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Assessment of Therapeutic Potential of Pearl Millet Iron Fortified Cookies through Animal Modeling

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Diet diversification and food fortification are successful strategies to combat micronutrient deficiencies and allied disorders. The current research study is an attempt to assess the therapeutic evaluation of pearl millet (*Pennisetum glaucum* L.) based iron-fortified cookies to combat anemia. In the first phase, pearl millet was characterized for nutritional composition through respective methods. Moreover, iron fortified cookies were prepared that composed of composite flour of pearl millet and wheat flour with the addition of iron fortificants *i.e.* NaFeEDTA and $FeSO_4$. In 2nd phase, the efficacy study was accomplished through animal modeling. Results showed that anemia was significantly reduced by both pearl millet iron fortified cookies treatments. In addition, pronounced increase in hemoglobin (Hb) level was examined for pearl millet iron fortified cookies with NaFeEDTA than pearl millet iron fortified cookies fortified with $FeSO_4$. The parameters for anemia study including red blood cell, hemoglobin and hematocrit level showed improvement both in anemic and non-anemic rats studies. Conclusively, the results from the current study revealed that NaFeEDTA salt is a better option for fortification than $FeSO_4$. In the nutshell, pearl millet composite flour with iron fortification (NaFeEDTA) should be included in dietary patterns for controlling anemia.

Keywords: Malnutrition, Iron deficiency anemia, Fortification, Diet-diversification, Pearl millet.

INTRODUCTION

In millennia, malnutrition is a major nutritional disorder worldwide particularly in the developing economies. Imbalance nutrient's intake and poor dietary patterns result in adverse effects on the normal body functions. The phenomenon of malnutrition is closely related with a malfunctioning of immune system *i.e.* phagocyte function, complement system, impairment of cell-mediated immune (CMI) responses and immunoglobulin, anatomic barriers and antibiotics. According to World Health Organization (WHO), food fortification and bio-fortification are important sustainable strategies for achieving the goals of controlling deficiencies of micronutrients [1,2].

Anemia is a derivation of Greek word anaimia meaning without blood [3]. An individual is considered anemic if his/her circulating blood concentration is less than 120 g/L. It depends upon age, sex, iron requirement and hemoglobin thresholds [4,5]. Anemia is a community dilemma that influences populace of the world and its prevalence is more dominant in developing countries as compared to developed economies. It can occur through life cycle in all human beings but pregnant women and young children are more prone to it. Globally, anemia is devastating for human health and reverses social and economic growth [6]. Deficiency of iron affects over 3.5 billion people and 2 out of 3 people in emergent nations. According to WHO, this is the most common nutritional disorder in world [7].

Pearl millet is one of the most important cereal grains in the world. Pearl millet crop belongs to a good adaptableness to local environments for its properties of being tolerant to drought and heat [8]. Millet being a healthy, nutritious and versatile grain can prove to be a valuable add-on to food. It is extremely palatable and is regarded as a good source of phenolic compounds, proteins, minerals and energy [9]. Millets don't contain gluten and is not an acidic food, hence is gentle and easily digestible. Millets have no allergenic reactions associated being warming grain aids the body to warm in rainy seasons and cold climates [10].

To overcome the nutrient deficiencies fortification of staple foods is an effective strategy. In case of the multiple mineral deficiencies, fortification of food with two or more minerals simultaneously is most appropriate. It is essential that forms of the added nutrient should be satisfactorily bio-available. However, addition of these minerals to staple foods is a challenging task because it may cause unacceptable organo-leptic changes and rejection of the product by population [11].

EXPERIMENTAL

Raw material *i.e.* two pearl millet varieties MB-87 and Sargodha Bajra-2011 were procured from Ayub Agricultural Research Institute, Faisalabad. Iron fortificants *i.e.* NaFeEDTA and $FeSO_4$ were procured from Sigma Aldrich. Samples were tested for red blood cell (RBC), hemoglobin (Hb) and hematocrit level.

