EFFECTS OF SAMBILOTO ETHANOL EXTRACT ON FATTY LIVER, SGOT/SGPT LEVELS AND LIPID PROFILE OF WISTAR STRAIN WHITE RAT (Rattus norvegicus) EXPOSED TO HIGH-FAT DIET

FX Himawan Haryanto Jong12, Ari Gunawan1, Mochamad Wirono Aman Santoso1, Susilowati Andajani3, Ni Wajan Tirthaningsih1, Ahmad Basori4
1Department of Anatomy and Histology, Faculty of Medicine, Universitas Airlangga, 2Department of Anatomy and Histology, Faculty of Medicine, Universitas Katolik Widya Mandala Surabaya, 3Department of Public Health and Preventive Medicine, 4Department of Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

The aim of this study was to analyze the effects of ethanol extract of sambiloto (Andrographis paniculata) on fatty liver percentage, serum glutamic oxaloacetic transaminase (SGOT) level and lipid profile of wistar strain white rat exposed to high fat diet. The study used randomized post test only control group design. Total sample was 50 male wistar strain rats (Rattus norvegicus) divided randomly into 5 groups (randomization). The normality test used was Kolmogorov Smirnov test (α=0.05). The homogeneity test used was Levene test (α=0.05). The correlation test was done using Pearson test (α=0.05). The administration of sambiloto ethanol extract with doses of 10, 200 and 400 milligrams (mg)/kilogram (kg) body weight (BW) decreased the percentage of fatty liver (r=-0.950), SGOT (r=-0.964)/ SGPT (r=-0.917) and increased HDL (high-density lipoprotein) (r=0,956) in white rats exposed to a high-fat diet. In conclusion, increased dose of ethanol extract of sambiloto can decrease the percentage of fatty liver, SGOT/SGPT and total cholesterol/TG/ LDL and increase HDL level of white rats exposed to high fat diet. (FMI 2018;54:89-95)

Keywords: Fatty liver; Andrographis paniculata; SGOT/SGPT; lipid profile

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) becomes a pandemic in the world nowadays. Around one billion people worldwide suffer from this disease. Epidemiological data shows that 25% of population in Asia Pacific, including in Indonesia, suffer from NAFLD, and about 10-20% of these numbers suffer from more severe forms of the disease, i.e. non-alcoholic steatohepatitis (NASH) and cirrhosis. More severe problem that threatens NAFLD patients is the increased risk of liver failure and liver cancer (World Gastroenterology Organization 2012, Mahady & George 2013).

Non-alcoholic fatty liver disease is a liver disease that is characterized by the appearance of ectopic fat in the
liver. Inflammatory reactions due to fatty liver accumulation in NAFLD are not caused by alcohol consumption (Machado & Cortez-pinto 2014, Kumar et al 2013). Non-alcoholic fatty liver disease has close correlation with metabolic syndrome. High-fat diets and carbohydrates, obesity and lack of motion are considered as the causes of this syndrome (Vornoli et al 2014). Non-alcoholic fatty liver disease is a form of metabolic syndrome in the liver that results from fat accumulation in the liver (Takaki et al 2013).

The multi-hit hypothesis is widely accepted by experts as the pathogenesis of NAFLD (Campana et al 2015). Insulin resistance is a major factor in this pathogenesis that causes the accumulation of free fatty acids in the liver. Fatty deposits give rise to oxidative stress and inflammatory processes in liver tissue. All of these factors apparently complicate the disease. The results of previous studies have not revealed the most effective treatment for the disease (Kumar et al 2013, Takaki et al 2013).

Male wistar strain rats exposed to a high-fat diet in several studies showed the accumulation of free fatty acids in the liver (Romestaing et al 2007, Kucera & Cervinkova 2014). Vornoli et al (2014) used animal models of male wistar rats exposed to a high-fat diet (29% fat). The histopathology of male wistar rat liver in this study demonstrated the fatty liver characterized by steatosis.

Results of SGOT and SGPT, blood glucose levels and lipid profile, total cholesterol, triglycerides (TG), and LDL (low-density lipoprotein) examinations showed significant increase, while the HDL (high-density lipoprotein) showed a decrease. These animal models resembled the condition of individuals with NAFLD.

Andrographis paniculata, known as sambiloto, is one of the popular medicinal plants in Indonesia for the treatment of various diseases. The useful bioactive components of the plant’s extracts are especially lactones and flavones, which are known to be hepatoprotective (Bardi et al 2014). Hepatoprotective properties are obtained from, among others, their capacity as an anti-oxidant and anti-inflammatory. The hepatoprotective properties present in the sambiloto extract are thought to play a role in reducing liver damage in NAFLD. Sambiloto’s bioactive agent also has a capacity to inhibit the accumulation of cholesterol and triglycerides in liver tissue since the agent has properties which may inhibit the transcription factor for cholesterol absorption (Jayakumar et al 2013, Negi et al 2008). The aim of this study was to analyze the effect of ethanol extract of sambiloto on fatty liver, SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvic transaminase) and lipid profile of male wistar rats exposed to high-fat diet.

