Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.56

RESEARCH ARTICLE

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Histopathological analysis of the liver in hypercholesterolemia rats treated with *Dillenia serrata* fruits

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ABSTRACT

Background: *Dillenia serrata* (locally known as singi) is a natural product with the potential to improve liver function in hypercholesterolemia, due to anticholesterol and antioxidant properties.

Objective: This study aimed to investigate the effect of *D. serrata* on the histopathological features of rat livers induced by a high-fat diet.

Methods: This study used a quasi-experimental *in vivo* with a post-test-only control group design. Rats were divided into four groups: normal, high-fat diet, as well as *D. serrata*, and simvastatin treatment. Hypercholesterolemia was induced by a high-fat diet for two weeks. Rat liver tissues were analyzed histologically using Hematoxylin-Eosin (HE) staining, and were observed under a light microscope at 40x magnification in five wide fields of view.

Results: The high-fat diet group had the most fat cells, while the *D. serrata* group had the least. Statistical analysis demonstrated a significant difference between the high-fat diet group and the other groups ($p < 0.05$). Interestingly, no significant difference between the *D. serrata* group and either the normal or simvastatin group ($p > 0.05$), suggesting the treatment of fruit may restore liver function comparable to normal and simvastatin group.

Conclusion: The *D. serrata* fruit reduces the number of fat cells in the histopathology of rats induced with the high-fat diet.

Keywords: *Dillenia serrata*, non-alcoholic fatty liver disease, high fat diet, histopathological of liver

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the world [1], with a worldwide prevalence ranging from 22-28% [2]. NAFLD is characterized by fatty infiltration, consisting mostly of triglycerides in hepatocyte exceeding 5% of the liver's total weight. Indonesia's prevalence of NAFLD is 30.6%, higher than India (24.6%) and China (20%) [3].

Between 44-64% of NAFLD cases will develop to non-alcoholic steatohepatitis (NASH) within 3-7 years. Among these NASH cases, 21-26% will develop cirrhosis within 8 years, and 8% of cirrhotic cases will develop hepatocellular carcinoma within 5 years [1]. The prevalence of NAFLD and NASH has increased over the past three to four decades, coinciding with the increasing prevalence of obesity and type 2 diabetes mellitus [4]. One of the main causes of NAFLD is hypercholesterolemia due to high-fat consumption, thereby increasing the accumulation of free fatty acids in the liver, which are then esterified into triglycerides [5].

Hypercholesterolemia can lead to to the production of free radicals in the body. Increased free radicals can cause of lipid peroxidation and decreased lipoprotein lipase activity, resulting in increased triglyceride levels in the liver cells and subsequent fat degeneration around liver cells [6]. Reduced lipoprotein lipase activity also impairs the conversion of very low-density lipoprotein (VLDL) to intermediate-density lipoprotein, causing VLDL to accumulate in the liver and promote fatty liver [7].

Statins are a class of drugs known to reduce LDL levels in the blood by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA Reductase). However, statin use can cause several side effects, with muscle pain (myalgia) being the most common [8]. Additionally, statins can cause asymptomatic elevations of ALT and AST, raising concerns about hepatotoxicity in patients with underlying liver disease [9].

Due to the side effets of statins, there are efforts to explore natural resources for treating hypercholesterolemia. One such alternative is the triterpenoid compound betulinic acid, which has been found to efficiently lower total cholesterol levels similarly to simvastatin. This compound also significantly increased levels of high-density lipoprotein (HDL) compared to simvastatin in hyperlipidemic rats [10]. Betulinic acid, known for its broad biological and pharmacological properties, is widely distributed in various plants, including *Dillenia serrata* [11].

D. serrata (locally known as singi) is member of the *Dilleniaceae* family, commonly found in forest and residential yards [12]. The fruit is characterized by a sour taste and vibrant color. In addition to its exotic appearance, *D. serrata* fruit contains over 84% vitamin C. Ethanol extract of *D. serrata* fruit contains alkaloids, flavonoids, tannins, saponins, phenolic compounds, and triterpenoid. Phytochemical tests have revealed high levels of betulinic acid in *D. serrata* [11]. The potential content of *D. serrata* fruit metabolites can be used to improve liver function in hypercholesterolemia conditions. The purpose of the study is to investigate the potential of *D. serrata* as an alternative treatment for hypercholesterolemia.

