Amla (*Phyllanthus emblica* L.) enhances iron dialysability and uptake in *in vitro* models

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***Phyllanthus emblica* L. (Indian gooseberry or *amla*) is a well-known dietary supplement (*Rasayana*) in Ayurveda used in the management of iron deficiency anaemia (*Pandu*). *Amla* is said to act by regulating the 'metabolic fire' (*agni*), which is important for proper digestion and absorption of nutrients. In the present study standard cell-free and cell-based models that are employed in biomedical sciences to study digestion and bioavailability of nutrients were used to examine the influence of *amla* fruit juice on iron dialysability and uptake. *Amla* juice contained 0.35% ascorbic acid (AA), 0.33% tannins (gallic acid equivalent), 0.13% gallic acid, 0.58% total organic acid and 0.002% iron on a w/w basis. *Amla* juice exhibited a dose response to iron dialysability with an optimum at 1 : 0.25 molar ratio of Fe : *amla* juice (AA equivalent) in the cell-free digestion model and 1 : 0.5 in both Caco-2 and HepG2 cell lines. *Amla* juice increased the dialysable iron three times more than the $FeSO_4$ alone control in the cell-free digestion model. Iron uptake in Caco-2 and HepG2 cell lines increased 17.18 and 18.71 times more than the control respectively, in the presence of *amla* juice. AA, a known Fe bioavailability enhancer, at the same molar ratios showed an enhancement only by 1.45 times in the cell-free model and 13.01 and 12.48 times in the Caco-2 and HepG2 models respectively. As a dietary supplement that enhances iron dialysability and uptake, *amla* fruits can be explored further as a low-cost intervention in the management of iron deficiency anaemia.**

Keywords: Anaemia, *amla*, iron bioavailability, *in vitro* digestion model.

IRON deficiency anaemia (IDA) is the result of long-term negative iron balance and is the most common nutritional disorder in the world. It is the only nutrient deficiency which is prevalent both in industrialized as well as developing nations, particularly where diets are based on cereals and legumes[1]. According to the biomedical understanding, mainly three factors determine the amount of iron absorbed from the diet, namely the amount of iron ingested, its availability for absorption in the body, and iron status of the individual. Bioavailability represents integration of processes whereby an ingested nutrient becomes available for digestion, absorption, transport, utilization and elimination[2]. The amount of ingested iron that is available for use in metabolic processes or deposition in storage forms like ferritin is a key factor in iron nutrition[3]. The bioavailability of iron can be significantly influenced by the presence of factors such as organic acids and phytates in food that may enhance or reduce its absorption and utilization. L-Ascorbic acid (AA) or vitamin C for example is a known iron bioavailability enhancer, while phytates in cereals and legumes impair absorption of native iron from foods as well as from soluble and poorly soluble iron salts[4].

Understanding the world views of traditional medical systems can help us gain new insights into disease diagnosis, etiology and management. IDA is correlated to a disease entity called *Pandu* in Ayurveda, an Indian medical system[5]. According to Ayurveda, one of the important causes of IDA is the derangement in *jataragni*, the digestive and absorptive 'fire' in the body due to which the nutrients are not absorbed. *Jataragni*, in turn is balanced by *pachakapitta*, the *pitta* that drives metabolic processes in the proximal gastrointestinal tract. *Pitta* is one of the three humors that control all metabolic transformations in the body[6]. Undigested or partially digested food by the deranged *agni* (~metabolism) causes the production of *ama* (~undigested materials) that blocks natural assimilative and eliminative processes. This is the root cause of most diseases[7].

One of the large-scale programmes to control IDA in modern times has been medicinal supplementation with iron (ferrous sulphate) and folic acid, which has not had much success[8]. One of the reasons for this could be that the iron is not properly assimilated by the body. In classical Ayurvedic texts, there is rich information on the management of *Pandu* using *Rasayana* herbs that 'enhance *agni*' (*agnivardhaka*). *Rasayana* is a branch of Ayurveda that explains methods to nourish body tissues (*dhatu*) by optimizing metabolism and eliminating wastes thus maintaining good health[9]. *Phyllanthus emblica* L. (Euphorbiaceae), commonly known as *amla*, is one such *Rasayana* which is used in the management of *Pandu*[10]. It is also used as an acidulant, diuretic and in the treatment of dysentery, jaundice, skin conditions, eye diseases and diabetes[11].

We hypothesize that certain *Rasayana* herbs like *amla* which are said to play a role in digestion and absorption can enhance the dialysability and uptake of iron. This could be one of the reasons why in the Ayurvedic management of *Pandu*, *amla* is used as a dietary supplement (*pathya*) and in drug formulations. For example, Dhatri loham is a herbo-mineral formulation containing *amla* and other herbs along with iron that is used for treating *Pandu*[12].

