Knowledge Consortium of Gujarat
Department of Higher Education - Government of Gujarat
Journal of Science
ISSN: 2320 - 0006

Continuous issue-7 | June - July 2016

Effect of feeding flaxseed and amaranth seed powder incorporated laddu on total antioxidant capacity and hematological status of young females (20-25 years)

Abstract

The present investigation was carried out to formulate laddu by incorporating flaxseed and amaranth seed powder. The formulated laddu were fed to young females (20-25 years) to assess its impact on the plasma total antioxidant capacity and hematological profile. Flaxseed and amaranth seed powder was incorporated in laddu at a concentration of 10gm and 20gm, respectively. The formulated laddu was used for feeding trial. 12 young females in the age group of 20-25 years were enrolled from P. G. Department of Home Science, Sardar Patel University. Sensory evaluation of laddu incorporated with varying levels of flaxseed and amaranth seed powder indicated the highest score of overall acceptability in sample containing flaxseed + amaranth seed powder at a level of 10 gm + 20 gm respectively. The mean of hemoglobin level before (initial) and after (final) supplementation were found to be 10.68 and 13.05 mg % respectively. A significant increase in hemoglobin concentration ($P \leq 0.05$) was observed after feeding flaxseed and amaranth seed powder incorporated laddu. Serum protein levels were not increased after supplementation of flaxseed and amaranth seed powder incorporated laddu. The present study revealed that incorporation of flaxseed and amaranth seed powder at a level of 10 gm + 20 gm respectively helps to improve the hemoglobin concentration and RBC count. Thus flaxseed and amaranth seed as a food supplement should be encouraged for anemic patients.

Introduction

Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children (Malhotra and Passi, 2007). Iron deficiencies compromise the immune system and are associated with limited cognitive development in children. Because iron is an essential element for the function of various organs, its deficiency may lead to impaired perception and learning difficulties ending up with declined school success (Soemantri, Pollitt and Kim, 1985).

According to the World Health Organization (WHO) (2006), anemia is defined as a condition in which the total circulating red cell mass (haemoglobin) content is below normal level and leading to tissue hypoxia. This situation occurs because of different pathophysiological mechanisms. The most prevalent types of anemia are due to nutritional deficiencies (malnutrition and iron, vitamin B12 and folic acid deficiencies) and non-nutritional (such as infection and hemoglobinopathies; chronic diseases such as cancer, kidney disease and congestive heart failure) that contribute to the onset of anemia (Sanap and Jadhav, 2014).

Given the role of iron in oxygen transport and the low levels of available iron in the diets of a large proportion of the global population, it is assumed that iron deficiency is one of the biggest contributing factors, one of the ten leading global risk factors in terms of its attributable disease burden of anemia (Sanap and Jadhav, 2014).

Children and women of reproductive age are most at risk, with global anemia prevalence estimates among preschool age children 23 % (Gegios et al, 2010), 47 % in children younger than 5 years, 42 % in pregnant women, and 30 % in non-pregnant women aged 15-49 years. Africa and Asia account for more than 85 % of the absolute anemia burden in high-risk groups (Benoist et al, 2008). In India, 79 % of children between 6-35 months and women between 15-49 yrs of age are anemic (Krishnaswamy, 2009). The prevalence of anemia is disproportionately high in developing countries, due to poverty, inadequate diet, certain diseases, pregnancy/lactation and poor access to health services. The nutritional anemia in this group attributes to high incidence of low-birth weight babies, high parental mortality and fetal wastage and consequent high fertility rates (WHO 1999).

The most common cause of anemia is iron deficiency and infection plays an important role. Infection results in decreased antioxidant enzymes and substances such as catalase, GPx, SOD, GSH, ascorbate, and plasma tocopherol which leads ROS generation; however, recent work has shown that reactive oxygen species (ROS) of erythrocytes are one of the principal causative factors of anemia. Elevation of ROS in erythrocytes could occur either by activation of ROS generation or by suppression of antioxidative/redox system. When erythrocytes experience an excessive elevation of ROS, oxidative stress develops. ROS are known to contribute to the pathogenesis of several hereditary disorders of erythrocytes, including sickle cell anemia, thalassemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency (Iuchi, 2012). In vitro and in vivo studies have demonstrated that treatment of blood cells with antioxidants decreases oxidative stress (Pace et al, 2003).

