <0.05) thus it can be said that there is significant increment on Serum Iron. The same was found to be 49.73% with the use of elemental iron. This probably might be due to better absorption of iron from the interventional combination as it was a biological form of iron and also

contains other macro and micro nutrients **[29]**.

The mean grade of Serum Ferritin on 1st day was 17.37 mcg/L which increased to 39.71 mcg/L at the end of 30th day. The mean increment was 22.338 mcg/L an improvement of 56.25% which is significant as observed by paired t test (as p value <0.05) thus it can be said that there is significant increment on Ferritin. Similar results were seen with use of iron ascorbate where the ferritin rose from 18.9 mcg/dL to 47.65 mcg/dL the difference of which was found to be 28.75 mcg/dL there was 60.34% improvement seen with the use of iron ascorbate, slightly higher than with the use of bio iron. Ferritin also rises during inflammatory conditions and so is considered as an inflammatory marker. In this study, inflammation was not ruled out on serological parameters, and so, it is difficult to comment whether there were any inflammatory conditions present in the subjects post completion of study, though visually there were none nor there were any conditions reported by the study subjects to suspect so.

The mean grade of TIBC on 1st day was 535.88 which decreased to 339.25 at 30th day. The mean improvementt in score was 57.96% which is significant as observed by paired t test (as p value <0.05) thus it can be said that there is significant fall in TIBC. The fall in Total Iron Binding Capacity was consistent with the findings of rise in haemoglobin levels and serum iron levels. Total Iron Binding Capacity is inversely proportional to the serum iron levels and haemoglobin levels. Similar observations were also seen with the use of iron ascorbate, where TIBC fell from 527.588 to 328.5.

CONCLUSIONS:

The increment in haemoglobin and serum iron level concentration and serum ferritin levels was found to be equally significant with the use of elemental iron and also with bio iron. The rise in haemoglobin and serum iron stores was marginally better with biological form of iron as compared to the elemental iron. The rise in ferritin levels and fall in TIBC were seen to be slightly better with the use of elemental iron. The trial combination does not give rise to any adverse/untoward/unwarranted reactions of the drug as are commonly seen with the commonly used iron salts.

Conflict of interest:

None.

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