Development of iron enriched rice flour pasta to address nutritional anemia in experimental subjects

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Abstract

According to WHO, it has been estimated that nearly 60% children and 30 to 40% women are suffering from iron deficiency anemia in Pakistan. Objective of this study was to address nutritional anemia and elucidate the bio-efficacy of iron Fortificant in rice flour-based pasta using rodent experimental model. An experimental study in which NaFeEDTA fortified rice pasta were developed with 10, 15, 20, 25, 30 ppm concentration whilst commercially available fortified cereal was also purchased to compare the effectiveness of developed product rice flour. Product was analyzed for cooking attributes, sensory evaluation, texture. Anemia was induced in selected animals using carbon tetrachloride (1.9 ml/kg of body weight twice per week for three successive weeks) administered intraperitoneally. For efficacy trials, 40 Sprague Dawley mice were divided into eight groups (5 in each group). Different levels of NaFeEDTA fortified diet was administered orally. At the end of the study, organ, and blood sample (cardiac puncture) were collected for different biochemical analyses. From results it was concluded that rice pasta fortified with 30 ppm NaFeEDTA has significantly addressed nutritional anemia.

Keywords: Anemia, Albino rats, Fortification, NaFeEDTA salt, Nutritional anemia.

1. Introduction

Microelement are playing foremost role to assist life and perform biological functions. Globally micronutrient deficiencies are prevalent in pregnant women and children under 5 years of age. Widespread nutritional deficiencies are iron, folate, zinc, iodine, vitamin A. It has been observed that these nutritional deficiencies lead to poor growth, perinatal complication, intellectual impairment and increased chances of morbidity and mortality (Bailey and Black, 2015)

Iron is vital component of biological function including energy production, cell proliferation, DNA synthesis and respiration (Hentze *et* al 2010). Persistent low amount of iron causes iron depletion that ultimately leads to physiological disorder like iron deficiency anemia (Camaschella, 2015). Iron deficiency is worldwide a critical public health problem and most prevalent nutritional deficiency. Internationally, iron deficiency is present twice time as compared to iron deficiency anemia (Lanzkowsky, 2016).

Worldwide, it has been reported that prevalence of anemia is 47%, 42% and 30% in children 5 years of age, in younger pregnant women and non-pregnant women of age 15-49, respectively. Therefore, anemia leads to about 115,000 maternal death and 591,000 prenatal death globally per year. It has been noticed that prevalent of anemia

in adolescent girls is 56%, (15-19 year), 30% in adolescent boy and about 80% among children that is under 3 year of age (Cheema *et* al., 2016) (Benoist *et* al., 2008). In Pakistan, it has been estimated that approximately 60% preschool suffers with iron deficiency. Similarly, almost 35 to 40% women are dealing with iron deficiency, whose 90% of pregnant women have dangerous low reservoir of iron that enhanced the chances of fetus improper development. But at somehow, the situation in Pakistan is less complicated than south Asia but present situation suggested that there are need of work and awareness to overcome the hidden hunger (WHO, 2012).

Iron fortification of rice is best treatment and long-term nutrition intervention strategy for reducing prevalence of iron deficiency anemia. In India rice is staple food and its consumption is 74.2kg per capita in 2016-2017 whilst worldwide average consumption is 61.2 (Azam *et* al., 2017).

The purpose of study is to overcome the iron deficiency anemia and promote the use of rice flour-based iron fortified pasta as dietary intervention. The objective of study is development of iron enriched rice flour pasta to have control on iron deficiency anemia, assess the impact of processing and storage on overall nutritional quality attributes of developed pasta and elucidation the efficacy of developed iron fortified pasta using rodent experimental model.

2. Materials and methods

2.1. Materials

Basmati rice flour for development of pasta were purchased from local market (Moon market) of Lahore, Pakistan. The chemicals and reagents were purchased from reputed companies. For bio-efficacy trial, female Sprague Dawley mice were purchase from Foot and Mouth disease research center at Bedian road Lahore, Pakistan. This study was done in the University of Veterinary and Animal Sciences, Lahore.

