Conference Paper

The Effect of Sappan Wood Extract (*Caesalpinia sappan*), Wheat grass and Vitamin E Treatment on the Liver Structure of Iron overload of Rat (*Rattus norvegicus*)

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Abstract

This research was carried out to detect the effect of Sappan Wood Extract (Caesalpinia sappan), Wheat grass and Vitamin E Treatment on the liver structure of iron overload rat (Rattus norvegicus).The method of experimental used Completely Random Design (CRD in triple repetition. The treatment had been carried out orally. Iron dextran with total dose of 1.5 g kg^{-1} of body were given to rat on the first, fourth, seventh, ten and thirteenth day. Sappan Wood Extract (Caesalpinia sappan)200 mg kg^{-1} bw, 400 mg kg^{-1} bw, Wheat grass extract 100 mg kg^{-1} bw and Vitamin E 60 mg kg^{-1} bw were given to rat everyday for 15 days. At the seventeenth, rat were killed and their liver were taken. The observed parameters are morphological abnormality including the colour, the contour, ratio between liver weight and body weight as well as histological destruction. The result showed iron dextran treatment was proved the abnormality on morphological and histological desruction. Futhermore, Sappan Wood Extract (Caesalpinia sappan), Wheat grass and Vitamin E Treatment can decrease the morphological abnormality and the liver histological destructionon of iron overload rat.

Keywords: Sappan Wood Extract, Wheat grass, Vitamin E, Iron, Morphological abnormality, Histological destruction.

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1. Introduction

In Indonesia, traditional medicine by utilizing plants has been carried out and used extensively from one generation to another. Generally, medicinal plants traditionally used for the prevention, treatment and increase endurance. Plants are used as medicine for generations have a distinct advantage, for instance, low toxicity, easy to obtain, relatively cheap and without adverse effects. However, research in potential utilization of plants in Indonesia as thalassemia therapy is very rare.

Thalassemia is a hereditary disease characterized by the occurrence of impaired synthesis of globin in hemoglobin chains [1]. *Thalassemia* occurs due to the inability of the bone marrow to form components of erythrocytes, namely hemoglobin, an iron-rich protein contained in the red blood cells and serves to carry oxygen from the lungs to all parts of the body [2].

Typically, the human red blood cells have a four months' lifespan, but in patients with thalassemia, in thalassemia, the red blood cells are destroyed at a faster rate, leading to anemia, a condition that can cause fatigue and other complications. To cope with this condition, usually blood transfusions is as possible solution. However, repeated blood transfusions lead to hematokromatosis, the condition of iron accumulation in the body that causes damage to organs such as liver, spleen, kidney and heart [3]. Iron is a transition metal ion and a source of endogenous free radicals that cause damage to organs and tissues, including liver damage, heart and endocrine glands [4]. Excess iron in the body will mostly accumulate in liver cells and bone marrow reticuloendothelial [5]. Therefore, the liver is the organ that will be exposed to the adverse effects of free radicals from iron overload. This can be observed in patients with thalassemia who perform blood transfusions continuously. In this case, liver fibrosis will occur in the second year since the first transfusion. Damage to the structure is characterized by the occurrence steatosit liver or fatty liver on hepatocytes, which can lead to fibrosis and 30% of patients with liver fibrosis will developed cirrhosis after 10 years [6]. Liver damage occurs due to an imbalance between the amount of free radicals and antioxidant system effectiveness that cause toxic effects on the liver [4]. Given the adverse effects of excess iron, patients with thalassemia require the intake of antioxidants and iron chelation.

