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# Hepatoprotective effect of Rhodomyrtus tomentosa fruit juice in rats fed with high fat high cholesterol diet

*by* Ernawati Sinaga

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## Hepatoprotective effect of *Rhodymyrtus tomentosa* fruit juice in rats fed with high fat high cholesterol diet

E Sinaga<sup>1,3\*</sup>, S Suprihatin<sup>2</sup>, P Sari<sup>3</sup>, M Iswahyudi<sup>3,4</sup> and V D Prasasty<sup>5</sup>

<sup>1</sup>Center for Medicinal Plant Research Universitas Nasional, Jalan Sawo Manila Pasar Minggu, Jakarta Selatan, Indonesia

<sup>2</sup>Faculty of Health Sciences Universitas Nasional, Jalan Sawo Manila Pasar Minggu Jakarta Selatan, Indonesia

<sup>3</sup>Faculty of Biology Universitas Nasional, Jalan Sawo Manila Pasar Minggu Jakarta Selatan, Indonesia

<sup>4</sup>Cipto Mangunkusumo National Central General Hospital, Jakarta Pusat, Indonesia

<sup>5</sup>Faculty of Biotechnology Atma Jaya Catholic University, Jakarta 12930, Indonesia

\*Corresponding author's email: ernawatisinaga@unas.ac.id

**Abstract.** In this study we conducted experiments to prove the effectiveness of *Rhodymyrtus tomentosa* fruit juice as hepatoprotective agent in rats fed with high fat high cholesterol diet (HFHCD). The experiments were carried out using male albino rats which were fed with HFHCD for 75 days and at the same time orally supplemented with *R. tomentosa* fruit juice (RTFJ) in doses of 500, 1000 and 2000 mg/kg bw daily for 75 days. Simvastatin is used as a positive control. At the end of the experiment, the liver function markers in serum, AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were determined as well as the histopathological image of the liver. Results of the experiments showed that HFHCD significantly induced hepatotoxicity showed by increase of sAST and sALT level. Supplementation of RTFJ significantly prevent the increase of sAST and sALT levels and maintained healthy histopathological image of liver tissue in rats fed with HFHCD, reduce fat accumulation and ballooning hepatocytes as seen in untreated rats. From the results we concluded that *R. tomentosa* fruit juice possess significant hepatoprotective activity in rats fed with high fat high cholesterol diet, and therefore could be develop further as functional food to prevent hepatic diseases due to high fat high cholesterol diet.

**Keywords:** NAFLD, *Rhodymyrtus tomentosa*, hepatoprotective, high cholesterol diet, functional food.

### 1. Introduction

Non alcoholic fatty liver disease (NAFLD) is a worldwide epidemic with increasing global prevalence. It is an alarming public health problem due to its potential as risk factor for other diseases such as steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma [1]. The non-alcoholic fatty liver disease (NAFLD) can be defined as accumulation of fat in liver in the absence of excessive alcohol consumption [2]. One main cause of NAFLD is daily lifestyle including high fat high cholesterol diet. Therefore, lifestyle modification, including diet modification and physical exercises and activities is the best treatment intervention for patients with NAFLD [3]. Diet modification is one of the best ways



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to prevent or manage NAFLD. Diet containing functional food or its ingredients such as dietary fibers and phenolic phytochemicals is a valuable concept to prevent or treat NAFLD. Therefore, nowadays there has been considerable interest in developing functional foods from natural products that may prevent or reduce the risk of developing liver diseases.

*Rhodomirtus tomentosa* is a wild fruit plant belong to Myrtaceae family, thrives in tropical region of Asia, Afrika, and America, including Indonesia. Its fruit are edible and rich in phenolic compounds, such as flavonoids, anthocyanins, and piceatannol [4-7]. These phytochemicals had been proved to have antioxidant and lipid regulatory activity, that make them potential to be developed as hepatoprotective agent especially to prevent and treat NAFLD [8, 9]. In this study we conducted experiments to prove the effectiveness of *R. tomentosa* fruit juice as hepatoprotective agent in rats fed with high fat high cholesterol diet.

