Hepatoprotective Effect of Papaya Seed Ethanol Extract on Rifampicin Isoniazid-induced Rats

Yessi Sunari Wahfar¹, I. Nyoman Ehrich Lister†* and Edy Fachrial¹

¹Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia.

ABSTRACT

Aims: Hepatotoxicity induced by anti-tuberculosis drugs, including rifampicin and isoniazid (AT-DILI, Anti Tuberculosis-Drug Induced Liver Injury), is an adverse reaction followed by significant morbidity. Several in vivo and in vitro research has confirmed that papaya seeds contain various non-essentials, minerals, and fiber. Carica papaya role in disease prevention through modulation of various processes, such as anti-inflammatory, anti-diabetes, immunomodulatory activity, and antioxidant activity, suggests a role in neutralizing free radical generation and ultimately preventing pathogenesis. This study aimed to determine the hepatoprotective effect of ethanol extract of papaya seeds on rifampicin and isoniazid-induced rats.

Study Design: This study is experimental study.

Methodology: The experimental animals in this study were divided into eight groups, including normal group, negative group 1, negative group 2, negative group 3, positive group, treatment group I (papaya seed ethanol extract dose of 100 mg/kgBW), treatment group II (papaya seed ethanol extract dose of 300 mg/kgBW), and treatment group III (papaya seed ethanol extract dose of 500 mg/kgBW), then the rats were dissected, and blood was taken for AST, ALT, ALP, GGT, and Bilirubin level measurements.

Results: The results showed that papaya seed ethanol extract could reduce ALT, AST, ALP, GGT, and Bilirubin levels that were significantly different (P <0.05) than those in the negative control group.

*Corresponding author: E-mail: nyoman@unprimdn.ac.id;
Conclusion: Flavonoid contains in the extract ethanol carica papaya has vita role to prevent the liver toxicity caused by isoniazid and rifampicin.

Keywords: Ripampicin; isoniazid; hepatoprotective.

1. INTRODUCTION

Tuberculosis (TB) is caused by bacillus bacteria known as Mycobacterium tuberculosis and is characterized by coughing and fever [1]. TB disease is a chronic infectious disease that causes severe problems globally and is still a significant infectious disease in Indonesia [2]. It requires a long-time comprehensive solution to treat, although various types of TB drugs have been found, as well as the Bacillus Calmette Guerin (BCG) vaccination. However, pulmonary tuberculosis is still a health problem in the world, and this disease yet cannot be eradicated. Based on data from the Ministry of Health, the prevalence of pulmonary tuberculosis cases in Indonesia in 2013 was 285 per 100,000 population, and the mortality rate was 27 per 100,000 people. Based on World Health Organization (WHO) report in 2014, the incidence of pulmonary tuberculosis in 2013 was estimated at 450,000 cases with 170,000 deaths [3].

Hepatotoxicity induced by anti-tuberculosis drugs (AT-DILI, Anti Tuberculosis-Drug induced liver injury) is a severe adverse reaction with significant morbidity. This form of toxicity has the potential to impact TB treatment outcomes in some patients. First-line anti-tuberculosis drugs, oxidative stress, and more broadly interfering with redox homeostasis along with mitochondrial dysfunction can contribute to hepatotoxicity caused by TB drugs. Some identified risk factors of poisoning, in addition to genetic factors including age, malnutrition, alcoholism, chronic hepatitis C and chronic hepatitis B infection, HIV infection, and preexisting liver disease. Importantly, these comorbid conditions are related to oxidative stress and antioxidant-related drugs, especially those for the management of mitochondrial dysfunction. Thus, joint pathogenetic mechanisms for liver injury due to the disease interactions with medications [4].

Papaya (Carica papaya Linn.), a versatile plant commonly called paw-paw, is a type of tropical evergreen tree originating from Mexico and Central America, Taiwan, and southern China, such as Hainan, Guangdong, Guangxi, Yunnan, and Fujian Province [5]. In addition to its delicious fruit flavor, papaya fruit that contains many nutrients is useful for the body and healthy digestive system. Further, the other plant parts are also used in traditional medicine systems [6].

This in vivo study on the hepatoprotective effect of carica papaya seed ethanol extract (EECP) on hepatotoxic-induced rats with Rifampicin and INH. TB drugs that are known hepatotoxic due to oxidative stress and redox homeostasis that cause mitochondrial dysfunction, and papaya seeds that contain high antioxidant ingredients [8,9]. In this study, a comparison test was performed on Curcuma FCT tablet supplement by conducting laboratory tests on liver enzymes such as SGOT, SGPT, Alkaliphosphatase (ALP), Gama-GT (GGT), and Bilirubin.