Various studies reveal that plants sappan (Secang: *Caesalpinia sappan* L.) contains flavonoids and polyphenols that have the potential to reduce free radicals and suppress the formation of reactive oxygen species (ROS) by inhibiting the enzymes that trigger the ROS formation and iron chelation [7]. Same author demonstrated that there are at least 5 different antioxidant compounds from the wood extracts of Sappan, namely brazilin, isomers brazilin, biflavonoid, 3 - [[4,5dihidroksi-2-(hydroxymethyl) phenyl] meti] -2-3-dihydro -3.6-benzofurandiol and 1 ′, 4′-dihidrospiro [Benzofurans-3 (2H), 3′ - [3H-2] benzopyran] -1 ′, 6′, 6 ′, 7′-tetrols. Therefore, in this study, extracts of Sappan wood is used as a source of antioxidants to reduce the effect of iron to liver damage. In this study, wheat grass is also used as a rich chlorophyll substance (up to 70%) and serves as a builder immune system and for detoxification. Furthermore, chlorophyll intake can increase the production of red blood cells and reduce toxic due to excess iron [8b].

Natural antioxidant serve to suppress the formation of reactive oxygen, trapping free radicals and bind metal ions such as Fe^{+} or Cu^{+} and inactivate prooxidant metals, and thus preventing radical initiator and free radicals production [9]. In vitro study demonstrated that Sappan wood extracts are antioxidants that inhibit the activity of the enzyme xanthine oxidase, the enzyme that catalyzes the formation of superoxide radicals with activity of 89.9%. Moreover, these antioxidants are also capable of scavenging superoxide radicals up to 100%, and a hydroxyl radical trapping ability of 62.89%. These results indicate that the cup can prevent the formation of superoxide radicals and hydroxyl radicals [7, 10b]. Additionally, Sappan wood extract contains brazilin as the main compounds that are chelating agents of iron [7]. In addition, by using natural antioxidant extracts such sappan and wheat grass, this study also used Vitamin E, recognized commercially as an antioxidant supplement. Vitamin E also has the ability to prevent lipid peroxidation that occurs in conditions of iron overload [11]. This vitamin is an antioxidant that is capable of capturing a wide range of reactive oxygen and nitrogen species. In addition, vitamin can reduce free radicals by radical reaction and formation of stable toccopheroxyl. Afterwards, the toccopheroxyl radical is converted back into an active form by synergistic work from other antioxidant compounds.

Chemically, chlorophyll in wheatgrass can neutralize free radicals due to its binder fibers in the cell walls of chlorophyll that is able to bind metal ions that exist in the cell body [12]. In patients with thalassemia, consumption of wheat grass juice as much as 100 ml day^{-1} could reduce transfusion requirements [8a]. Based on the description above, it is necessary to study the structure of the liver in iron overload conditions and the effect of antioxidant of Sappan wood extracts, wheat grass and vitamin E on liver structure with iron overload.

2. Materials and Methods

2.1. Materials

Sappan wood (Caesalpinia sappan L) extract (Hereafter named: Ekstrak kayu Secang (EKS)) wheat grass, Vitamin E, rat (Rattus norvegicus L) aged 3-4 months, weighing 200-300 g, Feed, PGA (Powder Gum Arabic), chloroform. Physiological NaCl 0.9%, Bouin fixative solution, Alcohol (70, 80, 96 and 100%), xilol, paraffin, dye (hematoxylin and eosin), Erlich solution and entelan.

2.2. Animal Experiment and Sample Collection

The study was carried out experimentally in the laboratory by using a completely randomized design with 13 different treatments and 1 control. Each treatment contains 3 replicates. Briefly, rats (Rattus norvegicus) were conditioned with excess iron. Tested animal were grouped into: 1) control group where only distilled water was given. Group with iron (Fe^3) dextran addition was conducted with dose of 75 mg kg-1 injection-1 that is given orally to reach a total of 1.5 g kg-1 wet weight. Group with iron and antioxidants addition consist of a) EKS with a dose of 50, 100 mg kg-1 day-1 wet weight, b) wheat grass extract 100 mg kg-1 day-1 wet weight, c) Vitamin E 60 mg kg-1 day-1 wet weight, d) combination of EKS and vitamin E, e) combination of wheat grass and vitamin E and f) combination of EKS, vitamin E and wheat grass. The parameters used in this study is the structure of rat liver, which consists of morphometric abnormalities in form of color aberration and the surface of the liver that were analyzed with Chi-square test with 95% confidence intervals. Comparison on the ratio between liver weight and body weight was analyzed using Analysis of variance (ANOVA) test, whereas histological abnormalities of the structure was analyzed by using a non-parametric test of Kruskal-Wallis with 95% confidence level.

