EFFECT OF SODIUM NITRITE (NaNO₂) TO ERITROCYTE AND HEMOGLOBIN PROFILE IN WHITE RAT (Rattus norvegicus)

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ABSTRACT

Sodium Nitrite (NaNO₂) is a food preservative that tend to be misused. Poisoning from Sodium Nitrite that often occurs reduces hemoglobin ability to bind oxygen and the formation reactive oxygen species (ROS) that can changes erythrocyte count and hemoglobin level. This was an experimental laboratories study using Post Test Control Group Design to identify the effect of Sodium Nitrite administration on the profile of erythrocytes, including erythrocytes count, and hemoglobin levels in white rat. Samples comprised 33 male rats (Rattus norvegicus) weight 175 -200 grams, divided into 3 groups, each consisting of 11 rats. Group 1 was the control group, group 2 received Sodium Nitrite of 0.5 mg/200 grams BW rats and group 3 received Sodium Nitrite of 1.5 mg/200gram BW rats for 30 days. Results of research showed that there was a high difference in means p=0.000 (p<0.05) on erythrocytes count and hemoglobin level. Sodium Nitrite of 0.5 mg/200gram BW for white rat, which was a safe dose, has already changed erythrocytes count, and Sodium Nitrite of 1.5 mg/200gram BW showed higher changes either in erythrocytes count and hemoglobin level. In conclusion, Sodium Nitrite administration of 0.5 mg/200gram BW/white rat, and 1.5 mg/200gram BW rats can decrease erythrocytes count and hemoglobin level of male white rats. (FMI 2012;48:1-5)

Keywords: Sodium Nitrite, erythrocyte, hemoglobin.
consumed according to FAO/WHO, namely: 0.07 mg/kgBW/day so for 70 kg body weight was 7 mg. Meanwhile, according to Depkes RI 0.4 mg/kg/day (Department of Health 2006, Afrianti 2010).

The purpose of this study is to obtain the number of erythrocytes and hemoglobin levels of white rats (Rattus norvegicus) males treated with sodium nitrite for 30 days. This study is an experimental laboratory that uses Post Test Control Group Design to determine the effect of sodium nitrite on erythrocyte profile ie the number of erythrocytes, levels of hemoglobin, hematocrit and reticulocyte in white rat.

MATERIALS AND METHODS

This research was experimental laboratories, using a random design complete with 2 treatments to adult male white rat (Rattus norvegicus) wistar strain with heavy 175-200 gram in 3-4 month age with physically health. Total 24 Rats white kept in cage, each cage contains 8 male rats white (total 24 lambs). Rats had acclimated first for 1 weeks. Rats were given feeds and drink et libitum and given oral solution sodium nitrite during the 30-day, with the dose per day for each group as follows.

In group one, rats gift feeds and drinking water standard 3 ml/200g BB rats/day (during the 30-day / sonde) without Sodium Nitrite. Then, group two gift Feeds standard plus Sodium Nitrite 0.5 mg in 3ml aquades per 200g BB rats/day (for a period of 30 hr/ sonde). While group three gift feeds standard plus Sodium Nitrite 1.5 mg in 3ml aquades/200g BB rats/hr (during the 30 hr/ sonde).

Technical data

Taking blood specimens to group 1-3 would be done on the 30th day after having been given preferential treatment to know the total amount erythrocytes and hemoglobin. Taking blood specimen came from the heart rats, where previously done anesthesia by using Ketamin Hcl (dosage 20-40 mg/kg BW in intraperitonial) as many as 1 ml. After taking blood specimens then decapitation done (Kusumawati 2004). Blood Specimens in all the groups, was included in the tiny reactions that have been given EDTA then given identity in accordance with the sequence specimens in the group and the cook in Balai Besar Health Laboratory Surabaya.

The checking procedures of erythrocytes and levels of hemoglobin analysis methods with a computerized with the instruments Sysmex KX- 21. Measuring result was visualized in numbers and histogram (Bakta 2006). Statistical analysis bothered by perfomed Pearson correlation test and Multi-analysis of variance (Manova) was used for post hoc analysis, p < 0.05 was considered as statistically significant

RESULTS

The following is the spread data based on age and the standard deviation of erythrocytes and levels of hemoglobin.