The present study investigates the effect of phytochemically standardized *amla* fruit juice on dialysability

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and uptake of iron using cell-free[13] and cell-based[14] (Caco-2) *in vitro* digestion and absorption models. Additionally, the effect of *amla* dialysates on the uptake of iron in HepG2 cell model was also studied, because hepatocytes play a crucial role in iron transport, storage and regulation of iron homeostasis. In IDA, iron depletion occurs not only in the serum but also in the liver where it is stored as ferritin[15]. Iron uptake in hepatocytes is normally mediated by transferrin (iron carrier protein). However, in instances when transferrin is unavailable or saturated due to iron overload, iron uptake is also facilitated by non-transferrin-bound iron (NTBI) forms[15]. Any enhancement in the level of dialysed iron is likely to proportionately enhance iron levels in the hepatocytes, provided the dialysed iron is taken up.

AA was purchased from HiMedia (Mumbai, India); 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulphonic acid (ferrozine), digestive enzymes porcine pepsin (800–2500 units/mg protein), pancreatin (activity equivalent to 4 × USP specifications (United States Pharmacopeia)), bathophenanthroline disulphonic acid, epidermal growth factor (EGF), triiodothyronine (T3), sodium selenite, insulin, hydrocortisone, PIPES, trypan blue, sulphorhodomine B (SRB), trichloroacetic acid, chelex-100, phosphate buffered saline (PBS) and Bradford reagent were purchased from Sigma Chemicals (St Louis, USA); bile salt (sodium tauroglycocholate) was purchased from Loba Chemie (Mumbai, India); hydroxylamine hydrochloride and sodium hydrogen carbonate were purchased from Fisher Scientific (Mumbai, India), and iron standard was obtained from Fluka Analytical. Minimum essential medium (MEM), 0.25% trypsin-EDTA (ethylenediamine tetracetic acid), Pen-Strep and foaetal bovine serum (FBS) were purchased from GIBCO (Auckland, New Zealand). All other chemicals, reagents and solvents were of analytical grade and purchased locally. Water used in the preparation of reagents was double deionized.

Fresh fruits of *amla* were purchased from local Bangalore market, India and identified by a qualified botanist and an Ayurvedic practitioner at FRLHT-I-AIM (Institute of Ayurveda and Integrative Medicine). A voucher specimen was deposited at FRLHT Herbarium, Bangalore. Fruits were washed, cleaned and made into pulp using a domestic blender and strained through muslin cloth to obtain juice. The juice was then aliquoted into 50 ml screw-cap Falcon tubes and stored at –80°C until use.

Qualitative analysis for alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, resins, phenolic acids and tannins, protein and amino acids, flavonoids, gums and mucilage in *amla* juice was performed using methods of Raaman[16]. *Amla* juice was also estimated for AA[17], tannins[18], total organic acid content[19] and iron[20]. Iron content in the dialysate was estimated colorimetrically using ferrozine according to the method of Kapsokefalou and Miller[21] and was taken as a measure of iron bioavailability.

Analysis was performed on TLC silica gel 60 F254 plates (Merck, USA) of uniform thickness. The plates were developed in the solvent system ethyl acetate : formic acid : acetic acid : water (7.2 : 1 : 1 : 0.8) for fingerprinting using the method of Patel and Telange[22]. After development, the plate was observed for bands under UV 254 and 366 nm. The plates were also derivatized with 5% methanolic sulphuric acid, heated to 110°C and observed under visible light.

HPLC fingerprint analysis and quantification of AA, citric acid, malic acid and gallic acid in juice were performed on a Thermo Scientific, Surveyor HPLC system, controlled by XCalibur software with a PDA detector. A reverse phase Purospher, Hibar (Merck, USA) C-18, 250 × 4.60 mm column was used.

In vitro digestion was performed according to the protocol given by Miller *et al.*[13], with modifications to suit a six-well plate.

Blank contained only 0.01 N HCl; $FeSO_4$ control mixture contained 5 ml ferrous sulphate ($FeSO_4 \cdot 7H_2O$) stock solution (with 2 mg Fe equivalent); *Amla* juice control group contained different molar ratios of Fe and *amla* juice (represented as AA equivalent) and pure compound AA control group equivalent to AA present in *amla* juice.

Test mixtures contained $FeSO_4$ + AA with 2 mg Fe and AA (different molar ratios); $FeSO_4$ + *amla* juice mixture contained 2 mg Fe and *amla* juice (different AA equivalents). AA or *amla* juices in the test mixtures were added just prior to the start of digestion to avoid oxidation of AA. Molar ratios 1 : 0.007 to 1 : 4 of $FeSO_4$: *amla* juice (Fe equivalent : AA equivalent) were tested to determine the dose response and to arrive at optimum molar ratio for further experiments. Using the optimum molar ratio 1 : 0.25 (see later in the text), the effect of *amla* juice on dialysable iron was studied in comparison with the positive control AA employing *in vitro* digestion model.

Each of the test and control mixtures and blank was incubated in a shaking water bath at 37°C for 10 min after adjusting the pH to 2.0 using 6 M HCl/1 M $NaHCO_3$. Pepsin solution, 0.64 ml (1.60 g of pepsin brought to 10 ml with 0.1 N HCl) was added to each of the samples which were then incubated at 37°C, shaking at 200 rpm for 2 h.

