INTRODUCTION

One of the most dangerous communicable illnesses in the world is tuberculosis (TB). According to a World Health Organization (WHO) data from 2015, the 10th biggest cause of mortality globally is still TB. In Indonesia, there were 297 new cases of TB for every 100,000 people in 2014. There were 9.7 million new cases of TB reported in 2014, with 480,000 of those cases progressing to multidrug-resistant (MDR) TB. Only 14,964 cases (7.7%) of the 194,853 pulmonary tuberculosis patients in Indonesia received full treatment, leaving only 161,365 patients (82.8%) with a cure rate for the disease.

The first-line standard therapies for TB are isoniazid (INH), rifampicin, pyrazinamide, and ethambutol. INH can also be used as a preventive therapy. Long-standing use of anti-tuberculosis therapy drugs (INH, rifampicin, and ethambutol) tends to have consequences of hepatotoxicity, gastrointestinal illnesses, neurological disorders, and hypersensitivity reactions. One of the most thoughtful and common consequences of anti-tuberculosis is hepatotoxicity. Among all the first-line therapies, INH is one of the drugs with the most serious effect of causing liver dysfunction and peripheral neuropathy. In 10-20% of patients taking INH, there is an increase in serum alanine transferase (ALT). Most patients can adapt to the condition, whereas in 1-3% of patients, it develops severe liver injury to liver failure. The occurrence of impaired function reduces the effectiveness of anti-tuberculosis drugs due to significantly decreased adherence to treatment, failure of therapy, and also increased rates of drug resistance. The hepatoprotector group is often used empirically to treat patients who experience side effects of liver dysfunction, one of which is herbal medicine. If the hepatoprotector is able to significantly reduce impaired liver function it is possible to increase adherence and decrease the prevalence of treatment failure.

According to earlier studies, bay leaf (Syzygium polyanthum) is used to manage gout, gastritis, diabetes mellitus, hypertension, and excessive cholesterol. Saponins, triterpenoids, flavonoids, polyphenols, alkaloids, tannins, and essential oils made up of sesquiterpenes, lactones, and phenol tannins, and essential oils made up of sesquiterpenes, lactones, and phenol are among the chemicals found in bay leaves. Plants produce flavonoids, which are polyphenol secondary metabolites, in a variety of plant components, including fruit, seeds, leaves, stems, and flowers. Anthocyanidins, flavones, flavanones, flavonolignans, isoflavones, isoflavonones, and chalcones are the eight groups of flavonoids. Another study claims that the active components in South Sulawesi
propolis include phenolic compounds like flavonoids, which have been shown to have hepatoprotective properties in rats. The test for total polyphenol and flavonoid content in propolis revealed that it contained 1.1% polyphenols and 2.7% flavonoids. The test for antioxidant activity revealed an IC₅₀ value of 9849 ppm, and the LCMS/MS analysis confirmed the existence of phenolic components in South Sulawesi propolis. In this study, this study aimed to determine whether the flavonoid content in the ethanol extract of bay leaves was able to act as hepatoprotective by examining the levels of SGOT, SGPT, and bilirubin in rats induced by rifampicin and INH.

MATERIALS AND METHODS

Animal preparation

Wistar rats weighing approximately 200 grams were obtained from Nahdlatul Ulama Surabaya University. Rats were fed regular animal pellets and unlimited amounts of water during their one-week adaptation period. We received authorization and ethical clearance from Nahdlatul Ulama Surabaya University. The animals were divided into six groups, each with six male rats (Table 1).

Extraction of bay leaves with 70% ethanol

The bay leaves were macerated in 70% ethanol for 3–5 days to extract the oil. The extract was contained after filtering with filter paper. Bay leaf extract was created by concentrating it over a water bath.

Determination of dosage

The dosage of INH was determined based on previous research, namely the INH dose used in this study was 200 mg/kg human body weight. Rifampin dosage is determined based on previous research, namely the dose of 200 mg/kg human body weight. The ethanol extract dosage of 70% bay leaf is 75 mg/kg, 150 mg/kg, and 300 mg/kg.

Determination of bilirubin, SGPT, and SGOT levels

Liver damage parameters can be observed by measuring serum bilirubin, SGOT, and SGPT levels. Each group was given the same treatment every day for 4 weeks, then at the end of the 4th week, the blood was drawn to measure the serum bilirubin, SGOT, and SGPT levels.

The SGOT examination procedure is as follows: 20 µl of serum sample was taken in a test tube, plus 1000 µl of SGOT reagent, mixed, and incubated at 37 °C for 1 minute. Read on absorbent on the photometer with wavelength 340, factor 1745, K 20 program.

The SGPT examination procedure is as follows: fill the test tube with serum samples of as much as 20 µl and added SGPT reagent as much as 1000 µl. Mix and incubate at 37 °C for 1 minute. Read the absorbent after exactly 1 minute, 2 minutes, and 3 minutes at a wavelength of 340, factor 1745, and on the K 20 program.

