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The Differences of HB Levels of Mice (Mus-musculus) Administered with Fe + Vit C Compared to the Ones Administered with Fe + Chitosan

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Abstract: *Hemoglobin levels affect a person's health status. Hemoglobin deficiency (anemia) has yet to be completely resolved, because of the many factors causing anemia indirectly as exposure to heavy metals and other oxidants. Giving Fe and antioxidants such as vitamin C is common but yet to get meaningful results, while the addition of Fe Chitosan pad has not been done yet. Some studies found that chitosan takes role in binding leads in industrial waste. Therefore, researchers wanted to examine the difference in hemoglobin levels between mice administered with Fe + chitosan compared to those administered with Fe + Vitamin C. The experiment was conducted at the Laboratory of Anatomy, Faculty of Dentistry, University of Jember. The design of this study was a True Experiment study with the use of the Post Test Only Control Group Design. The samples were 20 mice (Mus - muculus) as guinea pigs aged 8-10 weeks with approximately 25-30 grams weight and in good health condition. Data Analysis was using two independent T-test samples. The results of the study showed that there was an average difference on theHb levels ofmiceadministered with Fe + vit C and those administered with Fe+Chitosan, or can be inferred thatmice administered with chitosan has higher level of Hb than thoseadministered with Fe + Vitamin C. This is because chitosant has better ability as an antioxidant compared to Vitamin C, and better ability to facilitate the absorption of ferritin Ferro.*

Keywords: *Hb. Fe, Vitamin C, Chitosan.*

I. Introduction

Hemoglobin is the oxygen-carrying metaloporotein containing iron in the red blood cells in the blood of mammals and other animals. Hemoglobin molecule consists of globin, apoprotein, and four heme groups. The word hemoglobin is derived from the words heme and globin, reflecting the fact that each subunit of hemoglobin is a globular protein with a single patch of heme group; each heme group contains one iron of atom, and it is responsible for wrapping oxygen (Anonymous, 2009). Hemoglobin in red blood cells serves to bind oxygen (O2). With the amount of oxygen that can be tied up and carried by the blood, the presence of hemoglobin in red blood cells, the oxygen supply to every place throughout the body, even the most remote and isolatedarea will be achieved (Sadikin, 2002).

Hemoglobin is a widely used parameter to specify the prevalence of anemia. Hemoglobin is the oxygen-carrying compound in red blood cells. Hb can be measured chemically from the amount of hemoglobin /100 ml of blood which can be used as an index of oxygen-carrying capacity of the blood. Low hemoglobin thus indicating anemia (Supariasa, 2002). Some of the factors that cause hemoglobin decrease in the human body are the lack of substances needed in the formation of hemoglobin, theavailability and functioning of the enzymes required for the metabolism of hemoglobin or it could be because humans are always exposed to pollutants containing lead inhaled throughthe air as well as from food we eat. Leads in the synthesis of hemoglobin is an inhibitor that interferes with the functioning of enzymes that play a role in the synthesis of hemoglobin.

Iron is an essential element for hemoglobin synthesis, synthesis of catecholamines, heat production and as a component of certain enzymes needed to produce adenosine triphosphate involved in cell respiration. (Jordan, 2002: 271). The lack of hemoglobin in acute or chronical conditionmay be fatal for vital organs, and so if the shortage of hemoglobin occurs in pregnant women it may lead to complications. The mildest one ranging from mild to severe anemia which at the most severe level can cause malfunction of the body. Anemia in pregnant women can cause abortion or congenital defects (Pillitery, 2007) Anemia treatment programs have been conducted with supplementary feeding, fortification and the provision of iron tablets, but it has not shown any meaningful results. This condition is possible because of the iron consumed cannot be synthesized for due to the lead which disturb enzymes' work. Therefore it is necessary to prevent them from interfere the effectiveness of the enzyme. Chitosan and vitamin C can bind the lead so as not interfere with the working of the enzymes. Chitosan is a non-toxic natural substance that has the potential as a chelating agent for its ability to bind lead and form chitosan-lead complex. The ability of chitosan to bind lead is affected by the molecular weight (MW) and the degree of deacetylation (DD).

The effect of leadcan be decreasedby chitosan administration. There is a mechanism of chitosan to bind lead and formchitosan-lead complex with hydrophilic character so that it can be easily removed through urine (Suharsih, 2008). Vitamin C as an antioxidant also has the function of binding lead. Studies about the ability of Chitosan and vitamin C in binding lead had been conducted many times, but the study to compare the effect of Fe added with Vit C and Chitosan against hemoglobin concentration has never been done yet. Based on this consideration, the researchers wanted to find out the differences in Hb levels on mice (Mus muskulus) as guinea pigs administered with Fe + Vitamin C and Fe + Chitosan.

