Characterization and evaluation of the hepatoprotective activity of α-mangostin isolate in diabetic rats

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INTRODUCTION

Mangosteen (Garcinia mangostana L.) is a species of the genus Garcinia. This plant can grow in Southeast Asian countries, one of which is Indonesia. This plant is also widely cultivated in several provinces in China. Previous studies reported that mangosteen contains various bioactive compounds such as xanthone compounds, pigments, polysaccharides, and phenolic acids. Xanthones are the main active substances in mangosteen, where the isolated compound α-mangostin (α-MG; 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthan-9-one) is the most dominant compound found in the skin and trunk of the mangosteen fruit tree (Garcinia mangostana L.). These compounds are reported to have many pharmacological activities such as antibacterial, antifungal, antidiabetic, anticancer, and several other bioactivities.

Diabetes mellitus (DM) is still a health problem in the world with a high prevalence that continues to increase, especially in developing and newly industrialized countries. This disease can attack almost all systems of the human body, starting from the skin to the heart that can cause complications due to irreversible pathological conditions such as retinopathy, nephropathy, nephropathy, hepatoopathy, vasculopathy, and cardiovascular disease. Elevated levels of chronic transaminase enzymes describe the occurrence of insulin resistance. Elevated serum aminotransferase levels, such as those of the enzymes hepatic intracellular circulation (ALT) and aspartate aminotransferase (AST), show the presence of the enzyme. This is a main indicator of non-screening alcoholic steatohepatitis (NASH), which causes hepatocellular damage. Non-alcoholic fatty liver disease (NAFLD), often known as fat buildup or steatosis, is another condition that can impair liver function. NALFD affects 40 to 70 percent of DM patients.

Based on research (Bath, 2018) reported that the aqueous extract of Bixa orellana was able to show a significant reduction in enzyme levels in the liver in rats induced by diabetes so this extract could be indicated as having hepatoprotective properties. According to studies conducted by Fu et al., 2018, the isolated compound α-MG can reduce levels of liver malondialdehyde (MDA), serum alanine aminotransferase (ALT), aspartate transaminase (AST), tumor necrosis factor (TNF-), interleukin-1 and 6 levels (IL-1, IL-6), and hepatic glutathione recovery (GSH), superoxide dismutase (SOD).

So far, studies on the effect of α-MG isolate compounds on hepatoprotective bioactivity in diabetic conditions have not been found. This study aimed to...
characterize the isolated compound α-MG using 13C and 1H NMR (Nuclear Magnetic Resonance) and to find out whether this compound has bioactivity as hepatoprotective in diabetic rat models with parameters measured are alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, and liver histopathology.

METHODS

Study Design
The 36 rats used in this true-experimental study were divided into 6 groups: a positive control group, a negative control group, a standard drug group, and a treatment group that received -MG doses of 10 mg/kg body weight, 30 mg/kg body weight, and 50 mg/kg body weight after a week of acclimatization (adaptation) prior to treatment. The rats were taught in exposure cages while they were becoming acclimated.

Compound Characterization of α-MG
Garcinia mangostana L. rind is washed under running water to remove dirt and other foreign matter. Then it is dried in the open air, and the skin of the dried fruit is mashed then weighed 1 kg, and extracted using hexane at room temperature. Pure compound alpha mangosteen was isolated from mangosteen rind using column chromatography using n-hexane extract. The isolation process was carried out by a fractionation process using a mixture of n-hexane and ethyl acetate eluent with a gradual increase in polarity gradient. The isolates obtained were characterized by UV-Vis and infrared to determine the functional groups. The pure solid obtained was also characterized by 1H-NMR and 13C-NMR. It takes as much as 7-10 mg and is dissolved in 0.5 ml of proton-free solvent (CD3OD) which can dissolve completely. The sample solution was put in an injection tube and then placed in a Jeol JNM ECA 500 MHz NMR tool to measure 1H-NMR and 13C-NMR.

Measurement of Blood Biochemical Parameters AST, ALT, ALP, and Bilirubin
Thirty-six experimental animals that met the inclusion and exclusion criteria, were divided into 6 treatment groups with 6 mice in each group, namely: (1) Positive control group (diabetic): given a 20% fructose solution orally at a dose of 1.86 g/kg BW, (2) Negative control; given equates, (3) Treatment group I: given α-MG compound at a dose of 50 mg/kg BW, (4) Treatment group II: given the compound α-MG at a dose of 100 mg/kg BW, (5) Treatment group III was given α-MG compound at a dose of 150 mg/kg BW, (6) Standard drug group: given orally with glibenclamide at a dose of 10 mg/kg BW.