MATERIALS AND METHODS

This study used randomized post test only control group design. Total sample was 50 male wistar strain rats, divided into 5 groups randomly. Each group consisted of 10 rats. The first two groups were control groups. In control groups, the rats received standard diet from day 1 to 84. High-fat diet was administered to positive control group with the same duration as the first group. Three other groups received high-fat diet for 84 days using sonde with ethanol extract of sambiloto in doses of, respectively, 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW every two days from days 71-84.

Total rat cholesterol levels from all groups were examined on the first day of the experimental study. Total cholesterol levels were examined by cutting the rats’ tails as much as 0.5 mm and the blood was dripped on the sticks on analyzer. The same examination was performed again on days 70 and 85. The data of total cholesterol on days 1 and 70 was tested by Levene normality test (α=0.05), homogeneity test of Kolmogorov Smirnov (α=0.05), and One-way Anova (α=0.05) to observe normal and homogeneous distributions of all groups of rats before high-fat diet. Ethanol extract therapy was applied.

On the day 85, the rats were anesthetized and cardiac puncture was performed for blood sampling for SGOT, SGPT, TG (triglyceride), LDL (low-density lipoprotein) and HDL (high-density lipoprotein). Thereafter, the rats were sacrificed and the liver was removed to be put into formalin for the preparation of the histotechnical process. Histologic preparations were made from liver and observed under light microscope with 400 times magnification. The percentage of hepatocyte cells undergoing steatosis was calculated in 10 visual fields.

The rats were fasted for 10 hours before blood collection at all examinations. All data were collected to be presented as descriptive data and further processed for statistical analysis using Statistical Software for Social Sciences 16 (SPSS 16). Normality test was conducted using Kolmogorov Smirnov test (α=0.05) and homogeneity test using Levene test (α=0.05). If data distribution was normal (p>0.05) and homogeneous (p>0.05), the Anova test (α=0.05) was performed. If data distribution was not normal (p<0.05) and not homogeneous (p<0.05), the Brown-Forsythe (α=0.05) test was performed. The correlation test used in this study was Pearson’s correlation test (α=0.05) since all data were of ratio type.
RESULTS

Fatty liver percentage

The histological general description of the liver in negative control group indicated normal liver histology. A positive control group who received high-fat diet without therapy showed fatty liver histology with a seemingly inexact parenchyma and many hepatocyte defects. Histologic profile of the liver in group of rats receiving sambiloto ethanol extract therapy showed histological improvement. The histologic profile of liver of the white rats can be seen in Fig. 1.

Fatty liver percentage data were normal (p>0.05) but not homogeneous (p<0.05), so we used Brown Forsythe test to find difference between groups. It turned out that the differences were significant (p<0.05). Mean percentage of rats fatty liver in negative and positive control groups was 0.09±0.01% and 9.35±0.55%, while mean percentage fatty liver in rats receiving ethanol extract of sambiloto of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were 7.65±0.45%, 6.67±0.38% and 5.58±0.38% respectively. Pearson’s correlation test results showed that there was a strong correlation (r=-0.950) between increasing dose of sambiloto ethanol extract and decreasing percentage of fatty liver.

SGOT and SGPT levels

Data of SGOT and SGPT levels were normal (p>0.05) and homogeneous (p<0.05), so we used Anova test to find difference between groups. There were significant differences in both levels between groups (p<0.05).

The mean SGOT levels in negative and positive control groups were 159.90±4.77 IU (international unit)/L (liter) and 260.20±3.19 IU/L, while the mean SGOT levels in rats receiving sambiloto ethanol extract of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were respectively 213.20±4.64 IU/L, 190.30±3.56 IU/L and 174.30±2.79 IU/L.

The mean SGPT levels in negative and positive control groups were 53.80±3.55 IU/L and 102.90±4.82 IU/L, while the mean SGPT levels in rats receiving sambiloto ethanol extract of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were 85.80±4.13 IU/L, 82.90±3.96 IU/L and 68.90±4.68 IU/L, respectively.

Fig. 1. Histological profile of the liver in: a) negative control group, b) positive control group, c) group with 100 mg/kg BW sambiloto ethanol extract therapy, d) group with 200 mg/kg BW sambiloto ethanol extract therapy, e) group with 400 mg/kg BW sambiloto ethanol extract therapy, and f) blue arrows showed normal samples of hepatocytes and red arrows showed examples of steatotic hepatocytes.

