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Effectivity Comparison of Cow’s Horn and rhu-EPO as Anemia’s Medicine
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Abstract
Anemia is decrease of hemoglobin (Hb) or functional blood cell in body so the body will be hypoxia. Anemia not only in human, but in animals too. One way to overcome anemia is by injecting rhu-EPO, but the obstacle is the price of rhu-EPO are quite expensive. This study aims to examine the content of monoacylglyceride in cow’s horn solution and compare it with rhu-EPO (recombinant human eritropoietin) as a medicine for anemia. Beside that, this study aims to find a new cheaper medicine for anemia. Sample of this research are 15 mice. This study was designed to divide the sample (mice) into 3 groups; one group was not given any treatment (control); group 2 given cow’s horn solution; and group 3 was given rhu-EPO. Before given treatment, mice were aclimated and made into anemia. Treatment given for two weeks. Red blood cell count is done once a week using a hemocytometer. This research carried out for 2 months from May to June 2016. The results of this study showed an increase in red blood cells of group 2 were more significant than group 1 and group 3. Group 1 has increased from 6.726.000 µ/L (week 1) to 8.216.000 µ/L (week 2); group 2 increased from 9.046.400 µ/L (week 1) to 11.450.000 µ/L (week 2); while the third group was increased from 6.818.000 µ/L (week 1) to 7.284.000 µ/L (week 2). This Proves that the cow’s horns can increase the number of red blood cells quickly intervening and quite well to use as anemia’s medicine.

Keywords: anemia; cow’s horn; eritropoietin; mice; rhu-EPO;
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Effectivity Comparation of Cow’s Horn and rhu-EPO as Anemia’s Medicine

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Abstract

Anemia is decrease of hemoglobin (Hb) or functional blood cell in body so the body will be hypoxia. Anemia not only in human, but in animals too. One way to overcome anemia is by injecting rhu-EPO, but the obstacle is the price of rhu-EPO are quite expensive. This study aims to examine the content of monoacetyldiglyceride in cow’s horn suspension and compare it with rhu-EPO (recombinant human eritropoietin) as a medicine for anemia. Beside that, this study aims to find a new cheaper medicine for anemia. Sample of this research are 15 mices. This study was designed to divide the sample (mice) into 3 groups; group 1 was not given any treatment (control); group 2 given cow’s horn suspension; and group 3 was given rhu-EPO. Before given treatment, mice were aclimated and made into anemia. Treatment given for two weeks. Red blood cell count is done once a week using a hemocytometer. This research carried out for 2 months from May to June 2016. The results of this study showed an increase in red blood cells of group 2 were more significant than group 1 and group 3. Group 1 has increased from 6.726.000 µ/L (week 1) to 8.216.000 µ/L (week 2); group 2 increased from 9.046.400 µ/L (week 1) to 11.450.000 µ/L (week 2); while the third group was increased from 6.818.000 µ/L (week 1) to 7.284.000 µ/L (week 2). This Proves that the cow’s horns can increase the number of red blood cells quickly intervening and quite well to use as anemia’s medicine especially for regenerative anemia.

Keywords : anemia; cow’s horn; eritropoietin; mice; rhu-EPO;

Introduction

Anemia is one of the most common problems in all age groups in Indonesia, both in humans and animals, especially iron deficiency anemia. The problem of iron deficiency anemia is a widespread health problem associated with an increased risk of morbidity and mortality not only in humans but also in animals\textsuperscript{[1]}. The proliferation and maturation of red blood cells (erythocytes) is regulated by cytokines including erythropoietin as the most important factor in this mechanism. When the hypoxia happened, the kidney nephron responds by producing erythropoietin. Erythropoietin (EPO) is a glycoprotein hormone that has molecular weight 30-39 kD which will be attached to specific red blood cell progenitor receptors and further give a signal to stimulate proliferation and differentiation\textsuperscript{[2]}. Conversely, if there is an increase in the volume of red blood cells above normal due to transfusion, erythropoietin activity in the bone marrow will be reduced\textsuperscript{[3,4]}. To overcome anemia and reduce the need for recurrent transfusions in severe cases, some people considers the use of exogenous erythropoietin (recombinant human erythropoietin = rhu-EPO)\textsuperscript{[5]}. Rhu-EPO works to increase or maintain the RBC levels, thereby decreasing the need for blood transfusions. The half-life of administration ranges from 4-13 hours in patients with chronic renal failure...
through intravenous and approximately 27 hours on subcutaneous administration. Rhu-EPO is an expensive drug, so it costs more than 1 billion per year. Available rhu-EPO preparations are 1000 IU, 2000 IU, 4000 U (in 1 syringe) 2000 IU, and 4000 IU (in 1 ampoule) [6].