The experimental animals were then adapted for 14 days. During the acclimatization process, normal food and drink were given. Hyperglycemia occurred on day 55 after the induction of a high-fructose diet. After 56 days of administration of 20% fructose solution orally at a dose of 1.86 g/kg BW, the rats were fasted overnight and then the rat's blood glucose level was measured first, then the rat's blood glucose level was measured again. Mice with blood glucose levels of more than 150 mg/dl were used for research in the treatment and control groups of diabetics. Furthermore, the treatment group was given α-MG compound according to the dose of each group for 14 days. Fasting blood glucose levels were checked using a glucometer.

The rat serum was prepared, then determined using the enzymatic method with a photometric device. Then 250 mL of AST/ALT mono reagent was added with 25 mL of serum, then incubated for 50 seconds. Then measured with a wavelength of 340 nm at 37°C. then read the results of the AST/ALP readings that appear on the monitor of the device. 4-nitrophenol phosphate is hydrolyzed by alkaline phosphatase to produce phosphate and free 4-nitrophenol, which is colorless in aqueous acid solution. Under fundamental circumstances, 4-nitrophenol is changed into the highly concentrated yellow 4-nitrophenoxide ion. A recording spectrophotometer was then used to measure the rate of production of 4-nitrophenol at 405nm after the addition of alkaline phosphatase to 4-nitrophenol at 37°C.

Purple azo bilirubin is created by adding plasma to a sodium acetate and caffeine solution (sodium benzoate), which is then combined with diazotized sulfanilic acid. Acetate of sodium is resistant to diazotized sulfanilic acid's pH. When ascorbic acid is added, the process is stopped because the extra diazo reagent is destroyed. After that, blue azobilirubin was created by adding a strong base tartrate solution, and a spectrophotometer was used to detect the color intensity at 600 nm.

Data analysis
The output of this study was compiled, revised, input into a computer, coded, and cleaned. The collected data were entered using the Statistical Product for the Social Sciences (SPSS) data format version 20.0 (SPSS, Inc., Chicago, Illinois). The One-Way ANOVA test was used for analysis.

RESULTS

Characterization Results with UV-Vis
The UV spectrum of a compound with the x-axis is a wavelength (nm) and the y-axis is an absorbance (Figure 1) in methanol solvent shows the presence of 2 bands, namely band 1 shows a peak at a wavelength of 316 nm which indicates the presence of electron excitation π→π*, which is a typical chromophore for conjugated double bond systems (-C=CC=C-) or on the aromatic ring. The absorption in band 2 shows peaks at wavelengths of 204 and 243 nm which indicates the excitation of electrons n→π* which indicates the presence of conjugated heteroatoms with bonds (-C=C-C=O). The
an intensity (Figure 3) it was shown that there were several signal groups consisting of 11 protons with different chemical environments. This spectrum shows 4 singlet signals at H 1.77; 1.75; 1.59 and 1.58 which indicated the presence of 4 methyl groups. The singlet signal at H 3.68 is a typical signal for the methoxy group. The doublet signal at H 3.23 and 3.90 indicates a signal from a methylene proton, while the multiplet signal at H 4.85 and 18 indicates a signal from a methine proton. The singlet signal at H 6.18 and 6.61 indicates the presence of a signal from isolated aromatic protons. From the 1H-NMR spectrum data for compound (1), information can be obtained that there are 2 prenyl groups, 1 methoxy group, 2 methylene groups, 2 methine groups, and 2 isolated aromatic protons.

Characterization Results with 13C NMR
Based on the 13C-NMR data with the x-axis as a chemical shift (ppm) and the y-axis as an intensity (Figure 4), it shows that there are 24 carbons, namely 183.3; 163.7; 161.7; 158.0; 156.8; 156.3; 144.9; 138.6; 131.9; 131.8; 125.2; 123.9; 112.3;111.5;103.8; 102.8; 93.2; 61.4; 49.3; 49.1; 48.9; 27.2; 26.963 ppm, 22.3; 18.4; and 18.0. The spectrum at c 183.3 is carbon from carbonyl (C=O), absorption at c 131.9 and 131.8 indicates the presence of C=C in the prenyl group, at c 18.4 and 18.0 shows 2 methyl groups at the end of the prenyl group. From the data obtained from the 13C-NMR spectrum, it can be concluded that xanthone compounds are substituted by prenyl groups.

Characterization Results with 2D NMR Analysis of HMBC
To determine the position of the substituent, the 2D NMR analysis of HMBC was carried out as follows.

