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The Effects of *Caesalpinia sappan L.* Extract Granule to Antioxidant Activity In Blood Serum of Wistar Rat (Rattus norvegicus) With Excessive Iron Condition

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Abstract : This study aims to determine the effective dose and the granule formulation of sappan wood (*Caesalpinia sappan* L.) extract (SWE) to increase the antioxidant activity in blood serum in excessive iron condition. This study was complete random design with 11 treatments and was repeated three times. Male wistar rat (*R. norvegicus* L.) of 200 g, was given with iron dextran to induce a state of iron excessed. A total of eight groups were then given by two types of granule formulations, and each granule formulation consisting of SWE doses of 0, 100, 200, and 400 mg / kg bw, 3 treatment groups: group that was given by Iron Dextran, distilled water, and deferiprone as a comparison. The study was conducted over 15 days, the parameters observed including: activity of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and the levels of malondialdehyde (MDA). The results showed that administration of Iron Dextran 60 mg / kg caused increase of iron level which also caused the increasing activity of SOD at 90.54%, GPx at 12.25% and the levels of MDA at 31.82%, also decreased the catalase activity at 19.77%. The results also showed that SWE in granule formulation at 200 mg / kg body weight dosage can reduce the activity of SOD at 73.78,%, lower the MDA levels at 47.91% and increased the activity of GPx at 145.41% and catalase activity at 25.89 % .

Keywords : Sappan wood (*Caesalpinia sappan* L.), antioxidant activity, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) malondialdehyde (MDA).

Introduction

The long term side effects due to recurrence of blood transfusions could lead to excessive iron in various organs. This occurs because every blood unit that have been transfused contains 250-200 mg of iron, and the average of iron body daily intake as hemoglobin is about 25 ± 30 mg. This daily amount of iron is

released into the plasma from the breakdown of red blood cells through the reticuloendothelial system, especially the spleen and liver. Iron is released in a plasma derived from transfused red blood cells that can't be used in patients with iron overload condition. Human body is only able to release no more than 1 mg of iron per day. Patients who have done 10-12 times of blood transfusion will surely have excess iron condition[1].

Free iron in human body can act as a catalyst of non-enzymatic reaction of the Fenton and Haber-Weiss forming Reactive Oxygen Species (ROS) such as superoxide radical (O_2*), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH*)[2] Free radicals cause cell damage and cell components such as lipids, proteins and DNA, also cause mutations and carcinogenic[3]. Under normal conditions, iron in blood plasma is not present and superoxide anion (O− 2) is converted by the enzyme superoxide dismutase into H_2O_2. H_2O_2 then will be converted into harmless substances by the catalyze enzyme and peroxide glutation. However, in patients with thalassemia disease who experience iron overload condition in their internal organs, there are free iron (Fe^{2+}) in the plasma that can act as a catalyst of non-enzymatic reaction through Fenton, then react with H2O2 to form OH * and OH-, i.e very reactive hydroxyl free radical[4]. Free radical activity in human body can be reduced by antioxidants. Malondialdehyde (MDA) is a lipid peroxidation products generated as a result of a number of membrane-bound iron in erythrocytes of thalassemia patients. Iron is a trace metal which generates free radicals that can cause oxidative damage to erythrocytes.

Antioxidants in human body can be divided into two groups, the enzymatic antioxidant and non-enzymatic antioxidant. Enzymatic antioxidants known as deterrent, consists of dismutase superoxide, catalyze and glutathione peroxide. While the non-enzymatic antioxidants also called as antioxidants chain cutter. Antioxidants chain cutter consists of vitamin C, vitamin E and beta carotene[5]. Safitri[6] stated that there are five active antioxidant compounds in sappan wood extract (*C. sappan* L) (SWE), three of the compounds are brazilin, 1', 4'-dihidrospiro [benzofuran-3 (2H), 3' - [3H -2] benzopyran] -1', 6', 6', 7'-tetrols, and 3 - [[4,5-dihydroxy-2 (hydroxymethyl) phenyl] methyl] -2-3-dihydro-3,6- benzofurandiol able to inhibit xanthine oxidase activity, capture the superoxide anion radical, capture the free hydroxyl radicals, and indicated resources as chelating iron. All of the three compounds are classified as primary antioxidants that are capturing a radical and break the chain of oxidation and secondary antioxidants that are preventing radical formation. Two other compounds are isobrazilin, merely as a radical catcher or as a primary antioxidant, and a mixture of stereoisomers compounds (7R-, 7S-protosapanin B only act as radical catcher and radical deterrent but less effective. According Jullihar[7] sappan wood extract (SWE) is capable to increase the antioxidant activity in mice with iron overload condition. According to Attia et al.[8], children with thalassemia (age 4-17 years) who undergo iron chelation therapy with deferiprone (75mg / kg / day) have higher levels of SOD, MDA and GPx three times higher compared to children who are normal and healthy, but have lower level of catalase. Children with thalassemia who underwent antioxidant therapy using vitamin A, C and E, decreased SOD, MDA and GPx as well as increased levels of Catalase in blood. Some reports also suggested that deferiprone, causing neutropenia or agranulocytosis are reversible[9]. Therefore, it is needed to take the antioxidants that can scavenge free radicals and also chelate iron, just like the sappan wood extract.