To pepsin digest, 0.5 ml pancreatin–bile salt mixture (40 mg pancreatin and 250 mg bile suspended in 10 ml 0.1 M $NaHCO_3$) was added and titrated against 1 M $NaHCO_3$ till pH increased to 7.5. The volume of titratable 1 M $NaHCO_3$ was recorded.

Simulated pancreatin–bile digestion and dialysis were carried out in six-well plates (Becton Dickinson, USA). The opened dialysis membrane (nominal MW cut-off 6000–8000 Da, Fisher Scientific, Pittsburgh, USA) was fixed to the base of glass. Then 1 M $NaHCO_3$ equivalent to the volume of titratable acidity was added to each well and made up to 2.5 ml with distilled water. The pepsin digest (2 g) obtained was kept on the upper chamber of

the insert and was placed on the well with the membrane just immersed in 1 M $NaHCO_3$. The plates were incubated on a shaking water bath at 37°C for 30 min or until the pH of the digest increased to 5. Pancreatin–bile salt mixture (0.5 ml) was then added to the samples and incubation was continued for 2 h in the shaking water bath at 37°C. At the end of this incubation period, the insert with the digest was removed. The dialysate was collected from the well and stored as aliquots in 2 ml microfuge tubes at –80°C until further use.

Iron content in dialysate was estimated colorimetrically using ferrozine according to the method of Kapsokefalou and Miller[21]. This was taken as a measure of iron bioavailability.

The test (Fe + Am) and control dialysates (Fe/AA/Am alone and blank) were used to study the iron uptake in Caco-2 and HepG2 cells. The human colorectal adenocarcinoma cell lines (Caco-2) and human liver hepatocellular carcinoma (HepG2) cell lines were obtained from NCCS, Pune, India. The cells were maintained in MEM with 10% FBS and 1% antibiotic–antimycotic solution at 37°C in 5% CO_2. The protocol described by Glahn *et al.*[14] was followed with modification to study the effect on iron uptake. Dialysates were prepared as in the case of cell-free digestion model discussed above and this dialysate was filter-sterilized and added to the confluent cell monolayer.

The iron uptake study was carried out as follows. Caco-2 (passages 35–45) and HepG2 (passages 22–33) cells were seeded in six-well plates at a density of 1.0×10^5 and 6.0×10^4 per well respectively. The medium was changed every two days. After 5 days, the medium was removed and constituted with MEM medium containing growth factors (10 mmol/l PIPES, 4 mg/l hydrocortisone, 5 mg/l insulin, 5 µg/l sodium selenite, 34 µg/l T3, 20 µg/l EGF) and incubated for further 10 days. Post 10–13 days of incubation, when the cells reached 90% confluency, the medium was removed and cells were washed twice with PBS. MEM medium containing growth factors (1 ml) and 1 ml of the filter-sterilized control and test dialysates were added directly to the cells. The cells were then incubated at 37°C under 5% CO_2 – 95% atmospheric O_2 for 18–20 h.

Subsequent to incubation, the medium was removed from the wells and cells were washed with ice-cold saline (0.9% NaCl). Cells were then washed three times with 500 µl of stop solution (140 mmol/l NaCl and 10 mmol PIPES – pH 6.7, 4°C). This was followed by washing with 500 µl of removal solution (140 mmol/l NaCl, 10 mmol/l PIPES, 5 mmol/l bathophenanthroline disulphonic acid – pH 6.7). Cells were then solubilized in 0.5 M NaOH (500 µl per each well) and harvested. They were stored at –80°C until further processing (for total iron and total protein estimation).

Iron was estimated in cell lysate using ferrozine using the method described by Fish[20] with modifications. Briefly, 100 µl cell lysate was mixed with 100 µl distilled water and 100 µl iron-releasing agent (1.4 M HCl and 4.5% w/v $KMnO_4$ in water and freshly mixed in 1 : 1 ratio). The mixture was then incubated at 60°C for 2 h. It was then cooled to room temperature and 30 µl iron detection reagent (6.5 mM ferrozine, 2.5 mM ammonium acetate and 1 M ascorbic acid in water) was added. Absorbance was measured at 550 nm after 30 min incubation at room temperature. The amount of iron taken up by the cells was expressed as µg iron/mg total protein.

Total proteins in the cell lysate were estimated by Bradford assay[23].

Qualitative phytochemical analysis of *amla* juice indicated the presence of saponins, phenolic acids, tannins, flavonoids, gums and mucilage. Alkaloids, phytosterols, glycosides, resins, fixed oils and fats were found to be absent in *amla* fruit juice. *Amla* juice contained 0.35% w/w AA, 0.33% tannins (expressed as gallic acid equivalent), 0.13% gallic acid, 0.58% total acid content and 0.0021% iron.

