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Research Article

Development and Evaluation of Nutritious Supplementary Food Product in Phenyl Hydrazine Induced Anaemia in Wistar Rats

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Abstract

Objective- Nutritional deficiency anemia mainly results from a lack of iron, but it also associated due to lack of folate/vitamin B12 and low vitamin C intake. From various studies this is found anemia in people is due to poor eating habits, such as not eating enough fruits and legumes such as beans and peas. Proper nutritional supplementation may be useful in such condition. The aim of the present work was to select a suitable iron rich food material, and prepared a suitable dosage form for management of anaemic condition and to evaluate anti-anaemic potential of nutritious supplementary food. **Method-** Anemia was induced by phenyl-hydrazine. Animals were treated with nutritious supplementary food throughout the study for 30 days. The haemoglobin concentration was determined after 30 days. **Results-** Nutritious supplementary food was found to be rich in folic acid, ascorbic acid, and iron. Following the induction of anaemia, the haemoglobin concentration decreased by 30%. Administration of standard hematonic preparation and nutritious supplementary food 2gm/day in divided dose resulted in significant increase ($P \leq 0.05$) in haemoglobin concentration when compared to the untreated phenyl hydrazine-induced anaemic rats. **Conclusion-** Nutritious supplementary food effectively raised the level of haemoglobin. Vitamin and minerals found in nutritious supplementary food are most likely active ingredients responsible for its hematonic effects.

Keywords- Anaemia, nutrition, nutritional anaemia, iron, phenyl hydrazine

INTRODUCTION

Anaemia is a condition in which the blood does not have enough healthy Red Blood Cells. Anaemia results from a lack of RBC/ dysfunctional RBC in the body. This leads to reduce oxygen flow to the body's organs^{1,2}. Anaemia affects over 30% of the world's population, according to the World Health Organization (WHO). According to global data April 2018, India has the highest prevalence of anaemia and this even higher among Indian women with around 50% of women. It is often more common in pregnant women and children^{3,4,21}.

Nutritional anaemia is an important nutritional problem affecting large population groups in most developing countries. Nutrition deficiency anaemia is common issue that can happen if the body does not absorb enough of certain nutrients. It can results from an imbalanced diet or certain health condition. Iron, folate or vitamin B12 these nutrients can cause the nutrition deficiency anemia and low vitamin C intake can contribute to it. Nutrition deficiencies can lead to a low red blood cell count, low of haemoglobin in these cells, or red blood cells that do not function as they should. Iron deficiency can delay the development of unborn babies^{5, 6,17}.

From various studies this is found anaemia in people is due to poor eating habits, such as not eating enough fruits and

legumes such as beans and peas. So these nutrients are important for maintaining the wellbeing of human^{7,15,22}.

Dr. Stephen Defelice coined the term "Nutraceutical" from "Nutrition" and "Pharmaceutical" in 1989. Nutraceutical is a broad term that is used to describe any product obtained from food sources. Nutraceutical product is a food supplement and it provides medical benefits to human beings. Nutraceutical provides medical benefits such as it helps in improving health, delay the aging process, prevent chronic diseases, increase life expectancy, therefore nutraceutical known as medicinally / nutritionally functional food^{8,18}. When food is being cooked/prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, etc., needed for its healthy survival. When functional food aids in the prevention and /or treatment of disease(s) and /or disorders other than anaemia, it is called a nutraceutical^{8,19}.

In the present innovation, composition of nutritious supplementary food contains kidney beans, soybeans, corn flakes, oats, tomato powder, beetroot powder. The composite nutraceutical preparation tested for presence of iron and folic acid. These food materials are rich source of iron, folic acid, vitamins such as vitamin C. vitamin B12, and minerals.

Vitamin and minerals found in nutritious supplementary food are most likely active ingredients responsible for its hematinic effects⁹. The aim of this study was to develop and evaluate the product containing nutritious supplementary food for the management of anaemia.

