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An Evaluation of Bioavailability Enhancement of Organic Elemental Iron with BioPerine® in Rabbits

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ABSTRACT

Iron being an essential trace mineral is found in every cell of the body, the deficiency of iron is highly prevalent and is one of the foremost health concerns worldwide, with the cause of deficiency primarily being poor bioavailability and / or poor dietary intake. Beans being considered a potential source of iron, can have a considerable role in cases of iron deficiency. BioPerine® has been reported to increase the bioavailability of nutritional compounds owing to its thermogenic property and termed as a natural thermo-nutrient and bioavailability enhancer. The current novel study was designed to evaluate the improvement in the bioavailability of organic elemental iron in combination with and without BioPerine® in rabbits, taking into consideration the widespread iron deficiency and in order to establish and validate the property of BioPerine® in improving the bioavailability of organic elemental iron (BioIron). Rabbits were split into two groups to receive orally a single dose of either BioIron with BioPerine® (sample I) or BioIron without BioPerine® (sample II). Blood samples were collected at 0, 30, 60, 120, 240, 360, 480 minutes, 12, 24, 36, 48 and 60 hours after administration of the samples I and II. Samples and standards were prepared for estimation of iron and analysed by ICP-MS method. The iron content was determined by a calibration graph and the results were expressed in µg/ml at different time intervals. In conclusion, the iron bioavailability as observed in the serum was significantly higher in animals treated with BioIron containing BioPerine® (36.55 ± 9.97µg/ml at 8h) in comparison to treatment with BioIron without BioPerine® (4.730 ± 0.94µg/ml at 24 h); thus, validating the property of BioPerine® which can be used as a key ingredient in enhancing the bioavailability of organic elemental iron.

1. INTRODUCTION

Iron (Fe), an essential mineral found in the human body, is a part of the oxygen-carrying proteins haemoglobin in red blood cells and myoglobin in muscles [1]. Iron is also available in proteins and enzymes. Iron as an essential micronutrient plays a vital role in oxygen transport, oxidative metabolism, cellular proliferation and many other physiological processes. It is a redox metal participating in most of the reversible one electron oxidation-reduction reactions by switching between the two oxidation states, ferrous and ferric. The human body has therefore developed intricate but exquisitely controlled mechanisms to absorb, transport and store iron, thus ensuring a ready supply for cellular growth and function but limiting its participation in reactions that produce free radicals and its availability to invade pathogens. Iron can be stored in the body and utilized only when dietary iron is deficient. Iron deficiency in humans may occur when there is a low level of iron in the blood or when the body's iron storage goes low. Iron deficiency anaemia can be a result of numerous factors, such as low intake of dietary iron, low absorption and excessive blood loss. However, anaemia is widespread in India in spite of diversity in food habits, particularly in the consumption of cereals and such tight metabolic regulation. The causality between poor dietary iron density, bioavailability and high prevalence of anaemia in our population has not been well established, as anaemia has a multi-factorial aetiology [2].

The bioavailability of iron from foods is ultimately determined by interactions between iron and other components in the digestive milieu. Several factors contribute to iron bioavailability such as meal composition, oxidation status, promoter substances ("meat factors"), metabolic demand for iron and genetic inclination for iron absorption [3].

There are two types of iron that can be obtained from food: Heme and Non-heme. Heme iron is primarily found in meat, fish and poultry. Beans, lentils and grains are a good source of non-heme iron. However, non-heme is less bioavailable than heme iron [4].

Non-heme iron absorption is inhibited by phytic acid, which is found in whole grains and legumes, and by polyphenols such as tannins which are found in tea, coffee and red wines [5, 6]. In humans who consume moderate amounts of red meat, heme iron accounts for almost half of the iron absorbed in the body [6, 7]. In humans with low iron stores, non-heme iron is absorbed more than heme iron [6]. Although non-heme iron is less bio-available than heme iron, it has not

been established whether this has any adverse consequences – vegetarians typically have lower iron stores than omnivores, but they have no greater incidence of iron deficiency.[8]

However, a recent study that examined the bioavailability of iron in 24 genotypes of bean seeds containing a range of concentrations of iron, phytic acid and tannin showed that tannin and phytic acid concentrations did not affect bean iron bioavailability. [9]

Ferritin iron, a storage form of iron in legumes is highly bioavailable, even when the phytate (salt form) concentration is high [10-12].

BioIron is a natural organic iron supplement from green gram (*Phaseolus aureus*). Mung beans or green soy beans through the technology of hydroponics is enriched with iron by a soil-less process. This remains the source of elemental iron used in BioIron. Improved varieties of mung bean can contain 0.06 g of iron kg^{-1} raw grain [13] compared with traditional mung bean varieties with only 0.03 to 0.035 g [14]. A proprietary hydroponics process is used to enrich the beans to contain 15000-17000 ppm of elemental iron.