## 2. Materials and Methods

### 2.1. Preparation of fruit juice

Fresh ripe fruits of *R. tomentosa* (Figure 1) were collected from the wild plants grown along Simpang Teritip Coast at west part of Bangka Island, Indonesia in April 2019. The plants and the fruits were identified by Dr. Sri Endarti Rahayu, a taxonomist in Herbarium Center of Faculty of Biology Universitas Nasional, Jakarta Indonesia, and a voucher specimen was deposited in Herbarium Center of Faculty of Biology Universitas Nasional, Jakarta, Indonesia. After washing with clean water, the fruits were processed with *slow-juicer*, and the juice was collected and immediately freeze-dried and stored in a freezer. The frozen juice were freeze-dried at  $-50^{\circ}\text{C}$ , and then stored in a refrigerator until used in the experiment. For the experiment, various doses of the fruit juice were freshly prepared with 0.5% Na-CMC suspension.



**Figure 1.** The fresh ripe fruits of *Rhodomirtus tomentosa* were processed to get the fresh and freeze-dried juice.

### 2.2. Preparation of high fat high cholesterol diet (HFHCD)

HFHCD was prepared by adding 1.5% of cholesterol and 10% of duck egg yolk powder to standard diet. The duck egg yolk powder was prepared by boiled the eggs for 30 minutes, and the boiled yolk was mashed and dried at  $40-45^{\circ}\text{C}$  for 12 hours.

### 2.3. Animals and experimental design

The experiments were carried out using 30 healthy Sprague-Dawley rats, male, 6 weeks old, 190-220 g body weight. The rats were randomly allocated into six groups, consisting of 5 animals each, as follow: Group 1 (KS) served as healthy untreated or healthy control group; Group 2 (KH) served as hypercholesterolemic group, rats fed with HFHCD without treatment; Group 3 (KSIM) served as positive control group, rats fed with HFHCD treated with simvastatin; Group 4, 5, and 6 (KT1, KT2, and KT3) served as hypercholesterolemic groups treated with *R. tomentosa* fruit juice (RTFJ) *per oral* (0.5; 1; and 2 g/kg bw, respectively). All the rats, except the KS group were fed with HFHCD for 75 days and at the same time orally supplemented with *R. tomentosa* fruit juice in doses of 500; 1000 and 2000 mg/kg bw daily or 5 mg/kg bw simvastatin for 75 days. The KS group received the standard diet

<sup>1</sup> along the experiment. At the end of the experiment, the liver function markers in serum, AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were determined as well as the examination of histopathological image of the liver. All animal experimental procedures had been approved by the Animal Ethics Committee of Atma Jaya Catholic University, Jakarta, Indonesia.

#### 2.4. Blood and liver collection and processing

Before collecting the blood and liver, the rats were anesthetized using 80 mg/kg bw, i.p. injection of ketamine hydrochloride after kept in fasted state for 12 hours. The blood was collected via intracardiac puncture, and subsequently centrifugated to obtain serum. The serum obtained was used for sAST and sALT determination. The livers were quickly removed, rinsed with physiological 0.9% saline, and then fixed with 10% NBF (neutral buffered formalin) for histopathological examination.

#### 2.5. Determination of serum aspartate aminotransferase and alanine aminotransferase

The activities of hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were evaluated using the Reitman and Frankel method [10].

#### 2.6. Histopathological observation

Following a dehydration process with gradual series of alcohol, the specimen of the liver was inserted into the embedding cassette and embedded in paraffin wax using automatic tissue processor, and sectioned by rotary microtome at 4  $\mu$ m and then stained with hematoxylin-eosin (H&E). Histopathological examination of the liver specimen was carried out at a magnification of 400x with a light microscope to study the changes in histopathological image of the liver.

#### 2.7. Data Analysis

Data obtained from the experiment were represented as mean  $\pm$  standard deviation (SD). Comparisons between groups were performed using one way ANOVA (analysis of variance) followed by post hoc Tukey.  $P < 0.05$  was considered statistically significant. All statistical analyses were performed using SPSS (Statistical Package of Social Sciences) program version 16.