2.3. Liver Morphometry and Histological Observations

After 15 days of treatment, on 17th's day rat organs were dissected and the heart was taken. Subsequently, isolated heart was washed with 0.9% physiological saline. Morphological observations were performed by weighing the weight, color as well as disorders that appear on the surface of the liver. Additionally, morphometry observations were conducted directly (descriptive) with scoring method. Liver histology was observed with light microscope to observe the liver tissue including cell nucleus, sinusoids, central veins and parenchyma and liver tissue preparation was observed prior to scoring.

3. Result and Discussion

3.1. Morphometry of Liver

Effect of iron excess in the body on the liver structure was observedby heart morphometry comprises liver color, liver surface and the ratio of organ weight to body weight. Morphometric observations were also performed to determine the effect of antioxidant of EKS, wheat grass and vitamin E as an antioxidant against the effects of free radicals that caused by excess iron conditions.

Observations of color and surface of the liver was done by giving a score of 1 for the normal liver without any abnormalities and a score of 2 for the liver with color disorders. Normal liver is dark red and has a smooth surface. While liver with abnormalities has red color or pale brownish compared to normal liver and rough surfaces. Furthermore, color morphometry data and liver surface morphometry were statistically analyzed with Chi-square test. The results showed that no significance different was observed between treatments for both surface of the liver or liver color ($p > 0.05$). Although not significantly different, Table 1. shows that the color of the liver in groups of mice with the addition of iron dextran only (T1; score 1.3 ± 0.58, $p > 0.05$), and groups of mice were given Fe and Vitamin E (T6) (score of 1.3 ± 0.58, $p > 0.05$) had abnormalities in liver color, with red brownish color.

Excessive intake of iron in the body can induce oxidative stress in the liver due to iron induces free radicals such as hydroxyl radicals. The imbalance between the number of prooxidant (iron metal: iron dextrans) and the effectiveness of antioxidants may result in damage to cellular components. The effects of free radicals on the structure of the liver can be observed from the liver morphometric abnormalities, where the difference in liver color was observed between the group with iron intake only (T1) and the group with iron and vitamin E (T6) compared to control group (T0). The liver has a maximum capacity to accept metals, which at certain level, iron will accumulate in the liver and become toxic [13]. This toxic effect may further lead to changes in morphology due to changes in physiological and microscopic tissue structure. In addition, oxidative damage could cause shrinkage on the liver cells due to blockage of blood vessels in the liver [14].

In T6 group, combination of vitamin E that is an antioxidant showed morphometry abnormalities in the T6 group. This indicates that the addition of vitamin E merely less effective to prevent liver damage due to Fe^{3+} dextrans addition. It also indicates that under conditions of excess iron intake, the more antioxidants combination required to work synergistically and to provide better protection (synergism). Our results showed that in the T7 group (Fe + EKS 50 + Vit E), T8 (Fe + EKS 100 + Vit E), T9 (Fe + ERG + Vit E), T12 (Fe + EKS 50 + ERG + Vit E) and T13 (Fe + EKS 100 + ERG + Vit E), which combines vitamin E with other antioxidants such extract the sappan wood (EKS) and wheat grass extract, hepatoprotective effects were more optimal in preventing and repairing morphometric abnormalities that occur in conditions of iron overload. In addition, work of vitamin E that was less effective could also be caused by improper dose.

Liver morphometric abnormalities that can be observed from the surface of the liver might be due to a layer of dense connective tissue that covers the surface of the liver due to toxic damage caused by free radicals formed produced by iron in excess amount. However, no abnormalities or damage to the contour of liver surface contour generated from all treatments (Table 2). The invisibility effect on the contour conditions of excess iron could be caused by the delayed effect where the excess iron accumulated in the organs and tissues of the body were not directly visible but were accumulative and could appear in the long term [15]. In coherence with literature, damage or deterioration of liver function are sometimes not visible on the morphological structure of the liver and could be observed on the histological structure of liver [16]. In addition, the absence of morphometric abnormalities on the surface of the liver could be caused by the addition of wood Sappan extract, wheat grass, vitamin E and their combinations as an antioxidant with hepatoprotective effects that protect the liver from damage caused by the excess iron thus not affecting abnormalities on liver surface.