The blood of the mice taken through the veins then was deposited and separated from the plasma by centrifugation at 3500 rpm for 15 minutes. Blood and working reagents are mixed and let stand for one minute. The bilirubin level was determined on a spectrophotometer with a wavelength of 340 nm. The assay of serum bilirubin levels in test animals was carried out spectrophotometrically by mixing 25 µl of blood serum with 1000 µl of working reagent, incubated for 5 minutes then measuring the absorbance on a spectrophotometer with a wavelength of 340 nm, 1 cm thick cuvette, temperature 37°C. The bilirubin reagent consists of R1 (NaCl, buffer phosphate, detergents, and stabilizers) and R2 (HCl, detergent, and 2,4 dichlorophenyl diazonium salt). R1 plus R2 for each of these reagents and reacted with blood serum. The calculated serum bilirubin levels are expressed in units/Liter and calculated for each group of rats. Testing of serum bilirubin levels in test animals was carried out photometrically. The determination of bilirubin levels in this study used ready-to-use reagents without dilution, namely in the form of packaging obtained from the Laboratory of the Faculty of Medicine, Nahdlatul Ulama Surabaya. This study’s data analysis includes the normality test, two-way ANOVA if the data are normally distributed, and Kruskal-Wallis if they are not. The data obtained were evaluated statistically using a two-way ANOVA test with a confidence level of 95%.

Determination Malondialdehyde (MDA)
The Wills method was used to measure the levels of plasma MDA. About 200 L of the plasma sample was mixed with 1 mL of 20% trichloroacetate (TCA) and 2 mL of thiobarbituric acid (TBA) to make 200 L total. The mixture was heated in a bath for ten minutes after being thoroughly mixed. After the solution has cooled, it is centrifuged for 10 minutes at 3000 rpm (1 rpm = 1/60 Hz). The pink filtrate was measured at a wavelength of 532 nm using a UV-VIS spectrophotometer. Using conventional MDA curves with values of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 nmol/mL, MDA levels were determined.

RESULTS

Effect of ethanol extract of bay leaves on liver function test of rats induced by rifampicin and INH

Liver function parameters that were examined in this study included total

| Table 1. Distribution of experimental animal groups and their treatment |
|------------------------|------------------------|
| Group                  | Treatment |
| Group I (normal control) | Without treatment, that is, without any treatment for each mouse, the rats were only given food, drink, and placebo |
| Group II (positive control) | This group was administered a combination of INH-Rifampicin, with a dose of INH 200 mg/kg body weight (BW) of rats and rifampin at 200 mg/kg BW of rats. |
| Group III               | This group was administered INH-Rifampicin suspension and antihapatotoxic drugs (silymarin). |
| Group IV                | This group was administered a combination of INH-Rifampicin and 70% ethanol extract of bay leaves 75 mg/kg EEDS. |
| Group V                 | This group was administered a combination of INH-Rifampicin and 70% ethanol extract of bay leaves 150 mg/kg EEDS. |
| Group VI               | This group was administered a combination of INH-Rifampicin and 70% ethanol extract of bay leaves 300 mg/kg EEDS. |

Table 2. Mean of total bilirubin levels, SGOT, SGPT, and MDA among each group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Total Bilirubin</th>
<th>SGOT</th>
<th>SGPT</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I: Negative Control</td>
<td>0.14 ± 0.054</td>
<td>157.36 ± 45.696</td>
<td>69.18 ± 17.035</td>
<td>985 ± 69.332</td>
</tr>
<tr>
<td></td>
<td>Group II: Positive Control</td>
<td>0.32 ± 0.044</td>
<td>282.8 ± 56.465</td>
<td>65.16 ± 15.748</td>
<td>1102 ± 132.104</td>
</tr>
<tr>
<td></td>
<td>Group III: Standard Drug</td>
<td>0.25 ± 0.057</td>
<td>275.325 ± 16.709</td>
<td>69.375 ± 5.713</td>
<td>962.25 ± 215.647</td>
</tr>
<tr>
<td></td>
<td>Group IV: BLE 75 mg/kg</td>
<td>0.16 ± 0.089</td>
<td>226.98 ± 44.938</td>
<td>57.32 ± 10.562</td>
<td>795.2 ± 107.883</td>
</tr>
<tr>
<td></td>
<td>Group V: BLE 150 mg/kg</td>
<td>0.18 ± 0.045</td>
<td>207.58 ± 55.919</td>
<td>62.3 ± 9.113</td>
<td>915.75 ± 135.256</td>
</tr>
<tr>
<td></td>
<td>Group VI: BLE 300 mg/kg</td>
<td>0.2 ± 0.071</td>
<td>193.28 ± 16.024</td>
<td>64.66 ± 5.858</td>
<td>727.4 ± 367.997</td>
</tr>
<tr>
<td>Significant Value</td>
<td>0.900 (P&gt;0.05)</td>
<td>0.132 (P&gt;0.05)</td>
<td>0.384 (P&gt;0.05)</td>
<td>0.08 (P&gt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>

BLE = bay leaf extract

Table 3. Bay leaf ethanol extract’s effects on liver histopathology

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Score Average</th>
<th>Significant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Negative Control</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Group II: Positive Control</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Group III: Standard Drug</td>
<td>4</td>
<td>P&gt;0.01</td>
</tr>
<tr>
<td>Group IV: BLE 75 mg/kg</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Group V: BLE 150 mg/kg</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Group VI: BLE 300 mg/kg</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

*Description: Score 1-5 (minor damage – major damage)

Bay leaf ethanol extract’s effects on liver histopathology

Based on observations of rat liver tissue, the level of damage to liver tissue in each group can be obtained in table 3. Based on the data in table 3, the negative control group with a score average of 2.2 indicates that there is slight liver tissue damage, while the positive control group with a score average of 4, it indicates that liver tissue damage has occurred. Descriptively, in the treatment group with a dose of 300 mg/kg BW, there was a process of normalizing the liver tissue cells again compared to the positive control group.

