



Iron Between Stomach and Intestine

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Abstract

The present study is aimed at establishing the importance of iron absorption in stomach and intestine. The re-precipitation of absorbed iron in intestine may fail to offer any medical solace to patients who suffer from iron deficiency anemia despite taking iron based drugs. Use of herbs to prepare colloidal solutions of metals is known and therefore we have used juice of Eclipta prostrata and Citrus lemon is to prepare soluble iron. The iron prepared as per Siddha system of medicine showed greater bio-absorption and poor re-precipitation at higher pH when compared to commercially available ferrous fumarate. The findings clearly show the medical importance and superiority of Bekay formulated with bio-absorbable iron.

Keywords: Ferrous fumarate; Bekay; Iron; Bio-absorption; Eclipta; Lemon

Introduction

Iron is an important element for most of the vertebrates including human being. The required level of iron is obtained mainly from the food we eat. Deficiency of iron will have serious implications and such condition is called anemia [1-3]. Common reason for iron deficiency is mostly not due its poor presence in our food but due to its poor absorption. Re-precipitation of the absorbed iron in the stomach when it enters into high pH ecosystem of the intestine also affects its absorption.

In order to minimize the problems associated with poor absorption of iron, ferrous fumarate form of iron is widely used as it contains very high percentage of soluble heme iron.

Iron can be processed by several methods to increase its absorption. One of the commonly used methods is treating iron with herbal juices like citrus as it contains natural acid- Citric acid [4,5]. During such process, the iron undergo oxidation and the oxidized iron dissolves in the juice. Such dissolved iron is likely to have great bio-absorption. However, whether such bio-absorbable iron would re-precipitate in intestine due to the pH difference is not clearly known. If such precipitation and the subsequent rejection (excretion) can be stopped, even at low concentration of iron in food or drug, iron insufficiency can be avoided.

The present study is aimed at estimating the quantitative details of non-heme soluble iron present in natural juices that are treated with iron filings for a given period of time and to establish the likely rate of absorption of the iron in stomach and the re-precipitation possibility in intestine. The use of natural juices to enhance the absorption of iron is highly desired because of the natural substances are very safe. The present paper discuss about how the presence of iron and its absorption can vary and how iron insufficiency can be address by following certain formulation nuances that are documented in the ancient traditional system of medicine.

Materials and Methods

Ferrozine method for total iron

In this method, initially the sample is digested in a mixture of nitric acid and hydrochloric acid at 1:1 ratio in order to convert iron into ferric form then the sample is treated with ferrozine reagent [3-(2-Pyridyl)-

5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine, monosodium salt, monohydrate] to forms a purple colored complex with ferric ion, and the intensity of the color is always proportional to the iron content and which is read through colorimeter.

Extraction of soluble and ionizable iron

The 2 g of the sample (Iron prepared in the juice of Eclipta prostrata and Citrus lemon, Bekay, Ferrous fumarate) were mixed separately with 25 ml of pepsin-HCL. The pH of mixture was adjusted to 1.35 with distilled HCL and incubated in a 100 ml conical flask at 37°C in a metabolic shaker water bath for 90 minutes. At the end of incubation, the contents of the flask were centrifuged at 3000 rpm for 45 minutes and the supernatant was filtered using Whatman filter paper no. 44. The sample were divided into two equal halves to determine the soluble and ionizable iron at pH 1.35 and pH 7.5.

One the aliquots was adjusted to pH 7.5 with NaOH and incubated at 37°C for 90 minutes in a metabolic shaker water bath. At the end of this incubation period the contents of the flask was centrifuged at 3000 rpm for 45 minutes, and the supernatant was filtered again. The filtrate was used for the determination of soluble and ionizable iron [6,7].

Bio-absorbable iron

In vitro Bio-absorbable iron was estimated by using standard procedures (Narasinga and Prabhavathi).