Table 1. Average test and standard deviation erythrocytes profile

<table>
<thead>
<tr>
<th>Erythrocytes Profile</th>
<th>Controls (N=11)</th>
<th>NaNO2 = 0.5 mg (N=11)</th>
<th>NaNO2 = 1.5 mg (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average, SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes (x 10^6 µL)</td>
<td>9.61, 0.54</td>
<td>7.70, 0.84</td>
<td>5.99, 0.74</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.51, 1.23</td>
<td>12.27, 1.19</td>
<td>8.61, 1.20</td>
</tr>
</tbody>
</table>

Table 1 shows decreasing average test number of erythrocytes and levels of hemoglobin respectively in the control group, the group treatment NaNO2 dosage 0.5 mg/200gram BB rats, and the treatment NaNO2 dosage 1.5 mg/200gram BB rats. Changes can be seen in the figure 1 below:

Figure 1. Bar chart of average number of erythrocytes / RBC (x 10 6 per µL)

Results of The Analysis Multivariate Anova (Manova)

To know the difference between mean of each groups: number of erythrocytes and levels of hemoglobin in the control group, the group NaNO2 dosage 0.5 mg per 200gram BB rats and NaNO2 dosage 1.5 mg per 200 grams BB rats , then used tests Manova. Test result Manova showed the p=0.000 (p< 0.05), so it can be said that there are huge differences between ; number of erythrocytes as many as 72.1 (Sum of Square Squares
Type III) and levels of hemoglobin as many as 195.0. Whereas in Mean Square the number of erythrocytes are 36.0 and hemoglobin 97.5 in the current controls, the group NaNO2 dosage 0.5 mg per 200 gram BB rats and NaNO2 dosage 1.5 mg per 200 gram BB rats.

Figure 2. Bar Chart of average levels of hemoglobin/HGB (g/dl)

DISCUSSION

Effect of Giving Sodium Nitrite to Number of Erythrocytes

Nitric is chemicals that are not excreted by the human body so that it is accumulated and can cause health problems (Lundberg et al. 2008). Number of erythrocytes is one of the important parameters to assess health, considering his role is very big in carrying oxygen in the body. Blood circulation describes basic mechanisms chemical distribution throughout the body (Loomis 1978).

Results of research shows that there is a decline of the average value for the number of erythrocytes respectively: 9.61x10^6 µL; 7.7x10^6 µL; 5.99x10^6 µL. Manova test on the number of erythrocytes shows there is a big difference means with p= 0.000 (p<0.05) from the sodium Nitrite dosage 0.5 mg/200 grams BB white rat, and 1.5 mg/200 grams BB rats, as shown in table 1 and the figure 1

The Sodium Nitrite dosage 0.5 mg/200 grams BB white rat that is dosage conversion from safe dosage is allowed according to Permenkes 1988 is 0.4 mg/kg BW/day while safe dosage that nominated FAO, WHO is 0.07 mg/kg BW/day, but on the dose of 0.5 mg/200 grams BB rats has shown that there is a decrease in the number of erythrocytes. According to Lundberg et al. (2008), Sodium Nitrite if in consumption will continue to insist on and excessive cause stock exchange toxic substances in humans.

On the gift Sodium Nitrite dosage 1.5 mg/200 grams BB rats, effects on a decrease in the number of erythrocytes is greater. This is because Sodium Nitrite into the blood circulation will bond hemoglobin produce Reactive Oxygen species (ROS) namely anions superoxide (O2 - ). ROS cause oxidative stress to the proteins in cytoskeleton erythrocytes and damage membrane structures erythrocytes by ties crossing (cross linking) proteins, near break easily and cause erythrocytes (lysis) (May et al 2000). Hemolysis early triggers bone marrows to speed up the formation of new erythrocyte, up to 10 times the normal speed marked with the increase in the number of erythroblast and reticulocyte accelerated into blood so produce erythrocytes that has not been mature (in the normal situation, life expectancy erythrocytes are 120 days). The destruction of erythrocytes faster than producing it causes a decrease in the number of erythrocytes.