EKS can work effectively as an antioxidant because it contains flavonoids. Flavonoids are antioxidants that are able to capture free radicals, to inhibit xanthine oxidase enzyme activity and to act as an iron chelator, with the presence of catechol groups [17]. With the ability of flavonoid compounds contained therein, EKS could overcome morphometric abnormalities caused by free radicals due to iron overload condition.

Wheat grass also has the ability to prevent and repair damage on the liver cells caused by free radicals. This because wheat grass contains compounds that serve as free radicals scavenger. The most important component in the process is chlorophyll. Chlorophyll is quickly assimilated in the blood stream, helps cleanse the liver, improves blood sugar and blood flow, build the immune system and detoxification [18]. Natural antioxidants from wheat and sappan wood could be more optimal if coupled with vitamin E addition, which is an antioxidant that breaks the chain reaction of free radicals and prevent free radical activity [19].

Morphometric abnormalities of liver were also observed through comparison of liver weight per body weight (liver-body ratio). Statistical tests of organ-body weight ratio showed significant difference results ($p < 0.05$).

Change in organ weight is an indicative of abnormalities in organs [13b]. Table 2 showed that the treatment group with an iron (Fe) excess (T1) and combination of Fe and Vitamin E (T6) experienced reduction in organ weight per weight of the liver compared to control. T1 group, with the addition of Fe without antioxidants showed lower value of liver weight per body weight compared to control (2.99 ± 0.22 and 3.57 ± 0.37, $p < 0.05$, respectively). It could be caused by an excess amount of metal contained in the body acts as a metal prooxidant that generates free radicals in the body and causes oxidative stress [19]. Thus, causing abnormalities symptoms on liver cells, for instance, fats degeneration and blockage on blood flow. Therefore, the blood goes to the liver stuck, which causes the shrinkage on liver to constrict [14].

In T6 group, a slight decrease in liver weight per body weight was observed (2.95 ± 0.43, $p < 0.05$) compared to T1. This could be due to a single addition of vitamin E, so that the work was less effective. This is in accordance with the statement of Rahal et al., (2014) that the optimal work of antioxidants can be obtained through cooperation between the elements of antioxidants that work synergistically [19]. Changes in liver weight per body weight indicates loads of metal ions (Fe) in the body that triggers the formation of free radicals that interfere metabolism of digestive organs and cause abnormalities on liver cells.

In several groups of treatment, it showed that there was an increase in liver weight per body weight value compared to controls group and to the treatment of merely iron dextran. For example, in T8 group were given Fe, vitamin E and sappan wood extract (EKS) dose 100 mg/kg bw/day, which is 4.02 ± 0.97. It could be caused by steatosis, which is the occurrence of fats in liver cells and the formation of small vacuoles in liver cells that surround the cell nucleus that contains a large numbers of fatty liver, thus the volume of each hepatocyte swelling and enlargement of liver organs [14]. On the other treatment, the value of liver weight per body weight was close to the value of control. This could be due to other treatments with the addition of EKS, wheat grass, vitamins E and the combination, showed hepatoprotective effects that can protect the liver from abnormalities or free radical damage caused by excess iron. Adverse effects from the excess of Fe, which is a transition metal ion, was prooxidative and tends to produce reactive oxygen species (ROS) that can be overcome by the antioxidants contained in the sappan wood extract, vitamin E and wheat grass that are able to trap free radicals and iron binding.

