Original Article

Hepatoprotective Effect Of Hibiscus Sabdariffa L Extract On 7,12-dimethylbenz(α) antracene (dmba) Induced Rat

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants generated by incomplete combustion of fuels wood, and other organic matter. PAHs are present in cigarette smoke, grilled foods, automobile exhaust, and woodsmoke. DMBA is a prototypic PAH with carcinogenic, immunosuppressive effects in various species. DMBA has been reported to be hepatotoxic. The conversion of DMBA to its ultimate carcinogenic metabolites is mainly accomplished by the cytochrome P450 (CYP)1 family enzymes. In particular, CYP1A isoforms are responsible for its bioactivation in the liver, the major organ of DMBA metabolism, while CYP1B enzymes are reported to exert their activity predominantly in extra-hepatic tissues, such as the mammary gland.

Liver cells or hepatocytes contain various enzymes, some of which are important for the diagnostic of liver damage. This enzyme also excreted and found in the blood vessels. The enzyme aminotransferase including Serum Glutamic Pyruvic Transaminase (SGPT) and serum glutam oxaloacetic transaminase (SGOT) are specific indicators to determine the damage of the liver cells. Alkaline phosphatase (ALP) is enzyme produced by hepatocyte and hydrolyze ester phosphate on alkaline condition. Increasing levels of these enzymes reflect the existence of damage to the liver cells.

Rosella (H.sabdariffa) has been reported to have an antioxidant activity. This antioxidant properties expected to protect the liver damage caused by free radical (DMBA). Rosella also reported to have hepatoprotective effect against paracetamol , carbon tetrachloride , streptozotocin . The objective of this study was to explore the hepatoprotective effect of rossela extract on DMBA induced rat.

2. MATERIAL AND METHODS

2.1 Collection of material

The rossela calyx were collected from Madiun (East Java, Indonesia. After it had been dried were pulverized and stored in a desiccator before extraction.

2.2 Extraction

2.2.1 Determination of Total Phenolic Content (TPC).

Determination of TPC was carried out by Folin Ciocalteu method with gallic acid as a standard. The amount of 50.0 mg of ethanol extract of rossela was diluted to a volume of 50.0 ml with a 50% ethanol. Then, 300 uL solution were added with 1.5 ml of Folin-Ciocalteau reagent. After shaking the solution were then added by 1.2 ml of 7.5% Na2CO3 solution and allowed to stand in 10 minutes at room temperature. Absorbance was then measured by UV-Vis spectrophotometer at a 730 nm.

2.3 Determination of Total Flavonoid Content (TFC).

Determination of total flavonoids content was carried out using AlCl3 as reported by Chang et al. (2002) using quercetin as a standard. The amount of 1 gram rossela extract was added to a volume of 100 ml of ethanol. The solution of 0.5 ml was added with 1.5 ml ethanol, 0.1 ml of 10% AlCl3 reagent, 0.1 ml of Na-acetate, and 2.8 ml of distilled water. The mixture were stand at room temperature for 30 minutes. Furthermore the absorbance were determined using UV-Vis spectrophotometer at 430 nm.

2.4 Animal treatment
The experiment was performed on Sprague Dawley rats with 4 weeks age. The animal tests were adapted with the research environment for 1 week and fed ad libitum with commercially available feed and water. A total of 30 animals were equally divided in to five groups, each group consist of 6 animals. Group I was normal (base line), untreated, fed ad libitum with standard feed and water. Group II was induced with DMBA 15 mg/rat single dose through intragastric. Group III, IV and V are the treatment groups, induced with DMBA 15mg/rat and followed by administration of rosella extract with dose of 10, 50 and 100 mg/kg BW respectively. The extract were administered for 33 days.

2.5 SGPT, SGOT and ALP assay

Determination of SGPT, SGOT and ALP activities were carried out by enzymatic reaction using the reagent kit Dyasis®.

2.6 Histopathological observation

Histopathological examination of organs was conducted to determine the possibility of damage or changes in the organ tissue structure due to administration of the rosella extract. The histopathological observation was carried out using Haematoxilin & Eosin staining. The extraction of dried rosella calyx were performed using maceration method with 70% ethanol and followed by evaporation to get the concentrated extract.

3. RESULTS AND DISCUSSION

3.1 Total Flavonoid and Total Phenolic content of H. sabdariffa L extract

Anthocyanin one of the flavonoids group member is the major compound of H. sabdariffa and various color fruit and vegetables. Flavonoids have been reported to have antioxidant effect . The antioxidant properties of flavonoids were consider to deactivate the reactive metabolite of DMBA.

The previous research reported the high level of flavonoids and phenolics compound on H. sabdariffa L extract. It has been recognized that flavonoids show antioxidant activity. The mechanisms of action of flavonoids are through scavenging or chelating process . Phenolic compounds are a class of antioxidant agents which act as free radical scavenger. The total phenolic content was expressed as gallic acid equivalent. It was found that total phenolic content of rosella extract was 6.03 ±0.21 GAE/100g. The flavonoid contents of the extracts in terms of quercetin equivalent was 2.47 ± 0.11 %. Flavonoid is also reported to have a hepatoprotective activity .