### 3. Results and Discussion

#### 3.1. RTFJ prevent the increase of sAST and sALT levels in rats fed with HFHCD

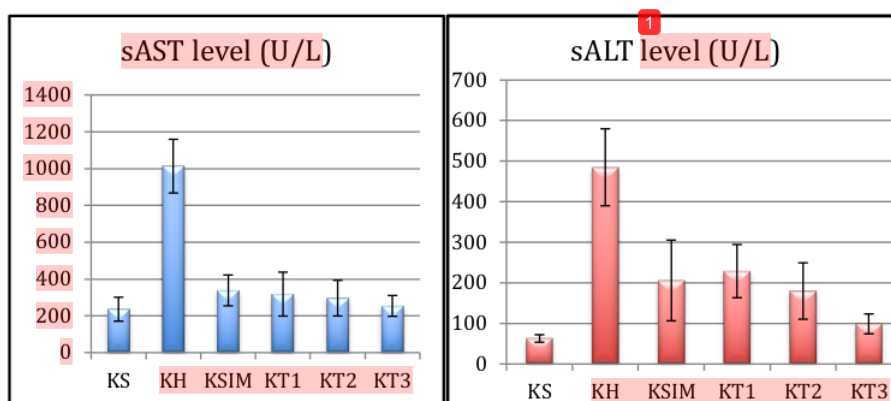
Results of the experiments showed that HFHCD (high fat high cholesterol diet) significantly induced hepatotoxicity showed by the increase of sAST and sALT level (Figure 2). The sAST and sALT level of the KH group (hypercholesterolemic group, rats fed with HFHCD without treatment) is very high compare to the KS or the healthy group. The average of sAST level in KH group is 1013 U/L while in healthy group the average level is 236 U/L, it means that the HFHCD increased the sAST level more than 400% higher compare to the healthy group. The effect of HFHCD in sALT level is more significant, the level of sALT in KH group almost 800% higher (485 U/L) compare to the healthy group (63 U/L). From this results it was confirmed that the HFHCD given in 75 days significantly induced hepatotoxicity in rats.

Supplementation of *R. tomentosa* fruit juice (RTFJ) significantly prevent the increase of sAST and sALT levels in rats fed with HFHCD. The sAST and sALT level of the rats fed with HFHCD treated with the fruit juice is significantly lower compare to KH group and statistically not significantly different with healthy group and group treated with simvastatin (KSIM) (Figure 2). However, with the doses uses in this experiment, we cannot see the dose-dependent effect of the fruit juice.

sAST and sALT are the aminotransferases enzymes which normally high in liver cells but very low in serum. The increase of their level in serum reflects damage or injury of hepatocytes. Although their use as biomarkers for hepatotoxicity has limitation, sALT and sAST are probably the most commonly used biomarkers in both clinical diagnosis and research involving liver damage [11]. The increase of sAST and sALT in rats fed with HFHCD indicates that administration of HFHCD not only increases

<sup>1</sup> fatty deposit in liver, but causes further damage or injury of hepatocytes so that the liver enzymes flow out into the blood.

The damage or injury of hepatocytes is indirectly caused by the fat accumulation in the liver. When considerable amount of fat accumulated in the liver, oxidative stress becomes significantly increased. Oxidative stress is the most important mechanism causing damage to the liver. Oxidative stress occurs when more oxidant species are produced than the antioxidant process the liver can carry out. The abnormal accumulated lipid compounds leads to the formation of ROS (Reactive Oxygen Species). The compensatory pathway for the excessive fat accumulation in the liver is activation of mitochondrial beta fatty acid oxidation due to desensitization of carnitine palmitoyltransferase (CPT-I) which is the gateway that regulates the entry of long-chain fatty acids into the mitochondria. Most of the electrons will be active in the respiration chain and migrate along the respiration chain to cytochrome c oxidase. The imbalance between the high electron input and the restriction of electron flow cause excessive reductions in complex I and III of the respiratory chain. The reduced complexes will then react with oxygen to form ROS [12]. ROS can initiate lipid peroxidation and indiscriminately oxidize all molecules in biological membranes and tissues, resulting in injury. This is the most likely mechanism by which liver cells are damaged by a build-up of fat in the liver.