Food-based intervention programs, dietary enhancement and diversification, and food fortification including biofortification improving the availability of, access to, and consumption of vitamin, mineral and antioxidants which not only improved intakes of specific nutrients but also improved overall diets and health status (Thompson, 2007).

Flaxseed and amaranth seed are not frequently used by people of Gujarat. It is low saturated fat food and an excellent source of omega-3 (alpha linolenic acid) essential fatty acid which has antioxidant activity (Bloendon, Phillipe and Scazapary, 2004). Flax seeds contain anti-oxidants and have high dietary fiber that can inhibit lipid peroxidation and helps in scavenging of hydroxyl radicals (Praasad, 1999). It also contains 21% protein and also is a good source of folic acid, vitamin B6; vitamin E as well as minerals like magnesium, phosphorous and copper (Anonymous, 2005). It has been observed that, consumption of amaranth seed is high among people only during fasting days, thus presenting significant potential to provide additional nutrition in the diet by using this in their regular diet. It contains fair quantity of fat, energy, fiber, iron, calcium, vitamins and minerals. Amaranth is an excellent source of protein which contains those amino acids that are usually found only in animal foods. It is also the best plant source of squalene, tocols (Johns and Eyzaguirre, 2007), rutin and quercetin (Kumar et al, 2009) a powerful antioxidant.

Thus, the present work was undertaken to assess the effectiveness of iron supplementation for improving haemoglobin levels of girls. The present study stridently supported the food-based therapy implying flaxseed and amaranth seed based laddus.

Materials & Methods

Locale of study:-

The research work was conducted at the laboratory of Foods and Nutrition, Jashbhai Khodabhai Patel Department of Home Science, Sardar Patel University, Vallabh Vidhyanagar, Anand.

Experimental Design:-

It is comprised of three phases.

Phase I:- Product development

✓ **Preparation of control and experimental laddu:**

Control laddu was prepared by using wheat flour, Jaggery, oil, ghee, poppy seeds etc. Experimental laddu were prepared by replacing known quantity of wheat flour with flaxseed and Amaranth seeds powder in different ratio. The composition of control and experimental laddu is presented in table 1.

Table: - 1 Composition of control laddu and experimental laddu

Control	Wheat flour	Flaxseed powder	Amaranth seed flour	Jaggery	Ghee	Oil	Cardamom	Poppy seeds
Expt. Sample I	100gm	-	-	80 gm	20 gm	15 ml	2 gm	5 gm
Expt. Sample II	90 gm	4 gm	6 gm	80 gm	20 gm	15 ml	2 gm	5gm
Expt. Sample III	80 gm	8 gm	12 gm	80 gm	20 gm	15 ml	2 gm	5 gm
Expt. Sample IV	70 gm	10 gm	20 gm	80 gm	20 gm	15 ml	2 gm	5 gm

✓ **Sensory trial**

Sensory evaluation carried out (Srilakshmi, 2007) to study the organoleptic characteristics like appearance, texture, flavour, overall acceptability.

Phase II:- Feeding Trial

12 young females in the age group of 20-25 years were enrolled from P.G. Department of Home Science, Sardar Patel University. The subjects were chosen on the basis of the following criteria:

1. Willingness to co-operate.
2. Free from any apartment complications.

Collection of blood sample:

The enrolled subjects were requested to report at the laboratory of Department of Foods and Nutrition, after an overnight fast (12 hours). Approximately 5ml of venous fasting blood sample was drawn from each subject and collected in a clean dry centrifuge tube containing dried Ethylene Di amine Tetra Acetic acid (EDTA). It was then centrifuged to separate the plasma. The plasma was stored at 20 °C temperature till used for further analysis.

Feeding laddu to subjects:

One laddu weighing 40 gm was fed to the subjects in the morning for a period of one month.

Phase III:- Bio -chemical Analysis

Hemoglobin*, Packed Cell Volume*, Red Blood Cell from whole blood (*Automated Hematology Analyzer "poch-100i" which was marketed by Transasia Biomedicals Ltd., INDIA) and Total Antioxidant Capacity (Benzie and Strain, 1996), Total protein (Auto kit supplied by sigma Diagnostics (I) pvt. Ltd.) from serum.