MATERIALS AND METHODS

Materials- Kidney beans, oats, corn flakes, soybean, powdered sugar, baking powder, butter are collected from local market. Tomato powder and beetroot powders are collected from food masters foods LLP, kondhwa Pune411048, India.

Methods 1-

Table 1: Composition of supplementary food for 100 gm

Content	Quantity (gm)
Kidney beans	7
Soybeans	7
Oats	15
Corn flex	20
Tomato Powder	20
Beet root powder	28
Mannitol	3

Table 3: Folic acid and iron content

Sr. No	Parameter	Result	LOQ	Units	Test Method
1	Iron	7.13	-	Mg/100g	AOAC 944.02
2	Folic Acid	< LOQ	25	Mcg/100 g	Based on SOP030

LOQ-Limit of Quantification

Method 2-

Procedure for Induction of experimental anaemia:

Anemia was induced by intraperitoneal injection of phenyl hydrazine (60 mg/kg, i.p., in divided doses daily, for 3 consecutive days. Anemia was considered to be induced when haemoglobin concentration of the blood reduced by about 30%¹¹.

Procedure:

Above all ingredients were collected and grind separately. All powders are sieved using sieve no.60. Each ingredient weighs and mixed well by geometric method. The final product is packed in air tight container¹⁰.

Evaluation-

1. Physical properties: Physical properties such as weight, colour, taste, density, Flowability are analysed.

Table 2: Evaluation of powder¹⁰.

Parameter	Characterization/amount
Colour	Brownish
Odour	Sweet
Taste	Sour-sweet
Bulk density	0.4167
Tapped density	0.4464
Flowability	1.0712

Study group design

After induction of anemia, rats were divided into groups, except Group I comprised of healthy rats. Grouping is as follows:

Table 4: Group of animal

S.N.	Category	Treatment	No. of animals (Female)	No. of animals (male)	Total
1.	Group 1	Normal control	3	3	6
2.	Group 2	No treatment(phenylhydrazine)	3	3	6
3.	Group 3	Phenyl hydrazine +Std. treatment (lohasav syrup)	3	3	6
4.	group 3	Phenyl hydrazine + Nutritious product 2.5gm/day in divided dose)	3	3	6
5.	Group 5	Phenyl hydrazine + Nutritious product 5gm/day in divided dose)	3	3	6

Bio-analytical studies:

The haemoglobin concentration was determined every 7 days for 30 days.

Evaluation:

1.Hb estimation –

Procedure for Hb evaluation: by using drab kin's reagent

i. Pipette into 2 test tubes

- Drab kin's reagent no. 1
- Sample (fresh blood)

ii. Mix well. Incubation at room temperature for 5 min.

iii. Optical density (O.D) read at 530-550 nM. The final color is stable for 30 min.

Formula: $\frac{O.D.TE\text{ST} * 15.06}{O.D. STD}$

O.D. STD

RESULTS:

Table 5: Result of haemoglobin concentration

Sr.No.	Group	Mean ± SEM
1.	Normal control	9.332±0.3260
2.	No treatment(phenylhydrazine 60mg/kg)	7.552±0.6478
3.	Phenyl hydrazine +Std. treatment (lohasav syrup)	9.897±0.3356
4.	Phenyl hydrazine + Nutritious product 2.5 gm/day in divided dose)	9.29±0.2197
5	Phenyl hydrazine + Nutritious product 5 gm/day in divided dose)	10.62±0.5098