GeneralObjective

To find out the difference of Hb levels increase in mice (Mus-musculus) of those administered with Fe and Fe + Chitosan

SpecificObjectives

1. To identify the increase of hemoglobin levels in mice (Mus-musculus) administered with Fe
2. To identify the increase of Hb levels in mice (Mus-musculus) administered with Fe + chitosan.
3. To analyze the increase difference of Hb levels in mice(Mus-musculus) on those administered with Fe and those with Fe + Chitosan

II. Research Methodology

Design:

The design of this research is a True Experimental research by using a Post Test Only Control Group design. This design was used because there was no initial measurement and all the groups ascribed to the same population.

Population, sample and sample size:

The population is all mice (Mus musculus) aged approximately 8-10 weeks of age weighing between 20-25 grams, with the inclusion criteria that they were in healthy condition, no history of illness or disability. The number of sample is 20.

Research variable

Independent variables: Fe + Vit C and Fe + Chitosan, dependent variables: hemoglobin (Hb)

Data analysis :

The data was checked or corrected after being collected, then it was analyzed using t-test analysis.

Hypothesis

1) Ho is accepted (Ha is rejected) if t-count ≤ t-table with significant error level of 0.05 or $p \geq \alpha$ which means there is no difference in hemoglobin levels between mice (Mus - musculus) administered with Fe + chitosan and thoseadministered with Fe + Vitamin C ,

2) Ho is rejected (Ha is accepted) when t-count> t=table with significant error level of 0.05 or $p \leq \alpha$ which means that there are differences in hemoglobin levels of mice (Mus - musculus) administered with Fe + chitosan and thoseadministered with Fe + Vitamin C.

III. Results

Hb level of miceadministered with Fe + Chitosan

The examination of Hb levels in miceadministered with Fe + Chitosan were performed after given treatment for10 days. It showed various results. Then, the average level was calculated. The results are shown in Table 1.

Table 1. the average level of Hb in mice administered with Fe + chitosan.

Group	Average level of Hemoglobin	Deviation standard	n
Fe + Chitosan	12,520	0,818	10

Hb level of miceadministered withFe + Vitamin C

Hemoglobin levels of miceadministered with Fe + Vitamin C for 10 days varies with a standard deviation of 1.197. Average levels of Hb of them can be seen in Table 2.

Table 2. The average level of Hb level of mice administered with Fe + Vitamin C

Group	Average level of Haemoglobin	Deviation standard	n
Fe + Vitamin C	10,680	1,197	10

The difference level of Hb between miceadministered with Fe + Chitosan with thoseadministered with Fe + Vitamin C

Hb level differences between mice administered with Fe + Vitamin C and those with Chitosan can be seen in Table 3. Based on the results of t-test, thep-value is 0.001 and less than $\alpha = 0.05$ (0.001 <0.05), then it can be

concluded that there is a difference between the levels of hemoglobin levels on mice administered with Fe+ Chitosan and Fe + Vitamin C. The hemoglobin level of mice administered with Fe + Chitosan is at the level of 12.520 mg%, whereas mice administered with Fe + Vitamin C is equal to 10.680 mg%. So, it can be concluded that the average Hb levelof mice administered with Fe + Chitosan is higher than those administered with Fe + Vitamin C.

IV. Discussion

Hb level of mice (Mum -musculus) administered with Fe + Vitamin C

The average level of Hb mice administered with Fe + Vitamin C is 12.52 mg%. The average hemoglobin concentration falls within the normal category. Hemoglobin concentration in the blood is determined by the metabolic processes and the availability of hemoglobin in the body of the mice. Hb metabolism is closely related to the Fe. Fe can be derived from deposits in the body and the intake of Fe derived from the intake which is absorbed in the small intestine and facilitated by Vitamin C

Hemoglobin is composed of protein (globin) and heme. Giving Fe continuously every day to mice supports the sustainability of heme formation. The availability of adequate heme allows adequate formation of hemoglobin. Sanghyl (2012) in his research found out that the administration of iron increases hemoglobin concentration of 1.17 mg% in developed countries and 1.13mg% in developing countries.

Heme is formed from a combination of protoporphyrin with Fe ++. Fe ++ absorption occurs in the intestine. Fe is more easily absorbed when it is the form of ferro. Its availability in the intestine is maintained by vitamin C as a reducing agent. Fe absorption results transfer to the liveris also aided by Vitamin C The important role of Vitamin C is to enable other enzymes that act as bio catalysator in the metabolism of hemoglobin. Availability of the enzyme increases Hb levels because the metabolism can take place properly. Vitamin C mechanism in activating the enzyme is by binding oxidants that inhibit the action of the enzyme especially the ALA enzyme.

Based on the description above, the administration of Fe + Vitamin C can prevent anemia. Amelda (2005) had invented the administration of Fe by giving orange syrup that can reduce the incidence of anemia and increases hemoglobin concentration on pregnant women. The condition also occurs in children, which can reduce anemia from 29.3% to 7.9%, as it also increases the levels of significance (Rocha, 2012). It is strongly associated with the availability of the substrate in the form of Fe and biocatalysator form of the enzyme.