Results and Discussion

Table 2: shows the sensory scores of control and experimental laddu.
Plate 1: shows the appearance of control and experimental and laddu.

The highest scores of appearance laddu was observed by sample I (control) (9.33) and the lowest by sample III (6.83). Among the experimental laddu the highest score was observed at highest incorporation level of flaxseed and amaranth seed. The highest score for texture of laddu was observed by sample IV (9.00) and the lowest by sample III (6.28). The highest score for flavour of laddu was observed by sample IV (18.94) and lowest by sample II (14.72). Sample IV (9.67) had significantly higher ($P \leq 0.05$) score for overall acceptability and the lowest was observed by sample II (6.28).

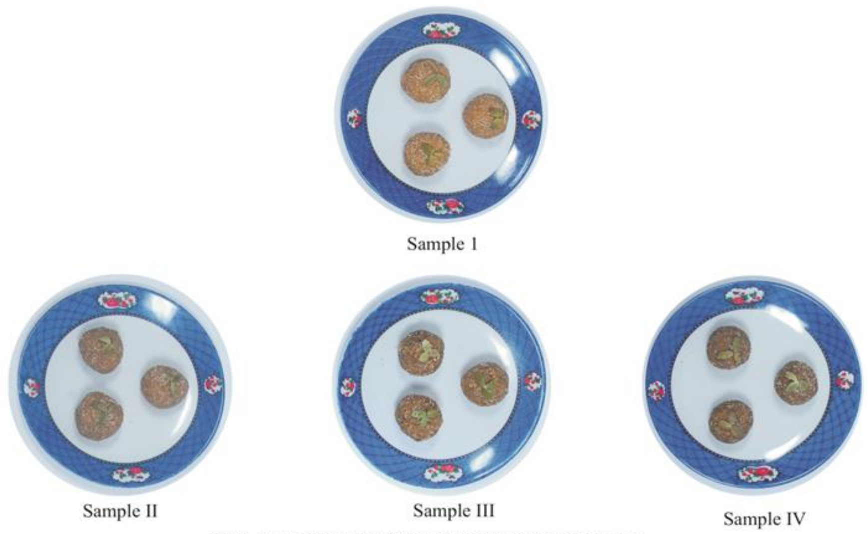

Plate 1 : Appearance of control and experimental laddu

Sample I - Control laddu
Sample II - 4 gm F.S. + 6 gm A.S
Sample III - 8 gm F.S. + 12 gm A.S.
Sample IV - 10 gm F.S. + 20 gm A.S.

Table 2: - Sensory scores of control and experimental laddu

	Appearance	**Texture**	**Flavour**	**Overall Acceptability**
Sample I	9.33 [b] ± 0.18	8.56 [b] ± 0.28	17.11 [b] ± 0.37	8.33 [c] ± 0.18
Sample II	7.17 [a] ± 0.34	6.89 [a] ± 0.21	14.72 [a] ± 0.53	6.28 [a] ± 0.24
Sample III	6.83 [a] ± 0.20	6.28 [a] ± 0.23	14.89 [a] ± 0.28	6.89 [b] ± 0.20

Sample IV	9.17 [b] ± 0.12	9.00 [b] ± 0.16	18.94 [c] ± 0.21	9.67 [d] ± 0.14
F-value	34.04*	33.65*	30.02*	62.16*

- Values are mean ± S.E.M three observations.
- Mean values with the different superscript within a column are significantly different ($p \leq 0.05$).
- *indicates significant difference at $p \leq 0.05$.

Table 3 depicts the mean initial and final levels of Hb, PCV, and RBC of subjects fed laddu and fig: 1 shows the percentage change in Hb, PCV and RBC levels.

Table 3: Initial and Final Levels of Hb, PCV, and RBC of subjects fed laddu

	Initial Level	**Final Level**	**t-value**
Hb (gm/dl)	10.68 ± 0.51	13.05 ± 0.62	0.0009*
PCV (%)	35.93 ± 2.04	37.16 ± 0.79	0.25
RBC (mill / cu.mm)	4.42 ± 0.19	4.61 ± 0.10	0.19

- Values are Mean ± S.E.M.
- *indicates significant difference.