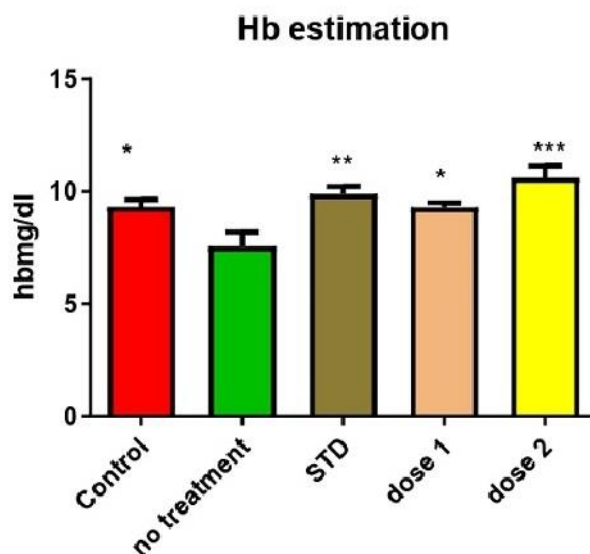


Figure 1: Effect of Nutritious Supplementary Powder on haemoglobin concentration in phenyl hydrazine induced anaemic rats.

The composite nutraceutical preparation tested for presence of iron and folic acid. The results indicates that the supplementary food/ composite nutraceutical preparation contains iron 7.13 mg / 100g and folic acid 25 Mcg/100 g. Data are presented as mean ± standard error mean and analyzed by one-way analysis of variance, followed by

multiple comparison test (post-test); $P \leq 0.05$ was considered as statistically significant in all analyses.

Nutritious supplementary food 2.5/5gm/day in divided dose resulted in significant increase ($P \leq 0.05$) in haemoglobin concentration when compared to the untreated phenyl hydrazine-induced anaemic rats.

2. Body weight-

Table 5: Result of body weight of animal

Sr.No.	Group	Mean ± SEM
1.	Normal control	313±0.3203
2.	No treatment(phenylhydrazine 60mg/kg)	268.66±0.6478
3.	Phenyl hydrazine +Std. treatment (lohasav syrup)	326±0.3356
4.	Phenyl hydrazine + Nutritious product 2.5 gm/day in divided dose)	365±0.2197
5.	Phenyl hydrazine + Nutritious product 5 gm/day in divided dose)	333±0.5099

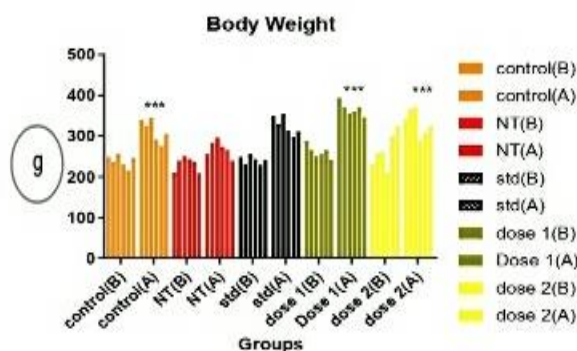


Figure 2: Effect of Nutritious Supplementary Powder on body weight of wistar rats in phenyl hydrazine induced anaemic rats.

Data are presented as mean \pm standard error mean and analyzed by one-way analysis of variance, followed by multiple comparison test (post-test); $P \leq 0.05$ was considered as statistically significant in all analyses. Nutritious supplementary food 2.5/5gm/day in divided dose resulted in significant increase ($P \leq 0.05$) in Body weight concentration when compared to the untreated phenyl hydrazine-induced anaemic rats.

DISCUSSION:

Prepared nutritious supplementary powder contains several nutritious food materials those are rich source of iron and folic acid. Therefore this nutritious supplementary powder will helpful in managing the anaemic condition. Other vitamins, minerals and proteins present in the nutritious supplementary food material will be helpful in increase the nutritious value of nutritious supplement by contributing health promoting effect.

CONCLUSION:

Nutritional deficiency anemia is due to poor intake of micronutrients such as mainly iron and folate. In the present innovation, composition of nutritious supplementary powder contains kidney beans, soybeans, corn flakes; oats, tomato powder, beetroot powder are rich source of iron and folic acid which help in managing anaemic condition in children below 5 years. Nutritious supplementary powder is rich in proteins hence it can be used in the treatment of malnourishment or nutrition deficiency. Above prepared nutritious supplementary powder tested on anaemic rats and result were found to be increase in Hb level of animal.

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