HPTLC profile of *amla* juice was observed under UV 254 nm, 366 nm and white light (Figure 1 *a*) after spraying 5% methanolic sulphuric acid. The densitometric scan of TLC plate was done at multiple wavelengths. Figure 1 *b* shows densitometric scan of *amla* juice at UV 255 nm.

HPLC fingerprint pattern of *amla* juice (Figure 2) showed clear peaks for malic acid (R_t 8.94 min), AA (R_t 10.05 min), citric acid (R_t 19.17 min) and gallic acid (R_t 42.72 min). Further, freezing of *amla* juice at –80°C did not alter the HPTLC fingerprint and AA content was estimated to be 0.35 ± 0.025% w/w, when tested over a period of six months.

Amla juice increased bioavailability of iron from $FeSO_4$ in a concentration-dependent manner from 1.17 ± 0.03% to 3.08 ± 0.03%, as shown in Figure 3 *a*, when added at molar ratios of 1 : 0.007–1 : 0.25 of $FeSO_4$: *amla* juice (AA equivalent) in the mixtures. At molar ratios of 1 : 0.50 and above, significant inhibition in iron bioavailability was observed. Thus the optimum molar ratio for studying the effect of *amla* juice on iron bioavailability was selected as 1 : 0.25 Fe : *amla* juice (as AA equivalent). At this ratio, iron bioavailability was enhanced from 1.09 ± 0.012% to 3.40 ± 0.029% by *amla* juice (3.09 times) and to 1.62 ± 0.023% by AA (1.45 times), as shown in Figure 3 *b*.

The iron uptake by Caco-2 cells increased in a dose-dependent manner from 0.55 ± 0.06 to 9.54 ± 0.14 µg/mg protein when 1 : 0.03 to 1 : 0.5 of *amla* juice (AA equivalent : Fe molar ratio) was added, peaking at 1 : 0.5, as shown in Figure 4 *a*. At higher molar ratios (e.g. at 1 : 1 to 1 : 4), iron uptake was inhibited. *Amla* juice increased iron uptake at the molar ratio of 1 : 0.5 of Fe : Am (AA equivalent) by 17.18 times (from 0.55 ± 0.11 to 9.54 ± 0.67 µg Fe/mg protein), while there was only 13.01 times (from 0.55 ± 0.11 to 7.11 ± 0.20 µg Fe/mg protein)

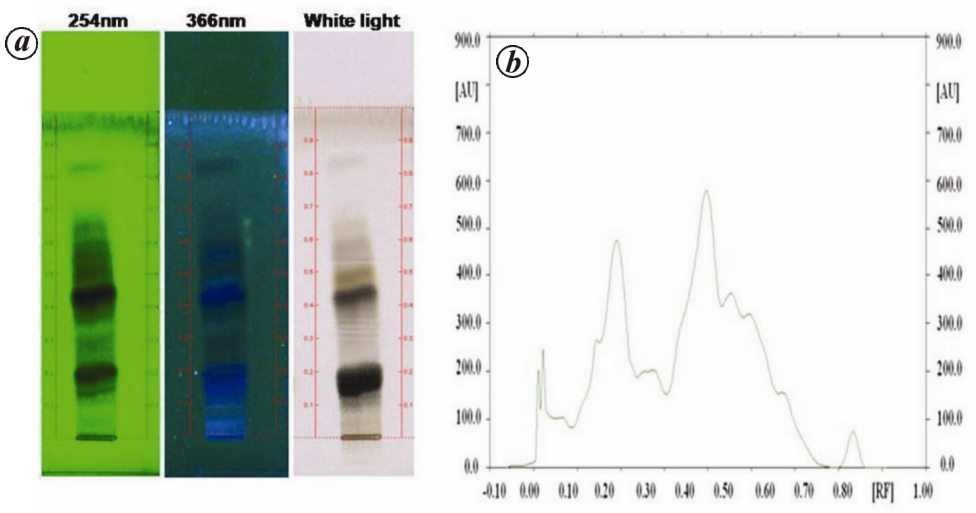

Figure 1. ***a***, HPTLC chromatogram of *amla* juice fingerprint at 254 nm, 366 nm and visible light. ***b***, HPTLC densitometric scan of *amla* juice at UV 255 nm.