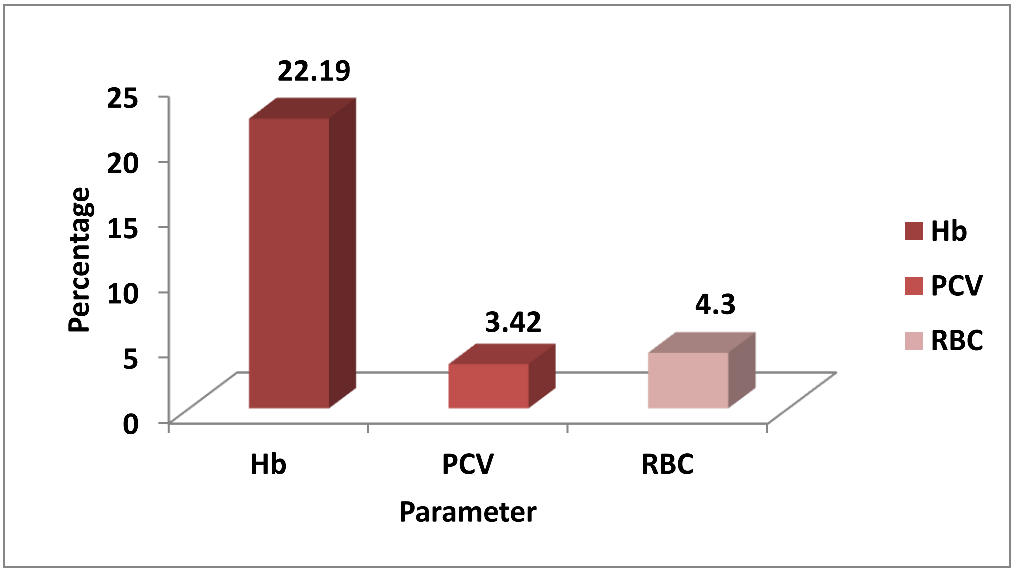

Figure 1:- Percent change in hemoglobin, PCV and RBC concentration of subjects after supplementation of laddu

Hemoglobin

The mean value of hemoglobin before (initial) and after (final) supplementation in the subjects were found to be 10.68 and 13.05 mg %, respectively. A significant increase in Hb concentration ($P \leq 0.05$) was observed after feeding flaxseed and amaranth seed powder incorporated laddu. A percentage increase of 22.19 was observed after supplementation for one month. The increase may be attributed to the present of copper, folic acid, vitamin B6 in flaxseed (Gupta, Sachdeva and Kochar, 2010). Copper is the chief component of the enzyme ceruloplasmin that helps in the transfer of iron to hemoglobin and is essential for hemopoesis. Folic acid and vitamin B6 help in the heme part of hemoglobin synthesis. Laddu also contains amaranth seeds which have a protein content of around 15 gm % (Kelly and Martin 2008). Protein helps in the formation of globin portion of hemoglobin and hence hemoglobin synthesis. Gupta, Sachdeva and Kochar (2010) have also reported an increase of hemoglobin concentration from 12.26 – 12.50 gm % of hemoglobin after 10 gm of flaxseed supplementation. De França Cardozo et al (2011) found that chronic intake of 25% flaxseed into diet alters hematologic indicators haemoglobin in adult Wistar rats.

PCV

The mean initial and final levels of PCV were found to be 35.93 and 37.16 %, respectively. A non significance increase was observed in the PCV after laddu supplementation. A percentage increase of 3.42 was observed after supplementation for one month. Gupta, Sachdeva and Kochar (2010) have reported a non significant increase in PCV value after flaxseed supplementation of 10 gm to patient suffering from CHD.

RBC

The mean count of RBC before (initial) and after (final) supplementation were 4.42 and 4.46 mill / cu. mm, respectively. A non significant increase was observed after 1 month feeding of laddu, which could be due to presence of phenolic acid which helps in the maturity of RBC and hence resulted in more RBC count. A percentage increase of 4.3 was observed after supplementation for one month. Similar non significant increase was observed by Gupta, Sachdeva and Kochar (2010) after supplementation of flaxseed at a level of 10 gm to patient suffering from CHD. Babu et al (2000) have also reported an increase in the RBC count after feeding flaxseed meal to female rats.

