Iron chelation ability and hematological effect of sappan wood (*Caesalpinia sappan*, L.) Extract tablet on iron overload condition of rats

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Research of iron chelation ability on sappan wood (*Caesalpinia sappan*, L) extract tablet of rats (*Rattus norvegicus*, L) on iron overload condition aims to obtain optimum dose sappan wood extract (CSE) tablet effective as iron chelator herbal. The research was performed using a completely randomized design (CRD) to 27 rats female Wistar 8 weeks old with an average weight of 200 grams. Research is divided into 9 treatments with 3 repetitions. Negative control (KN1), the control formula (KN2), Tablet of sappan wood extract (CSE) dose of 100 mg/kg BW/d (P1), dose of 200 mg/kg BW/d (P2), dose of 300 mg/kg BW/d (P3), dose of 400 mg/kg BW/d (P4), a satellite group (P5), deferiprone dose of 75 mg/kg BW/d (P6). Data were statistically analyzed using analysis of variance (ANOVA) level of 95% (α = 0.05), if there is a significant difference, then followed by the Duncan multiple comparison tests. The results showed that the sappan wood extract tablet is in the normal values range at each measurement of the blood parameter. It indicates that administered of sappan wood extract tablet only binds the excess of iron body and does not destabilize of the eritrosit.

Keywords: *Caesalpinia sappan*, L., iron chelation, tablet, thalassemia

INTRODUCTION

Thalassemia is a hereditary disease associated with a proportion of globin chain synthesis (Vanichsetakul, 2011). Hemolysis and erythropoiesis ineffective is the main cause of anemia in thalassemia disease (Kotze et al., 2011). Blood transfusion therapy for thalassemia should maintain hemoglobin in the normal range, in order to prevent deleterious effects of anemia. It is also important to restrict iron overload by adequate chelation to prevent organ damage due to the free radical formation (Sharaf et al., 2014). Iron overload in thalassemia is the side effect of ineffective erythropoiesis, increased gastrointestinal absorption of iron, lack of the physiological mechanism for excreting the excess of iron and multiple blood transfusions resulting in hemochromatosis (Jeon and Sin, 2013). Complication by iron overload is common and its toxic effect progressively damage heart, liver and endocrine glands at later age (Coates et al., 2014).

Iron chelation therapy is the only method of iron overload. Iron chelation therapy involves the
use of lighting drugs which is able to coordinate with iron forming complex to reduce exaggerated levels of iron (Maskoen et al., 2016). Currently, desferrioxamine, deferiprone, and deferasirox clinically used for the treatment of iron overload (Hoffbrand, 2012). Due to its side effect like agranulocytosis and neutropenia (Hoffbrand, 2012), as well as its high cost, the third chelator is considered.

Phenolic and flavonoid compound is commonly used as the chelating agent (Gupta et al., 2011; Ebrahemzadeh, 2009). The heartwood of Caesalpinia sappan, L (CS) contains various types of phenolic components, such as flavones, chalcones, xanthones a brazilin (Nirmal et al., 2014). Brazilin in C. sappan, L has chelation activity due to hydroxyl functional (Nirmal et al., 2014) and catechol group in its structure (Safitri et al., 2016).

Dosages form to play the important role of thalassemia patient. According to most of the patient is in childhood age, a tablet can be administered due to its advantages. Tablet can be given for systemic effect, patient compliance, and less expensive to manufacture. Tablet can provide high precision dosing. Tablet form is the most widely used dosage form because of self-administration and ease to manufacture (Jaimi and Rawat, 2013).

In this study, extract of CS heartwood was made in tablet that consist in different doses 0 mg/kg BW/d, 100 mg/kg BW/d, 200 mg/kg BW/day, 300 mg/kg BW/d, and 400 mg/kg BW/d and satellite dose 400 mg/kg BW (P5). The state of an iron body known by measurement red blood cells parameter (hematocrit level, hemoglobin, total erythrocytes, and Erythrocytes index) on rats (Rattus norvegicus L.) with iron overload. Determining blood parameter is helpful in assessing the thalassemia Iron status. This study aims to get an effective dose of CSE tablet as iron chelation in rats with iron overload condition by observing red blood cells parameters

**MATERIALS AND METHODS**

**Material**
The material used in this study were distilled water, deferiprone (Ferriprox®), ethanol 96%, iron dextran, dried powder of CS heartwood, chloroform, rats feed type of CV 151 (Laboratory standard), 8 weeks old female rats (weight between 150 – 200 g). Tablet excipient consist, citric acid, Avicel PH 102, lemon oil. Na CMC, sodium cyclamate, and Lactose.

**Tablet Preparations**
About 2.5 kg dried powder of CS heartwood placed into macerator, macerated using ethanol 96% for 3 x 24 hours. Collect all macerate, and dried using rotary evaporator at 40°C to form the crystalline powder. Sappan wood extract tablet made by wet granulation method, in which citric acid, sodium cyclamate, lactose, Avicel PH 102 and powder extract mixing with Na-CMC mucilage. The mixture was homogenized and granulated (mesh 12), then dried in the oven (temperature ± 40°C) until constant weight (2 days). The granules were mixed with Mg Stearate, Avicel PH 102 and lemon oil, until homogeny, and compressed using single punch machine.

