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Iron fortificants wheat flour and iron bioavailability on rats.

Sunanda Bera*

Dept. of Nutrition, Bajkul Milani Mahavidyalaya, Purba Medinipur, West Bengal, India.

***Corresponding author**

Abstract

Wheat flour fortification with iron has long been recognized as an effective tool in the global problem against iron deficiency anemia. Fe absorption in rats and humans are similar thus the use of rat in this experiment helps to predict human responses. This study evaluates the in vivo bioavailability of iron from wheat flour fortified with iron and iron absorption promoters.

Key words: Fortification, wheat flower, iron, rat, anemia.

Introduction

Food fortification (adding micronutrients to food) programs are usually considered the most cost-effective and sustainable approach to combat iron deficiency. Iron fortification and supplementation are considered the major approaches to the control of iron deficiency (ID) and iron deficiency anemia (IDA) (Huang et al., 2009). However, food fortification with iron is difficult because uses of iron compounds that are poorly bioavailable in fortification programmes; since the best absorbable forms of iron are chemically reactive and often produce undesirable side effects in the food vehicle. Inert iron compounds on the other hand do not lead to flavor or color changes but are usually poorly absorbed (Fidler, 2003). The public health impact of food fortification depends on a number of parameters, but predominantly the level of fortification, the bioavailability of the fortificants, and the amount of fortified food consumed. Several recent interventions using innovative approaches to dietary fortification that ensure the delivery of adequate quantities of bioavailable iron have demonstrated that iron fortification of food can be an effective and implementable strategy for controlling nutritional iron deficiency (Flour fortification initiative, 2004).

Fortification of wheat flour has long been recognized as an effective tool in the global problem against iron deficiency anemia (Yameen et al., 2013; Ahmed et al., 2010; Hurrell et al., 2010; Olsson etal. 1997; Milman etal. 2002; Muthayya et al., 2012). It increases iron intake (Horton, 2006) without requiring consumers to modify behaviors. Wheat flour-based foods are widely consumed around the world (Curtis, 2002), and the technology for flour fortification is well-established. The recurring cost of adding iron to wheat flour during milling is low at USD 0.62/metric ton of flour when ferrous sulfate is added as the iron compound to USD 2.00/metric ton when Na, Fe and EDTA are added to flour (Aaron et al., 2012). Iron is very stable in wheat flour during production and storage. Iron losses are negligible in the baking and processing of wheat-based foods. Other micronutrients can be easily added at the same time as iron (Nestle and Nalubola, 2000). Wheat-based meals—especially those using breads and pastas—are often quicker to prepare than foods made from other staples.

The use of an animal model experiment (as in this study on rat) to predict human responses has been tried often and reliably. There is strong evidence that humans and rats absorb similar percentages of Fe when Fe statuses and Fe intakes relative to

requirements are similaar. The biochemical mechanisms of haem and non-haem Fe absorption in rats and humans are similar. The mucosal enzymes systems (haem oxygenase) involved in the release of Fe from the haem ring are found in humans and rats. Thus, whatever result is obtained from this study will be basis for a reasonable prediction of human response ((Ologunde et al, 1994).

The overall aim of this project is to optimize iron bioavailability from iron fortificants with wheat flour on rats. Further effort were given to evolve technology wheat flower fortified with iron or iron absorption promontories; to elevate the in vivo bioavailability of iron from foods prepared with wheat flour fortified with iron; to assess the determination during packaging and storage and to assess the heart and liver weight after consuming fortified diet.

Materials and methods

The study was designed to evaluate the in vivo bioavailability of iron from wheat flour fortified with iron and iron absorption promoters. The methodologies adopted for the experiment are described under the following heads.

- Preparation of iron fortified wheat flour food for rats.
- Gross behavioral study of rats.
- Estimation of the hemoglobin level (in vivo bioavailability).

Preparation of iron fortified wheat flour food for rats:

Processing of wheat:

A single batch of 20 kg 0f wheat (normal variety) purchased locally was cleaned of extraneous particles. The extent of iron contamination was checked in wheat grain sample. The unwashed wheat was ground to a fine in a flour mill.