Hb level of mice (mus-muscular) administered with Fe + Chitosan

The results of the study on Hb level of mice administered with Fe + Chitosan was12.52 mg% in average. The administration of Fe as one of the elements forming heme takes role in the determination of Hb levels in mice. A Research by Nancy (2002) found out that Fe supplementation can improve hemoglobin levels and can prevent pregnant women suffering from anemia. Giving Fe regularly can ensure its availability in the body so that the Hb metabolism can run well. This is consistent with the results of research by Mungen (2003) who found out that by giving Fe on regular basis can prevent anemia in pregnant women. Increased levels of hemoglobin can occur from 11 ± 14 mg / L to 31 ± 18 µ / L in supplementation for 6 months (Lindsay , 2000).

Adequate Fe provision can form heme. Heme formation depends on the availability of the catalyst (enzyme). Kinetic properties of enzymes influenced their inhibitor in the form of the lead that inadvertently enteringthe body through food or inhalation. Leads can be bound by the chitosan so as not to interfere with the function of enzymes in the metabolism of hemoglobin especially the ALA synthesis (Wu, 2010). Poerwaningsih (2008) in his research found out that the activity of Î'-ALAD enzyme addedwith chitosan increasesespecially in the control group.A research abouthow chitosan improves the function of the enzyme was also conducted by Prabu (2012). The effect of the rising activity of the enzyme is the increasing of Hb level. Joao (2012) said that antioxidants from chitosan can prevent hemolysis and DNA damage. The increase amount of chitosan increases iron absorption and the amount of water-soluble Ferro (Ayer, 1999). Ozsoylu (2000) suggests that increasing absorption occurs in the form of ferritin iron, not only on the normal cases but also on thalassemia cases. Increasing absorption of ferritin was higher in cases of thalassemia with the value of 10.7% while in normal conditions it only reaches 7.5% with the same amount of chitosan (20mg).

The difference level of Hb on Mice administered with Fe + Chitosan and those with Fe + Vitamin C

Two mean different test results got $p \geq \alpha$, so it can be concluded that there are differences in Hb levels between mice administered with Fe + Vitamin C with those with Fe + Chitosan. Hb level is influenced by the availability of the components that play roles in the metabolism of hemoglobin. The important components include: Protein, Fe and enzymes that act as catalysts in the metabolism of hemoglobin. On the experiment,protein component almost becomes homogeneous because of the similar nutrition intake to all guinea pigs. They also got similar Fe components intake. Therefore, the distinguishing component was vitamin C with Chitosan.

The average difference in Hb levels between mice administered with Chitosan + Vitamin C of 1.85 mg / dl. This value has a quite high meaning because it affects the ability to bind oxygen used for the body's metabolism. So we can conclude that the administration of Fe + chitosan effect on Hb levels were higher compared to the provision Fe + Vitamin C. Vitamin C and Chitosan have the same role to increase the absorption of ferro and ferritin and function as antioxidants that makes enzymes function optimally.

Chitosan higher ability to increase levels of hemoglobin compared with Vitamin C can be seen from the findings of Ozsoylu (2000) which state that chitosan not only increases blood hemoglobin levels in normal conditions but also on the condition of thalassemia. Another advantage of the chitosan is that it is able to increase hemoglobin levels in the case of thalassemia higher than in the normal case.

V. ConclusionAndRecommendation

Conclusion

Based on the results of research and discussion it can be concluded that:

1. The average level of Hb on mice administered with Fe + Vitamin C was 10,.80g / dl. The addition of Fe increases the availability of Fe so that it also increases the absorption of Ferro as the component of heme.Besidesthat Vitamin C can increase Ferro absorption and improve the work of enzymes involved in the metabolism of hemoglobin especially for ALA synthase.
2. The average level of Hb on mice administered with Fe + Chitosan was 12.520g / dl. Fe addition increased Fe levels in the colon so it can be absorbed in the form of ferrous and chitosan facilitate the bonding with ferritin. Another role of chitosan is as antioxidant to bind leads that interfere with the work of theenzymes.
3. Hb level on mice administered with Fe + Vitamin C was different to mice administered with Fe + Chitosan, where Hb level of the mice given Fe + chitosan was 1. 85g/dl higher. This difference is caused by higher ability of chitosan compared to vitamin C, both in facilitating absorption and its ability as an antioxidant. Chitosan not only play role in normal blood condition but also in the case of thalassemia which can function better.

VI. Recommendation

1. For Service Provider

Hb levels were higher in mice administered with Fe + Chitosan is one of the ways to prevent and handle anemia that needs to be disseminated and applied in health care especially for midwifery services.

2. For Further Research

This study did not examine the effect of chitosan and vitamin C against the action of the enzyme. Therefore, to ensure and clarify this fact then it is necessary to do research on the effects of vitamin C and chitosanagainst enzyme activity.