Nutritional composition of pearl millet: Samples were tested for moisture content, crude protein content, crude fat, crude fiber, ash content and mineral profile (Fe) through respective methods.

Preparation of iron fortified cookies: Different treatments of the cookies using two iron fortificants *i.e.* NaFeEDTA and $FeSO_4$ were prepared according to the method No. 10-54 as mentioned in AACC [12] with some modifications.

Efficacy study: Sprague Dawley rats were purchased from National institute of Health (Veterinary Division), Islamabad and brought to the laboratory at Animal Room of Department of Physiology, Govt. College University, Faisalabad, Pakistan. The ethical approval for conducting such studies was taken from ethical review committee (ERC), Government College University, Faisalabad. Biological essay was conducted according to the model adopted [13].

The standard conditions for the animal handling during the entire study durations were adjusted according to standard guidelines of Animal Institute of Nutrition (AIN), USA. These conditions include temperature (23 ± 2 °C), relative humidity (55 ± 5 %) and 12 h light-dark cycle. The rats were randomly divided into 2 main groups for anemic and non-anemic rats. Each main group was again divided into 3 subgroups (with 8 rats each) based on their diet plan for consuming control diet and diet containing cookies fortified with iron compounds NaFeEDTA and $FeSO_4$ coded as D_0, D_1 and D_2 consecutively. Each member of the group was marked a number.

At the end of rodent modeling study, rats from each group were decapitated for blood collection through neck and cardiac puncture. Initially some rats were killed to calculate the base line values, while the rest were scarified at the end of study. The collected blood samples were analyzed for further assays and details are mentioned herein.

Statistical analysis: Statistical design complete randomized design (CRD) was used for data analysis. The values presented in tables are means ± standard deviation. The technique of analysis of variance (ANOVA) was applied to check the level of significance. Least significant difference test (LSD) further clarified the effects of diets in a comprehensive manner [14].

RESULTS AND DISCUSSION

The results for physico-chemical analysis of pearl millet (Table-1) showed that maximum moisture content (7.63 ± 0.53 %) was found in MB-87 whilst, the minimum (6.62 ± 0.47 %) was reported in Sargodha Bajra-2011. These results are compatible with earlier findings reported by [15] who found moisture content of the millet cultivars ranged from 5.9 to 7.7 % which was well below the maximum moisture content limit (13 %) intended for human consumption as recommended by FAO/WHO [16]. The results showed that higher ash content (2.30 ± 0.17 %) was found in Sargodha Bajra-2011 while the lower (1.74 ± 0.11 %) was exhibited in MB-87. The fat content in whole flours of pearl millet ranged from 3.81 ± 0.27 % to 4.53 ± 0.33 % (Table-1). Higher fiber content (2.62 ± 0.10 %) was found in Sargodha Bajra-2011. Because of high fiber content it can be useful for the preparation of healthy foods for people who needs high fiber diet [17]. The results indicated that the protein content (11.70 ± 0.72 %) was found maximum in Sargodha Bajra-2011 whilst the lowest (10.14 ± 0.67 %) was exhibited by MB-87. The results indicated that the maximum iron contents (3.27 ± 0.15 mg/100 g) were found in Sargodha Bajra-2011. The iron content (2.43 ± 0.12 mg/100 g) was exhibited minimum by MB-87.

Hematological parameters

Red blood cell count (RBC): Means (Table-2) related to red blood cells (RBC) count in trial I, study I *i.e.* non-anemic rats showed values for control group (D_0), NaFeEDTA fortified cookies (D_1) and $FeSO_4$ fortified cookies (D_2) as 6.9 ± 0.21, 7.18 ± 0.16 and 7.15 ± 0.19 respectively. For trial I, study II *i.e.* anemic rats values for (D_0), NaFeEDTA fortified cookies (D_1) and $FeSO_4$ fortified cookies (D_2) were observed as 4.12 ± 0.17, 7.09 ± 0.11 and 6.79 ± 0.21. Data for red blood cell count in 2nd trial, study I *i.e.* non-anemic rats showed values for D_0, D_1 and D_2 as 6.96 ± 0.22, 7.27 ± 0.25 and 7.21 ± 0.0.27 respectively. For trial II, study II *i.e.* anemic rats values for D_0, D_1 and D_2 were recorded as 4.01 ± 0.24, 7.11 ± 0.09 and 6.72 ± 0.32 cells/µL. During the study trials, a highly significant increase was recorded in RBC count for both anemic and non anemic rats. Rats NaFeEDTA fortified cookies (D_1) showed maximum increase in RBC count.