Fortification of wheat flour with iron absorption promoters:

Wheat flour was dry mixed with inorganic iron salt $FeSo_{4,}H_2O$ as well as with iron absorption promoters ,EDTA, citric acid and lemon juice. A total 4 combination of the fortified wheat flour were prepared according to the given below.

Quantity of different fortification used for preparing a 5 times concentrated premixes and the expected iron content:

Sample	Ingredient	Amount
1	**A)** wheat flour	1.0kg
	B) $FeSo_{4,}7H_2O$ (5X)	1.5gm
2	**A**) Wheat flour	1.0kg
	B) $FeSO_{4,}7H_2O$	1.5gm
	C) EDTA (5X)	2.7kg
3	**A**)Wheat flour	1.0kg
	B) $FeSo_4$, $7H_20$ (5X)	1.5gm
	C) Citric acid	40gm
4	**A)** Wheat flour	1.0kg
	B)$FeSO_4$,$7H_2O$ (5X)	1.5gm
	C) Lemon juice	40ml

Packaging and storage

The fortified sample were stored in 500gm polythene bag sealed air tight and kept at room temperature in the dark. Periodic observation were made with the ingredients viz.bengal gram flour, milk powder,nut paste, oil along with each type of fortified wheat flour. Those are mixed properly under identical condition. Necessary care was taken to avoid iron contamination during preparation of food. The pots were made in iron free utensils.

Gross behavioral study of rats:

The effects on the gross behavioral of rats after a single dose of drug, a brief period of sedation was observed only after 20 mg/kg dose hyper motor activity of the animals was observed. It has been documented that behavior changes of animals is indicated by its movement. Any motor activity of the animals in relation to the surroundings or any microscopically observed by the motor cortex as well as psychic motivation of the organism. In the present experiment of drug suspension did not appear to interfere with the normal psychomotor control of

the motor activity of the animals (Roy & Mukherjee, 1990).

Principle

The effects on the gross behavior of the rats after administration of iron fortified wheat flour (10 mg/day).

SL. No.	Materials
1	12 Albino rat
2	24 Porcelain pot
3	Iron fortified wheat flour
4	Milk powder
5	Bengal gram flour
6	White oil
7	Nut paste
8	Weight machine

Procedure

At first the rats were fed the unfortified wheat flour with Bengal gram flour, milk powder, nut paste, oil (one drop) that means normal diet for 7 days. After 7 days, the gross behavior of the rats were also studied. Then for the next 15 days, the fortified diet was given to the rats and the gross behaviour was studied.

- Intake of food.
- Heart beat per min.
- Weight of rat.
- Frequency of fecal discharge within 24 hrs.
- Blinking.
- Feature of fecal matter.
- Disturbed or not disturbed

Estimation of the hemoglobin level (in vivo bioavailability).

Estimation of haemoglobin by cyanmethaemoglobin method :

Principle

In solution the ferrous ions (Fe2+) of the haemoglobins (Hb) are oxidized to the ferric state (Fe3+) by potassium ferric State (Fe3+) by potassium ferric cyanide to form methemoglobin. In turn methemoglobin reacts with the cyanide ions (CN) provided by potassium cyanide to form cyanmethemoglobin, which has the absorbance at 540 nm.

Reagents

Cyanmethemoglobin solution (Drabkin,s solution): Dissolve 0.05 g potassium cyanide, 0.200g potassium ferric cyanide and 0.140 g potassium phosphate in 1 lit. of distilled water. Add 1 lm of Triton x 100 and mix stable for at least 6 months.

Haemoglobin solution: Lyophilised human methemoglobin (Supplied by Sigma USA). Each vial is equivalent to haemoglobin concentration of 18g/dl whole blood when reconstituted in 50 ml of Drabkin' s solution. Stable for 6 months when refrigerated at 2-6^0 C.