2.2. Preparation of iron fortified rice flour-based pasta

Dough of fortified rice flour-based pasta was prepared by adding 2tsp oil, 2 cup water, and 2tsp iron salt into 4 cup rice flour. Dough was passed through pasta maker machine, five levels of treatments was prepared having 10ppm, 15ppm, 20ppm, 25ppm, 30ppm concentration iron salt. Furthermore, formulated pasta was placed in dehydrator. Afterward, rice flour-based pasta was cooked in non-stick pan by using traditional recipe method.

2.3. Texture analysis

For each test, the developed pasta was placed in dehydrator at 158°F about 2 hours. After cooking, the pasta was mildly be smudge and surrender to firmness and stickiness analysis, performed by a model FRTS Texture Analyzer (model: FRTS-100N-I IMADCO, LTD. Made in Japan). In this context, hardness of developed pasta was performed by used a stainless-steel cylinder probe (Jalgaonkar & Jha, 2016)

2.4. Color measurement

The products surface color, a* (–a greenness; +a redness), L* (lightness), and b* (–b blueness; +b yellowness) were measured through hand held Minolta chromo meter (Model CR-200 Minolta corporation, Tokyo, Japan). The samples were kept in a translucent petri dish and color parameter values of a*, L*, b*, chroma (c*) and hue angle were recorded (Limroongreungrat, Huang, & Technology, 2007).

Chroma (C*) = [a*2 + b*2]1/2

Hue angle (h) = tan-1 (b*/a*)

2.5. Efficacy trial

Effectiveness of fortified rice flour-based pasta was analyzed through animal trial including forty experimental mice (Sprague Dawley) age 4 weeks which bought from Foot and Mouth Disease research center at Badian road Lahore Can't.Mice were housed in animal room available at University of Veterinary and Animal Sciences Lahore. They were shed in room providing 12-hour light and 12-hour dark period, whilst room temperature was maintained around

20°C to 25 °C. For acclimatization, mice were kept on basal feed for two weeks before initiation of experiment to ensure normal growth and behavior. Nonetheless, ethical acceptance (No. DR/998) for conducting animal model study was taken from Office of Research, Innovation & Commercialization (ORIC), at UVAS Lahore. Afterward mice were randomly divided into eight group (5 in each group). Anemia was induced in selected animals using carbon tetrachloride (1.9 ml/kg of body weight twice per week for three successive weeks) administered intraperitoneally. However, G_o and G_7 served as control and anemic control, respectively. Whilst G_1, G_2, G_3, G_4 and G_5 served as anemic which were treated with 10, 15, 20, 25, 30 ppm NaFeEDTA fortified diet, respectively. Alongside, G_6 was fed on commercially available fortified cereal based diet (Table No.1)

Table: 1 Iron fortificants levels and treatments for efficacy trial

Groups	Treatments	Iron Fortificant	Induced anemia
G_0	Fed on unfortified diet	NaFeEDTA salt	N/A
G_1	Fed on 10 ppm fortified diet	NaFeEDTA salt	CCl_4 (1.9 ml/kg of body weight)
G_2	Fed on 15 ppm fortified diet	NaFeEDTA salt	CCl_4 (1.9 ml/kg of body weight)
G_3	Fed on 20 ppm fortified diet	NaFeEDTA salt	CCl_4 (1.9 ml/kg of body weight)
G_4	Fed on 25 ppm fortified diet	NaFeEDTA salt	CCl_4 (1.9 ml/kg of body weight)
G_5	Fed on 30 ppm fortified diet	NaFeEDTA salt	CCl_4 (1.9 ml/kg of body weight)
G_6	Fed on cereal fortified diet	Nestle corn Flex	CCl_4 (1.9 ml/kg of body weight)
G_7	Fed on unfortified diet	N/A	CCl_4 (1.9 ml/kg of body weight)

N/A = not applicable, CCl4= Carbon tetrachloride

Meanwhile, water and feed intake were measured on daily basis whilst weight were recorded on weekly basis. At termination day, blood sample were collected in EDTA coated tube and vacuum tube (bio-Vac) with help of cardiac puncture to study hematological parameter and serum biochemistry. Afterward, mice were slaughter for liver and kidney sample to determined iron content. The collected sample were inspected for serum ferritin, serum iron, hemoglobin, MCH, MCV and Total Iron Binding capacity.