**Animal Test and Treatment**
The research was performed using a completely randomized design (CRD) to 27 rats female Wistar (Rattus norvegicus, L.) 8 weeks old with an average weight of 200 grams. Animal initially acclimated to the laboratory environment for a week. During this acclimatization, rats only get rat feed type CV 151 and tap water ad libitum. All treatment was administered orally, therefore any given substance was made in solution. CSE Tablet extracts, deferiprone, and iron dextran dissolve in distilled water beforehand. Treatments given for 28 days were performed with oral gavage needle. Iron dextran was given 60 mg/kg BW in every 3 days, whereas deferiprone and tablet extract was daily given. Treatments given are presented in table 1.

**Measurement of Parameters**
Blood samples were carried on day 29. Hemoglobin (Hb) measured use Sahli method, whereas Hb concentration can be determined by reading the scale on dilution tube. Hematocrit or pack cell volume (PCV) measured by the microhematocrit method and the value of hematocrit expressed in percentage. The erythrocyte number measured use hemocytometer while red blood cells number performed under the microscope with magnification 45 x 10. Erythrocyte number can calculate using the formula.

\[
\text{Erythrocyte number} = \text{number of erythrocyte in 5 block R x dilution x 50}
\]

**RESULTS**

**Hemoglobin Level**
Hemoglobin is the main substance of the
erythrocyte. It’s consist of protein part (globin) and the non-protein part (heme). Hemoglobin level is one of the parameters to determine anemia. Hemoglobin is a compound containing iron, so the availability of iron will affect hemoglobin levels in the blood. Based on the results of analysis of variance (ANOVA) test, showed that there is a significant difference from each treatment to hemoglobin levels of rats with iron excess. Then proceed with Duncan’s multiple range test to know the significant differences in each treatment. The measurement results can be seen in figure 1.

Hematocrit Level

Hematocrit (Hct), the proportion of blood volume occupied by red blood cells, is a major determinant of blood viscosity. Hematocrit levels also serve as an indicator of health conditions. Based on the result analysis of variance (ANOVA), it showed that CSE tablet in each treatment gave the significant effect on the rat’s hematocrit number (p> 0.05), so the Duncan distance test is performed. Graph rats erythrocyte number with excess iron given by CSE tablet can be shown in Figure 3.

**Figure 1** Hemoglobin level of rats (*Rattus norvegicus* L) with iron overload condition, given CSE tablet.

Note: Based on Duncan test different letters in the same column showed a significant difference in the level of confidence 95%

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<th>Table 1. Treatment of animal test</th>
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Note: KN1 = Rats were given distilled water (negative control); KN2 = Rats were given formula without extract / dose 0; KP = Rats were given iron dextran dose of 60 mg/kgbw (positive control); P1 = Rats were given iron dextran dose of 60 mg/kg BW and tablet sappan wood extract dose of 100 mg/kgbw/d; P2 = Rats were given iron dextran dose of 60 mg/kg BW and tablet sappan wood extract dose of 200 mg/kg BW/d; P3 = Rats were given iron dextran dose of 60 mg/kg BW and tablet sappan wood extract dose of 300 mg/kg BW/d; P4 = Rats were given iron dextran dose of 60 mg/kg BW and tablet sappan wood extract dose of 400 mg/kg BW/d; P5 = Rats were given iron dextran dose of 60 mg/kg BW and tablet sappan wood extract dose of 100 mg/kg BW/d; Then allowed to be left for 14 days post-treatment; P6 = Rats were given iron dextran dose of 60 mg/kg BW and deferiprone 75 mg/kg BW/d.

Figure 2 Hematocrit level of rats (*Rattus norvegicus* L) with iron overload condition, given CSE tablet.

Note: Based on Duncan test different letters in the same column showed a significant difference in the level of confidence 95%

Figure 3 Erythrocyte number (*Rattus norvegicus* L) with iron excesses condition, given CSE tablet.

Note: Based on Duncan test different letters in the same column showed a significant difference in the level of confidence 95%
DISCUSSION

Duncan test results showed that rats hemoglobin levels, which were given iron dextran (16.33 gr / dl) had higher levels and significantly different than the negative control group which was only given distilled water (12 g / dl), but not significantly different from the group of rats who were given CSE tablet at the dose of 100 mg /kg BW/d and in group of rats given only tablet formula. Hemoglobin levels of rats which were given Fe addition in the feed were higher than the group without Fe addition. Despite having lower hemoglobin levels than iron dextran group, but the rat’s hemoglobin level which was given the tablet formula was slightly higher than the negative controls group. This is presumably because the content of lemon oil in the tablet formula helped the rats body in absorbing iron contained in the feed. It is known that vitamin C in lemon oil can help the body in improving iron absorption. Meanwhile, in the administration of CSE tablet at doses of 200, 300 and 400 mg/kg BW/d and satellite groups showed values of hemoglobin not significantly different than deferiprone and control groups. This indicates that administration of CSE has chelation effect as well as deferiprone.