Methods

Animals

This study used a quasi-experimental research with a post-test-only control group design. The subjects were white rats (*Rattus novergicus)* of Wistar strain, aged 6-10 weeks and weighing between 150-250 grams. Prior to the study, all subjects underwent a 10-day acclimatization period with standard laboratory rat feed (composition: carbohydrates 53–57%, water max 12%, crude protein min 15%, crude fat 3–7%, crude fiber max 6%, ash max 7%, calcium 0.9–1.1 % , phosphorus 0.6–0.9%) [12].

The research received ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine, Halu Oleo University (ethical eligibility letter number 240/UN29.17.1.3/ETIK/2021).

Treatment

Twenty-four rats divided into four groups: the normal group, the high-fat diet group, the *D. serrata* treatment group, and the simvastatin treatment group. Hypercholesterolemia was induced by feeding the rats a high-fat diet for two weeks, consisting of 10% beef fat, 20% cooking oil, and 20% quail egg yolk, mixed in 120 mL of mixture. Total serum cholesterol level were measured to confirm the increase in cholesterol levels, using the CHOD-PAP (enzymatic photometric test) method and a reagent kit (Cholesterol FS from Diasys) [13].

Fresh and ripe *D. serrata* fruits were administered at a dose of 60 mg/kg body weight. The fruits were mixed with distilled water, chopped into fine pieces, and given orally to experimental animals via a feeding tube. The treatment group received *D. serrata* fruit for two weeks. The simvastatin dosage was 0.36 mg/200 gram, administrated for two weeks (Figure 1).

Histopathology analysis

At the end of study, histopathological preparations of the Wistar rats livers were created using the

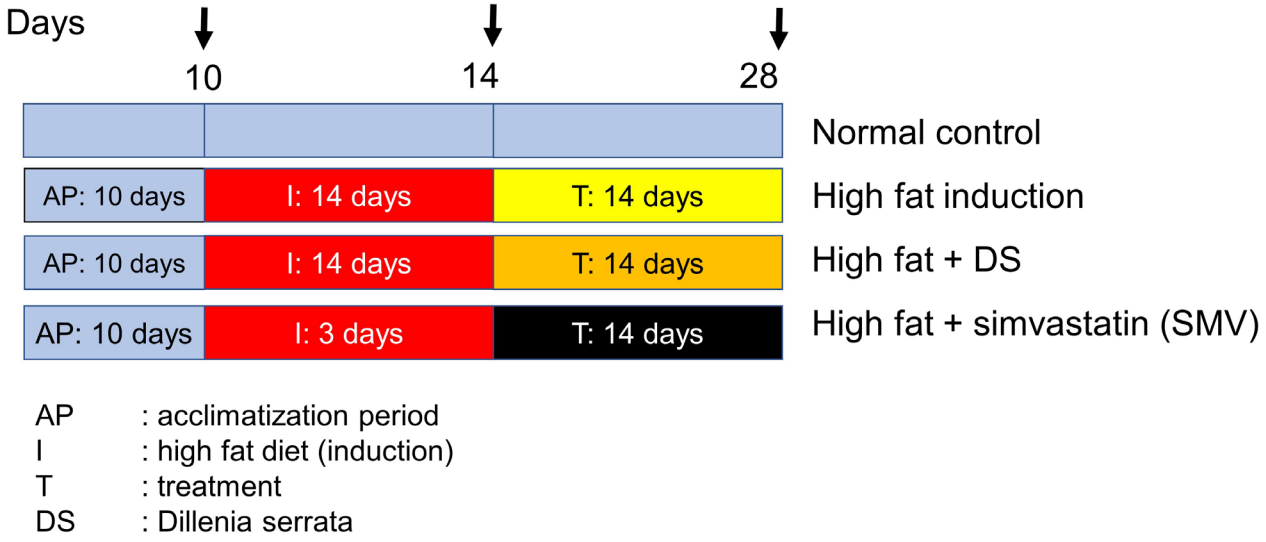

Figure 1. The research flow diagram

Hematoxylin-Eosin staining method. The samples were observed under a light microscope at 40x magnification across five large fields of view. Fatty cells were identified by their clear cytoplasm and peripheral cell nucleus [14]. Fat vacuoles were characterized by clear cytoplasm and a nucleus pushed to the edge. Liver damage was indicated by the presence vacuolization.