Soluble iron

The soluble iron was determined by Tennat and Greenman. The aliquot was digested with potassium permanganate to oxidize ferrous to ferric iron, decolorized with ascorbic acid and filtered. The iron content in the filtrate was determined by colorimetric method using alpha- alpha dipyriddy solution.

By using ferrous sulphate as a standard we have estimated the presence of iron in iron prepared in lemon juice to be 1% the two batches of Bekay formulated with iron prepared in lemon juice showed the presence of iron to be 0.05 and 0.08 mg/ml respectively (Figure 1; Table 1).

Bio-absorption of Iron Using Pepsin

When we checked the percentage presence of non-heme iron in iron prepared in the juice of Eclipta and lemon at pH 1.3, showed the percentage of bio-absorbable iron present is 34.4. The percentage presence of bio- absorbable iron in commercially available ferrous

fumarate tablet was 50. The above percentage of iron was calculated by colorimeter by keeping the ferrous fumarate tablet as standard.

The percentage presence of bio-absorbable iron in iron prepared in the juice of Eclipta and lemon when re-evaluated by adjusting the pH of sample to 7.5, it was found that only 24.3 could be detected. At alkaline pH, 10% reduction in iron was observed in the above sample (Table 2).

Two separate batches of a Bekay prepared with iron in iron prepared in the juice of Eclipta and lemon at 4% when tested showed that the percentage presence of bio-absorbable iron to be 15.5 and 16.5 respectively at pH 1.3. When the above two samples tested at pH 7.5 the percentage of bio-absorbable iron were 18 and 22.2% respectively (Table 2).

Determination of Ferric Iron

When all the above samples at both pH 1.3 and 7.5 checked for oxidation possibility with potassium permanganate, we found that the percentage of ferric iron was 40.8 and 21.0 respectively for pH 1.3 and 7.5 for iron prepared in the juices of Eclipta and Lemon. When the two batches of Bekay were tested by the above method we found the percentage of ferric iron to be 11.8 and 12.8 at pH 1.3 and 10.3 and 8.6 at pH 7.5. The commercially available ferrous fumarate tablet showed the presence of ferric iron to be 50 and 48% respectively for pH 1.3 and 7.5 (Table 3).

Discussion

The present study has clearly demonstrated the fact that it is not the mere presence of iron in the drug/food alone is important but its bio absorption in the stomach as well as its likely non-re-precipitation in intestine only can address the problem of iron deficiency anemia.

We have prepared a bio-absorbable iron as per the traditional method by treating iron filings in lemon and eclipta juice (ref). The iron prepared in lemon and eclipta juice was studied further as well as a traditional drug Bekay formulated with the iron prepared in the juices of lemon eclipta.

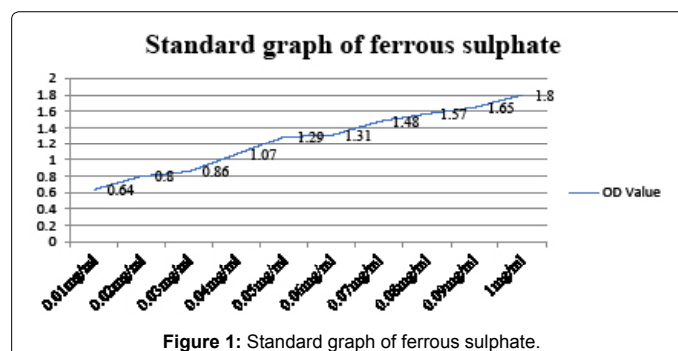
When we tested the bio- absorbable iron prepared in lemon and eclipta juice for the presence of total iron using ferrozine, we found the presence of total iron to be 1%. Similarly, the commercially available ferrous fumarate also showed the presence of total iron to be 1% iron.

Since the total iron tested can be either Fe²⁺ or Fe³⁺ we have decided to study the bio-absorbability of iron *vis-à-vis* pH and the presence of ferric iron.