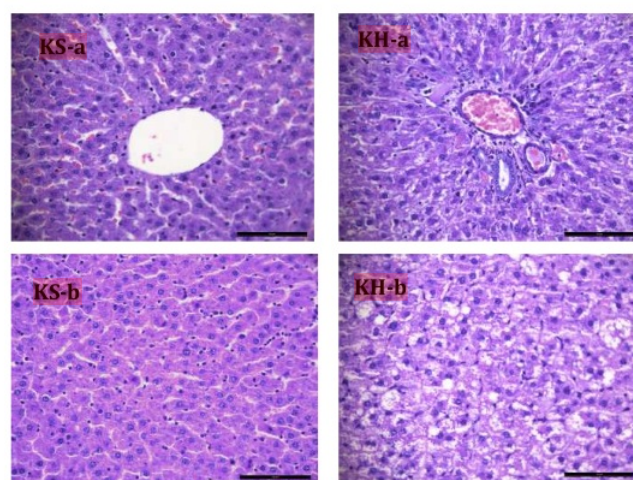


**Figure 2.** Serum aspartate aminotransferase (sAST) and serum alanine aminotransferase (sALT) levels in rats fed with high fat high cholesterol diet. (KS=healthy control group; KH=Rats fed with HFHCD without treatment; KSIM= Rats fed with HFHCD treated with simvastatin; KT1, KT2, KT3= Rats fed with HFHCD treated with RTFJ doses 0.5; 1, and 2 g/bw respectively).

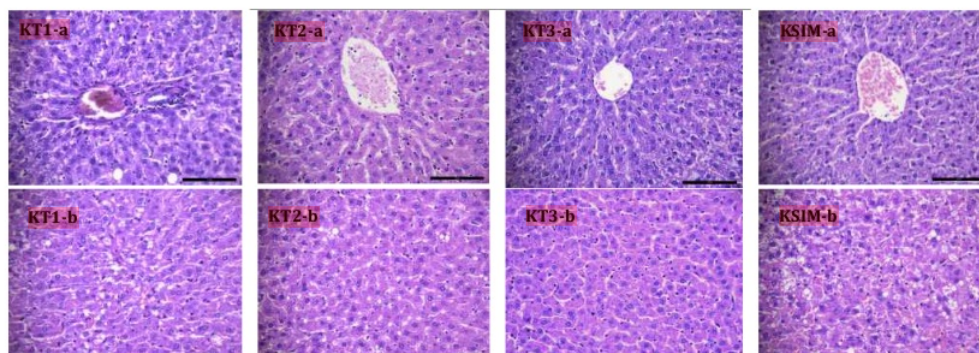
### <sup>1</sup> 3.2. RTFJ maintained the healthy histopathological image of liver tissue in rats fed with HFHCD

Histopathological examination of the rat's liver tissue corroborates the result of liver enzyme determination in serum. Figure 3 shows the the microscopic appearance of liver tissues of healthy rat and rat fed with HFHCD without any other treatment. The KS (healthy group) group showed normal healthy hepatic histopathological image of the liver with hepatocytes radiating from the central vein, with normal sinusoidal space and portal triad, well-preserved cytoplasm and large round and well-defined nucleus. Hepatocytes remained intact with clear lumen. No lipid vacuoles and lobular inflammation was seen. In contrast, histological examination of the KH group (hypercholesterolemic group, rats fed with HFHCD without treatment) showed loss of architecture with dilatation of sinusoidal space, intense macrovesicular steatosis, abundant lipid vacuoles in lobule cells, and infiltration of inflammatory cells, as well as numerous Kupffer cells infiltration were seen. In addition, ballooning degeneration of hepatocytes were observed.

**1** The supplementation of RTFJ remarkably alleviated the liver damage caused by HFHCD, especially in the rats supplemented with highest dose of RTFJ (Figure 4). The histological image of the liver of rats treated with RTFJ showed reduced lipid vacuoles and reduced swollen or ballooning hepatocytes, as seen in untreated rats. In contrary with its effect on aminotransferase enzymes level in serum, supplementation of RTFJ has a dose-dependent effect in maintaining the histopathological architecture of the liver. As shown in Figure 4, the higher the dose of RTFJ given to the rats the better the histopathological liver image observed. Surprisingly, simvastatin has a weaker effect compare to RTFJ. The rats fed with HFHCD and treated with simvastatin still had a considerable fat accumulation in liver tissue as shows in Figure 4-KSIM-b, even though the histological architecture of the liver is maintained with hepatocytes neatly radiating from the central vein and only a mild dilatation of sinusoidal space were observed (Figure KSIM-a). However, we confidently confirm that the hepatoprotective effect of RTFJ is stronger than simvastatin.