BioPerine® is a standardized extract from the fruits of *Piper nigrum* L (black pepper) or *Piper longum* L (long pepper). It contains a minimum piperine content of 95% compared to the 3-9% found in raw forms of *Piper spp*. BioPerine® may be co-administered with various nutrients for both human and animal health [15].

A thermo-nutrient such as BioPerine® would potentially improve the process of nutrient absorption by enhancing thermogenesis. In view of these findings, it is proposed that BioPerine® ingested in relatively small amounts would act as a thermo-nutrient. Localized thermogenic action on the epithelial cells would in turn increase the rate of absorption of supplemented nutrient(s) [16].

Hence, the current study was performed to evaluate the bioavailability of organic elemental iron (BioIron) with and without BioPerine®.

2. MATERIALS AND METHODS

2.1. Chemicals

Standard iron solution of 10 ppm concentration and aqua regia (3:1 Nitric acid: Hydrochloric acid). BioIron tablets (containing BioPerine®) with batch/lot no. FD/JJ0715/SD-14 (sample I) and Bio-Iron tablets (without BioPerine®) with batch/lot no. FD/JJ0815/SD-04 (sample II) was provided by the sponsor (M/s. Sami Labs Ltd). All the reagents used were of analytical grade.

2.2. Procedure for preparation of standards for estimation of Iron (Fe).

2.2.1. Preparation of Standard iron (Fe) solution 10ppm:

1ml of the readymade 1000ppm stock solution was pipetted out, transferred into 100ml volumetric flask and volume made up to the mark with millipore water.

1) Preparation of calibration standard iron (Fe) solutions:

An appropriate volume of above 10ppm standard solution was pipetted out into 5 different standard volumetric flasks and diluted up to the mark with millipore water to obtain 1, 2, 5, 10 and 25 ppb of Fe standard solutions for calibration.

2) Preparation of sample and blank:

Equal quantity of samples was taken into microwave digester Teflon vessel and 9ml of aqua regia (3:1 Nitric acid: Hydrochloric acid) was added. Microwave digestion was carried out as per instrument protocol and the volume made up to 10ml with Millipore water. The digested sample was injected into Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the iron (Fe) estimation. As an internal control, blank sample was prepared in a same manner without serum.

3) Estimation of iron (Fe) in Bio-Iron tablets by ICP-MS.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was utilized for estimation of iron (Fe) in serum. ICP-MS instrument (Make-Agilent, Model-7700) was tuned by aspirating the tuning solution. The instrument was calibrated by aspirating the calibration standard solution in sequence to have Correlation coefficient >0.995. Further sample analysis was carried out and the iron content was calculated from the calibration graph. The results obtained were expressed as µg/ml at different time intervals.

2.3. Animals

For evaluation of bioavailability, twelve in house breed rabbits were selected and grouped manually. No computer generated randomization program was used. All the study animals were acclimatized under laboratory conditions for 5, 7 and 9 days for Step I, Step II and Step III respectively after veterinary examination. Only animals without any visible signs of illness were used for the study.

The animals were identified by unique cage number and individual animal numbers marked with indelible marker pen on the tail. The animals were marked (towards the tip of tail) with the temporary animal numbers at start of acclimatization. The animals were marked with permanent animal numbers (towards the base of tail) with different colour indelible marker pen before the start of test item administration.

2.4. Ethics

The bioavailability study was performed in accordance with the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility, and as per OECD guidelines.

2.5. Dosing procedure

The test substance was administered to each rabbit as a single dose through the feeding tube orally. The blood samples were collected through the marginal ear vein of the rabbits in centrifuge tubes at 0, 30, 60, 120, 240, 360, 480 minutes, 12 hours, 24 hours, 36 hours, 48 hours and 60 hours after the test substance administration. The blood samples were centrifuged at 5000 rpm for 10 min to separate the plasma for analysis. The plasma samples were stored in sterile tubes at -80^{o}C until analyzed.

3. RESULTS

In this bioavailability study, all the rabbits of Group I were administered test sample I with batch/lot no. FD/JJ0715/SD-14. It was observed that by 8 hours maximum iron levels of 36.55 ± 9.97 µg/ml (Fig 1) were observed in serum. While rabbits in Group II were administered sample

II with batch/lot no. FD/JJ0815/SD-04, the maximum iron levels in serum were found to be 4.730 ± 0.94 µg/ml (Fig 1) at 24 hours.

4. DISCUSSION

Iron deficiency anaemia is a major public health concern. The high incidence is either due to insufficient intake of iron or poor bioavailability. Enhancing the bioavailability of iron is as important as increasing the intake [17]. The current study was planned in rabbits, wherein the bioavailability of Bioiron (derived from mung beans) was evaluated in the presence and absence of BioPerine® (pepper standardized extract).