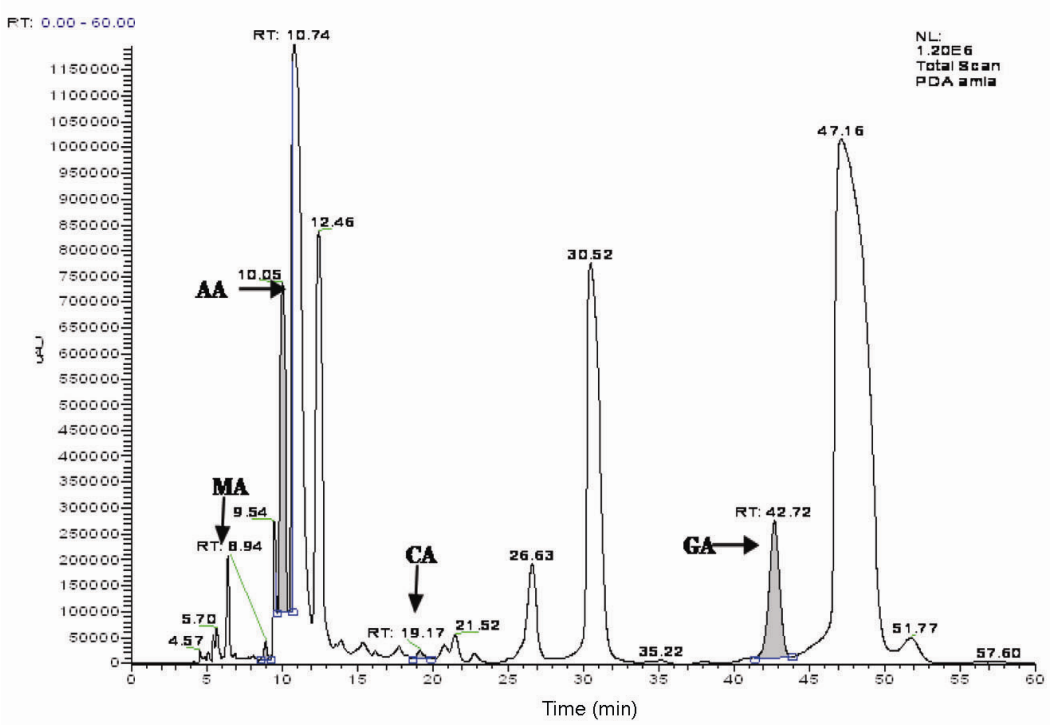

Figure 2. HPLC fingerprint of *amla* fruit juice showing malic acid (MA), ascorbic acid (AA), citric acid (CA) and gallic acid (GA).

increase in iron uptake in the presence of AA at the same molar ratio (Figure 4 *b*).

Amla juice enhances iron uptake by HepG2 cells: *Amla* juice enhanced the uptake of iron in HepG2 cells significantly from 0.56 ± 0.06 to 11.64 ± 0.28 µg/mg protein at molar ratio 1 : 0.03 to 1 : 0.5, where it was highest (Figure 5 *a*). The iron uptake increased from 0.62 ± 0.06 to 11.78 ± 0.21 µg/mg protein (19 times) in the presence of *amla* juice at 1 : 0.5 molar ratio of Fe : *amla* juice (AA equivalent) in comparison to the group containing only $FeSO_4$ (Figure 5 *b*). There was only 12.64 times (0.62 ± 0.06 to 7.84 ± 0.17 µg/mg protein) increase in iron uptake in the presence of AA at the same molar ratio. At molar ratios greater than 1 : 0.5 of Fe : *amla* juice (AA equivalent), iron uptake showed a significant decline, even though it was still higher than the $FeSO_4$ alone control.

Exploring the fundamental principles behind traditional medicine, using appropriate transdisciplinary models can unearth new perspectives to biomedicine that would be of contemporary relevance. According to Ayurveda,