3.2. Histological Structure of the Liver

Histological structures observed were central venous, parenchymal cell composition, sinusoids and liver cell nuclei. Based on observations, the most severe liver histological damage occurred in the T1 group (score: 2.6 ± 0.58, $p < 0.05$) with the addition of Fe dextran alone. Variations in damage level ranged from the mild to severe damage (Table 3). Damage in T1 group was characterized by tightening on central vein (Figure 1), irregular composition of parenchymal cells (image 2.) and picnotic in cell nucleus (Figure 4.4). Thus, it was categorized as severe damage. In Figure 4.2b, visible abnormalities were the tightening of central venous, irregular parenchymal cell structure and picnotic in cell nucleus. Inflammation or abnormalities on liver began on central venous blood as blood reservoir that comes from the hepatic artery and portal vein [20]. Central venous narrowing might be due to the blockage on blood vessels that carry blood from the rest of the body, caused by the presence of toxic or metal that accumulates in the body. Thus, the settings in the hepatic plates and sinusoids obstructed [14].

Inflammation could be caused by excess of free iron concentrated in the portal vein, which could enter into the interstitial tissue and eventually entered the capillary sinusoids and affected the composition of the central venous and parenchymal. Inflammation that follows the pattern of blood flow appeared to be the most frequent cause of hepatitis or liver inflammation [15].

Irregular parenchyma cells composition in several treatments could be caused by the damage on hepatocytes cell membrane that reduce cells size or death due to Fe excess that provoke free radicals, enter sinusoids and trigger irregular arrangement of hepatocytes in the liver lobule. This could be due to abnormalities of hepatocyte such cytoplasmic vacuolization in the liver lobule because of toxic free radicals [15].

Hepatocytes or liver parenchymal cells play significant role in the metabolic processes that occur in the liver. Liver receives all the materials that are absorbed through the intestinal portal vein. Portal vein carries all compounds and metals into the liver which then will be excreted [14]. However, the body does not have a natural mechanism for removing excess iron, thus Fe that is a transition metal will be a source of endogenous free radicals in the body Free radicals *(Reactive Oxygen Species:* ROS) are formed with the help of iron as catalyst. ROS cause damage to the membrane or lipid peroxidation in the liver [21]. Difference in damage level from different treatments was shown in Figure 1, 2 and 3. The entire group was given iron-dextran to create the excess amount of iron. The most severe damage was observed in T1 where no antioxidant was added.

In the group that received a single antioxidant intake showed varying levels of damage. In the T4 group rats (1 ± 0), addition of iron dextran and antioxidants of sappan wood with 100 mg of dose showed normal liver histological structure. Therefore, it can be concluded that the sappan wood with a dose of 100 mg appeared to be the most effective as an antioxidant. According to Safitri (2016), sappan wood extracts contain at least five antioxidants compounds that can reduce free radicals and suppress the formation of ROS either by inhibiting enzymes involved in the formation of ROS or by chelate metal ions including iron serves as a catalyst in the formation of ROS [22].

In T3 group (score 1.6 ± 0.58), T5 (Score of 1.6 ± 0.58) and T6 (2.3 ± 0.58) had a slight damage. This indicates that sappan wood extract with dose of 50 mg kg^{-1} bw day^{-1}, vitamin E and wheat grass could act as hepatoprotective effects that can protect the liver from free radical damage caused by iron overload conditions. Nevertheless, the hepatoprotective effect of each antioxidant is not optimal because given alone [15].

Vitamin E has the ability to prevent lipid peroxidation that occurs in conditions of iron overload and to prevent ROS as well as to reduce free radicals by means of reacting with the radicals and form a fairly stable toccopheroxyl. Furthermore, theoccopheroxyl radical is converted back to its active form through synergistic work with vitamin C [19]. Therefore, vitamin E could work optimally if it's coupled with other antioxidant compounds.

Wheat grass contains 70% chlorophyll that has the ability to prevent the adverse effects of carcinogens, to neutralize free radicals and to bind with metal ions [8b]. In addition, the content of the wheat grass can assist work of liver and protect the liver [23].