Analysis using 2D-HMBC NMR was carried out to strengthen the position of the carbon atom because it can explain the long-range correlation between protons and carbon as shown in Figure 5. From the above spectrum, it can be seen that the methylene proton at C-11 (3.23 ppm, m) correlates with C-12 (123.9 ppm), C-13 (131.8 ppm), and C-1 (161.7 ppm), and C-2 (111.5 ppm). It can be seen that 1 prenyl group is located at C-2. In addition,
Characterization Results with 2D NMR Analysis of HMQC

Based on the HMQC analysis, it can be seen in Figure 6 the correlation between methylene protons at C-16 (3.39 ppm, m) correlated with C-9a (112.3 ppm), C-17 (125.2 ppm), C-18 (131.9 ppm), C-7 (144.9 ppm), and C-8 (138.5 ppm) which indicated that 1 other prenyl group was substituted at C-8. The methoxy proton at the proton shift of 3.38 is correlated with C-7 (144.9 ppm) so it can be suggested that at C-7 a methoxy group is substituted.

Blood Chemical Analysis

In the positive control group, the negative control group, the standard glibenclamide drug group, and the α-MG treatment group at doses of 10, 30, and 50 mg which were stained with HE (Hematoxylin Eosin) showed no necrosis of hepatocytes. The following are the results of liver histopathology observations from each group which are presented in Table 2 below.

DISCUSSION

Previous research conducted by Fu, 2018 has identified the ability of α-MG compounds as hepatoprotectors in rats induced by lipopolysaccharide/D-galactosamine in mice, but research on the ability of a-mangosteen compounds as hepatoprotectors in diabetic conditions has not been found. Research conducted by Wulandari, 2020 explains that α-MG compounds can reduce levels of malondialdehyde (MDA) which is a marker of lipid peroxidation due to diabetes conditions and this compound can repair cell necrosis in pancreatic tissue due to streptozotocin induction through antioxidant mechanisms. Diabetes can be a risk factor for the development and progression of liver disease. On the other hand, diabetes develops as a complication of cirrhosis known as “hepatogenic
diabetes. Excessive intake of fructose has been proven to enhance de novo lipogenesis. Conversion of fructose to glucose will increase the risk of type 2 diabetes. In addition, fructose can also contribute negatively to blood glucose homeostasis by causing insulin resistance. In human studies, fructose has been shown to cause insulin resistance. Insulin resistance is a condition in which cells fail to respond normally to insulin. Several studies have shown an increase in cases of insulin resistance with fructose consumption. The administration of fructose in animal experiments induces insulin resistance and obesity caused by hyperinsulinemia. Fructose can enter cells via GLUT5, membrane carrier enterocytes so that they are not dependent on sodium and do not require energy. Then most of the fructose will be absorbed through the intestines into the portal vein and the hepatocytes via GLUT2. In hepatocytes, fructose is rapidly converted by the enzymes fructokinase to fructose-1 phosphate and then converted to triose phosphate by the enzyme aldolase B. These two enzymes are not affected by both insulin and the energy status of the cells. An increase in the concentration of triose phosphate in hepatocytes will trigger a higher rate of hepatic ATP consumption high at the start of fructose phosphorylation, so that when high fructose intake triggers depletion ATP, AMP formation and adenosine degradation to uric acid. The accumulation of triose phosphate will be converted to lactate or glucose which will be released into the circulation (gluconeogenesis). Therefore, the accumulation of triose phosphate can stimulate the synthesis of glycogen and fatty acids from carbon dioxide on fructose through a metabolic pathway known as de novo lipogenesis. Process de novo lipogenesis will trigger the formation of adipose tissue in the abdomen which is an important component in the development of dyslipidemia, hyperglycemia, and hypertension.

Liver function tests are used clinically in practice to detect liver failure, monitor the progress of liver disease, and monitor the potentially hepatotoxic effects of a substance. The most commonly used liver function parameters include ALT (alanine aminotransferase), AST (aspartate aminotransferase), and total bilirubin. Table 1 shows the result of blood biochemical analysis for different treatments.

### Table 1. Result of Blood Biochemical Analysis

<table>
<thead>
<tr>
<th>Blood Biochemical Parameter</th>
<th>Positive Control</th>
<th>Negative Control</th>
<th>Standard Drug (Glibenclamide)</th>
<th>α-mangostin 10 mg/kg BB</th>
<th>α-mangostin 30 mg/kg BB</th>
<th>α-mangostin 50 mg/kg BB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT/AST</td>
<td>244.27 ± 27.94</td>
<td>145.5 ± 22.86</td>
<td>186.5 ± 19.33</td>
<td>156.67 ± 16.45</td>
<td>183.33 ± 41.02</td>
<td>117.33 ± 25.7</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>SGPT/ALT</td>
<td>73.55 ± 10.48</td>
<td>67.33 ± 12.32</td>
<td>65.5 ± 14.93</td>
<td>58.54 ± 8.42</td>
<td>85.66 ± 6.04</td>
<td>72 ± 13.29</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Bilirubin Total</td>
<td>4.77 ± 0.34</td>
<td>3.77 ± 0.77</td>
<td>2.735 ± 2.17</td>
<td>2.25 ± 0.76</td>
<td>1.90 ± 0.44</td>
<td>1.735 ± 0.51</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>69.27 ± 6.15</td>
<td>41.5 ± 7.17</td>
<td>31.76 ± 10.73</td>
<td>29.78 ± 7.07</td>
<td>29.36 ± 4.48</td>
<td>27.08 ± 7.14</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