The exact form of drug or the proper drug formulation in processing natural ingredients into a dosage form that can be easily accepted by consumers is expected to improve the practicality and public interest in consuming herbal medicines[1011]. In this study SWE was made into granule formulation that is instantly and easily dissolved so that it can be used to be consumed orally[10]. This study aims to get the formula and the effective dose of sappan wood granulated extract as an antioxidant to improve the antioxidants in thalassemia patients with iron overload condition.

Experimental

Preparation of Sappan Wood Extract Granule

The experimental animals are 8 weeks old wistar male rats (*R. norvegicus*) with average weight of 200 grams, aquabides, ethanol 96%, superoxide dismutase (RANSOD-Randox), tiobarbiturat acid (TBA) / $C_4H_4N_2O_2S$, Glutathione Peroxide Kit (BACKPACK -Randox), a solution of trichloroacetic acid (TCA) / CCl_3COOH, tetraetoksipropane solution (TEP) / $C_{11}H_{24}O_4$, Catalyze Assay Kit Item No. 707002 and rat feed types CV 551. The materials used for the manufacture of granules are Fumaric acid, citric acid, Avicel, sappan wood extract (*C. sappan* L.) (SWE) in powder form, mannitol, $NaHCO_3$, NaCl, NaCMC, and Na-cyclamate.

Extract Granules of sappan wood were dried in the open air and sheltered from direct sunlight. Once dried, the bulbs were crushed using a blender, then macerated using 96% ethanol for 3 x 24 hours. Macerate then was concentrated using a rotary evaporator at 40°C to obtain powder extract.

Granular material was made with mixed acid, citric acid, Fumaric acid, cyclamate, mannitol, Avicel PH 102 and Na-CMC. The mixture was then homogenized using a mixer and then dried in an oven (temperature ± 40°C) for 2 days, Followed by slugging process using a molding tool punch tablet size diameter of 20 mm, then granulated (mesh '12). The granules were then mixed with powder extract and sodium bicarbonate and then dried in an oven (temperature ± 40 ° C) to obtain constant weight. The difference of the two types is the amount of Na-CMC content.

2.3 Provision Treatment of Experimental Animals

All the treatment of wistar rats were given orally, therefore any given substance in the solution form were prepared in advance. Iron dextran (Fe^{3+}) at a dose of 12 mg / rat / injection beforehand dissolved in distilled water and then given once every 3 days for 15 days. Deferiprone at a dose of 15 mg / rat / days and instant granules dissolved in a cup of distilled water extract was administered daily for 15 days. Rat blood was taken on day 16 and added EDTA to prevent clotting, then the separation of red blood cells, followed by testing the activity of SOD, GPX and catalyze and also MDA using TBARS in red blood cells.