2. MATERIALS AND METHODS

2.1 Materials

Rifampisin (kimia farma), INH (kimia farma), Curcuma FCT (kimia farma), Na CMC 0.5%, reagen kit ALT Dialab®, reagen kit AST Dialab®, rutin (Sigma Aldrich), kit alkalifosfatase, kit gama gt, dan kit bilirubin, serbuk seng, toluen, zat warna (hematoksilin dan eosin). Microplate Reader, pH meter (OHAUS Starter300 Portable) Beaker glass (IWAKI CTE33), Multiskan Go Reader (Thermo Fisher Scientific 1510), analytic measure, Eppendorf tube, vial 1 ml, Spatula, Micropipet (1-10 μL, 50-200 μL, 100-1000 μL) (Eppendorf), Termometer, automated plate washer, Tumeric, Vitamin E, Ketamine (Sigma P-4417).

This study used 24 Rattus norvegicus weighed 150 – 200 gr. Before treatment, the rats were acclimated with a 12-hour light/dark cycle for one week at a room temperature (22-25 °C). Rats were given food ad libitum in the form of pellets and water.

2.2 Extraction of Carica Papaya

The sample used in this study was papaya seed simplicia. As much as 500 g dry powder papaya
RESULTS AND DISCUSSION

The results show that the positive control 3 group with ALT 384.33 ± 13.19 U/L was significantly different (p <0.05) from the normal group with ALT 27.33 ± 2.05 U/L. The Curcuma treatment group with ALT 26.33 ± 1.24 U/L did not differ significantly (p > 0.05) from the normal group. The treatment group I with AST 250 ± 24.85 U/L was significantly different (p <0.05) from the normal group. Treatment group II with ALT 250 ± 24.85 U/L was significantly different (p <0.05) from the normal group. Treatment group III with the ALT 122 ± 9.89 U/L was significantly different (p <0.05) from the normal group. Based on Table 1, the positive control 3 group with AST 637.37 ± 44.28 U/L was significantly different (p <0.05) from the normal group with AST 105.33 ± 5.24 U/L. The Curcuma treatment group with AST 133.33 ± 6.23 U/L did not differ significantly (p > 0.05) from the normal group. Treatment group I with ALP 265 ± 8.60 U/L was significantly different (p <0.05) from the normal group with ALP 57.66 ± 1.24 U/L. The Curcuma treatment group with ALP 54 ± 6.37 U/L did not differ significantly (p > 0.05) from the normal group. The treatment group I with ALP 159.33 ± 12.76 U/L was significantly different (p <0.05) from the normal group. Treatment group II with ALP 250 ± 24.85 U/L was significantly different (p <0.05) from the normal group. Treatment group III with ALP 112.67 ± 7.58 U/L was significantly different (p <0.05) from the normal group.

The negative control group 3 with GGT 72 ± 12.02 U/L was significantly different (p <0.05) from the normal group with GGT 13.33 ± 3.68 U/L. The Curcuma treatment group with GGT 20.33 ± 4.10 U/L did not differ significantly (p > 0.05) from the normal group. The treatment group I with GGT 48.66 ± 3.09 U/L was significantly different (p <0.05) from the normal group. Treatment group II with GGT 33.33 ± 3.85 U/L was significantly different (p <0.05) from the normal group. Treatment group III with GGT 13.66 ± 2.49 U/L was significantly different (p <0.05) from the normal group.

The negative control group 3 with bilirubin 19.06 ± 0.30 U/L was significantly different (p <0.05) from the normal group with bilirubin 3.81 ± 0.50 U/L. The Curcuma treatment group with bilirubin 5.41 ± 0.37 U/L did not differ significantly (p > 0.05) from the normal group. The treatment group I with bilirubin 10.71 ± 0.78 U/L was significantly different (p <0.05) from the normal group. Treatment group II with bilirubin 7.50 ± 0.49 U/L was significantly different (p <0.05) from the normal group. Treatment group III with
bilirubin 4.79 ± 0.40 U/L was significantly different (p < 0.05) from the normal group.

Rifampicin is known to cause hyperbilirubinemia and liver dysfunction due to liver injury (DILI, Drug-Induced Liver Injury) and can ultimately be diagnosed as clinical exclusion. However, histologic specimens of the liver are often not obtained. Other causes of liver damage, such as acute viral hepatitis, must be sought methodically. Usually, the onset of the acute injury is within a few months after taking the drug. Agent withdrawals are allegedly offending more than double the serum alanine aminotransferase (ALT) elevation, and cessation that cause a decrease in ALT are the most reliable confirmation of diagnosis [16].