Data analysis

Data were statistically analyzed using GraphPad 9.0 (San Diego, US). A Shapiro-Wilk normality test was conducted, followed by one-way Anova. A p-value < 0.05 was considered statistically significant.

Results

Fatty liver generation

Fatty liver was observed in the normal group, the high-fat diet group, *D. serrata* treatment group, and simvastatin treatment group. Figure 2 displays the fatty liver cells in each group. The high-fat diet group had the highest number of fatty liver cells compared to the other three groups, indicating the successful induction of fatty liver (in addition the highest cholesterol level, *data not shown*). However, a significant difference was observed between the *D. serrata* treatment group and the high-fat diet group, as indicated by the lower average number of fatty liver cells in the *D. serrata* group. This finding indicates that *D. serrata* can improve liver function by reducing fatty liver cells.

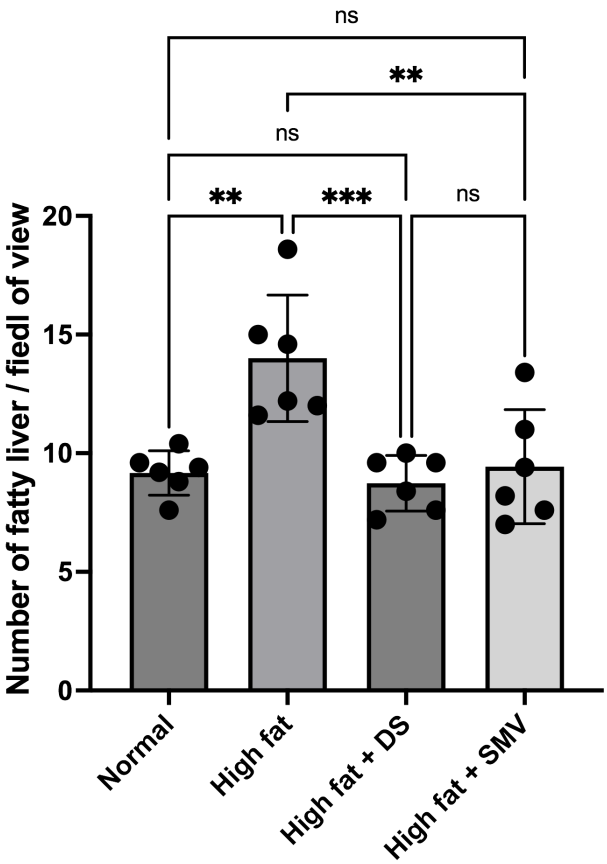

Figure 2. Number of fatty liver in each group. A Shapiro-Wilk normality test was conducted, followed by one-way Anova. *p < 0.05 was significant