From T7 to T13, treatment with various combinations of antioxidants was performed. Treatment with a combination of antioxidants was appeared to be more effective in reducing visible damage to the liver histological structure compared to the treatment with a single antioxidant. T9 group (Fe + ERG + Vit E) and T10 (Fe + EKS 50 + ERG) showed the best histological liver conditions where they were almost similar with normal heart, which means the combination of antioxidants showed a hepatoprotective effect which protects liver disruption from tissue damage caused by free radicals conditions of iron overload. Reparation of liver cells damaged by the toxic effects of free radicals subsequent to addition of wheat grass extract and vitamin E might be caused by the presence of vitamin C in wheat grass, which is a water-soluble antioxidant that works in the extracellular fluid.

Table 1: Morphometry Color Wistar rat liver and Wistar Rat Liver Surface.

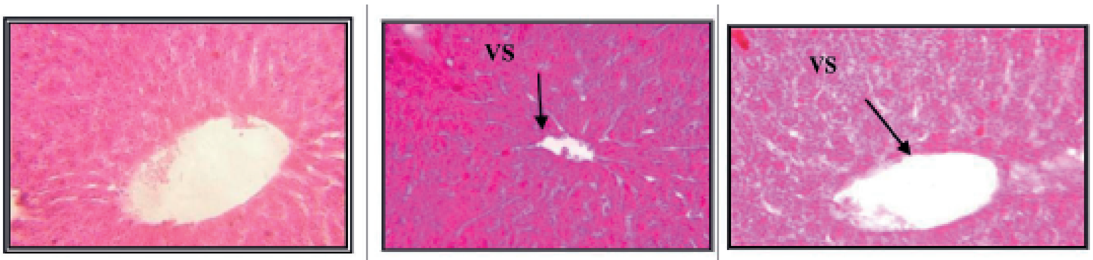
Treatment	Average Color	Scores color	Mean ± SD*	Surface	Score	Mean ± SD*
Control	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe	Brownish red	2	1.3 ± 0.58	Nothing subtle abnormalities	1	1 ± 0
Fe + desferal	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 50 mg/kg bw/day	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 100 mg/bw/day	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + ERG	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + Vit E	Brownish red	2	1.3 ± 0.58	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 50 + Vit E	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 100 + Vit E	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + ERG + Vit E	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 50 + ERG	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 100 + ERG	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 50 + ERG + Vit E	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Fe + EKS 100 + ERG + Vit E	Dark red	1	1 ± 0	Nothing subtle abnormalities	1	1 ± 0
Description: EKS (Wood Extract Secang)						
	ERG (Wheat Grass Extract)					
	Vit E (Vitamin E)					
*) Standard Deviation Not significantly different (p> 0.05), with the Chi Square						

Whereas vitamin E is a fat-soluble vitamin constituted in phospholipid membranes [8b]. Therefore, the combination of vitamin E and wheat grass is effective in protecting and curing liver tissue damage.

Histological Abnormalities. Description:To Control; T1 Fe; T2 Fe + desferal; T3 Fe + EKS 50; T4 Fe + EKS 100; T5 Fe + ERG;T6 Fe + Vit E; T7 Fe + EKS 50 + Vit E; T8 Fe + EKS

Table 2: Measurement of liver weight and body weight of rats.

Treatment	The mean liver weight (g)	Body Weight (G)	Average liver wg / Body Wg (%) ± sd
Controls	8.63	239.6	3. 57 ± 0.37
Fe	8:26	274.3	0.22 ± 2.99 *
Fe + desferal	7.83	240.0	3.26± 0.31
Fe + EKS 50	8:06	218.3	3.65 ± 00:45
Fe + EKS 100	7.65	206	3.72 ± 0.62
Fe + ERG	8.66	279.66	3:08 ± 0:13
Fe + Vit E	7.63	261	0:43 ± 2.95 *
Fe + EKS 50 + Vit E	7.1	275.33	3:55 ± 0:17
Fe + EKS 100 + Vit E	9.93	243	4:02 ± 0.97
Fe + ERG + Vit E	8:33	246.66	3.41 ± 0.37
Fe + EKS 50 + ERG	11:46	278.66	3.55 ± 0.56
Fe + EKS 100 + ERG	9.3	259.66	3.55 ± 1.06
Fe + EKS 50 + ERG + Vit E	7.8	230.66	3.40 ± 0.46
Fe + EKS 100 + ERG + Vit E	7.2	223.66	3.25 ± 0.57
Description: EKS (Sappan Wood Extract)			
	ERG (Wheat Grass Extract)		
	Vit E (Vitamin E)		
*) Significantly different (p< 0.05), Duncan test			