2.6. Sensory evaluation

The developed fortified rice pasta with the mixture of chili and tomato sauce cooked vegetables and chicken were checked for sensory interpretation which is assorted from exceedingly liking to disliking (9 = like extremely; 1 = dislike extremely) by applying nine-point hedonic scale system according the guidelines of Meilgaard et al., (2007). Randomized participants were joined for evaluation. Sensory response of fortified rice pasta of various traits like appearance, color, taste, texture and overall were obtained.

2.7. Statistical Analysis

Obtained data were analyzed statistically using SPSS (Statistical Package for Social Science) Version 22.0 (Steel & Torrie, 1980). Although, technique of one-way analysis of variance (ANOVA) was used to check level of significance ($p < 0.05$). Alongside, data was analyzed for means ± standard deviation.

3. Results and discussion

3.1. Results

Color of different concentration fortified pasta were measured at baseline and termination days of storage study (2 month). From result it has been concluded that brightness values (L*) was higher for 25 ppm fortified pasta than 10 ppm, 15 ppm, 20 ppm and 30 ppm fortified pasta. Redness (a*) values were decreased for fortified than unfortified pasta as noted in 30ppm level. Moreover, storage study revealed that maximum value exists for 30 ppm fortified pasta. Furthermore, hue angle values were showing non-significant trend among fortified and unfortified pasta. Overall color difference between unfortified and 10ppm, 15ppm, 20ppm, 25ppm and 30ppm fortified pasta were very little over 2month storage study.

Texture attribute values were non-significant among unfortified and fortified pasta ($p<0.05$) (figure no.1)

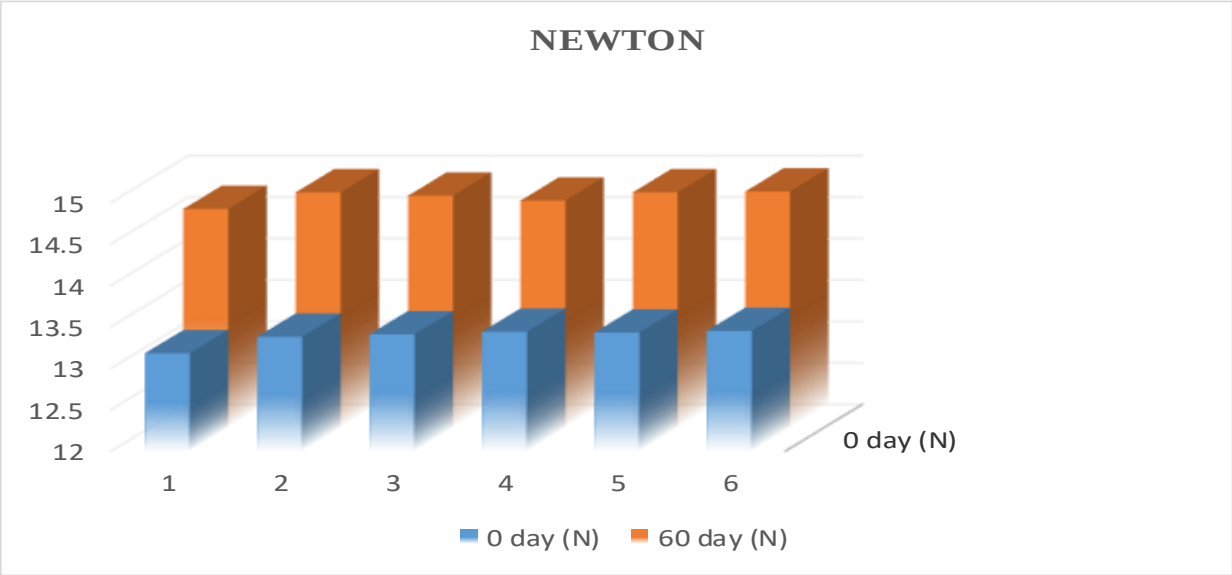

Fig No.1: Graphical representation of texture analyzer of rice pasta (Newton)