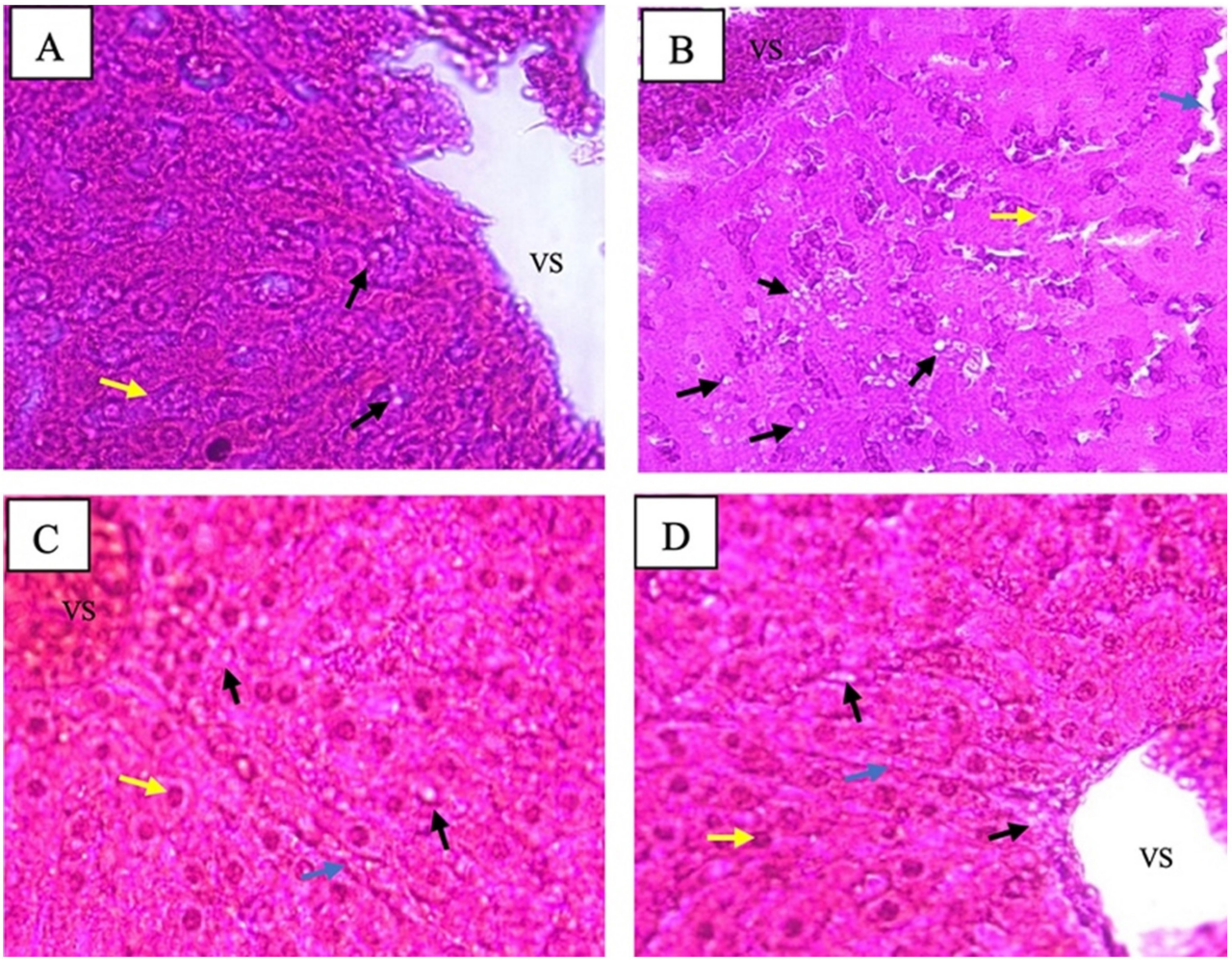

Figure 3. Histopathological of liver with 40x magnification using Hematoxyline-Eosin.

In this study, no significant difference in the number of fatty liver cell between the *D. serrata* and normal groups, implying that *D. serrata* can reduce fatty liver cells to a level comparable to that of the normal group. Furthermore, a significant difference was found between the simvastatin group and the high-fat diet group, with fewer fatty liver cells in the simvastatin group. Interestingly, no significant difference was observed between the *D. serrata* group and simvastatin group, suggesting that *D. serrata* has a similar ability as simvastatin to reduce fatty liver cells.

Histopathology analysis results

The normal group (A) displayed normal liver histology, with hepatocyte arranged radially centered on the central vein. Hepatocytes had normal boundary and shape. The high-fat diet group group (B) exhibited imperfect liver lobes, unclear boundaries of the hepatocyte structure, and widened sinusoids due to the increased fat cells disrupting hepatocyte structure.

In the *D. serrata* group (C), there was a reduction of fat-filled vacuoles, hepatocyte draining into the central vein, sinusoids and Kupffer cells with a large number of granule nuclei, and darker cytoplasm than hepatocyte. The simvastatin group (D) revealed visible liver lobules, hepatocyte arranged radially centered on the central vein, sinusoids present between the hepatocytes, and a few fat vacuoles (Figure 3).

Discussion

Diet playas a substantial role in fat accumulation within the liver. In this study, all groups were fed rat laboratory feed standard feed, which affected

fat accumulation in the liver. Despite not being fed a high-fat diet, the normal group still exhibited the appearance of fat vacuoles, albeit in lesser amounts than the high-fat diet group.

A significant difference was observed between the *D. serrata* treatment group and the high-fat diet group, with the former having a lower number of fatty livers. This result suggests that *D. serrata* has the potential to prevent fatty liver cells. High-fat diets rich in quail egg yolk and beef can contribut to increased cholesterol levels, exacerbating fatty liver condition. Quail egg yolk contains 65.50% triglycerides and 5.20% cholesterol, or contains cholesterol of 270 mg/130g quail egg yolks, while cholesterol levels in beef fat was about 1.75 mg/gram beef fat [15].