Results and Discussion

Effects of Sappan Wood Extract In Instant Granules Against Superoxide Dismutase (SOD) of Rat Blood Serum In Iron Excessive Condition

The measurements of SOD enzyme activity were performed to determine the ability of sappan wood extract (SWE) granules in scavenging the superoxide radicals in the body. As shown in Table 1, statistical analysis SOD activity showed significant difference in each treatments. Control rats has the lowest SOD activity (407.38 U / ml), that shows absence of induction substances that can trigger increase of free radicals in the body [8]. Increased SOD activity could indicate the existence of excess iron in the body. Treatment of Iron Dextran can cause excess iron to the highest SOD activity (776.25 U / ml) increased to 47.52% compared to controls. Excess iron can cause a chain reaction through the Haber-Weiss O2 * - + H2O2 → OH- + OH * + O2 and OH * + H2O2 → O2 * - + H2O + H +, which increases the amount of superoxide radical[2]. Superoxide radical is a substrate of SOD enzyme. Increased activity of an enzyme is affected by increased concentrations of the substrate. Increased activity of SOD is an action of cell protection against superoxide radicals that can cause oxidative stress. This is in line with the research conducted by Abdalla[11], which stated that SOD activity increased in β-thalassemia major patients compared to normal subjects. Increased SOD activity in patients with thalassemia is caused by continuous blood transfusions that led to iron overload state.

Table. 1Effects of Sappan Wood Extract Instant Granules On Superoxide Dismutase Activity [U / ml] In Wistar Rats Blood Serum With Iron Overload Condition

Treatments	SOD Activity[U/ml]
Control	407,38 [a]
Iron Dextran	776,25 [c]
Deferiprone	562,34 [b]
Iron Dextran + Fomula 1	707,94 [c]
Iron Dextran + Formula 1 dose 100 mg/kg bw	467,74 [ab]
Iron Dextran + Formula 1dose 200 mg/kg bw	478,63 [ab]
Iron Dextran + Formula 1 dose 400 mg/kg/bw	537,03 [b]
Iron Dextran + Formula 2	524,81 [ab]
Iron Dextran + Formula 2 dose 100 mg/kg bw	594,54 [b]
Iron Dextran + Formula 2 dose 200 mg/kg bw	446,68 [a]
Iron Dextran + Formula 2 dose 400 mg/kg bw	501,19 [ab]

Note : Based on DMRT, different superscript letters in the same column indicate significant differences at the level of 95%.

Deferiprone is the common drug used in thalassemia with iron overload condition. The results showed that administration of deferiprone can reduce the activity of SOD by 27.56%. Deferiprone chelation ability causes a reduction of superoxide and free radicals formed due to excess iron. According to Patrick *et al*[12]. deferiprone can effectively lower iron levels in the body and prevent the redox reaction of Fe 2+ into superoxide radicals. A decrease in superoxide radical gives impact on the activity of SOD[13].

Table 1 shows that both formulation 1 and 2 of SWE granules can decrease the activity of SOD in general. Administration of SWE granule at doses of 100 mg / kg bw, 200 mg / kg bw and 400 mg / kg bw reduced the activity of SOD close to the control and deferiprone. However, a dose of 200 mg / kg bw in formulation 2 is more effective than the other dose of Iron Dextran. These results might be due to higher level of sodium carboxymethyl cellulose (CMC Na) contained in the formulation 2 than the formultion 1. Sodium carboxymethyl cellulose serves as a binder in granules that can increase the viscosity of the solution. The better solubility of the granules, the bigger the possibility of active substances that can be easily dissolved [14] [15]. Referring to research done by Wijayakusuma [15], the antioxidant activity in SWE granule formulation 1 and 2 in vitro showed that the formulation 2 has a value of antioxidant activity better than formulation 1. Decrease in SOD activity in Wistar rats fed by SWE granules is mainly caused by flavonoids in SWE. Flavonoids have been confirmed to possess antioxidant properties that decreased SD avtivity [16]. The ability of flavonoids in scavenging free radicals depend on its structure [17]. Hydroxyl groups contained in flavonoids can react with superoxide free radicals by giving H atom (hydrogen) to superoxide radicals to become more stable. Reduced superoxide free radicals as a substrate to be tempered by SOD causes decrease in SOD activity. The ability of flavonoids in scavenging free radicals caused by hydroxyl groups that can react as follows: F-OH + R * → F-O * + RH. The hydroxyl group in ring B is the most significant in the capturing Reactive Oxygen Species (SOR) and change the SOR to be more stable. The reduced activity of SOD from application of SWE granules is also supported by Attia *et al*. [8] research which showed decrease of SOD activity in patients with β-thalassemia after consuming vitamin A, C and E as an antioxidant.