Treatment for low-income groups requires a lot of cost, it is necessary to find an alternative drug of nature that is easy, cheap and familiar in society that allegedly can increase the number of red blood cells one of them is by using a horn [7].

Horn is one of the product from livestock slaughter that can produce a huge added value if it can be processed optimally. Currently the horn has been widely used by humans to make handicraft materials such as decoration (accessories), souvenirs, goggles, combs, and other tools. In the field of health and pharmacy, horn of deer are occasionally used to increase the number of blood cells especially in cases of anemia caused by chemotherapy processes. Its cause by monoacetyldiglycerides in horn. The results showed that in the horn there is a monoacetyldiglyceride compound that is believed to stimulate the bone marrow to increase red blood cell production [8].

Monoacetyldiglyceride is a compound contained in horn, monoacyl diglyceride structure is divided into 8 parts. Monoacetyldiglycerides may have significant clinical potential for accelerated hematopoiesis.

Animal Slaughter House (RPH) of Makassar City is the only one slaughter house which located in Makassar, slaughter of cattle in RPH Makassar about 50-60 cattle / day [9].

By seeing the number of cows that has been cut per day then how much cow’s horn produced from RPH Makassar. So the average is about 120 horns produced from the cuts each day. This amount is a considerable amount and can be utilized in various fields [10].

Materials and Methods

The sample of this research is mice obtained from Faculty of Pharmacy, Hasanuddin University Makassar which is selected with quota sampling technique. The sample size was 15 mice with criteria: male; 8-11 weeks old; and the weighs from 30-35 grams.

The control technique in this study was to classify the sample into three groups to be compared i.e the group of mice that were not given cow horns or erythropoietin (control), a group of mice given only cow horn as anemia, and a group of mice given only erythropoietin as an anemia’s drug.

The dependent variable of this research is the increase of production and the number of red blood cells while the independent variables in this study is giving different type of anemia’s drug in each treatment group.

The procedures performed in this study are:

Making of Horn Powder:

Boil the horns until the outer and inner layer structures are separated; Drying the horns under the sun until a dry structure is obtained; Smoothing the horns to obtain a structure that resembles a powder.

Preparation of Horn Suspension:

Boil water until the temperature is above 70 °C; Mixing water as much as 50 cc with 45 gram of powder of cow horn solution into measuring flask; Closing and shaking the measuring flask until a suspension is obtained; Silence the horn suspension until cool and obtained a suspension of horn which is ready to be consumed on the experimental sample.

Injecting Recombinant human Erythropoietin (rhu-EPO) and Provision of Cow Ox Suspension:

Each treatment group used 5 mice. White mice were acclimatized for 10 days with commercial pellets feeding and drinking ad libitum. Subsequently grouped into three groups according to the treatment; Mice in accordance with the group is then sustained for 3 hours to take his blood, in accordance with procedures proposed by Parasuraman (2010). Blood collection is done with the assumption that white mice will have anemia. For treatment, Group 1 is not treated as a control group; Group 2 was given treatment in the form of 1 cc horn suspension once a day for 2 weeks; Group 3 was given the treatment of Recombinant human Erythropoietin (rhu-EPO) of 13.65 IU / 0.0046 ml once in 3 days for 2 weeks; After a week each group was being sampled to test their red blood cell production by using a haemocytometer observed under a microscope and compared the results each group. Blood sampling will be taken every week and will be seen whether there is a significant increase in the number of erythrocytes each week. In addition, comparisons
were also made on the production of red blood cells of mice given cow's horn solution with erythropoietin.