Sensory attributes values were significantly ($P>0.05$) different for unfortified and fortified pasta. For color, appearance, and taste trait, 30 ppm NaFeEDTA fortified pasta had higher score when compared with unfortified and 10ppm, 15ppm, 20ppm and 25ppm fortified pasta score (figure No. 2, 3, 4, 5)

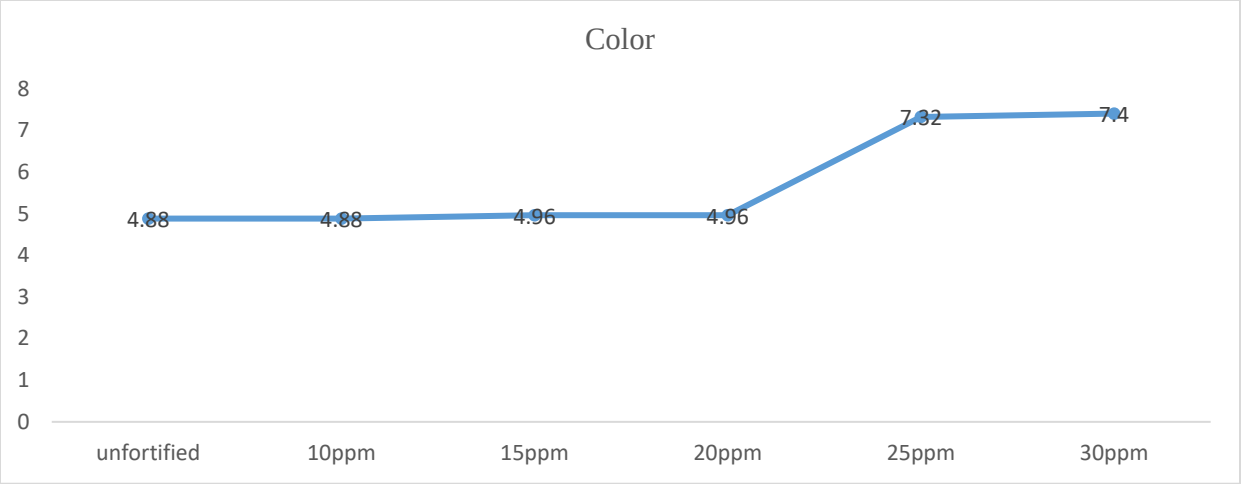

Fig 2: Graphical depiction of Panelist score for color of fortified rice pasta

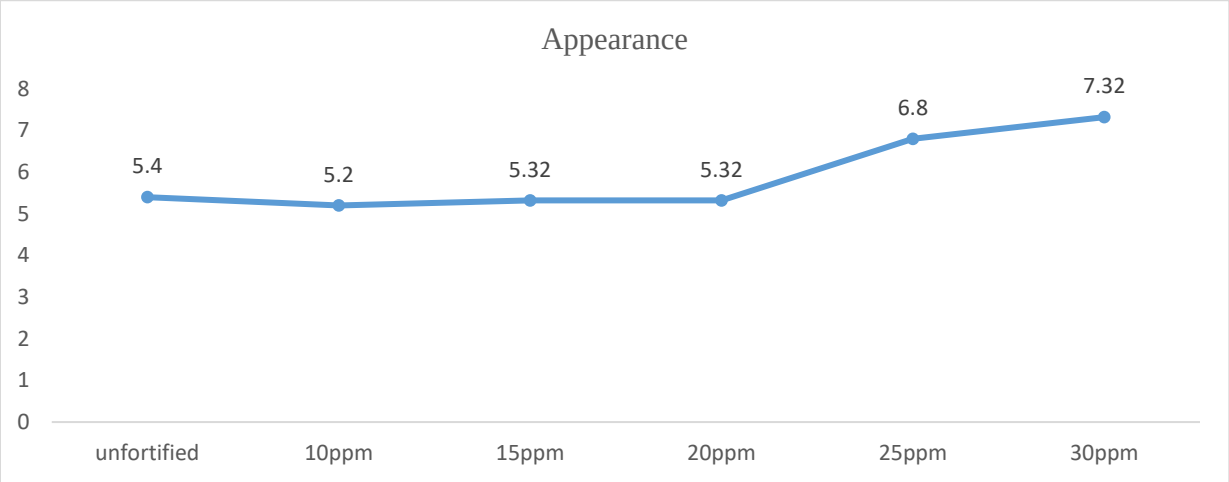

Fig 3: Graphical depiction of Panelist score for appearance of fortified rice pasta