Decreasing in the number of erythrocytes, affects the formation of the occurrence of eritropoietin and stimulate eritropoiesis, but because erythrocytes that is newly formed, then hemoglobin level that is in erythrocytes has yet to reach the optimum level. Number of hemoglobin that had not been stable, cause hemoglobin ability to tie was far from optimal oxygen. According to Dera ve and Taes (2009), the number of oxygen that decreased will lead to liver secretes more globulin, the kidneys producing more renal eritropoietin factors. In the blood, globulin and factors eritropoietin kidney will hold an interaction formed eritropoietin that will stimulate the occurrence of eritropoiesis.

Speed of eritropoiesis stated in such a manner so that a number of erythrocytes in the circulatory system there is more or less constant. Every time the number of oxygen transported to tissue become low usually will increase the speed formation of erythrocytes (Murray et al 2009). Research done by Hord et al (2009), the Sodium Nitrite with great dosage will speed up destruction the red blood cells, so reducing the number of erythrocytes circulation and tends to cause anemia. Based on the research Umbreit (2007) stated that all chemicals that were used as preservatives is the poison, but toxicity that is determined by the number of that is needed to produce the effects and health problems or sick.

Effect of Giving Sodium Nitrite to Levels of Hemoglobin

From the result of the research, that is obtained the decreasing average hemoglobin level with the sequence: 14.51 g/dL; 12.27 g/dL; 8.61 g/dL. Based on statistic
test Manova on the number of hemoglobin there is a significant difference with $p = 0.000$ ($p<0.05$) from the Sodium Nitrite dosage 0.5 mg/200 grams BB rats, and 1.5 mg/200 grams BB rats, as can be seen in table 1 and the figure 2.

Hemoglobin is an erythrocytes component and a conjugation protein in transport oxygen and carbon dioxide. The determination levels of Hemoglobin often used to know that there is an anemia (Tahono & Wuryaningsih 2000). In this research the sodium Nitrite dosage 0.5 mg/200 grams BB rats and 1.5 mg/200 grams BB rats showed average test levels of hemoglobin that decreased. This condition caused Nitric bound with hemoglobin forms methemoglobin, skin allergic that is hemoglobin is oxidized and is a form of hemoglobin which bind water, not oxygen; NaNO2 + hbfe2+ $\rightarrow$ NO + hb fe3+ + O2 $-$ , formation of methemoglobin, skin allergic cause blood hemoglobin capacity to carry oxygen is decreased, carbonation level so it can cause hypoxia or lack of oxygen on body tissues (Hoffbrand et al 2005, Murray et al 2009).

The gift with a dosage of Sodium Nitrite 0.5/200gram BB rats cause experienced haemoglobin in normal limit (11.1-18g/dl) but cause the Sodium Nitrite with the dose 1.5 mg/200gram BB rats drop levels hemoglobin under normal limit (<11,1g/dl), it shows that there is a rats tendency to anemia. Sodium nitrite is chemicals that usually used to preservative meat and fish to get a good color and prevent the growing microbes, but for a continual intake can cause harmful effects for the health and can cause anemia.

Research done by Faisol (1987) apparently anemia affected power concentration, perception and attention elementary school children this caused by hemoglobin is the primary protein in the human body that has a function to carry the oxygen from the lungs to the peripheral and carrying carbon dioxide from peripheral tissue to the lungs, to maintain retention or the capacity to concentrate, there is required energy that is available in the body. Energy was obtained from foods that go into the body through a series of processes metabolism.

Factors that are very affected supplies oxygen to tissues of the body is the number of the red blood cells and the number of hemoglobin that were found in it, so that those who are suffering from anemia, oxygen supply to the tissue of the body will experience disruption since the internal organs is less, automatically oxygen that is carried will decreased. With less oxygen in the tissue so the body metabolism process would be disturbed and the implication is the need for the learning process energy had problems. The more levels of Hemoglobin in the blood, the more oxygen that can be transported to various tissues vice versa (Ama 1987).

CONCLUSION

The sodium nitrite (NaNO2) dosage 0.5 mg per 200 gram BB in rats (Rattus norvegicus) during the 30-day decrease the number of erythrocytes, levels of hemoglobin, The sodium Nitrite (NaNO2) dosage 1.5 mg per 200 gram BB in rats during the 30-day higher decrease the number of erythrocytes, levels of hemoglobin.

REFERENCES