Fig. 2. The correlation of extract dose increase with fatty liver percentage.
Table 1. Results of the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative control</th>
<th>Positive control</th>
<th>100 mg/kg BW extract dose</th>
<th>200 mg/kg BW extract dose</th>
<th>400 mg/kg BW extract dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>%1,2,3</td>
<td>0.09±0.01</td>
<td>9.35±0.55</td>
<td>7.65±0.45</td>
<td>6.67±0.38</td>
<td>5.58±0.38</td>
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<tr>
<td>SGOT1,2,3</td>
<td>159.90±4.77</td>
<td>260.20±3.19</td>
<td>213.20±4.64</td>
<td>190.30±3.56</td>
<td>174.30±2.79</td>
</tr>
<tr>
<td>SGPT1,2,3</td>
<td>53.80±3.55</td>
<td>102.90±4.82</td>
<td>85.80±4.13</td>
<td>82.90±3.96</td>
<td>68.90±4.68</td>
</tr>
<tr>
<td>Kol1,2,3</td>
<td>102.90±2.88</td>
<td>140.90±2.42</td>
<td>122.20±3.79</td>
<td>116.50±2.07</td>
<td>110.30±3.62</td>
</tr>
<tr>
<td>TG1,4</td>
<td>48.90±7.59</td>
<td>108.00±3.68</td>
<td>83.00±3.80</td>
<td>71.80±2.10</td>
<td>54.30±4.85</td>
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<tr>
<td>LDL1,2,3</td>
<td>12.40±1.71</td>
<td>38.80±2.30</td>
<td>32.80±1.62</td>
<td>28.60±1.43</td>
<td>22.50±1.58</td>
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<tr>
<td>HDL1,2,3</td>
<td>62.10±3.14</td>
<td>41.90±2.33</td>
<td>51.00±1.94</td>
<td>55.80±2.25</td>
<td>61.70±1.64</td>
</tr>
</tbody>
</table>

Note: %: percentage of fatty liver, SGOT: SGOT levels on day 85, SGPT: SGPT levels on day 85, Kol: cholesterol levels on day 85, TG: triglyceride levels on day 85, LDL: LDL levels on day 85, HDL: HDL levels on day 85, 1: normal data distribution, p>0.05, 2: homogeneous data distribution, p>0.05, 3: Anova test results, p>0.05, 4: Brown-Forsythe test results, p<0.05

Pearson correlation test results showed that there was strong correlation between increasing dose of sambiloto ethanol extract with decreasing SGOT (r=-0.964) and SGPT (r=-0.917) levels.

**Lipid profile**

Distribution of total cholesterol data on day 1 and 70 was normal (p>0.05) and homogeneous (p>0.05) and there was no significant difference between groups (p>0.05). This test proved that experimental white rats were in normal and homogeneous conditions before the treatment of high-fat diet and sambiloto ethanol extract therapy (on day 71) began.

Data on total cholesterol level on day 85, LDL level and HDL level were normal (p>0.05) and homogeneous (p>0.05), so that the different test between groups used was Anova test. Data of TG level were normal (p>0.05) but not homogeneous (p<0.05), so that we used Brown Forsythe test to find the difference between groups.

Mean total cholesterol level in negative and positive control groups were 102.90±2.88 mg/deciliter (dl) and 140.90±2.42 mg/dl, while mean total cholesterol level of rats receiving dose of sambiloto ethanol extract of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were 122.20±3.79 mg/dl, 116.50±2.07 mg/dl and 110.30±3.62 mg/dl respectively.

Mean TG levels in negative and positive control groups were 48.90±7.59 mg/dl and 108.00±368 mg/dl, whereas the mean TG levels of rats receiving sambiloto ethanol extract of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were 83.00±3.80 mg/dl, 71.80±2.10 mg/dl and 54.30±4.85 mg/dl, respectively.
Mean HDL levels in negative and positive control groups were 62.10±3.14 mg/dl and 41.90±2.33 mg/dl, whereas the mean HDL levels of rats receiving sambiloto ethanol extract of 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW were 51.00±1.94 mg/dl, 55.80±2.25 mg/dl and 61.70±1.64 mg/dl, respectively.

The results of Pearson’s correlation test showed that there was a strong correlation between increased dose of sambiloto ethanol extract with decreased total cholesterol (r=−0.923), TG (r=−0.973) and LDL (r=−0.960) levels on day 85. There was a strong correlation between increased dose of ethanol extract of sambiloto with increased HDL levels (r=0.956).