In addition to Mung beans value as a protein rich food, it also has relatively high iron content. Since mung beans contain neither inhibitors nor enhancers that influence iron bioavailability [18], the iron bioavailability of mung beans can in turn be enhanced by combining it with a bioavailability enhancer such as BioPerine®.

The leading theory of food-induced thermogenesis relates to the autonomous nervous system. The autonomous nervous system is represented by two main receptors in the gastrointestinal tract, the alpha and beta adrenergic receptors. Most of the food or thermo nutrient-induced thermogenesis is facilitated by beta receptors, such a cyclic adenosine 3', 5' monophosphate (cAMP). The role of cAMP as a "second messenger" to the hormonal and enzymatic actions in the body is well recognized. When thermogenesis occurs, the demand for fresh nutrients to sustain the metabolic processes rapidly increases.

Piperine has been found in independent studies to stimulate the release of catecholamines, thermogenic hormones whose action is made possible by the presence of cAMP. However, the nature of the thermogenic response mediated by catecholamines is relatively short-lived. Therefore the window of opportunity for piperine-induced thermogenesis and enhanced nutrient absorption is narrow.

These thermogenic properties may explain how a small amount of BioPerine® (5mg) can afford such a profound effect on serum nutrient levels (as shown in our studies on water soluble, fat soluble and botanical ingredients). It is possible that when piperine is ingested, it has a localized thermogenic effect on epithelial cells which increase the uptake of nutrients. Other mechanisms

by which piperine stimulates nutrient absorption include increased micelle formation, stimulation of active transport of amino acids (gamma-glutamyltranspeptidase), and epithelial cell wall modification due to the affinity of piperine towards fats and fatty substances[16].

Piperine (1-piperoyl peperidine) is responsible for the bioenhancing effect of BioPerine®. It has been shown to possess bioavailability-enhancing property that may be attributed to increased absorption of organic elemental iron (Bioiron) due to alteration in membrane lipid dynamics and change in the conformation of enzymes in the intestine [19].

Hence in the current study, the group of rabbits receiving BioIron along with BioPerine® showed greater iron bioavailability in comparison to the group receiving BioIron alone.

5. CONCLUSION

From the results, it is evident that the animals from Group I receiving sample I with batch no FD/JJ0715/SD-14 showed more iron bioavailability in comparison to animals from Group II receiving sample II with batch no. FD/JJ0815/SD-04. Hence, it can be concluded that the sample I of BioIron containing BioPerine® has greater iron bioavailability.

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Conflict of interest

The authors declare that they have no competing interests.

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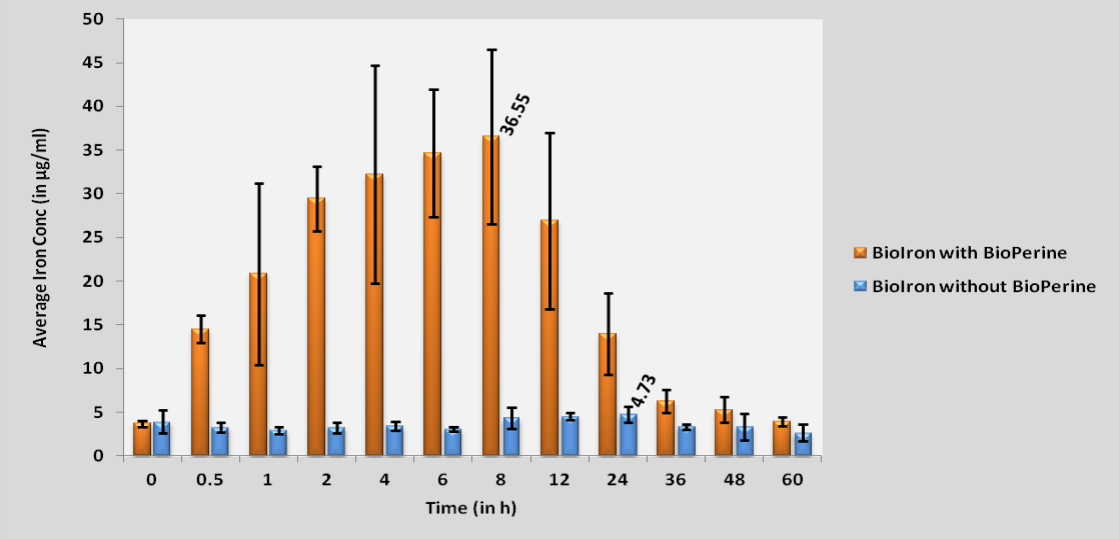

Fig 1: Iron levels in the serum samples from animals treated with BioIron with BioPerine® (FD/JJ0715/SD-14) and BioIron without BioPerine® (FD/JJ0715/SD-04)