Procedure

Transfer 0.02 ml of blood using a calibrated hemoglobin pipette, into a tube containing 0.5 ml of Drabkins reagent. Rinse the pipette several times with the reagent. Allow diluted hemoglobin solution to stand for at least 5 min to achieve full colour development. Measure the absorbance at 530-550 nm of the unknown sample and that of a standard of known hemoglobin content (ASTD) against a reagent black (Dacie and Lewis, 1968)

Calculation

Blood Hb in gm% =(O.D. test/O.D. std.)× Conc. of std in mg%×0.251

=(O.D. test/O.D. std)×60×0.251

=(O.D. test/O.D.std)×15.06

Note

- Drabkins solution should be stored in almber colour bottle. If any precipitate is formed the reagent should be discarded.
- Since the dilution is enormous (25 times) accurate measurement of 20ul of blood is absolutely essential for reproducibility. Hb pipettes must be cheeked for their accuracy by using pure mercury upto the mark.

RESULT:

The difference in percentage of food intake (gm) was most decreased in $FeSo_4$ fortified diet and less decreased in EDTA fortified diet (Table 1). Food intake capacity of different fortified materials graphically shown on fig 1. Food intake capacity was higher in normal diet and EDTA treated rats. The heart beat rate of both EDTA fortified and non fortified treated rat was high (Fig 2). In case of heart beat, difference between fortified and normal diet in percentage was high in EDTA fortified diet and low in citric acid treated diet (Table 2). Weight of lemon juice treated rat was high when normal diet was continued. During continuation of fortified diet, the weight of EDTA and lemon juice treated rat was high (Fig 3). Difference after fortified diet in percentage, citric acid treated rat's weight was heavy than any other diet treated diet and the weight of lemon juice treated rat was lowest than any other treated rat (Table 3). The frequency of faecal discharges was high in $FeSO_4$ and less in lemon juice treated rat during fortified diet (Fig 4). Difference after fortified diet it was observed that frequency of faecal discharge was increased in $Feso_4$ treated rat and decreased in citric acid treated rat (Table 4). By the study of gross behavior of rats after feeding fortified diet, there is no change in general visible feature (Table 5). The Hb level of the rats which were fed sample of wheat flour+$Feso_{4,}7H_2o$+lemon juice was greatly increased than the others (Fig 5). So this was the best quality of sample among other. Haemoglobin level of rat in different sample shown in table 6. The heart weight of rats which were fed sample of wheat flour+$FeSo_{4,}7H_2O$ was greatly increased than the others (Fig 6). Heart weight of rat in different sample shown in table 7 which indicate food containing wheat flour+$FeSo_{4,}7H_2O$ increases heart weight more than other food sample used in the experiment. The liver weight of rats which were fed sample of wheat flour+ $FeSo_{4,}7H_2o$ +citric acid was greatly increased than the others (Fig 7). Liver weight of rat in different sample shown in table 8.

Table 1.Intake of food (gm)

Sample	Sample 1 (Wheat flour+FeSO$_4$, 7H$_2$O)	Sample 2 (Wheat flour+FeSO$_4$, 7H$_2$O+ EDTA)	Sample 3 (Wheat flour+FeSO$_4$, 7H$_2$O+ Citric acid)	Sample 4 (Wheat flour+FeSO$_4$, 7H$_2$O+ Lemon juice)
1	5.55±6.07	6.30±6.83	5.42±5.86	4.99±6.06
2	6.31±6.51	6.60±6.68	5.76±5.79	5.72±5.77
3	-12.87	- 4.54	-5.90	-12.76

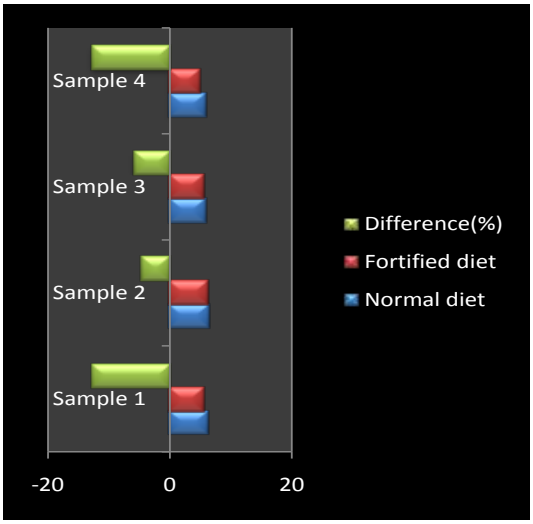

Fig1.Graphical representation of intake of food of different sample (%).