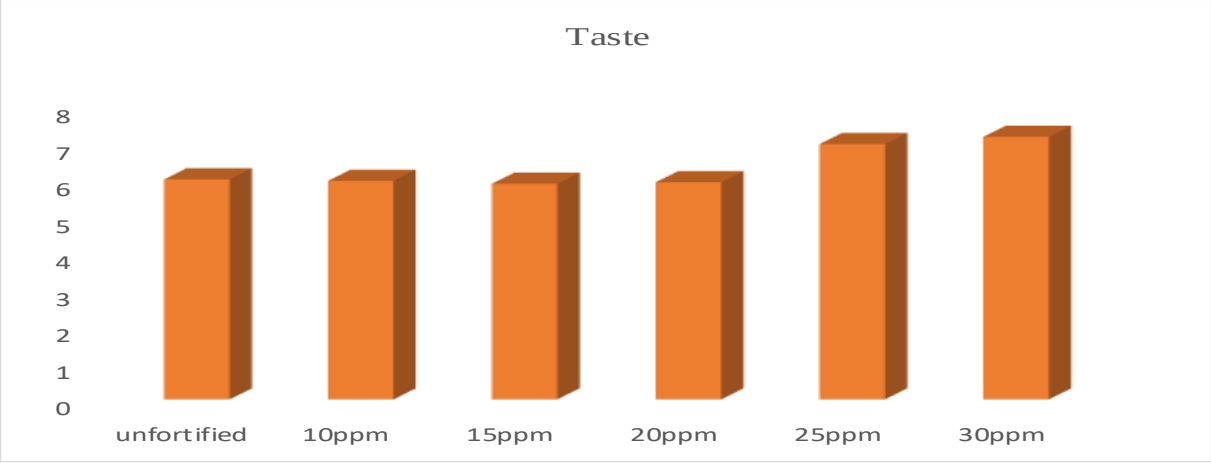

Fig 4: Graphical depiction of Panelist score for taste of fortified rice pasta

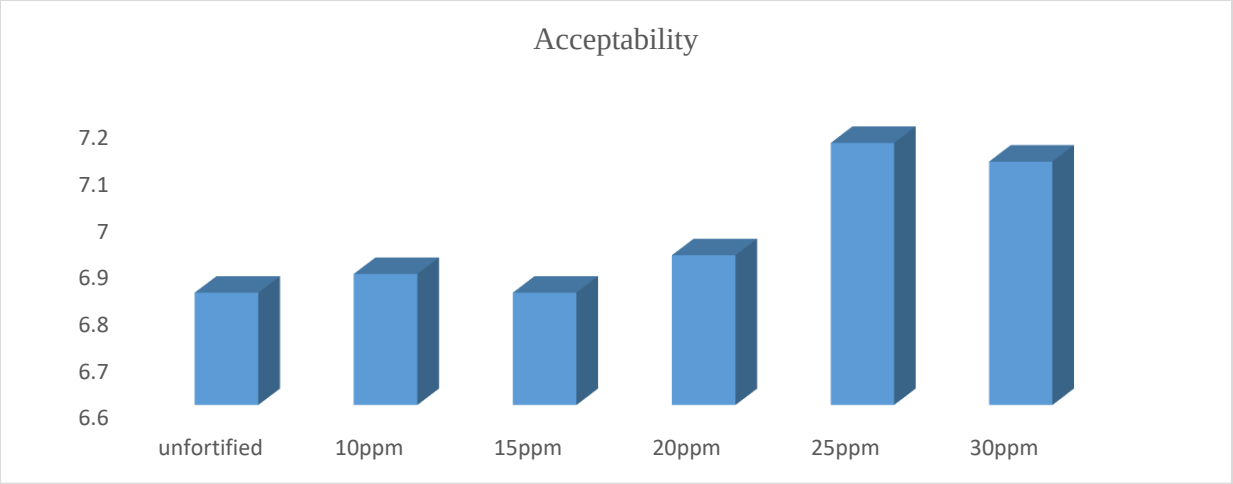

Fig 5: Graphical representation of Panelist score for acceptability of fortified rice pasta

Furthermore, [Table 2] depicted that levels of liver and kidney iron content were significantly improved in G_5 than G_0, G_1, G_2, G_3, G_4, G_6 and G_7. Result elucidated that treatment given to G_5 was more efficiently improved iron content of liver and kidney than G_6 (commercially available fortified cereal). Nonetheless,

Table: 2. Effect of NaFeEDTA fortified diets on liver and kidney iron content of anemic female mice

	Liver µg/g	**Kidney µg/g**
G_0	106.89±1.82	17.25±0.29
G_1	95.10±1.62	10.94±0.18
G_2	96.75±1.65	12.20±0.20
G_3	99.59±1.69	12.76±0.21
G_4	105.35±1.79	13.32±0.22
G_5	106.35±1.81	15.43±0.26
G_6	97.07±1.65	13.74±0.23
G_7	57.93±0.98	7.85±0.13

Value = *mean ± SD, G0 = normal (control), G1 = 10ppm NaFeEDTA fortified rice flour, G2 = 15ppm NaFeEDTA fortified rice flour, G3 = 20ppm NaFeEDTA fortified rice flour, G4 = 25ppm NaFeEDTA fortified rice flour, G5 = 30ppm NaFeEDTA fortified rice flour G6 = commercially available fortified cereal, G7 = Anemic