Effects of Sappan Wood Instant Granules Against levels of malondialdehyde (MDA) (mol / GHB) In Rat Blood Cells With Excess Iron Condition

Measurement of levels of malondialdehyde (MDA) was conducted to determine the ability of SWE granules in preventing lipid oxidation. The results showed that SWE granules influence the levels of MDA (Table 2).

Table 2.Effects of Sappan Wood Extract Instant Granules On Levels of Malondialdehyde (MDA) (mol/ g Hb) In Rat Blood Serum With Iron Overload Condition

Treatments	MDA Level (µmol/gHb)
Control	4897,79 ^{ab}
Iron Dextran	6456,54 ^{cd}
Deferiprone	6165,95 ^{bcd}
Iron Dextran + Fomula 1	7413,1 ^{d}
Iron Dextran + Formula 1 dose 100 mg/kg bw	5888,44 ^{abcd}
Iron Dextran + Formula 1dose 200 mg/kg bw	4677,35 ^{a}
Iron Dextran + Formula 1 dose 400 mg/kg/bw	5754,39 ^{abcd}
Iron Dextran + Formula 2	6309,57 ^{bcd}
Iron Dextran + Formula 2 dose 100 mg/kg bw	5495,41 ^{abc}
Iron Dextran + Formula 2 dose 200 mg/kg bw	4365,16 ^{a}
Iron Dextran + Formula 2 dose 400 mg/kg bw	6918,31 ^{d}

Note : Based on DMRT, different superscript letters in the same column indicate significant differences at the level of 95%.

MDA is a highly reactive compound and used as a biomarker in lipid peroxidation for assessing oxidative stress. Iron Dextran at a dose of 60mg / kg increased the levels of MDA as much as 24.14% as compared to controls. This is due to the provision of Iron Dextran caused excess iron (Fe^{2+}) condition which can lead to the formation of hydroxyl free radicals by acting as a catalyst for the Haber reaction Weiss: Fe 2+ + H2O2 → Fe^3 + * OH + OH - .. The hydroxyl radical (• OH) which is formed when joining the unsaturated fatty acids (LH) will produce a lipid radical (L •). The process of the ongoing series initiated lipid peroxidation[18]. Increased levels of MDA in the group of Iron Dextran is in line with the research done by Patne[19] who stated that an increase in MDA levels were significantly higher in patients with thalassemia compared to normal

subjects. Giving deferiprone as an iron chelator can reduce levels of MDA by 4.5% compared with the provision of Iron Dextran. This is because the decreased levels of free iron in the body. Decreased levels of iron can stop the chain reaction that produces hydroxyl radicals. Inhibition of hydroxyl radicals formation can prevent the initiation and propagation of lipid peroxidation[20]. However, administration of deferiprone is less effective in lowering levels of MDA. This is presumably because deferiprone only act as iron chelator and prevent the formation of free radicals, but does not reduce the free radicals that have been formed.

The level of lipid peroxidation can be suppressed by the presence of antioxidants which decreased levels of MDA. The SWE granules addition in formulation 2 showed better activity than the formulation 1 in lowering levels of MDA. This is caused by differences in granule composition of formula 1 and 2 on the content of the solvent that are CMC and Avicel. In formulation 1 used only 650 mg CMC and formulation 2 used 975 mg. This leads to a difference of solvent solubility difference granules. The more soluble in the solvent of a preparation it is expected that the active substances contained in such preparations can also dissolved better[15].

Based on research conducted by Wijayakusuma[15], measuring the antioxidant activity of the formula 1 and 2 in vitro using DPPH method showed that the formulation 2 have IC_{50} values of 44.6 ppm and 56.6 ppm formulation 1 IC50. Antioxidant activity of compound is measured by IC_{50} less than 0.05 mg / ml (50 ppm) as very strong, between .05 to .10 mg / ml (50-100 ppm) as strong, while if the value of IC50 ranged from .10 to .15 mg / ml (100-150 ppm), is undermined when IC50 values ranged from 0.15 to 0.20 mg / ml (150-200ppm) [21]. Based on the classification, formulation 2 belongs to a very strong antioxidant and Formulation 1 belongs to the strong antioxidant.