In this study, the results showed that the EECP dose of 100 mg/kgbw (group I) still showed quite high levels of ALT, AST, ALP, GGT, and Bilirubin. when compared with a dose of 300 mg/kgbw (group II) there was a decrease in ALT, AST, ALP, GGT, and Bilirubin. when compared with a dose of 300 mg/kgbw (group I) still showed quite high levels of ALT, AST, ALP, GGT, and Bilirubin. when compared with a dose of 300 mg/kgbw (group II) there was a decrease in ALT, AST, ALP, GGT, and Bilirubin. when compared with a dose of 300 mg/kgbw (group III)

Although it contains very high unsaturated fatty acids, papaya seeds do not seem to be good oil seeds. Other essential nutrients also found in papaya seeds for oil and extract production is very beneficial.

Papaya seeds offer benefits in eliminating intestinal parasites, detoxifying the liver (hepatoprotective), reducing fever and typhus, and as an anti-skin irritant, anti-worm, and anti-amoebic [17]. Papaya seeds contain balanced nutrition consisting of fatty acids (71.30% oleic acid, 16.16% palmitic acid, 6.06% linoleic acid, and 4.73% stearic acid), protein (24.3%), fat oil (25.3%), and total carbohydrate (32.5%) [18].

In the study by Adeneye, a dose-dependent (100 - 400 mg/kg/day/oral route) and a protective effect of time of 400 mg/kg/oral route from aqueous seed extracts from the Carica papaya fruit (CPE = Carica papaya fruit) was investigated in hepatotoxic carbon tetrachloride (CCl4) rats for 72 hours [20]. The results showed that the extract caused significant (p < 0.05, p < 0.001) dose-related attenuation in elevated liver enzyme markers in acute hepatocellular injury (ALT, AST), serum lipids (TG, TC, HDL-c, LDL-c, and VLDL-c), and serum proteins (TP and ALB). Maximum hepatoprotection is offered at an oral dose of 400 mg/kg/day extract. The biochemical results obtained are validated by improvements in the liver histological changes induced by CCl4. In addition, maximum hepatoprotection is offered at 400 mg/kg CPE up to 3 hours after CCl4 induction.

Table 1. ALT, AST, ALP, GGT, and bilirubin levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ALT ± SD (U/L)</th>
<th>Mean AST ± SD (U/L)</th>
<th>Mean ALP ± SD (U/L)</th>
<th>Mean GGT ± SD (U/L)</th>
<th>Mean Bilirubin ± SD (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>27.33 ± 2.05</td>
<td>105.33 ± 5.24</td>
<td>57.66 ± 1.24</td>
<td>13.33 ± 3.68</td>
<td>3.81 ± 0.50</td>
</tr>
<tr>
<td>Positive control 1</td>
<td>333 ± 14.23</td>
<td>587 ± 15.57</td>
<td>278.33 ± 20.43</td>
<td>58.66 ± 1.69</td>
<td>16.51 ± 0.98</td>
</tr>
<tr>
<td>Positive control 2</td>
<td>318.33 ± 19.70</td>
<td>410.33 ± 11.14</td>
<td>287 ± 9.79</td>
<td>64 ± 9.41</td>
<td>17.47 ± 0.78</td>
</tr>
<tr>
<td>Positive control 3</td>
<td>384.33 ± 13.19</td>
<td>637.37 ± 44.28</td>
<td>265 ± 8.60</td>
<td>72 ± 12.02</td>
<td>19.06 ± 0.30</td>
</tr>
<tr>
<td>Curcuma treatment</td>
<td>26.33 ± 1.24</td>
<td>133.33 ± 6.23</td>
<td>54 ± 6.37</td>
<td>20.33 ± 4.10</td>
<td>5.41 ± 0.37</td>
</tr>
<tr>
<td>Group I</td>
<td>250 ± 24.85</td>
<td>355 ± 8.52</td>
<td>159.33 ± 12.76</td>
<td>48.66 ± 3.09</td>
<td>10.71 ± 0.78</td>
</tr>
<tr>
<td>Group II</td>
<td>122 ± 9.89</td>
<td>217 ± 27.79</td>
<td>136 ± 6.68</td>
<td>33.33 ± 3.85</td>
<td>7.50 ± 0.49</td>
</tr>
<tr>
<td>Group III</td>
<td>52.3 ± 4.10</td>
<td>112.67 ± 7.58</td>
<td>66.33 ± 5.73</td>
<td>13.66 ± 2.49</td>
<td>4.79 ± 0.40</td>
</tr>
</tbody>
</table>
4. CONCLUSION

It can be concluded that extract ethanol of Papaya seed have various flavonoid and antioxidant activity that can be a hepatoprotection on rifampicin and isoniazid-induced rats by reducing liver biomarker such as ALT, AST, GGT, ALP, and bilirubin. Extract ethanol of papaya seed dose 500 mg/kgbw is the most effective agent as hepatoprotective.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study has received ethical clearance from the Ethics Commission of health and science commission, Universitas Prima Indonesia, Medan, Indonesia. (Ethical number 001/KEPK/UNPRI/II/2020).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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