A. central venous control (Width). *B. Central venous with addition of Fe 3 + dextran (Narrow).* *C. Central venouswith addition of Antioxidants (Width).*

Figure 1: Transversal incision of Wistar rat Heart (Rattus norvegicus). A: central venous control (Width). B: Central venous with Fe 3 + dextran (Narrow). C: Central venous with Antioxidants (Width). 400x magnification with HE staining. VS = central vein.

100 + Vit E; T9 Fe + ERG + Vit E; T10 Fe + EKS 50 + ERG; T11 Fe + EKS 100 + ERG; T12 Fe + EKS 50 + ERG + Vit E; T13 Fe + EKS 100 + ERG + Vit E

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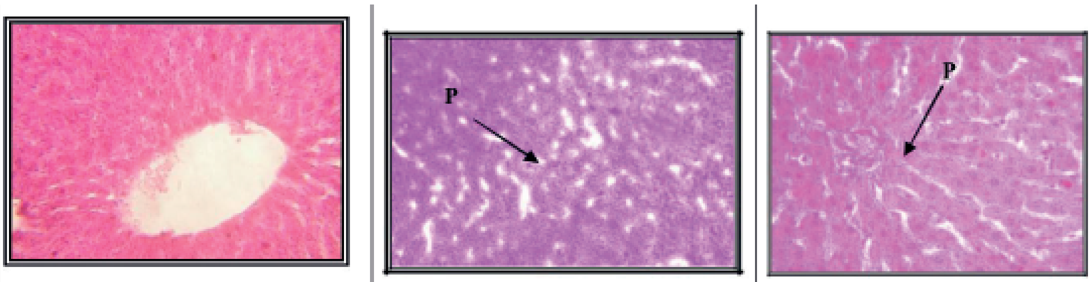
Table 3: Liver Histology of Wistar rat.

Treatment	Central venous	Sinusoid	The composition of the cell	Cell nucleus	scores	The average "score ± sd
Controls	Width	Whole	Regular	Normal	1	1 ± 0
Fe	Narrow	Whole	Irregular	Picnotic	3	2.6 ± 0.58*
Fe + desferal	Narrow	Whole	Regular	Picnotic	2	1,6±0,58
Fe + EKS 50	Width	intact	Irregular	Picnotic	2	1.6 ± 0.58
Fe + EKS 100	Width	intact	Regular	Normal	1	1 ± 0
Fe + ERG	Narrow	intact	Regular	Picnotic	2	1.6 ± 0.58
Fe + Vit E	Narrow	intact	Regular	Picnotic	3	2.3 ± 0.58
Fe + EKS 50 + Vit E	Narrow	intact	Regular	Normal	2	2 ± 0
Fe + EKS 100 + Vit E	Width	intact	Regular	Picnotic	2	1.6 ± 0.58
Fe + ERG + Vit E	Width	intact	Regular	Normal	1	1 ± 0
Fe + EKS 50 + ERG	Width	intact	Regular	Normal	1	1 ± 0
Fe + EKS 100 + ERG	Narrow	intact	Regular	Picnotic	2	1.3 ± 0.58
Fe + EKS 50 + ERG + Vit E	Narrow	intact	Regular	Picnotic	2	1.3 ± 0.58
Fe + EKS 100 + ERG + Vit E	Narrow	intact	Regular	Normal	2	1.3 ± 0.58
Description: EKS (Sappan Wood Extract)						
	ERG (Wheat Grass Extract)					
	Vit E (Vitamin E)					
Significantly different (P <0.05) with the Kruskal Wallis						