Results of SWE granule addition at a dose of 200 mg / kg can reduce levels of MDA both in formulation 1 (27.55%) and the formulation 2 (32.4%). In line with research conducted by Sarumathy [22], a dose of 200 mg / kg bw is the best dose of SWE that can lower the levels of MDA in the liver and kidneys of wistar rats induced by acetaminophen. Decreased levels of MDA is mainly caused by brazilin compound in SWE to capture the hydroxyl radical. The hydroxyl radical can initiate lipid peroxidation reaction. According to Coal et al. in Djulaika[23], SWE has active flavonoids and phenolic compounds, ie 4-0-metilsapanol, protosappanin A, protosappanin B, protosappanin E, brazilin, brazilin, caesalpinia, brazilide A, neosapanone, 7,3,4-trihydroxy-3 -benzil-2H. As already known in advance that flavonoids have a hydroxyl group that can donate electron to free radicals such as superoxide radicals, hydroxyl radicals and peroxyl radicals to be more stable.

Lipid peroxidation can be prevented by the flavonoids that also can reduce free radicals, which indicates that flavonoids react with peroxyl radicals (ROO *) and proceed to the termination stage of autoxidation reaction[24-25]. This is in line with the study by Hu[26] who tested the inhibition of hydroxyl radicals in vitro, the results showed that brazilin can capture hydroxyl radicals and converting it into a stable product.

The Activity of Glutathione Peroxidase (GPx)

Hydrogen peroxide is a free radical that is less reactive, but the accumulation of hydrogen peroxide can react with metallic iron (Fe 2+) that later lead to the formation of hydroxyl radicals through the Fenton reaction and the Haber-Weiss[27,2]. GPx activity was measured to determine the ability of the granules in SWE in converting hydrogen peroxide in the body into the water. GPx activity measurement results can be seen in Table 3.

Table 3.Effects of Sappan Wood Extract Instant Granules On Glutathione Peroxidase Activity [nmol / ml / min] In Rats Blood Serum With Iron Overload Condition

Treatments	Activity of GPx (nmol/ml/min)
Control	2,04 [a]
Iron Dextran	2,29 [a]
Deferiprone	3,71 [a]
Iron Dextran + Fomula 1	3,09 [a]
Iron Dextran + Formula 1 dose 100 mg/kg bw	2,95 [a]
Iron Dextran + Formula 1dose 200 mg/kg bw	4,67 [a]
Iron Dextran + Formula 1 dose 400 mg/kg/bw	4,36 [a]
Iron Dextran + Formula 2	2,88 [a]
Iron Dextran + Formula 2 dose 100 mg/kg bw	2,29 [a]
Iron Dextran + Formula 2 dose 200 mg/kg bw	5,62 [a]
Iron Dextran + Formula 2 dose 400 mg/kg bw	2,88 [a]

Note : Based on DMRT, different superscript letters in the same column indicate significant differences at the level of 95%.

Provision of Iron Dextran at 60 mg / kg body weight had lower GPx activity. This could be the result of glutathione depletion (GSH), which function as co-substrates needed by GPx enzyme to curb radical hydrogen peroxide. GPx enzyme requires 2 moles of GSH for eliminating 1 mole of hydrogen peroxide and produces 2 moles of water and sulphide glutathione (GSSG) as the following reaction: $H_2O_2 + 2\ GSH \xrightarrow{GPx} 2H_2O +$ GSSH. The excess of iron can lead to decreased levels of GSH due to excessive use of GSH by GPx enzyme, and also decreased levels of GSH [28]. This is in line with research done by Patne *et al*. [18] and Waseem *et al*.[29] which stated that a decline in GPx activity in patients with thalassemia who allegedly resulting from the influence of GSH levels.

Deferiprone at a dose of 75 mg / kg bw increased GPx activity wistar rats. Deferiprone as iron chelator can inhibit the formation of hydrogen peroxide caused by iron overload. Inhibition of hydrogen peroxide formation for deferiprone can prevent the occurrence of redox reactions on Fe^{3+} to Fe^{2+} in the blood plasma through the binding of one of Fe^{3+} molecule by every 3 molecules. Therefore, this prevention leads to prevention does non-depletion of GSH levels indicated by the increased activity of GPx. It has been reported that combination of deferiprone and Deferoxamine can increase GSH levels in red blood cells[2].