DISCUSSION

Fatty liver percentage

Oxidative stress plays an important role in causing steatosis in hepatocytes. The accumulation of free fatty acids triggers mitochondrial action to maintain the stability of hepatocyte cells. Continuous accumulation will cause mitochondrial damage that ends with the accumulation of free radicals in the liver tissue. The accumulation of free radicals will lead to cell damage and ultimately cell death (Vornoli et al 2014, Takaki et al 2013).

The bioactive components of lactones and flavones contained in sambiloto extract can decrease the production of TNF-α which is triggered by intercellular adhesion molecule-1 and endothelial monocyte adhesion that occurs when the cells are undergoing an inflammatory process. In addition, these bioactive substances in the extract have anti-inflammatory capabilities by preventing NF-κB bonds by DNA and pro-inflammatory cytokine production will be inhibited (Jarukamjorn & Nemoto 2008, Jayakumar et al 2013).

Sambiloto ethanol extract through its bioactive component also has antioxidant effect. This effect is thought to be obtained through its capacity as a free
radical scavenger in tissues by providing electron donors to these free radicals. Another possibility is the bioactive substance of ethanol extract of sambiloto improves the function of mitochondria and other cell organelles as the defense of the cells against free radicals (Koh et al. 2013, Jayakumar et al. 2013).

**SGOT and SGPT levels**

NAFLD diagnosis based on laboratory blood testing only is still not possible, but the use of SGOT and SGPT, as they are actually serum transaminase, can be applied as a first step (Berlanga et al. 2014). The accumulation of free fatty acids in the liver that results in inflammatory reactions and oxidative stress causes hepatocytes to leak out of the plasma membrane. Hepatocyte cell damage can be indicated by elevated levels of transaminase enzyme in the blood (Koh et al. 2013). Several studies have demonstrated the potential of sambiloto ethanol extracts in improving liver function in various conditions, such as hepatitis B disease or exposure to toxic substances to the liver, such as acetaminophen or carbon tetrachloride. Liver transaminase enzyme in the blood may decrease with the administration of the extract (Jayakumar et al. 2013).

Provision of sambiloto ethanol extracts in white rats exposed to high-fat diet in this study was able to reduce the levels of SGOT and SGPT in the rats’ blood. Decreased levels had a strong correlation with increasing doses of the extract. A study conducted by Bardi et al. (2014) using experimental animal models with liver damage due to induction of thioacetamide indicated that the bioactive substance of sambiloto ethanol extract may have hepatoprotective effect through its capacity to inhibit various factors that cause cell death (apoptosis). Hepatoprotective effects are also obtained through anti-inflammatory and antioxidant activity of bioactive components.

**Lipid profile**

Sterol regulatory element-binding protein is a major transcription factor in tissue fat storage bound to cell membranes. Hepatic lipogenesis is governed by type 1c of this transcription factor (SREBP1c). Some studies have shown that increased expression of SREBP1c in liver tissue is associated with NAFLD (Berlanga et al. 2014). Free fatty acids will be stored in the form of triglycerides in liver tissue under high-fat dietary conditions. Increased lipogenesis in non-adipose tissue such as the liver can cause tissue destruction because saturated fats may have free radical characteristics that cause inflammatory reactions and oxidative stress (Chen et al. 2014).

Increased blood cholesterol levels are associated with an increased risk of cardiovascular disease and atherosclerosis. Sambiloto ethanol extract has been widely used in the study to show the effect of extracts that have hypolipidemic biological activity that can reduce total cholesterol, TG and LDL (Jayakumar et al. 2013). Total cholesterol, TG and LDL levels of white rats in the treatment groups were found to decrease in comparison with those in positive control group exposed to a high-fat, non-therapeutic diet. Decreased free fatty acids in circulation is followed by a decrease in free fatty acids leading to the portal vein which reduces fat accumulation in the liver tissue.

Ding et al (2014) conducted a study showing the result that the sambiloto ethanol extract through its bioactive component was able to decrease SREBP expression on the tissue. This can be used to reduce fat accumulation in liver tissue as a cause of NAFLD. High-density lipoproteins have anti-atherogenic activity that can be useful to induce cholesterol out of tissue (Lund-Katz & Phillips 2010). Provision of sambiloto ethanol extracts in this study proved to be able to reduce HDL levels in white rats exposed to a high-fat diet. A study conducted by Ding et al (2014) also demonstrated the ability of bioactive substances of sambiloto ethanol extracts in improving the lipid profile of white rats exposed to a high-fat diet. The sambiloto ethanol extract is thought to increase the activity of LCAT (lecithin-cholesterol acyltransferase) enzyme which is a key factor in HDL formation.

**CONCLUSION**

The increased dose of sambiloto ethanol extract decreases the percentage of fatty liver, SGOT/SGPT and total cholesterol/TG/DL and increases HDL level.

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