Table 4 shows the mean initial and final values of total antioxidant capacity and total protein of subjects fed laddu and fig: 2 shows the percentage in total antioxidant capacity and total protein of subjects after supplementation of laddu.

TAC

The mean values of TAC before (initial) and after (final) supplementation were found to be 27.85 and 19.44 mg TE/ 100 ml, respectively. Inspite of presence of antioxidant rich ingredients in laddu, our results do not indicate an increase in the plasma TAC after laddu supplementation. A percentage decrease of 30.2 was observed after supplementation for one month. A low effect of flaxseed and amaranth seed on plasma TAC may be attributed to a short duration of the study. Jesper et al (2005) have also reported a decrease in FRAP values from 818 to 802 umol / Ltr after 6 weeks supplementation of flaxseed on total antioxidant capacity of plasma of healthy post menopausal women.

Table 4: - Initial and Final Levels of total antioxidant capacity and total protein of subjects fed laddu.

	Initial Level	Final Level	t-value
TAC (mg TE / 100 ml)	27.85 ± 0.62	19.44 ± 0.41	2.30
Protein (gm %)	5.76 ± 0.12	5.67 ± 0.45	0.41

- Values are mean ± S.E.M of two observations.

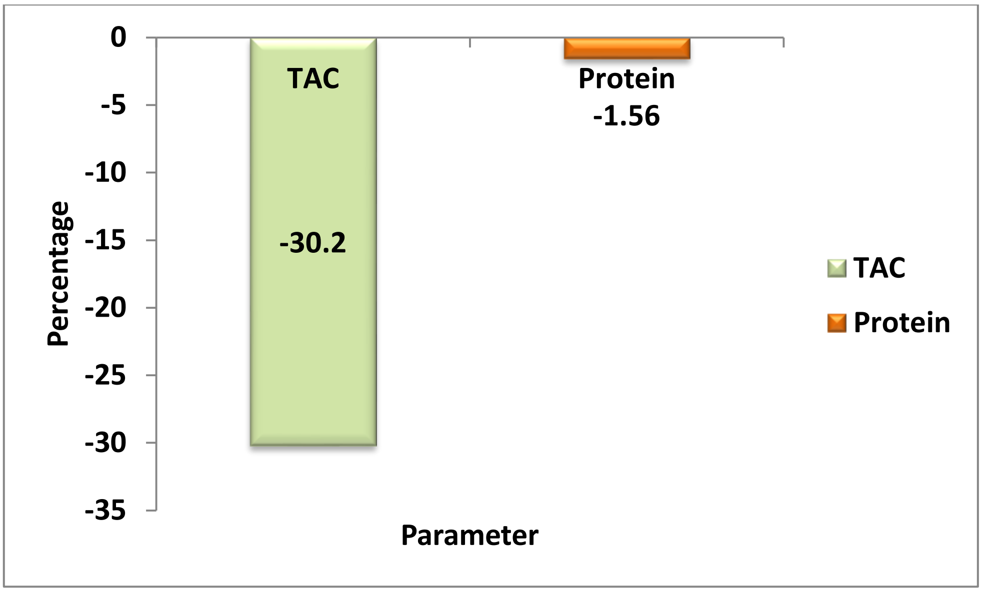

Figure 2:- Percent change in total antioxidant capacity and total protein of subjects after supplementation of laddu.

Total protein

The mean initial and final levels of total plasma protein were 5.76 and 5.67 gm % respectively. A percentage decrease of 1.56 was observed after supplementation for one month. No change was observed after supplementation of flaxseed and amaranth seed incorporated laddu.

Correlation data indicated a significant correlationship of hemoglobin with PCV ($r = 0.577$, $P \leq 0.05$) and serum TAC ($r = 0.700$, $P \leq 0.05$).

De França Cardozo et al (2011) did not observe alterations in total protein level at 250 days old Wistar rats after chronic intake of 25% flaxseed in diet.

Conclusion

The present study revealed that incorporation of flaxseed and amaranth seed at a level of 10 + 20 gm respectively in laddu helps to improve the hemoglobin concentration & RBC count. Thus flaxseed and amaranth seed as a food supplement should be encouraged for anemic patients.