Table 2.Heart beat of rat per minute.

Sample	Normal diet	Fortified diet	Difference after fortified diet(%)
Sample 1 (Wheat flour+$FeSO_4$, $7H_2O$)	112.28±2.01	108.14±4.06	-3.68
Sample 2 (Wheat flour+$FeSO_4$, $7H_2O$+ EDTA)	118±4.89	135.28±2.65	+14.64
Sample 3 (Wheat flour+$FeSO_4$, $7H_2O$+ Citric acid)	112.28±2.01	107.85±2.65	-3.94
Sample 4 (Wheat flour+$FeSO_4$, $7H_2O$+ Lemon juice)	108.71±6.31	108.14±4.06	-0.52

Table3.Weight of rat (gm)

Sample	Normal diet	Fortified diet	Difference after fortified diet (%)
Sample 1 (Wheat flour+$FeSO_4$, $7H_2O$)	125.5±0.5	125.75±0.83	+0.19
Sample 2 (Wheat flour+$FeSO_4$, $7H_2O$+ EDTA)	162.5±2.5	163.5±2.29	+0.61
Sample 3 (Wheat flour+$FeSO_4$, $7H_2O$+ Citric acid)	152.5±2.5	159±15.5	+4.26
Sample 4 (Wheat flour+$FeSO_4$, $7H_2O$+ Lemon juice)	172.5±2.5	163.5±2.29	-5.21

Table4. Frequency of faecal discharges within 24 hours.

Sample	Normal diet	Fortified diet	Difference after fortified diet(%)
Sample 1 (Wheat flour+$FeSO_4$, $7H_2O$)	3.85±0.38	4.21±0.4	+9.35
Sample 2 (Wheat flour+$FeSO_4$, $7H_2O$+ EDTA)	3.99±0.4	4.21±0.4	+5.51
Sample 3 (Wheat flour+$FeSO_4$, $7H_2O$+ Citric acid)	4.27±0.4	4.13±0.4	-3.27
Sample 4 (Wheat flour+$FeSO_4$, $7H_2O$+ Lemon juice)	3.85±0.38	4.01±0.3	+3.89

General visible feature:

Table 5.General visible feature of all sample:

	Types of food	Food administration(days)	Blinking	Feature of faecal discharge	Disturbed/not disturbed
Sample 1 **Sample 2** **Sample 3** **Sample 4**	Normal diet	7	Normal	Normal	Not disturbed
	Fortified diet	15	Normal	Normal	Not disturbed

Estimation of haemoglobin level of rats:

Table 6.Haemoglobin level of rat in different sample:

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Hb level after fortified diet(gm/dl)	17.1 ±0.1	20.82 ±0.5	17.67 ±0.4	21.09 ±0.7
Percentage(%)	22.14	48.71	26.21	50.64

Normal range of Hb level- 11 to 17gm/dl. Average- 14gm/dl. (Raghuramulu et al., 2003)

Estimation of heart weight of rats:

Table7.Heart weight of rat in different sample:

Sample	Sample1	Sample 2	Sample 3	Sample 4
Heart weight of fortified diet(gm)	0.83±0.1	0.62±0.7	0.62±0.7	0.74±0.01
Percentage (%)	38.33	3.33	3.33	23.33

Normal heart weight (gm) - 0.60±.10 (Viswanatha Swamy et al., 2011)

Estimation of liver weight of rats:

Table 8.liver weight of rat in different sample:

Sample	Sample1	Sample2	Sample3	Sample4
Liver weight after fortified diet	8.61±0.11	8.72±0.10	8.87±0.24	8.78±0.24
Percentage (%)	**0.46**	**1.75**	**3.50**	**2.45**