Simvastatin, which works by inhibiting the HMG-CoA reductase, significantly reduced the number of fatty liver cells in comparison to the high-fat diet group. By inhibiting HMG-CoA reductase, simvastatin prevents endogenous cholesterol production. Simvastatin also results in inhibition of apoB-100 synthesis in the liver and reduced synthesis and secretion of triglyceride-rich lipoprotein [16]. Moreover, the *D. serrata* group had slightly fewer fatty liver cells than the simvastatin group, suggesting that *D. serrata* fruit might prevent fatty liver cells more effectively than simvastatin.

There was no significant difference among the *D. serrata* group with normal group. This finding indicates that *D. serrata* can improve liver function, resulting in histopathological morphology similar to that of a normal liver.

D. serrata's ability to reduce fat vacuoles because of the role of antioxidants and antihypercholesterol compounds present in the fruit, especially flavonoids [17,18]. Flavonoids are known to inhibit lipid peroxidation by capturing free radicals that can otherwise cause excessive damage to cell macromolecules. The resulting excess of free radicals can lead cell damage, inflammation, and cell death [19]. Flavonoids have a role as hepatoprotectors against liver damage [20,21]. They achieve this by by reducing the activity of HMG-CoA reductase and by reducing cholesterol absorption in the digestive tract, which helps regenerating damaged cells. Flavonoids can also reduce triglyceride levels by increasing LPL enzyme activity [22,23]. Consequently, LPL converts VLDL into IDL, leading to decrease in the accumulation of VLDL in the liver and reducing fatty liver cells [24].

Vitamin C is another powerful antioxidant found in *D. serrata* fruit, with the ability to donate electrons and prevent other compounds from being oxidized. Vitamin C prevents lipid peroxidation by reducing reactive oxygen species (ROS) and inhibiting the lipid peroxidase process [25]. *D. serrata* fruit contain fiber, that can increase the excretion of cholesterol through the feces.

The triterpenoid compounds of betulinic acid found in *D. serrata* fruit also have a significant effect on fatty liver cells. Betulinic acid is as efficient as simvastatin in reducing total cholesterol levels, but can increase HDL levels more efficiently than simvastatin, making it a better alternative option for treatment [26]. Betulinic acid has an important role in reducing lipid accumulation in the liver by modulating the AMPK-SREBP signaling pathway. AMPK is a regulatory enzyme that works by inhibiting the anabolism pathway (lipogenesis) and stimulating the catabolism pathway (lipolysis). Treatment with betulinic acid can activate AMPK, which then reduces the level of SREBP1, a key inducer of lipogenesis in the liver. Activation of AMPK suppresses SREBP1 mRNA expression and inhibits SREBP1 target gene expression in primary hepatocytes, leading to reduced lipogenesis and lipid accumulation in the liver [27].

This study has limitations, including uncontrollable variables like the content of rat laboratory feed standard and the physical activity of the experimental animals. Additionally, only one dose of *D. serrata* flesh was used, so the optimal dosage for reducing total cholesterol levels in experimental animals remain unkown.

Conclusion

Treatment of *D. serrata* fruit improved the liver histopathological of male rats induced by high-fat diet. The results suggest *D. serrata* treatment could

restore normal liver function and has comparable activity as simvastatin in reducing fatty liver cells.

Acknowledgment

Acknowledgments are conveyed to the Institute for Research and Community Service at Halu Oleo University for providing research funds through the UHO Internal Beginner Lecturer Research in 2021 with a contract number 1455P/UN29.20/PG//2021.

Author contributions

T: conceptualization, methodology, formal analysis, validation, investigation, writing original draft, writing review and editing, funding acquisition, project administration. TWU: writing original draft, formal analysis, investigation, provision, visualization, writing review and editing, project administration. PA: conceptualization, methodology, funding acquisition, project administration. LK: conceptualization, methodology, funding acquisition. S: conceptualization, methodology, funding acquisition. ANKS: funding acquisition, project administration. MS: contributed to the visualization of the manuscript.

Declaration of interest

There was no competing interest.

Received: 07 June 2022
Revised: 05 August 2022
Accepted: 24 October 2022
Published online: 31 December 2022

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