References

1. Malhotra A and Passi SJ (2007). Diet quality and nutritional status of rural adolescent girl beneficiaries of ICDS in North India. Asia Pacific Journal of Clinical Nutrition, 16 (01): 08-16.
2. Soemantri AG, Pollitt E, Kim I. Iron deficiency anemia and educational achievement. Am J Clin Nutr 1985;42(6):1221–8.
3. Sanap Y and Jadhav K (2014). Effect of supplementation of poha ladoo on haemoglobin level in tribal anaemic adolescent girls. Journal of ready to eat food,1 (1):18-21.
4. Gegios .A, Author, R., Maziva-Dixan, Mallow.S, children consuming cassava as a staple food are at risk for inadequate zinc, iron & vitamin A intake. Plant foods for Human Nutrition 2010; 6:64-70.
5. Benoist B D, McLean E, Egll I, Cogswell M (2008). Worldwide Prevalence of Anemia 1993-2005: WHO Global Database on Anemia. Geneva: World Health Organization.
6. Krishnaswamy, K. 2009, The problem and consequences of the double burden-A brief overview Programme & abstracts symposium on Nutritional Security for India-Issues & way forward. Indian Nutritional Science Academy. New Delhi pp.5-6 c.f. Int.Food Res.J 18: 653-661.
7. Iuchi, Y. (2012). Anemia caused by oxidative stress. INTECH Open Access Publisher. www.intechopen.com.
8. Pace, B. S., Shartava, A., Pack-Mabien, A., Mulekar, M., Ardia, A. & Goodman, S. R. (2003). Effects of N-acetylcysteine on dense cell formation in sickle cell disease. Am. J.Hematol. 73(1), 26-32.
9. Thompson LU, Rickard SE, Cheung F, Kenaschuk EO, Obermeyer WR. Variability in anticancer lignan levels in flaxseed. Nutrition and Cancer 1997; 27:26-30.
10. Bloendon L, Phillipe O, Scazapary MD 2004.Flaxseed and cardiovascular risk. Nutr Rev, 62:18-27.
11. Anonymous 2005. Official home page of flaxseed Pro. Retrieved on 21.2.2007 from http://www.flaxseedpro.com/index/php.
12. Srilakshmi, Food Science, Fourth Edition, 2007.
13. Gupta, Rajiv Sachdev and Anita Kochar. Effect of flaxseed supplementation on nutrient adequacy and iron status of coronary heart disease patients. Ethno Med 2010;4(1):1-8.
14. Kelly and Dr. Martin L. Price. Amaranth grain & vegetable types. Echo Technical note 2008.
15. De França Cardozo, L. F. M., Soares, L. L., Brant, L. C., Chagas, M. A., Pereira, V. A., Velarde, L. C., & Bonaventura, G. T. (2011). Hematologic and inmunological indicators are altered by chronic intake off flaxseed in Wistar rats. Nutrición hospitalaria: Organo oficial de la Sociedad española de nutrición parenteral y enteral, 26(5), 1091-1096.
16. Babu US, Mitchell GV, Wiesenfeld P, Jenkins MY, Gowda H. Nutritional and hematological impact of dietary flaxseed and defatted flaxseed meal in rats. Int J Food Sci Nutr. Mar 2000;51(2):109-17.
17. Jesper Haullund, Gitte Ravn Haren, Susanne Bugel, Tine Tholstup, and inge Tetens. A lignan complex isolate from flaxseed does not effect plasma lipid concentrations or antioxidant capacity in healthy postmenopausal women. American Society for Nutrition 2006.

18. World Health Organization. Programming for adolescent health and development. Technical Report Series No.886;1999 c.f Ind.J of Com.Med. vol.31, No. 4,Oct-Dec, 2006.
19. whqlibdoc.who.int.

****

Priyal Panchal
M.Sc. Student
P. G. Department of Home Science
Sardar Patel University
Vallabh Vidyanagar

Vimal B. Jayswal
Research Scholar
P. G. Department of Home Science
Sardar Patel University
Vallabh Vidyanagar

V. H. Patel
Professor and Head
P. G. Department of Home Science
Sardar Patel University
Vallabh Vidyanagar

Neeta Rupesh Dave
Associate Professor
P. G. Department of Home Science
Sardar Patel University
Vallabh Vidyanagar