Normal liver weight- 8.57±0.42 (Viswanatha Swamy et al., 2011)

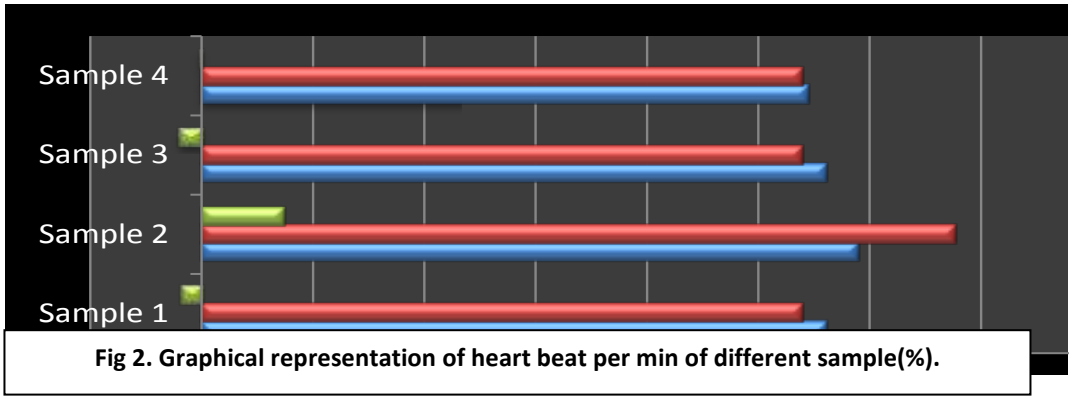

Fig 2. Graphical representation of heart beat per min of different sample(%).

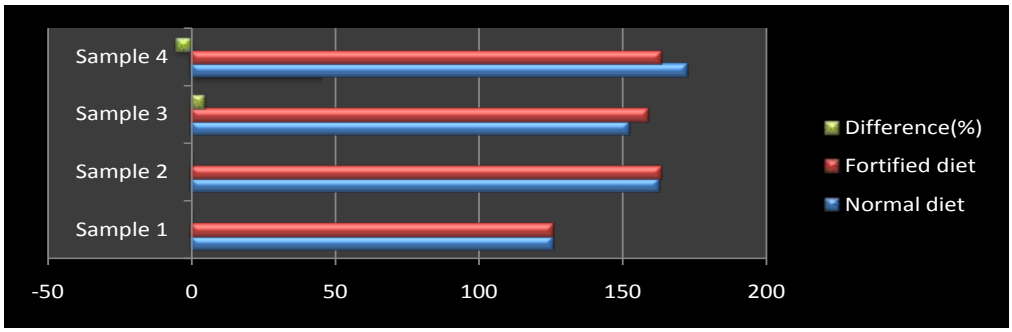

Fig 3.Graphical representation of weight of rat of different sample (%).

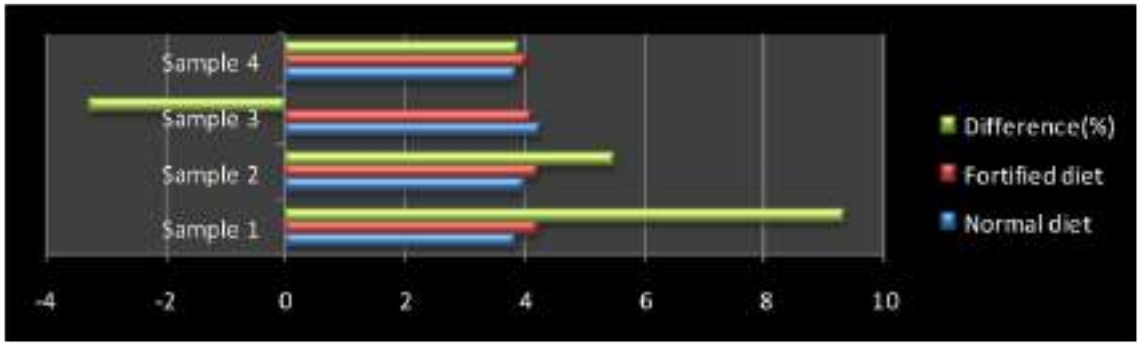

Fig 4. Frequency of faecal discharge within 24 hrs of different sample(%).