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic</th>
<th>Total flavonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosella extract</td>
<td>6.03 ±0.21 GAE/100g</td>
<td>2.47 ± 0.11 %</td>
</tr>
</tbody>
</table>

3.2 SGPT, SGOT and ALP level

Enzymatic activation of PAHs leads to the generation of active oxygen species such as peroxides and superoxide anion radicals, which induce oxidative stress in the form of lipid peroxidation. Certain aldehyde such as malondialdehyde (MDA), the end product of lipid peroxidation (LPO) arising from the free radical generation leading to the degradation of polyunsaturated fatty acids. Formation of reactive oxygen species (ROS) in hepatocytes is reported to undergo hepatotoxicity. The oxidative stress play an important role in liver injury. Many of the members of the PAH family are toxic, carcinogenic and immunotoxic . The DMBA was metabolized in hepar into a more toxic form and involved the activation of the cytochrome P450 enzyme into a reactive intermediate, epoxides dihydrodiol and radical cations. Epoxide dihydrodiol exocyclic will bind the amino group of purine DNA adducts covalently into a stable form, while the radical cation would bind purine N7 or C8 becomes unstable form adducts. Cytochrome P450 enzyme CYP1A1 or CYP1B1 and microsomal hydrolase enzyme change into DMBA-3,4-diol-1,2-epoxide (DMBA-DE) in the metabolism of DMBA. These enzymes were found in hepatic cells and extra hepatic such as lung, mammary gland, spleen, kidney, prostate, uterus, and heart . DMBA-DE elimination from the body is accelerated in the presence of the antioxidant enzyme glutathione-s-transferase (GST).

The result of rosella extract on SGPT, SGOT and ALP level on DMBA induced rat were shown on Table II.

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT (U/L)</th>
<th>SGOT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base line</td>
<td>43 ± 2.9</td>
<td>86 ± 10</td>
<td>13 ± 6.9</td>
</tr>
<tr>
<td>Negative control</td>
<td>52 ± 2.2</td>
<td>137 ± 15</td>
<td>25 ± 12</td>
</tr>
<tr>
<td>Extract 10 mg/kgBW</td>
<td>47 ± 5.8</td>
<td>123 ± 5</td>
<td>22 ± 7.1</td>
</tr>
<tr>
<td>Extract 50 mg/kgBW</td>
<td>40 ± 2.1</td>
<td>117 ± 6.7</td>
<td>21 ± 11</td>
</tr>
<tr>
<td>Extract 100 mg/kgBW</td>
<td>35 ± 4.4</td>
<td>91 ± 15</td>
<td>20 ± 3.4</td>
</tr>
</tbody>
</table>

The result showed that DMBA caused the hepatotoxicity as the SGPT, SGOT and ALP level after DMBA induced was increase . The SGPT activity on negative control was increase 21% after DMBA administration. Treatment with rosella extract 10, 50 and 100mg/kg BW were decrease the SGPT 10%, 23% 33% respectively. The hepatoprotective effect of rosella extract was shown on dose dependent manner.

The SGOT level also was found to increase after DMBA administration. The SGOT activity of negative control was 59% more than base line group. Increasing of SGOT activity was more than SGPT due to SGOT enzyme was found in some tissues including heart, muscle, kidney and brain. The SGPT is more specific indicator to liver function than SGOT. Treatment with rosella extract was found to decrease the SGOT level get near to the base line group.

ALP is the prototype of the enzymes that reflects the pathological alteration in the biliary flow. DMBA induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The ALP level was found to increase after DMBA administration by 92%. The treatment of rosella extract for 33 days cannot decrease ALP activity significantly.
The hepatic toxicity of DMBA was also reported. They reported that chronic DMBA treatment increase the SGPT, SGOT, and ALP level, and treatment with turmeric was decrease the SGPT, SGOT and ALP level by 22.5%, 21.4%, 19% respectively. Oral administration of ethanolic extract of rosella was reported to decrease of SGPT, SGOT, and ALP on CCl4-induced liver damage mice. They also reported that rosella extract also increase the activity of endogenous antioxidants including, SOD, Catalase and GSH and conclude that hepatoprotective activities observed in the study could be due to the ability of phenolic compounds to neutralize the free radicals produced from the metabolism of CCl4. The hepatoprotective of rosella extract on streptozotocin induced rat was related to the antioxidant activity and flavonoids content.

Some of Hibiscus family plant were also reported to have hepatoprotective activity. Hibiscus esculentus Linn ethanolic extract was reported to have a hepatoprotective activity by lowering ALP activity. Hibiscus hispidissimus ethanolic extract was reported to reduce ALP, bilirubin on CCl4-induced rat. In the in vitro test this extract also has antioxidant activity by reducing DPPH.

### 3.3 Histopathology of hepar

Histopathological observation of hepar was shown on Figure 2. Histopathological observation was carried out by Haematoxilin & Eosin staining.

Figure 2. The histopathological observation (magnification 400x) of liver after rosella extract treatment on DMBA induced rat, A, DMBA (negative control) showed inflammation, characterized by infiltration of inflammatory cells (lymphocytes) in the liver; B, DMBA (negative control) group marked degradation of fat-vacuoles. C, rosella extract group of 100 mg/kg BW, no specific pathological changes, D, rosella extract group of 50 mg/kg BW have foci necrotic.

The result showed that DMBA (negative control) showed inflammation, characterized by infiltration of inflammatory cells (lymphocytes) in the liver (Fig 2A). The DMBA (negative control) group also marked degradation of fat-vacuoles characterized by vacuoles of various sizes with clear boundaries (Fig 2B). Treatment wit rosella extract was returned the damage of the cells, which there is no specific pathological changes after treatment with rosella extract of 100 mg/kg BW (Fig 2C). The antihepatotoxic effect of rosella extract showed to be dose dependent due to the treatment with lower dose, (50 mg/kg BW) cannot repair the liver damage completely (Fig 2D). The result showed foci necrotic, marked by cell death in the core area of the hepatocytes, lymphocytic infiltration and picnotic.

### 4. CONCLUSION

Rosella extract showed the hepatoprotective activity on DMBA induction by lowering the SGPT, SGOT and ALP level.

### ACKNOWLEDGEMENTS

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### REFERENCES