*Analysis was carried out using the One-Way ANOVA test. Results were considered significant if the p-value ≤ 0.05.
aminotransferase), alkaline phosphatase (ALP), and total bilirubin.\textsuperscript{14} Hepatocellular disease is indicated by a rise in ALT and AST that is out of proportion to ALP and bilirubin. On the other hand, a cholestatic pattern will appear if ALP and bilirubin levels increase disproportionately along with ALT and AST. AST and ALT are examples of aminotransferases. They serve as indicators of hepatocellular damage. By facilitating the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to form oxaloacetic acid or pyruvic acid, respectively, they contribute to gluconeogenesis. Bilirubin, -glutamyl transpeptidase, and alkaline phosphatase are indicators of biliary function and cholestasis. In individuals with type-2 diabetes, elevated transaminases are frequently discovered.\textsuperscript{15}

Proteins, lipids, and nucleic acids are among the cell components that experience damage from oxidative stress. The major hepatotoxicity mechanism of the toxicant is the generation of free radicals since the liver is constantly subjected to oxidative stress. The equilibrium between the production of reactive oxygen species and the levels of antioxidants is upset in oxidative stress. Because they can prevent and neutralize free radicals and have the potential to protect organs from hepatotoxins through their free radical scavenging activities, prevention of lipid peroxidation, and prevention of cell damage, \textit{α}-MG compounds are known as antioxidants with free radical scavenging activity. The antioxidants prevent reactive oxygen species (ROS) from damaging DNA, which is linked to carcinogenesis, coronary heart disease, and many other age-related health issues.\textsuperscript{16} The compound \textit{α}-MG has been shown to reduce blood glucose levels, reduce malondialdehyde (MDA) levels, and can repair cell damage in pancreatic cells through its antioxidant mechanism.\textsuperscript{6} Antioxidants are substances that when present in low concentration significantly delay or reduce the oxidation of the substrate.\textsuperscript{17}

Observations were made microscopically on liver histopathological preparations of strain \textit{Rattus novergicus} Wistar rats using HE staining (Hematoxylin Eosin) in the negative control group, positive control group, standard drug, treatment with \textit{α}-MG doses of 10, 30, 50 mg/kg BW, were found. There were no necrotic cells on the histopathological picture of the liver. Based on previous studies, administration of a diet with 25\% sucrose (containing 50\% fructose) can increase liver ALT and AST levels for 18 days. Fructose metabolism differs from glucose metabolism. Early fructose metabolism involves the phosphorylation of fructose to fructose-1-phosphate by fructokinase (keto hexokinase, KHK) utilizing the substrate ATP before entrance into the glycolytic pathway. Hepatic ATP depletion can occur as a result of fructokinase’s increased activity in phosphorylating fructose to fructose-1-phosphate in the liver.\textsuperscript{18} However, another study showed that samples of Ossabaw pigs in which the pigs were fed a 6000 kcal/day moderate fructose-rich diet (20\% energy) with higher choline levels (1200 ppm) showed no difference between groups on histological observations or steatosis after 24 weeks. This shows that the administration of fructose, which is supported by dietary choline and the provision of foods with high-fat content, will produce changes in liver histopathology. This interpretation is supported by a recent study comparing a high-fructose diet alone and with a choline/methionine diet it was found that only a high-fructose and choline diet caused steatosis.\textsuperscript{19} This study still has several limitations such as still consists of compounding variables and uncontrollable variables during the experiment.

### CONCLUSION

\textit{α}-mangostin from \textit{Garcinia mangostana} \textit{L.} isolate in diabetic rats has a hepatoprotective through evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, but there were no necrotic cells on the histopathological observation of the liver. In further research, it is recommended to establish clinical toxicity and safety tests as a follow-up to develop mangosteen compounds as an alternative treatment for impaired liver function due to diabetes mellitus.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

### ETHICAL STATEMENT

This study has been declared ethically feasible by the Health Research Ethics Committee, the Universitas Nahdlatul Ulama Surabaya.

### AUTHOR CONTRIBUTION

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### REFERENCES