The results from this study are in accordance with the previous work [18] found a significant increase in the RBC of the rats fed with iron fortified rice. Similar results were reported [19] for iron fortified maize fed to school children in Kenya.

TABLE-1
MEAN VALUES FOR CHEMICAL COMPOSITION OF PEARL MILLET FLOURS

Varieties	Ash content (%)	Moisture content (%)	Fat content (%)	Fiber content (%)	Protein content (%)	Iron content (mg/100 g)
MB-87	1.74 ± 0.11[a]	7.63 ± 0.53[a]	3.81 ± 0.27[a]	2.27 ± 0.09[a]	10.14 ± 0.67[a]	2.43 ± 0.12[b]
Sargodha Bajra-2011	2.30 ± 0.17[a]	6.62 ± 0.47[b]	4.53 ± 0.33[a]	2.62 ± 0.10[a]	11.70 ± 0.72[b]	3.27 ± 0.15[a]

Means carrying same letters are significantly identical: a = Precentage; b = mg/100 g

TABLE-2
MEANS FOR EFFECT OF TREATMENTS ON RBC (cells/μL)

Groups	Non-anemic rats			Anemic rats		
	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies
Trial I	6.9 ± 0.21^b	7.18 ± 0.16^a	7.15 ± 0.19^a	4.12 ± 0.17^c	7.09 ± 0.11^a	6.79 ± 0.21^b
Trial II	6.96 ± 0.22^b	7.27 ± 0.25^a	$7.21 \pm 0.0.27^a$	4.01 ± 0.24^c	7.11 ± 0.09^a	6.72 ± 0.32^b

Means carrying same letters are significantly identical

Hemoglobin concentration (Hb conc.): Results (Table-3) pertaining to Hb concentration in trial I, study I *i.e.* non-anemic rats showed values for cookies without iron fortificant (D_0), NaFeEDTA fortified cookies (D_1) and $FeSO_4$ fortified cookies (D_2) as 12 ± 0.78, 12.49 ± 0.71 and 12.42 ± 0.81 respectively. For trial I, study II *i.e.* anemic rats values for D_0, D_1 and D_2 were recorded as 6 ± 0.27, 12.35 ± 0.19 and 11.94 ± 0.42. Results for Hb concentration in trial II, study I *i.e.* non-anemic rats showed values for D_0, D_1 and D_2 as 11.55 ± 0.76, 12.04 ± 0.69 and 11.96 ± 0.72 respectively. For trial II, study II *i.e.* anemic rats values for D_0, D_1 and D_2 were recorded as 7 ± 0.31, 12.24 ± 0.22 and 12.03 ± 0.37 g/dL, respectively. During the study intervals, an increase was recorded in Hb concentration for both anemic and non anemic rats. Rats fed NaFeEDTA fortified cookies (D_1) showed maximum increase in Hb concentration in anemic rats after consumption of iron fortified diets. Ebuehi and Oyewole [18] also found a significant increase in the Hb concentration of rats fed with and without iron fortified rice.

In another study, Salgueiro *et al.* [20] determined the Hb concentration variations owing to supplementation of ferrous sulfate and stabilized iron (II) sulfate groups. Ferrous sulfate and stabilized iron(II) sulfate groups showed positive changes in Hb concentration than that of the control group. They concluded that the iron provided by these fortified diets is efficiently incorporates resulting in improved hemoglobin level.