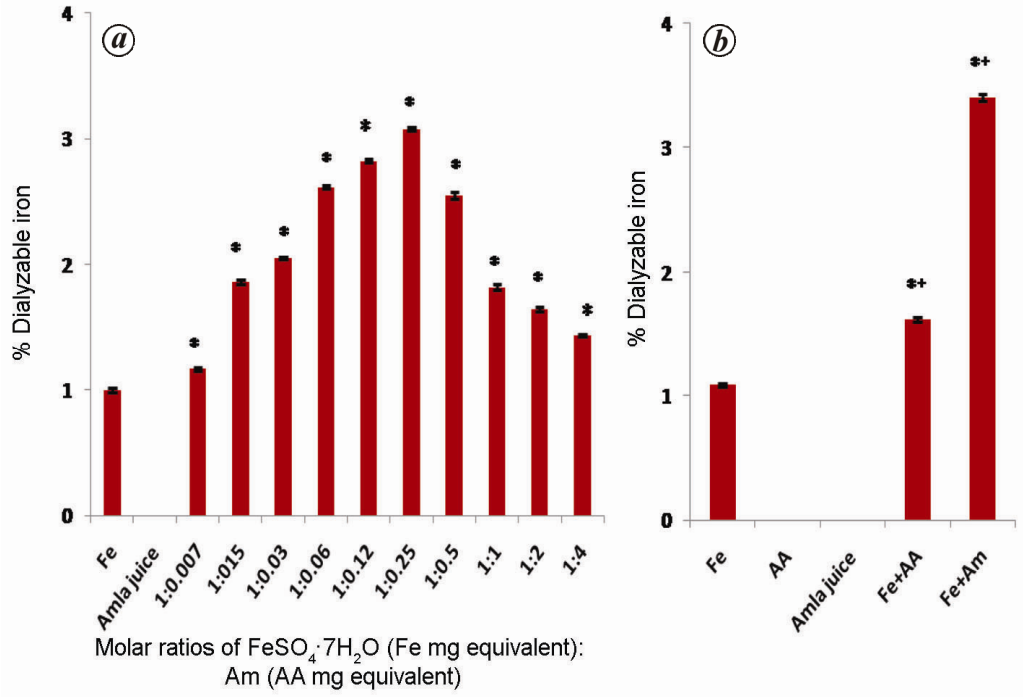

Figure 3. Effect of *amla* juice on bioavailability of iron (Fe) in cell-free digestion model. ***a***, Dose response of iron dialysability to *amla* juice. Iron dialysability was studied at different Fe : *amla* juice (AA equivalent) molar ratios. ***b***, Dialysable iron in the presence of 1 : 0.25 molar ratio of Fe : AA equivalent in *amla* juice. This was compared with dialysability in the presence of AA at the same molar ratio (Fe : AA = 1 : 0.25). Error bars represent standard error of mean values of the respective groups ($n = 8$). '*' and '+' show P values <0.05 for *amla*-treated groups compared to the Fe alone control and AA-treated groups respectively.

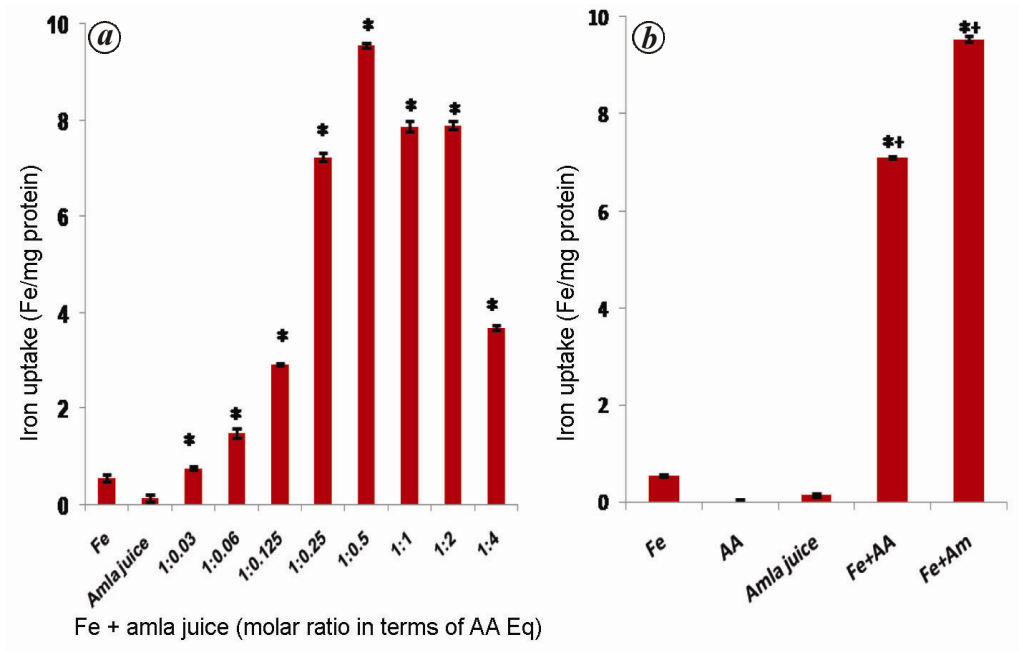

Figure 4. Effect of *amla* juice containing dialysates on iron uptake by Caco-2 cells. ***a***, Dose response of iron uptake by Caco-2 cells in the presence of *amla* juice containing dialysates. The effect of *amla* juice on the uptake of Fe was studied at different molar ratios of Fe and AA mg equivalent in *amla* juice and compared with Fe or *amla* juice alone. ***b***, Effect of AA and *amla* juice (AA mg equivalent) containing dialysates on iron uptake by Caco-2 cells at 1 : 0.5 molar ratio. Bars represent mean per cent dialysable iron calculated according to the formula. Error bars represent standard error of mean values of the respective group ($n = 9$). The basal cellular iron and iron content increment in the presence of blank meal were subtracted from the test groups. '*' and '+' show P values <0.05 for *amla*-treated groups compared to the Fe alone control and AA-treated groups respectively.