Generally, the addition of SWE granules with two types of formulations can increase the activity of GPx. From the results in Figure 3, the administration of the SWE granules in formulation 2 has higher GPx activity compared with formulation 1. This is caused by differences in granule composition of Formulation 1 and 2 and the content of the solvent are CMC and Avicel which cause solubility difference granule. Formulation 2 has better solubility compared to Formulation 1 so that it can affect the solubility of the active substance of SWE[15]. Increased activity of GPx third highest dose variation is at a dose of 200 mg / kg formulation 2.

Increased GPx activity in rats fed by the SWE granules for flavonoid content in SWE. Flavonoid content in SWE is expected to increase GSH levels which functions as co-enzymes GPx substrate [30]. Based on in vitro research conducted by Mosgauk[31], flavonoids can increase GSH concentration in muscles by triggering the expression of an enzyme that plays an important role in the antioxidant defence of cells. Previous study showed an increase in the activity of GPx best after the SWE with a dose of 200 mg / kg in the liver and kidneys of rats induced by acetominophene[22].

Catalase Enzyme Activity

Measurement of catalase activity was conducted to determine the ability of instant granules in SWE converts hydrogen peroxide into water and oxygen. The result of the calculation of catalase activity can be seen in Table 4. The results showed a decrease catalase activity after Iron Dextran intake at a dose of 60 mg / kg bw (141.25 nmol / min / ml) compared with controls (173.78 nmol / min / ml). These results are consistent with previous studies which stated that a decline in activity of catalase happened at 2.5 to 3 times in patients with β-thalassemia major who experience iron overload compared with controls[8] . Decrease in catalase activity may be caused by Phosphate Nicotinamide adenine dinucleotide (NADPH), which is very important in the activity of the enzyme catalase. NADPH deficiency can affect the action of the enzyme because of the catalase containing

monomer binds to the high affinity with NADPH. Decrease levels of NADPH might happened to result from free hemoglobine and iron due to lysis of erythrocytes.

Table 4.Effects of Sappan Wood Extract Instant Granules On Catalase Activity [nmol / ml / min] In Rats Blood Serum With Iron Overload Condition.

Treatments	Catalase Activity(nmol/ min/ ml)
Control	173,78 [a]
Iron Dextran	141,25 [a]
Deferiprone	177,82 [a]
Iron Dextran + Fomula 1	165,95 [a]
Iron Dextran + Formula 1 dose 100 mg/kg bw	158,48 [a]
Iron Dextran + Formula 1dose 200 mg/kg bw	177,82 [a]
Iron Dextran + Formula 1 dose 400 mg/kg/bw	158,48 [a]
Iron Dextran + Formula 2	151,35 [a]
Iron Dextran + Formula 2 dose 100 mg/kg bw	154,88 [a]
Iron Dextran + Formula 2 dose 200 mg/kg bw	177,82 [a]
Iron Dextran + Formula 2 dose 400 mg/kg bw	169,82 [a]

Note : Based on DMRT, different superscript letters in the same column indicate significant differences at the level of 95%.

Provision of deferiprone with a dose of 75 mg / kg bw as iron chelating drug can increase catalase activity close to normal. Increased activity of catalase allegedly as a result of iron chelation ability of deferiprone. Excess iron can cause an increase in hydrogen peroxide in the body. The increase in hydrogen peroxide can strengthen the action of the enzyme catalase.

In general, the intake of SWE granules with a variety of formulations and doses (100 mg / kg, 200 mg / kg, and 400 mg / kg) led to increase in catalase activity in all treatments whose value close to normal. Each treatment of SWE granule exhibited insignificant difference in catalase activity, however, the administration of the SWE granules at a dose of 200 mg / kg bw in Formulatin 1 and 2 had the highest catalase activity, which is 177.82 nmol / min / ml. This is due to the antioxidant content of SWE contained in granules. According to research conducted by Hu[23], SWE contains protosappanin A and B that can inhibit hydrogen peroxide. Increased activity of catalase has also been proven by previous studies by Jullihar[7] where an increase in catalase activity significantly in the provision of SWE at a dose of 100 mg / kg. Formulation 2 has a better activity than the formula 1, it is suspected because formulation 1 has a viscosity and better antioxidant activity compared with formulation 1[15].

In summary, SWE in granule formulation at 200 mg / kg is an effective formulation of sappan wood extract granule (*C. sappan* L.) that decreased the activity of SOD and MDA levels and increased the activity of GPx and catalase activity in the rat (*R. norvegicus* L) in conditions of iron overload.

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