DISCUSSION

One of the causes of acute hepatotoxicity in tuberculosis patients is the combination therapy of INH and rifampicin. The primary toxin is INH, and rifampicin increases its toxicity by altering the metabolite dynamics. Overall, the exposed group of patients experienced considerable or covert hepatocyte functional changes as a result of the INH and rifampicin combo treatment, which could have resulted in the activation of hepatic stellate cells. When the hepatocyte cell membrane is damaged, various enzymes such as SGOT and SGPT are released into the blood from the cytosol. This increase in serum enzyme levels is an indication of a cellular leak and loss of functional integrity of liver cell membranes. Estimation of this enzyme is a quantitative marker that is useful for assessing the level and type of liver cell damage. Additionally, because of bilirubin’s dysfunctional absorption, conjugation, and excretion, severe liver damage can result in hyperbilirubinemia. If the serum’s bilirubin concentration is markedly elevated, it diffuses into the surrounding tissue and results in jaundice, or a yellowing of the skin and mucous membranes. In this study, the administration of ethanol extract from bay leaf to rifampicin and INH-induced rats was carried out to determine its potential hepatotoxicity. The parameters of liver function damage that were examined included total bilirubin levels, SGOT, SGPT, MDA, and liver histopathological observations. Based on the examination’s findings, it was determined that administering an ethanol extract of bay leaves might, descriptively, lower SGOT levels while having no appreciable impact on total bilirubin and SGPT. Based on previous research argues that the decrease in SGOT activity and no effect on total bilirubin levels shows protection against liver damage as a hepatitis model but has no effect on the secretory system of liver cells. MDA levels in the treatment group at a dose of 300 mg/kg BW decreased descriptively compared to the positive control group. This is because the bioactive compounds contained in bay leaves include flavonoids, tannins, essential oils, saponins, alkaloids, and polyphenols which act as antioxidants. Rifampicin and INH have a hepatotoxic effect on mice that results in the production of highly reactive free radicals, a direct...
impact on polyunsaturated fatty acids, and a direct alteration of the liver microsomal membranes. Giving ethanol extract from bay leaves can reduce levels of SGOT and MDA descriptively through the antioxidant pathway. It is well known that phenolic compounds are effective at scavenging free radicals. Due to their oxidation-reduction abilities, which are crucial in absorbing and neutralizing free radicals, singlet and triplet oxygen quenching, or peroxide breakdown, polyphenol compounds are known to have antioxidant activity. The polyphenolic compounds contained in the ethanolic extract of bay leaves have the ability to donate protons and can function as free radical scavengers, which may act as primary antioxidants.

Lei’s research (2021) showed that the INH-Rifampicin combination used for the treatment of tuberculosis induces steatosis, hepatocyte apoptosis, and mitochondrial dysfunction, which all contribute to acute hepatotoxicity. Based on the results of liver histopathology observations in table 2, it shows that there was liver cell damage in the positive control group and no liver cell repair in the standard drug group, namely the 75 mg/kg and 150 mg/kg BLE treatment groups. However, there was a slight improvement in the 150 mg/kg BLE treatment group. This indicates that phenolic compounds in the ethanolic extract of bay leaves which have antioxidant activity plays a role in repairing liver cells damaged by rifampicin and INH induction.

CONCLUSIONS

Administration of ethanolic extract of Syzygium polyanthum has no significant effect on total bilirubin, SGOT, SGPT, and MDA, but this extract can reduce MDA although not significantly. Same with the results of histopathology, ethanolic extract of Syzygium polyanthum could not cure the liver tissue of rats. It means that ethanolic extract of Syzygium polyanthum can help to reduce oxidative stress in liver tissue. In further research, it is recommended to develop other bioactivity tests from bay leaf isolates and conduct more specific studies to explain the mechanism of action of the extract at the cellular and organismal levels that are more complex, as well as toxicity tests to determine pharmacological effects.

ACKNOWLEDGMENTS

We appreciate the University of Nahdlatul Ulama Surabaya for making its research facilities accessible and funding, and also thanks to the Central Drugs Evaluation Analysis Laboratory University of Surabaya for supporting to collect the data.

FUNDING

Universitas Nahdlatul Ulama Surabaya financed this study.

ETHICAL APPROVAL

Universitas Nahdlatul Ulama Surabaya has approved the ethical clearance for this study.

CONFLICTS OF INTEREST

No conflicts of interest exist, according to the authors.

AUTHOR CONTRIBUTIONS

This article’s authors all contributed equally.

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