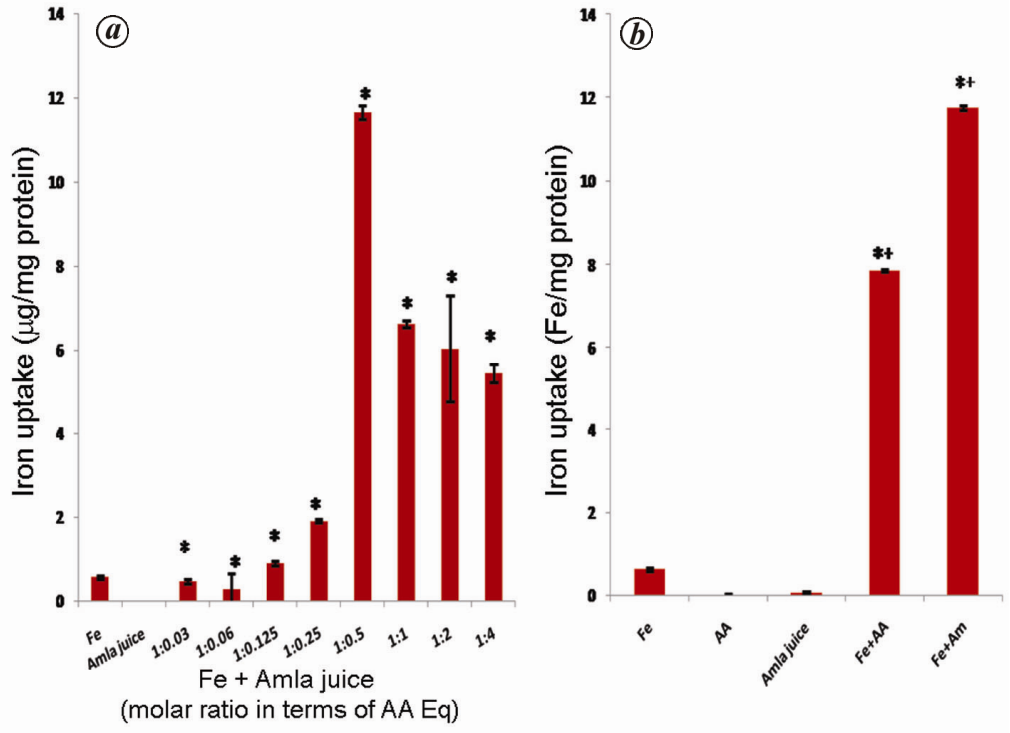

Figure 5. Effect of *amla* juice containing dialysate on iron uptake by HepG2 cells. ***a***, Dose response of iron uptake by HepG2 cells in the presence of *amla* juice containing dialysates. ***b***, Effect of *amla* juice containing dialysates on iron uptake by HepG2 cells in 1 : 0.5 molar ratio (Fe : AA eq). Bars represent mean per cent dialysable iron calculated according to the formula. Error bars represent standard error of mean values of the respective group ($n = 9$). The basal cellular iron and iron content increment in the presence of blank meal were subtracted from the test groups. '*' and '+' show P values <0.05 for *amla*-treated groups compared to the Fe alone control and AA treated groups respectively.

malfunctioning *agni* in an individual is one of the root causes of all diseases[24]. In conditions like iron deficiency anaemia, *jataragni*, one of the 13 types of *agnis*, which deals with the metabolism in gastrointestinal system is said to be affected. Rectifying the *agni* through detoxification procedures and supplementing with certain dietary herbs is an important focus of most Ayurvedic treatments[6].

It is well documented in biomedicine that poor availability and uptake of iron by enterocytes is one of the reasons for IDA[25]. It is also known that interaction between iron and dietary factors plays a significant role in enhancing as well as inhibiting iron absorption from the gastrointestinal tract[26]. The cell-free and cell-based models simulating the stomach and intestinal conditions as described by Miller *et al.*[13] and Glahn *et al.*[14] are well-accepted for studying iron dialysability, uptake and bioavailability. Hence, these models were selected to study aspects of *jataragni*, looking at *amla* as an iron dialysability and uptake enhancer.

Amla fruit is mentioned as an important *Rasayana* and forms a major constituent in several Ayurvedic formulations that promote health and longevity[27]. It contains several essential nutrients which account for its antioxidant and other biological activities[28]. It is also a rich source of AA, a known iron bioavailability enhancer[29]. Our study showed that *amla* juice enhances iron dialysability three-fold over the control in the cell-free digestion model. In addition, the presence of *amla* juice increased iron uptake in Caco-2 and HepG2 cell lines by 17.18 and 18.71 times respectively, which was more than that in the control. In the presence of *amla* juice, iron dialysability and uptake were also better than those seen in the positive control, i.e. AA + $FeSO_4$ mixture. AA at an equivalent molar ratio as that found in *amla* juice increased iron dialysability only by 1.45 in cell-free models and 13.01 and 12.48 times respectively, in Caco-2 and HepG2 models.

The better dialysability of iron obtained with *amla* juice than that with AA at the same molar ratio indicates that factors other than AA in *amla* juice could be involved. Organic acids, including citric acid, gallic acid, malic acid, tartaric acid and several others are also known to increase iron bioavailability[4]; these are present in *amla* juice[30]. The acids are known to maintain Fe in the Fe^{2+} form, which is favoured for absorption[31]. There are other factors in gastrointestinal digestion such as mucin that can influence iron absorption, presumably by acting as a reductant at acidic pH and a low-affinity ligand at neutral pH, thus aiding release of iron from food and maintaining it in a soluble, low molecular weight form[3,32].