The administered of CSE from low to high doses shows the decreased of hemoglobin levels. The Low level of hemoglobin is given by CSE dose 400 mg/kg BW/d that is 11 gr/dl or decrease about 48.45% compared to iron dextran group. The decrease of Hemoglobin level which was given of CSE showed the ability of CSE as iron chelation, its mean that iron-induced into rats body cannot be used for hemoglobin formation. Winter, 2014 mentions that the iron absorbed from the intestinal lumen will be bound directly to apotransferrin which will bring Fe to the liver and be used for hemoglobin formation (Winter and William, 2014). However, in all treatments in rats, it still showed a normal range of hemoglobin levels because it was still in the normal state, which is about 11-18g /dl. Murphy, W.G (2014), it is not the same in thalassemia patients who received blood transfusions due to hemoglobin formation disorders (Murphy, 2014).

The percentage of hematocrit is the ratio of erythrocytes to total blood volume. Hematocrit is a measure representing of erythrocytes in 100 ml of blood, so the results are expressed in percentages (Andrew et al., 2007). Based on Figure 2, it is a known that the rat’s hematocrit level in general in each group showed no significant difference. However, the iron dextran group had the highest hematocrit level which is 52.67%. The administration of iron dextran can increased rats Hematocrit level 10.49% compared with control rats group which is given distilled water.

According to Murphy, W.G (2014), the greater amount of iron in the body, the higher of hematocrit level. The main reason is iron play important role in the red blood cells (Erythrocytes) formation (Murphy, 2014). Figure 2 showed that in administered of CSE tablet with doses of 100, 100, 200, 300 and 400 mg/kg BW/d and deferiprone 75 mg/kg BW/d have lower hematocrit levels than dextran iron. The normal level of rat hematocrit is between 36-48%, which means the administered of CSE tablet did not decrease the red blood cells number. This shows that CSE tablet only bind the excess of iron body and not disrupt the stability of the erythrocytes.

In figure 3, it is known that erythrocyte number is given iron dextran (12.13 x 106 / μl) showed the higher amount of erythrocytes and significantly different from control group (9.07 x 106 / μl). This can explain that the excess iron from iron dextran use to forming red blood cell in rat body, so it can increase the erythrocyte number. While in the administration of deferiprone cause decreased of erythrocytes number lower than rats given iron dextran. This may occur because some of the iron ion excesses from iron dextran have bound to deferiprone molecule, so only a few iron ions are used for forming erythrocytes.

CSE tablet administration at dosage 100 and 200 mg/kg BW/d was not different from the control treatment. Meanwhile, at the higher dose which is 400 mg /kg BW/d give significant effect compared with control group. Although not significantly different from the iron dextran group, the rat has received orally CSE tablet at 400 mg /kg BW showed higher amounts of erythrocytes than control rats treated only with distilled water. This may be due to low doses (100 and 200 mg/kg bb) have been able to bind iron excess in rat with iron overload condition, So it can't be able to form red blood cells. While at higher doses (400 mg/kg bb), there is also an ability to stimulate the formation of erythrocytes with characterized by the increasing of higher erythrocytes number than the control group.

In this study, the total amount of erythrocytes ranged between 9.07 to 12.13 106/μl, higher than the normal erythrocyte range. According to Luciana et al., (2015), normal erythrocytes number in rats ranged from 7.2 to 9.6 x 106 /μl (da SilveiraCavalcante, 2015). The amount of
Erythrocytes is affected by several factors including age, sex, hormones, weight and other factors (Jeon et al., 2013; Galanello and Origa, 2010) add that nutritional factors, blood volume also affects the number of erythrocytes.

The results showed that the sappan wood extract tablet is in the normal values range at each measurement of the blood parameter. It indicates that administered of sappan wood extract tablet only binds the excess of iron body and does not destabilize of the erythrocyte.

CONCLUSION
Based on the research, it can be concluded that iron content through iron dextran administration can increase hemoglobin and hematocrit levels however CSE at various doses can decrease hemoglobin, hematocrit and erythrocytes to the same level as normal rat condition. Administration of CSE at various doses binds excess iron, but does not disrupt the stability of the amount of erythrocytes, even CSE can stimulate the formation of erythrocytes. The results showed that CSE able to bind iron excess and does not disrupt the stability of hemoglobin formation, hematocrit and erythrocytes. CSE produces just as safe as depheriphrone.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS
RS and AMM, designed the study and carried out the collecting sample. The RS carried out the experimental in-animal research, biochemical testing. AMM interpreted the results and statistical analysis, helped complete the draft manuscript. all authors read and approved the final version. RS

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