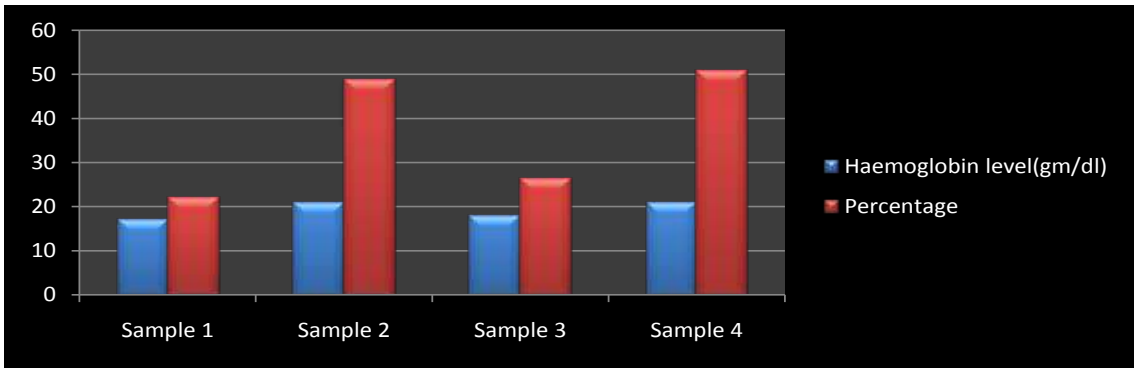

Fig 5.Graphical representation of haemoglobin level of rat of different fortified sample(%).
Sample 1: Wheat flour+$FeSO_4$,$7H_2O$; Sample 2: Wheat flour+$FeSO_4$,$7H_2O$+EDTA; Sample 3: Wheat flour+$FeSO_4$,$7H_2O$ + Citric acid; Sample 4: Wheat flour+$FeSO_4$,$7H_2O$+Lemon juice.

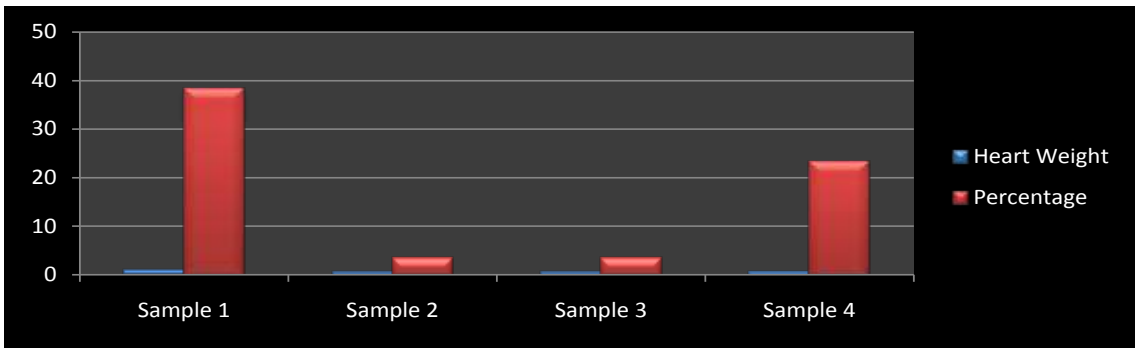

Fig 6.Graphical representation of heart weight of rat of different fortified sample.
Sample 1:Wheat flour+Feso4,$7H_2O$; Sample 2: Wheat flour+FeSO4,$7H_2O$+EDTA; Sample 3: Wheat flour+FeSO4,$7H_2O$ + Citric acid; Sample 4: Wheat flour+FeSO4,$7H_2O$+Lemon juice.