Likewise, [Table: 3] depicted that levels of serum iron and serum ferritin were significantly enhanced in G_5 (fed on 30ppm iron fortified based diet) as compared to G_0, G_1, G_2, G_3, G_4, G_6 and G_7. Nevertheless, relationship of TIBC is inversely proportional to serum ferritin and serum iron. Hence, [Table 2] shown that TIBC levels were significantly decreased in G_5 whilst increased in G_7 who depicted that 30ppm fortified diet has potential to combat with iron deficiency anemia. Furthermore, maximum improvement in levels of hemoglobin, MCV, MCH and hematocrit were seen in G_5 than G_0, G_1, G_2, G_3, G_4, G_6 and G_7. Nevertheless, it has been concluded that G_5 diet has more potential to improved hematological indicator as compared to G_6 diet

Table: 3 Effect of NaFeEDTA fortified diets on hematological and iron status of anemic female mice

	Serum iron µg/dl	Serum ferritin ng/ml	TIBC µg/dl	Hb g/dl	Hct (%)	MCV (fl)	MCH (pg)
G_0	218.43±3.72	35.62±0.60	535.06±9.12	14.84±0.25	38.38±0.65	47.30±0.82	21.64±0.36
G_1	194.38±3.31	27.48±0.46	625.24±10.66	11.32±0.19	22.05±0.37	37.18±0.63	19.63±0.33
G_2	208.41±3.55	28.55±0.48	599.19±10.21	11.62±0.19	23.02±0.39	38.19±0.65	20.16±0.34
G_3	224.44±3.82	29.81±0.50	573.14±9.77	12.02±0.20	22.15±0.37	38.98±0.66	21.55±0.36
G_4	245.49±4.18	31.89±0.54	550.09±9.38	13.52±0.23	27.07±0.46	39.38±0.67	22.14±0.37
G_5	258.51±4.40	33.60±0.57	519.03±8.85	14.22±0.24	28.76±0.49	39.98±0.68	23.02±0.39
G_6	194.38±3.31	29.20±0.49	584.16±9.96	13.58±0.23	26.11±0.44	36.19±0.61	19.94±0.34
G_7	114.22±1.94	21.00±0.35	705.40±12.02	8.21±0.14	16.88±0.28	28.66±0.48	16.24±0.27