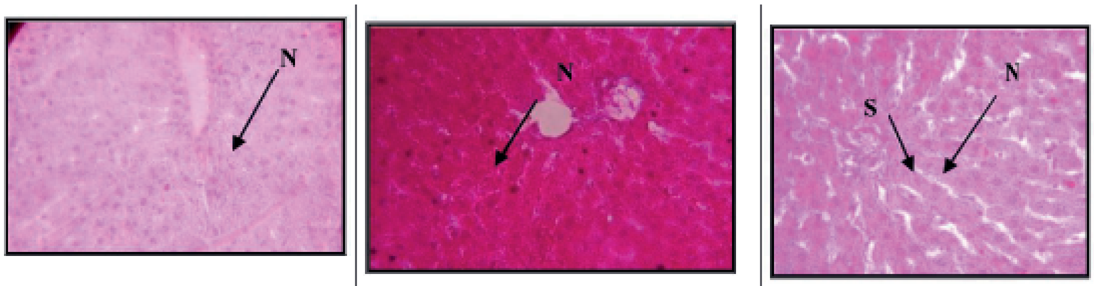


A. parenchyma control (Regular).

B. Parenchyma with Fe^{3+} dextran addition (not regular).

C. Parenchyma with Antioxidants addition (Regular).

Figure 2: Transversal incision of Wistar rat hearts (Rattus norvegicus). A: parenchyma control (Regular). B: Parenchyma with Fe 3 + dextran (not regular). C: Parenchyma with Antioxidants (Regular). 400x magnification with HE staining. P = parenchyma.



A. Nucleus cells in control (Normal). | B. Nucleus cells with Fe^{3+} dextran (Piknotis). | C. Nucleus cells with Antioxidants (Normal).

Figure 3: Transverse incision of Heart Wistar rat (Rattus norvegicus). A: Nuclues cells (Normal). B: Nucleus cells with Fe3+ dextran (Piknotis). C: Nucleus cells with Antioxidants (Normal). 400 × magnification with HE staining. N = Core Cells; S = Sinusois (Whole).

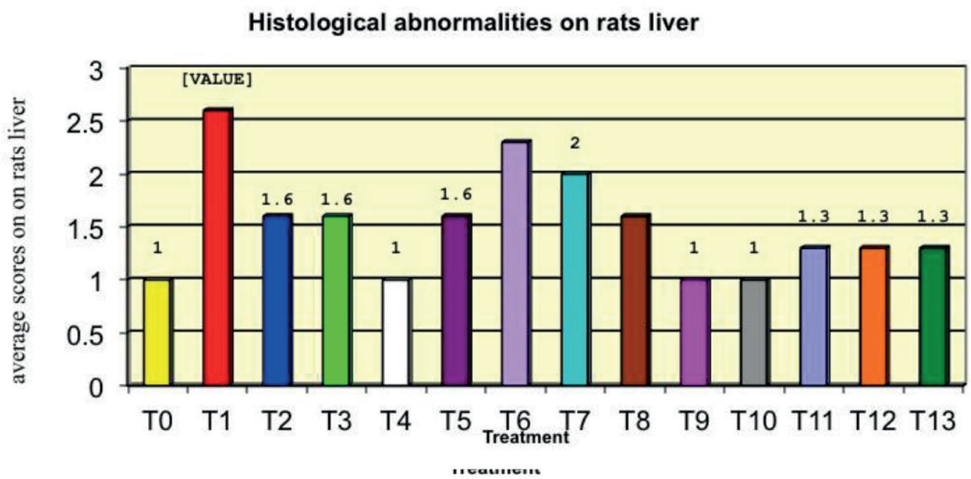


Figure 4: Relationship between treatment (with Fe and Antioxidants addition) and Rat Liver.

4. Conclusion

Conditions of excess iron can cause liver histological damage. This is because the Fe metal ions can catalyze the formation of free radical reactions and leads to oxidative stress that can damage cellular components and cause the lipid peroxidation resulting in steatosit, fibrosis to cirrhosis of the liver tissue. Addition of antioxidants such as, sappan wood extract (EKS) with dose of 50 and 100 mg kg-1 bw day-1, vitamin E, wheat grass and their combinations was capable of reducing the level of liver damage caused by excessive amounts of iron addition.

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