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The effects of iron and vitamin B_{12} on body weight and hematobiochemical parameters in mice

M. N. Hasan[1,2], M. S. Jahan[3], M. S. Kamal[1], M. F. Islam[1], S. Ahamed[2], M. M. Islam[4], M. E. R. Bhuiyan[1,*]

[1]Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[2]Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea.
[3]Department of Pharmacology and Toxicology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Agargaon, Dhaka 1207, Bangladesh.
[4]Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea.

Abstract

Background

Vitamin and mineral supplementation is common practice in both human and animal health, But indiscriminate, overdose and long term used may cause toxicity. The research work was designed to evaluate the effect of iron (Fe) &vitamin B_{12} (Vit-B_{12}) on body weight, hematological parameters: Total Erythrocyte Count (TEC), Hemoglobin (Hb) and Total Leukocyte Count (TLC), and biochemical parameters: serum creatinine (SC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in mice.

Methods

One-month old 20 female Swiss Albino mice (*Rattus norvegicus*) having 19.52±0.54 g body weight were divided into four equal groups (n=5). After acclimatization, the mice of group A was treated with Fe@ 0.2 mg/kg body weight (bwt) in pellet, group B was treated with Vit-B_{12} @ 5 mg/kg bwt, group C was treated with combination of Fe and Vit-B_{12} and group D was fed with standard pellet and was considered as control.

Results

The result showed significant (P<0.05) increase in body weight of treatment groups compared to the control group. The values of TEC, Hb and TLC in the treated groups were increased significantly (P<0.05). ALT, AST and SC values were decreased significantly (P<0.05) in the treated groups compared to the control group.

Conclusions

It is concluded that administration of Fe and Vit-B_{12} significantly (P<0.05) increased body weight and hematological (TEC, Hb, and TLC) parameters however biochemical parameters (ALT and AST) were decreased significantly (P<0.05). In addition, combination of Fe&Vit-B_{12} showed better effects compared to their single uses.

Key words: Fe, Vit-$B_{12,}$ Body Weight, Hematological parameters, Biochemical parameter

*Correspondence: eliushvet@yahoo.com

Introduction

Iron (Fe) is an essential metal involved in a wide spectrum of physiological functions in the body. It plays an important role in biology and forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates. It is also the metal at the active site of many important redox enzymes dealing with cellular respiration, oxidation and reduction in plants and animals. Fe deficiency is the most common nutritional deficiency and the leading cause of anemia, decreased immune function, glossitis and as well as lower level can disrupt brain development (Chang et al. 2013). On the other hand, Fe overload is a disease characterized by excessive Fe deposition in tissues and damage to vital organs including heart, liver, and kidney. It can be caused by hereditary hemochromatosis or repeated blood transfusions for diseases such as beta thalassemia, bone marrow failure, or myelodysplastic syndrome (Bird *et al.* 2012).

Vitamin B_{12} also called cobalamin, is a water-soluble vitamin that has a key role in the formation of red blood cells, normal functioning of the brain and nervous system. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism. Vit-B_{12} deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, and weight loss (Bernard *et al.* 1998).Vit-B_{12} deficiency is often associated with pernicious anemia, as it is the most common cause. Vit-B_{12} deficiency can also cause symptoms of mania and psychosis (Masalha *et al.* 2005).Combined administration of Vit-B_{12}, folic acid, Fe, and erythropoietin could decrease transfusion requirements in extremely low birth weight infants. Combined therapy with Vit-B_{12}, folate, erythropoietin, and orally and intravenously administered Fe seemed more effective in stimulating erythropoiesis among premature infants, compared with erythropoietin, Fe, and low-dose folate alone (Haiden *et al.* 2006).But indiscriminate, overdose and long term use of Vit-B_{12} and Fe may cause toxicity. In addition, it is better to estimate the cost effectiveness of long term administration of Vit-B_{12} and Fe. Based on the above consideration the research was carried out to study the effects of Fe and Vit-B_{12} on body weight and haemato-biochemical parameters of mice.