Value = *mean ± SD, G0 = normal (control), G1 = 10ppm NaFeEDTA fortified rice flour, G2 = 15ppm NaFeEDTA fortified rice flour, G3 = 20ppm NaFeEDTA fortified rice flour, G4 = 25ppm NaFeEDTA fortified rice flour, G5 = 30ppm NaFeEDTA fortified rice flour, G6 = commercially available fortified cereal, G7 = Anemic

3.2. Discussion

One peer accompanied with Yogeshwari (2019) studied on fortified rice analogues. Although, purpose behind this study was to analyze the feasibility of fortified rice analogues with zinc and iron. However, brightness of control (unfortified) was significantly higher than micronized ferric pyrophosphate fortified rice. Hence, they shown that micronized ferric pyrophosphate fortification was decreased L* value and increased in yellowness (Yogeshwari *et* al., 2019). Overall acceptability for texture was found to be non-significant among unfortified and fortified pasta. Likewise, a study has been conducted (Bouasla *et* al., 2017) who studied physiochemical properties, texture and sensory attributes of gluten free precooked rice pasta enriched with legumes flour. They revealed that rigidity of dry rice pasta was higher as 12.12N than rice pasta enriched with legumes flour as in range of 2.73 to 4.94, correspondingly. Furthermore, instant research has harmony with (Paudel *et* al., 2018) they studied prevalence, hematological status and risk factor among children suffering from IDA in a tertiary care center, Pokhara. Although, they founded that serum ferritin, serum iron and transferrin were low in IDA children whilst TIBC was high in IDA children. Result depicted that mean hemoglobin was 7.96±1.1gm/dl in IDA and 8.59±0.88gm/dl in non-IDA. Likewise, MCV (58.16fl), MCH (18.53pg) and MCHC (31.4%) were less in iron deficient anemic children than MCV (64.3±7.57), MCH (21.36±3.26) and MCHC (33.1±1.93) of non-anemic children.

Result of present study for liver and kidney iron content was in accordance to (Song *et* al., 2016) who investigated secure effect of lachnum YM226 melanin-iron complex (LM-Fe) on iron deficiency anemia in mice. Hence, contrasting dose of LM-Fe were administrated to anemic mice, with FeCl3 and FeSO4 as positive control. After that, it examined that LM-Fe was significantly improved hematological parameter. Alongside, they depicted that iron content of liver and kidney was significantly decreased in iron deficient mice than control group. Whilst iron content of liver and kidney in LM-Fe, FeCl3 and FeSO4 group were substantially higher than IDA model group ($P< 0.05$). Nevertheless, hyperplasia of kidney, liver and spleen had been shown in iron deficiency anemia.

Present result for serum iron and ferritin is in accordance with (da Conceiçao *et* al., 2001) as they investigated iron supplementation prevent the development of iron deficiency in rat with omeprazole induced achlorhydria. Nevertheless, iron supplementation was substantial enhanced serum iron than control as from 146.04±9.92 to 173.97±8.60µg/dl.

4. Conclusion

Concluded that, iron fortification rice has potential to diminish iron deficiency anemia. iron fortification in dietary staple are effective way to encounter hidden hunger. In instant research, 30 ppm NaFeEDTA fortified rice pasta were prominently enhanced serum iron and serum ferritin in experimental subjects. Similarly, provision of 30ppm iron fortified diet were considerably improved TIBC, hemoglobin, hematocrit, MCH and MCV. Although it has

been observed that 30 ppm fortified diet effectively improved serum iron, serum ferritin and hematological parameter than commercially available fortified cereal and other fortified diet.

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