Iron arriving at the site of absorption will normally be a mixture of ferrous/ferric ions and low molecular weight complexes[33]. The nutritional iron that primarily gets oxidized to the Fe^{3+} form in the peptic digest needs to get reduced by the enzyme ferric reductase present on the brush border of the enteric cells before being absorbed. The divalent metal transporter 1 (DMT1) aids in the transport of the reduced Fe^{2+} form into the cells[34]. The dialysability of iron from $FeSO_4 \cdot 7H_2O$ (Fe mg equivalent), is attributed to water solubility and its instantaneous dissolution in dilute acid of gastric juice. Hence, those factors that maintain Fe^{2+} in the soluble phase of the digestive milieu enhance iron bioavailability[35]. Dietary factors and reducing agents like ascorbate increase iron in the bioavailable form by altering iron solubility or oxidation state[29].

It is interesting to note from the present study that the levels of increase in iron uptake by Caco-2 and HepG2 cells are comparable. The similarity of results obtained in Caco-2 and HepG2 cells may be due to the proportionate increase in the dialysed iron from the Fe + *amla* or Fe + AA mixtures. The levels of cytosolic labile iron pool (LIP) are normally sensed and controlled by iron responsive elements and proteins (IRE/IRP) in all cells and mediated through transferrin-iron complex and transferrin receptors, as in hepatocytes[15,36]. However, it is also known that when transferrin is unavailable or saturated, iron uptake also happens through non-transferrin-bound iron (NTBI) form[37,38]. While the role of AA in increasing the intestinal absorption of iron is well known[29], that on the liver cells is not conclusive. However, there are reports on the role of citric acid as a ligand in the NTBI form of iron transport in hepatocytes[15].

Unlike an earlier report of the inhibitory effect of *amla* on iron bioavailability[39], we observed that co-digestion of *amla* juice with $FeSO_4$ had an iron bioavailability enhancing effect up to 1 : 0.25 and 1 : 0.5 (Fe : AA equivalent) molar ratios in both cell-free and cell-based models, beyond which it inhibited iron uptake. The inhibition of iron uptake beyond the optimum ratio could be attributed to high levels of tannins in *amla* juice, which in the present study was found to be 0.325% w/w, corroborating the earlier report[39]. The hydroxyl groups of the phenolic compounds form insoluble complexes with the iron molecule, thereby inhibiting intestinal absorption of Fe[26].

The study shows that *amla* can be explored further as a dietary supplement that can contribute to the management of iron deficiency anaemia in a cost-effective way. It is a well-known Indian fruit used in snacks, pickles and jams. This study also opens up the possibility of using cell-free digestion and cell-based absorption models to study the Ayurvedic principle of *Jataragni* and also use them to screen herbs that are said to rectify the malfunctioning *agni* (metabolic disorders). While a substantial body of literature is available on the herbs used in Ayurveda, studies on the fundamental principles of Ayurveda are few and far between, mainly because of the lack of appropriate bioassay models. This is important to bridge the traditional knowledge (*Sastra*) and modern life sciences.

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Evaluation of weather-based crop insurance products for *kharif* groundnut

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Weather-based crop insurance scheme (WBCIS) products proposed by four insurance providers was compared and evaluated using historical weather data for piloting WBCIS on *kharif* groundnut in Coimbatore, Dharmapuri, Theni, Tirunelveli and Virudhunagar districts of Tamil Nadu. Water deficits during the vegetative phases of groundnut crop generally delay flowering and maturity thereby reducing the crop growth and yield. The study revealed that the deficit rainfall risk was more pronounced in all the above-mentioned districts, whereas the risk of excess rainfall impact could be clearly observed in Theni district. Though the occurrence of strike events was for phase-I of deficit rainfall cover, the rate per mm of rainfall fixed by IFFCO–TOKYO was quite low. The product designed for HDFC–ERGO and MS–Cholamandalam was similar, whereas the product for AIC and IFFCO–TOKYO was designed with little variation in context to excess rainfall cover and consecutive dry days. The compensation benefit realized by the farmers of Virudhunagar and Dharmapuri districts was higher followed by Theni because the compensation rate per mm of rainfall fixed by the company was higher, which favours the farmers.

Keywords: Insurance companies, payout, product design, strike events.

AGRICULTURE provides livelihood for 60% of rural population in India and contributes to 35% of the country's gross national product (GNP). The greatest risk to crop yields in Indian agriculture is attributed to the variability of seasonal rainfall as well as the uncertainty in the amount and its distribution in a given season[1]. On an average, 12 m ha of crop area is affected annually by calamities, severely impacting the yields and total agricultural production in India[2]. Groundnut is a legume that belongs to the pea and bean family. More than half of the production area, which accounts for 70% of the groundnut growing area falls under arid and semi-arid regions, where peanuts are frequently subjected to drought stress for different durations and intensities[3].

The Government of India (GoI) had introduced the Comprehensive Crop Insurance Scheme (CCIS) in 1985. Subsequently this was replaced by the National Agricultural

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