**Result and Discussion**

Treatment for each group of mice was performed, which the group was given daily horn solution, the rhu-EPO group was injected once every three days, and the control group given only food and drink without any special treatment.

Blood sampling and examination of the amount of erythrocytes in each mouse have also been performed, where blood collection is done through the facialis vein which is then diluted with hayem solution using a haemocytometer tool and performed under microscope.

Calculation of the amount of erythrocytes has also been performed based on observations under a microscope. The results showed that the horn oxidation given to the group (B) gave significant results, where the rate of increase in the amount of erythrocytes shown was higher than in the other treatment groups. The results of the amount of erythrocytes obtained differ because of this is influenced by several factors that exist one of them is based on FCR (Feed Conversion Rate), the immune system of each individual mouse, and the environment of mice that can affect the number of different erythrocytes.

The results of the calculation of the amount of erythrocytes for each treatment in the first and second week can be seen in table below:

<table>
<thead>
<tr>
<th>Mice</th>
<th>Number of Erytrocyte</th>
<th>Mice</th>
<th>Number of Erytrocyte</th>
<th>Mice</th>
<th>Number of Erytrocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.040.000/µl</td>
<td>1</td>
<td>8.972.000/µl</td>
<td>1</td>
<td>7.270.000/µl</td>
</tr>
<tr>
<td>2</td>
<td>6.510.000/µl</td>
<td>2</td>
<td>9.070.000/µl</td>
<td>2</td>
<td>6.120.000/µl</td>
</tr>
<tr>
<td>3</td>
<td>6.230.000/µl</td>
<td>3</td>
<td>9.390.000/µl</td>
<td>3</td>
<td>7.010.000/µl</td>
</tr>
<tr>
<td>4</td>
<td>7.580.000/µl</td>
<td>4</td>
<td>9.880.000/µl</td>
<td>4</td>
<td>6.770.000/µl</td>
</tr>
<tr>
<td>5</td>
<td>6.270.000/µl</td>
<td>5</td>
<td>7.920.000/µl</td>
<td>5</td>
<td>6.920.000/µl</td>
</tr>
<tr>
<td>Mean</td>
<td>6.726.000/µl</td>
<td>Mean</td>
<td>9.046.400/µl</td>
<td>Mean</td>
<td>6.705.000/µl</td>
</tr>
</tbody>
</table>

From the result, we can see the cows horn suspension has a highest effect to produce the erytocyte than another group. Its caused by the effect of monoacetyldiglycerides in cow’s horn suspension. The number of increase from first week to second week by the cow’s horn suspension is 2.403.600/µl. In the field of health and pharmacy, horns of livestock are sometimes used to increase the number of blood cells especially in cases of anemia caused by the chemotheraphy process. Horns on cattle / animals can be interpreted as a bulge of two horns that grow in the head of cattle / animals. This definition is more focused on certain cattle such as cow, buffalo and...
The main constituent of the horn is the protein in this case keratin and collagen. Keratin is a type of fibrous protein with less digestibility and low levels of palatability. Horns have different sizes, colors, shapes and arches.

In control group, the increase only 1,490,000. The increase of red blood cell caused by the erythropoietin that produced by kidney.

In rhu-EPO group, the number of increase is 2,047,000. rhu-EPO work like erythropoietin, it stimulate the forming of erythrocyte in bone marrow. Recombinant human erythropoetin (rhu-EPO) contains small proteins composed of 165 amino acid glycoproteins which are genetically engineered (recombinate of DNA technology) identical to endogenous erythropoetin, an amino acid and have the effect of stimulating reticulocytes maturation into erythrocytes in stem cells in the bone marrow . Increasing the amount of erythrocytes and further increasing the oxygen reserves as well as improving the oxygen spreading capacity. In this research, the increase number of erythrocyte is lower than the horn’s suspension because the erythropoietin in mice still working. Beside that, erythropoietin use more in anemia nonregenerative case.

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References