Materials and Methods

One month old, 20 (twenty) female albino mice obtained from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. They were reared in a compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30)°C and lighting (12hour light /12hour dark). The mice were randomly divided into 4 equal groups (n=5) as A, B, C, D and supplied with standard rat pellet (5 g/mice/day) and fresh drinking water *ad libitum*. Group D was considered as the control group and fed with normal pellet. Mice of group A was fed with 0.5mg Iron/kg body weight with rat pellet, group B was injected with 5mg Vit-B_{12}/kg body weight intramuscularly and group C was treated with both Fe and Vit-B_{12}. At the end of the experiment (21day) body weights of mice were recorded. Blood samples were collected by cardiac puncture and half of blood samples were placed in anticoagulant (EDTA) containing tubes and half of blood samples were placed into clean dry tubes for serum separation. The tubes were then incubated overnight in the refrigerator (4°C). The serum samples were separated and centrifuged at 1500 at rpm for 15 minutes to get rid of unwanted blood cells if necessary.

Hemoglobin concentration was determined by Acid hematin / Shahli method and result was expressed in g/dl. Red and White blood cells counts were counted per cubic millimeter of blood by Hemocytometer and result were expressed in million/cumm and thousand/cumm respectively (Lambert and Rotheistein, 1977).

The serum biochemical parameters like AST, ALT, creatinine were performed in central laboratory of Bangladesh Agricultural University, Mymensingh.

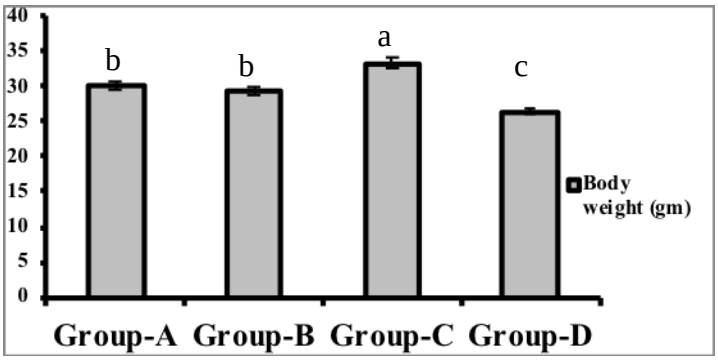
Statistical analysis

The data were calculated and analyzed using statistical SPSS program by one-way ANOVA followed by Duncan's multiple range test. (Bruning and Kintz, 1977)

Results and Discussion

Effect on body weight

The body weight(Mean ± SE) of mice is presented in figure 1. Experimentally treated group of mice showed increase body weight and significantly higher weight were recorded in group A, B, and C at the end of experiment. The highest weight was recorded (33.42 g±0.78) in group C and lowest weight was (26.50 g±0.32) in group D. The weight was significantly ($P<0.05$) higher in combined treated group than the other treated groups. Fe treated group showed increase body weight may be due to adequate concentration of iron increases the oxygen supply to the cell that increases the cellular respiration, oxidation and reduction leading to increase the total metabolism. On other hand, Vit-B_{12} indirectly responsible for the synthesis of DNA, RNA, hormones, proteins and lipids. It may influence the metabolic activities especially fat and protein. This may influence the body weight gain. Previously reported that Fe supplementation significantly increased body weight gain in calves (Baustad and Tollersrud, 1996), in lambs (Wenzlaf and Erhardt, 1991) and increased carcass yield in calves (Lind and Blum, 1994).



Values with different superscripts (a, b, c) indicates significance at 5% level.
Figure 1. Effect of Fe and Vit-B12 on Body weight (Mean ± SE) of mice at 21th day of experiment.