When we tested the bio-absorbable level of iron in iron prepared in the juices of lemon and eclipta, the sample showed 33.4% of bio-absorbable iron at pH 1.3 and 24.3% at pH 7.5. Nearly 10% dip in the value of bio-absorbable iron was observed at pH 7.5 which indicate the likely possibility of re-precipitation of the iron at pH 7.5 which further suggest such possibility in intestine where its pH is 7.5.

The above findings suggest that the iron prepared in lemon and eclipta juice is not fully bio-absorbable and there is every possibility that at least 10% iron may get re-precipitated in intestine and may get rejected. Therefore, it is not the presence but the bio-absorption of iron and the possibility of no to least re-precipitation at higher pH is more important.

When we tested the commercially available ferrous fumarate tablet we found that the rate of re-precipitation of iron at high pH (7.5) was 46.7 from its initial value of 50% at pH 1.3. The above findings clearly show that the rate of non-absorption possibility of iron in ferrous



Details of the Sample	OD value	Concentration (mg/ml)
Bekay (Batch 161)	1.2	0.05
Bekay (Batch 164)	1.5	0.08
Iron prepared in Eclipta and lemon juice	1.74	1.0
Ferrous fumarate tablet	1.77	1.0

Table 1: Determination of the presence of total iron by ferrozine method.

Details of the Sample	pH/OD value at 480 nm and % non heme			
	1.3	%	7.5	%
Bekay (Batch 161)	0.48	15.5	0.52	18*
Bekay (Batch 164)	0.51	16.5	0.64	22.2*
Iron prepared in Eclipta and lemon juice	1.03	33.4	0.70	24.3
Ferrous fumarate tablet	1.54	50	1.44	46.7

*Increased OD value of Bekay 161 and 164 at pH 7.5 was due to the colour interference of the herbal moiety and hence it as reflected as higher presence of iron. The absolute correction value was taken therefore from the bio-absorbable iron solution prepared in lemon juice that was used in Bekay

Table 2: Determination of % presence non- heme iron by Alpha- Alpha Dipyriddy solution.

Details of the Sample	pH/OD value at 480 nm and % non heme			
	1.3	%	7.5	%
Bekay (Batch 161)	0.35	11.8	0.3	10.3
Bekay (Batch 164)	0.38	12.8	0.25	8.6
Iron prepared in Eclipta and lemon juice	1.21	40.8	0.61	21
Ferrous fumarate tablet	1.48	50	1.45	48

Table 3: Determination of ferric form of iron.

fumarate is much higher than the iron that we have prepared in lemon juice by following the ancient traditional scripture. This suggests that the iron prepared in lemon juice by us has greater absorption of iron barring of the pH interference.

We have tested a Siddha drug Bekay which is formulated using the iron prepared in lemon juice. The findings show that the rate of re-precipitation of iron at higher pH (7.5) from Bekay was relatively less. We could not accurately calculate the level of iron that got re-precipitated at pH 7.5 because of the strong color interference of Bekay which had affected the colorimetric reading.

We have tested the ferric form of iron present in iron prepared in the juice of Eclipta and lemon using potassium permanganate. We found that the percentage of iron that could be oxidized in iron prepared in lemon juice was 40.8 and 21 respectively for pH 1.3 and pH 7.5.

In ferrous fumarate, the percentage of iron that could be oxidized

was 50 and 48 respectively at pH 1.3 and pH 7.5. The above findings reconfirm the fact that the iron prepared in lemon juice was superior over ferrous fumarate.

The Siddha drug Bekay formulated with iron prepared in iron prepared in the juice of Eclipta and lemon at 4% when estimated, the presence of bio-absorbable iron in Bekay was 15% irrespective of the pH whether it is acidic or alkaline.

The present study clearly shows that the ancient Siddha system is rich in science, vibrant and has perfect understanding about human physiology, digestion, assimilation etc., and also the chemistry of iron as well. Nevertheless, the traditional drug Bekay formulated with iron prepared in the juice of Eclipta and lemon lemon juice indeed superior and therefore it can play a significant role in addressing iron deficient anemia as the rate of re-precipitation and rejection of bio-absorbable iron in Bekay is very less.

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