**Figure 3.** Histopathological liver images of healthy rats (KS-a and KS-b) and rat fed with HFHCD (KH-a and KH-b) (HE staining, 400x magnification).



**Figure 4.** Histopathological liver images of rat's fed with HFHCD and received treatments. KSIM= rats fed with HFHCD treated with simvastatin; KT1, KT2, KT3= Rats fed with HFHCD treated with RTFJ doses 0.5; 1, and 2 g/bw mg/bw respectively (HE staining, 400 x magnification).

NAFLD is a very common form of chronic hepatic disease which is not caused by heavy alcohol intake with manifestation of over-accumulation of fat in liver [2]. It often develop liver damage ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH) with the characterization of hepatocellular damage, fibrogenesis and lobular necro-inflammation which may evolve to hepatic cirrhosis and hepatocellular carcinoma [13, 14]. With the constantly increasing prevalence of NAFLD in the world, it is urgent to develop functional foods from natural products that may prevent or reduce the risk of developing liver diseases as a simple and convenient way to combat these diseases. There is increasing evidence that the consumption of phenolic-rich fruits have many benefits in reducing the risk of NAFLD [15, 16]. That is why in this study we investigated the effectiveness of *Rhodomyrtus tomentosa* fruit juice as a hepatoprotective agent in rats fed with high fat high cholesterol diet as animal model of NAFLD.

*Rhodomyrtus tomentosa* is an invasive plant, robust and fast growing, thrives in many tropical parts of the world, including Indonesia. Its abundant and edible fruits are very rich in phenolic phytochemicals, and from this study we report for the first time the beneficial effect of *Rhodomyrtus tomentosa* fruit juice as hepatoprotective agent against high fat high cholesterol diet induced NAFLD in rat model. The result of this study provides a rationale for the development of *Rhodomyrtus tomentosa* fruit juice as functional food to prevent liver damage especially related to high fat high cholesterol diet. Combining this juice with another lipid-lowering natural product may be a promising strategy in the prevention and treatment of NAFLD or NAFLD associated diseases.

#### 4. Conclusion

From the results of the experiment we concluded that *R. tomentosa* fruit juice possess significant hepatoprotective activity in rats fed with high fat high cholesterol diet, and therefore could be develop further as functional food to prevent liver damage especially related to high fat high cholesterol diet.

#### Acknowledgement

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#### References

- [1] Lindenmeyer CC, McCullough AJ 2018 The natural history of nonalcoholic fatty liver disease- An evolving view *Clin. Liver Dis.* **22**(1)11-21
- [2] Huang TD, Behary J and Zekry A 2019 Non-alcoholic fatty liver disease (NAFLD): A review of epidemiology, risk factors, diagnosis and management *Intern. Med. J.* **2019** 10.1111/imj.14709. doi:10.1111/imj.14709
- [3] Sarwar R, Pierce N and Koppe S 2018 Obesity and nonalcoholic fatty liver disease: Current perspectives *Diabetes Metab. Syndr. Obes.* **11** 533-42
- [4] Sinaga E, Rahayu SE and Suprihatin S 2019 *Potensi Medisinal Karamunting (Rhodomyrtus tomentosa)* (Jakarta: Unas Press)
- [5] Abd Hamid H, Mutazah SSZR and Yusoff MM 2017 *Rhodomyrtus tomentosa*: A Phytochemical and pharmacological review *Asian J. Pharm. Clin. Res.* **10**(1) 10-16
- [6] Liu G, Guo H and Sun Y 2012 Optimization of the extraction of anthocyanins from the fruit skin of *Rhodomyrtus tomentosa* (Ait.) Hassk and identification of anthocyanins in the extract using High-Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (HPLC-ESI-MS) *Int. J. Mol. Sci.* **13** 6292-6302
- [7] Lai TNH, Herent M, Quetin-Leclercq J, Nguyen BT, Rogez H, Yvan L and Andre CM 2013 Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa* *Food Chemistry* **138**(2) 1421-30
- [8] Van De Wier B, Koek GH, Bast A, and Haenen GRMM 2017 The Potential of flavonoids in the treatment of Non-alcoholic fatty liver disease *Crit Rev Food Sci Nutr* **57**(4) 834-55

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