In another study, Akhtar *et al*. [21] also found that Hb level of the NaFeEDTA group was increased significantly from 2nd month to 6th month. Hemoglobin level of the $FeSO_4$ group became significantly higher at month 4 and month 6 than that of baseline and control group. The results from this study also showed that even when NaFeEDTA was added at a lower level, it has better effects than $FeSO_4$ and elemental iron in controlling iron deficiency anemia and improving iron status in anemic children. Although, some other researchers reported similar results [22] but the concertations of Hb were on the higher side. They used different fortificants for the preparation of Egyptian bread, casein diet and grain amaranth cereal and observed that NaFeEDTA performs better than $FeSO_4$ or $FeSO_4$ + NaFeEDTA cereals.

Hematocrit percentage: Means (Table-4) pertaining to hematocrit percentage in trial I, study I *i.e.* non-anemic rats showed values for cookies without iron fortificant D_0, NaFeEDTA fortified cookies D_1 and D_2 as 36 ± 1.65, 37.16 ± 1.45 and 37.08 ± 1.56 %, respectively. For trial I, study III (anemic rats) values for D_0, D_1 and D_2 were recorded as 38 ± 1.72, 39.22 ± 1.32 and 39.14 ± 1.39 %. Results for hematocrit percentage in trial II, study I *i.e.* non-anemic rats showed values for D_0, D_1 and D_2 as 29 ± 1.11, 36 ± 1.01 and 35.10 ± 1.19 % respectively. For trial II, study II *i.e.* anemic rats values for D_0, D_1 and D_2 were recorded as 28 ± 1.18, 34.23 ± 1.52 and 33.66 ± 1.67 % respectively. During the study intervals, an increase was recorded in hematocrit percentage for both anemic and non-anemic rats. Rats fed NaFeEDTA fortified cookies (D_1) showed maximum increase in hematocrit percentage. Similar results were found by Whittaker and Ologunde [22] who found a slightly more increase in hematocrit percentage in Egyptian bread, casein diet and grain amaranth cereal for NaFeEDTA than for $FeSO_4$ or $FeSO_4$ + NaFeEDTA.

Conclusion

Diet diversification and fortification are ideal strategies to combat micronutrient deficiencies. In the present research,

TABLE-3
MEANS FOR EFFECT OF TREATMENTS ON HEMOGLOBIN (g/dL)

Groups	Non-anemic rats			Anemic rats		
	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies
Trial I	12 ± 0.78^b	12.49 ± 0.71^a	12.42 ± 0.81^a	6 ± 0.27^c	12.35 ± 0.19^a	11.94 ± 0.42^b
Trial II	11.55 ± 0.76^b	12.04 ± 0.69^a	11.96 ± 0.72^a	7 ± 0.31^c	12.24 ± 0.22^a	12.03 ± 0.37^b

Means carrying same letters are significantly identical

TABLE-4
MEANS FOR EFFECT OF TREATMENTS ON HEMATOCRIT (%)

Groups	Non-anemic rats			Anemic rats		
	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies	Cookies without iron fortificant	NaFeEDTA fortified cookies	$FeSO_4$ fortified cookies
Trial I	36 ± 1.65^b	37.16 ± 1.45^a	37.08 ± 1.56^a	29 ± 1.11^c	36.00 ± 1.01^a	35.10 ± 1.19^b
Trial II	38 ± 1.72^b	39.22 ± 1.32^a	39.14 ± 1.39^a	28 ± 1.18^b	34.23 ± 1.52^a	33.66 ± 1.67^a

Means carrying same letters are significantly identical

the pearl millet that is neglected cereal crop was used to prepare wheat-millet flour blends due to its nutritional value. Moreover, the NaFeEDTA and $FeSO_4$ were used as iron salt to control anemia. The results indicated that NaFeEDTA showed good performance and is more acceptable by the community. The serological aspects *i.e*. liver functioning tests; AST, ALT, ALP and renal functioning test (serum urea and creatinine) showed no sign of toxicity. In efficacy study, anemic rat's red blood cells count, hemoglobin concentration and hematocrit percentage increased significantly showing that these pearl millet iron fortified cookies are the suitable option for anemic patients.

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