Effect on hematological parameters

The hematological parameters such as TEC, Hb and TLC are presented in table 1. On the final day of experiment (21thday) the values of TEC in groups D A,B and C were 6.97±0.04 million/cu.mm, 8.44±0.2million/cumm,7.31±0.16million/cu.mm and 8.80±0.14 million/cumm respectively. The values of Hb concentration in control group D were 7.16±0.07 g% and in treated group A, B and C were 7.98±0.20 g% 7.32±0.15 g% and8.27±0.22 g% respectively. At the final day of experiment, the value of TLC count in control group D was 7.81±0.06Thousands/cumm, treated group A- 8.13±0.06, B- 7.97±0.09 and C were 8.36±0.07 Thousands/ cumm respectively. In all parameters, the values of all treated groups were increased significantly than that of control group. The values of TEC, Hb and TLC in group A and C were significantly ($P<0.05$) higher than that of group B.

Table 1: Effect of Iron, Vitamin B_{12} and combination of Iron and Vitamin B_{12} on TEC, Hb and TLC in mice.

Hematological parameters	Mean ± SE			
	Group-A	Group-B	Group-C	Group-D
TEC (million/cumm)	8.44 ± 0.24^{a}	7.31 ± 0.16^{b}	8.80 ± 0.14^{a}	6.97 ± 0.04^{c}
Hb (g %)	7.98 ± 0.20^{a}	7.32 ± 0.15^{bc}	8.27 ± 0.22^{a}	7.16 ± 0.07^{c}
TLC(Thousand/cumm)	8.13 ± 0.06^{b}	7.97 ± 0.09^{c}	8.36 ± 0.07^{a}	7.81 ± 0.06^{c}

* Significant at 5% level of significance. a,b,c -Values with different superscripts in the same row differ significantly (P<0.05).

This may be happened due to availability of essential nutrient such as (Fe and Vit-B_{12}) which have crucial role in erythropoiesis, for RBC production requires adequate supplies of substrate mainly Fe, Vit-$B_{12}$and folate. Previous studies suggested that iron supplementation is essential to maximize the erythropoiesis(Adamson, 1996). In addition, iron supply plays an equally important role in the control of erythropoiesis (Hillman and Henderson, 1969). Combined therapy with Vit-B_{12}, folate, erythropoietin, and Fe seemed more effective in stimulating erythropoiesis(Haiden *et al*. 2006).

Effect on Biochemical parameters

The effect of Fe, Vit-B_{12} and combination of Fe and Vit-B_{12} on biochemical parameters is presented in Table 2. From the table it could be depicted that ALT and AST values were decreased significantly (p>0.05) in the treatment group A, B and C compared to control group D but serum creatinine (SC) level were increased significantly (P<0.05) in the treatment group compared to control group.

Biochemical parameters in present findings are resembled to that of Dong et al. 2010 and Kumar and Rawat, 1976. They revealed that the ALT values decreased with advance age of birds. A variety of reasons have been postulated for the decline of ALT with old age, including alteration in liver volume, function, and immune response with age (Goh *et al*. 2015).

Table 2: Effects of Iron, vitamin B_{12} and combination of Iron and Vitamin B_{12} on AST (U/L), ALT (U/L) and SC level (mg/dl) in mice.

Parameters	Mean ± SE			
	Group A	Group B	Group C	Group D
AST (U/L)	135.43 ± 1.14^{bc}	139.77 ± 2.05^{b}	130.83 ± 1.29^{c}	145.00 ± 2.06^{a}
ALT (U/L)	50.18 ± 1.01^{bc}	52.12 ± 0.83^{b}	47.88 ± 1.04^{c}	55.00 ± 0.87^{a}
SC (mg/dl)	0.50 ± 0.05^{b}	0.46 ± 0.04^{ab}	0.53 ± 0.07^{b}	0.37 ± 0.03^{a}

Values with different superscripts (a, b, c) differ significantly at 5% level.

Conclusions

Administration of Iron and Vitamin B_{12}improved body weight and hematological parameters and decreased the values of ALT and AST in mice. Combination of Fe&Vit-B_{12} showed better effects compared to their single uses.

Conflict of interest

The authors declare that they have no competing interests.

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