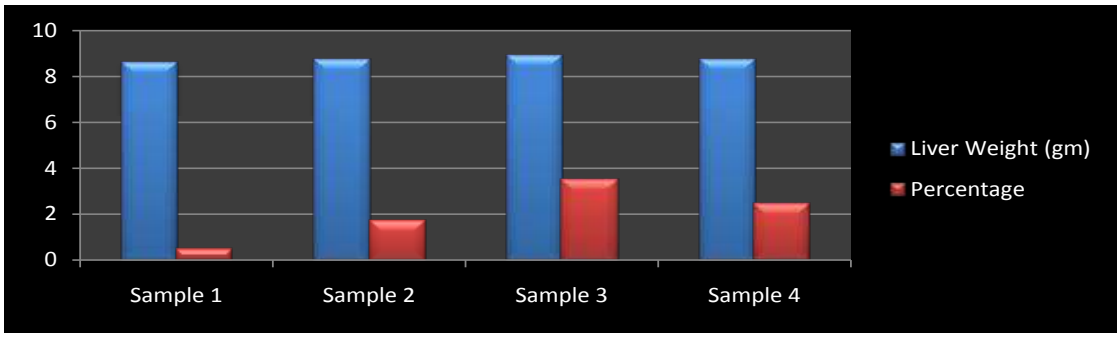

Fig 7.Graphical representation of rat of different fortified sample.
Sample 1:Wheat flour+$FeSO_4,7H_2O$; Sample 2: Wheat flour+ $FeSO_4,7H_2O$ +EDTA; Sample 3: Wheat flour+ $FeSO_4,7H_2O$ + Citric acid; Sample 4: Wheat flour+ $FeSO_4,7H_2O$ +Lemon juice.

Discussion:

Food fortification is very effective in iron deficiency anemia and fortification of wheat flour has long been recognized as an effective tool to global struggle against iron deficiency anemia. Since wheat flour is widely consumed in India. Elemental iron powders are perhaps the most commonly used iron fortification because of low cost, stable self life and test quality. It can be used with wheat flour to treat iron deficiency population. Studies have shown that the wheat flour fortification with iron produced some positive effect on rat's hemoglobin counts, digestion and other physiological activities. The biochemical mechanisms of haem and non-haem Fe absorption in rats and humans are similar; humans and rats absorb similar percentages of Fe when Fe statuses and Fe intakes relative to requirements are similar and mucosal enzymes systems (haem oxygenase) involved in the release of Fe from the haem ring are found in humans and rats. Thus, whatever result is obtained from this study will be basis for a reasonable prediction of human response.

Despite a strong interest by flour millers and national governments in the use of wheat flour fortification to combat iron deficiency and iron-deficiency anemia, it would appear that only 9 of the 78 national wheat flour programs could expect to have the desired nutritional impact. Most millers do not follow the Cuernavaca (Flour Fortification Initiative, 2009) guidelines for wheat flour fortification. In many countries, guideline for optimization of flour fortification programme in a multi stage process. First, Through evaluation of the prevalence of iron-deficiency in population should be established on the basis of survey; next the form, level and absorption of the fortificant should be optimized to provide the maximum acceptable amount of bioavailable iron without side effect; efficacy studies should demonstrate that programme is effective in decreasing the prevalence of anemia in the country. Finally, modify national regulations for wheat flour fortification so that only recommended iron compounds are added at concentrations necessary to achieve a satisfactory impact. Once the millers have clear guidelines for the efficacious fortification of wheat flour with iron, the small extra cost will be a price worth paying for the meaningful health benefit to women and children.

This experiment proved that any insufficient mineral intake still represents one of the most relevant causes of growth impairment. Thus, it is evident the importance of consuming a diet with an adequate mineral content in order to prevent or eliminate the severe consequences associated with their deficiency. Present study showed that the combined

mineral mix composed by iron and wheat, has some effects on animals that consumed it as part of their diet. Moreover, it has also a positive effect in animals recovering from a period of nutritional depletion.

So, it has been said that food fortification can make an important contribution for the prevention of iron deficiency and iron anemia and prevention is better than cure.

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