Animal Models of Alcoholic Liver Disease for Hepatoprotective Activity Evaluation

Hidayah Dwi Renggani¹, Triana Hertianti², and Retno Murwanti³*
1 Master of Pharmaceutical Science Program, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
2 Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
3 Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Background: The reported statistics suggest that alcoholic liver disease is on the rise. Furthermore, medications used to treat the disease have unpleasant effects, and this necessitates the need to continuously investigate hepatoprotective agents. This study investigates animal models of alcoholic liver disease used to evaluate hepatoprotective activity. Content: A good number of published articles evaluating hepatoprotective activity were summarized. The studies used three ethanol-induced liver injury models: the acute ethanol-induced liver injury model, the chronic ethanol-induced liver injury model, and Lieber–DeCarli model. Summary: Wistar rats were primarily used in the ethanol-induced liver injury model. High levels of alanine transaminase (ALT) and aspartate transaminase (AST) and histopathological alterations were found in all animal models (acute ethanol-induced liver injury, chronic ethanol-induced liver injury, and Lieber–DeCarli models). Severe steatosis was shown in both chronic ethanol-induced liver injury and Lieber–DeCarli models. However, fibrosis was undetected in all models. Keywords: Alcoholic liver disease, animal models, hepatoprotective activity, liver injury, ethanol

1 Introduction

For centuries, alcohol drinking has been a part of social activities in most countries (1). Alcohol use disorders affect millions of individuals worldwide (2). Heavy alcohol consumption is a causal factor for over 60 diseases (3), ranging from simple steatosis to more advanced alcoholic liver diseases, such as steatohepatitis, progressive fibrosis, cirrhosis, and hepatocellular carcinoma (4,5). Alcohol abuse is responsible for 5.3% (three million deaths) of global deaths (6). A previous study revealed that 75 million people were diagnosed with alcohol use disorders and were at the risk of developing alcohol-associated liver diseases (7). The number of patients with alcoholic liver diseases will continue to increase.

The FDA approved no therapy for alcoholic liver disease (8,9). Bifendate, tiopronin, and potenline are some of the available drugs for the clinical treatment of alcoholic liver diseases (10). While most medications used to treat liver diseases have unpleasant side effects or show limited beneficial effects, such as propylthiouracil, colchicine, corticosteroid, lecithin, and anabolic steroid (9,11), pentoxifylline (PTX) shows improved outcomes for alcoholic liver diseases and reduces the mortality rate (8,12). Nevertheless, side effects such as nausea and vomiting are an obstacle (12), causing lack of adherence. For this purpose, researches on hepatoprotective agents are continuously conducted.

Natural products are one of the major sources of new drug molecules. Many natural products have been traditionally used for the treatment of liver injury (13). Since the last decade, natural hepatoprotective agents have been prominently developed. Silymarin is a well-known hepatoprotective agent with a high safety profile, derived from Silybum marinum (14).

Animal experiment are still widely used in drug discovery and drug development. For example, pharmacological activity and preclinical studies are evaluated using animal experiments. Preclinical studies are one of the steps of drug discovery and development to ensure its safety and efficacy (15). Many animal models of alcoholic liver disease have been developed. Regarding the excellent conduct of the experiments, information regarding the laboratory animal used and hepatotoxicity induction in laboratory animals from previous study are necessary (16). Therefore, the present study describes the animal models of alcoholic liver disease used for evaluating hepatoprotective activity.

2 Method

We searched PubMed using the keyword “alcohol induced hepatotoxicity.” The search was performed on January 2021, and conditions were set as follows: “free full text” and “10 years” for the publication date. The search showed 365 articles, which were subjected to screening. Review articles, conference articles, thesis,
Rodents were used in all in vivo alcoholic liver disease models in the publications. Wistar rats were the most frequently used, followed by Sprague–Dawley rats, Kunming mice, and C57BL/6 mice, respectively. Using rodents in the alcoholic liver disease model provides several benefits. Rodents are small in size, have a short life cycle, and offer abundant genetic resources and high numbers of progeny. They are easy to breed and maintain. There are inbred strains, transgenic models, and many knockouts (KO). Moreover, a vast number of commercial immunological reagents are available for rodents (17,18).

In rats, ethanol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (19,20). A sufficient amount of ADH is needed in animals, which helps ethanol metabolize faster in animals than humans (21,22). ADH activity in rats is two times higher than that in humans. Thus, this will be the limiting factor in ethanol intoxication study in rats. Normally, rats do not experience alcohol addiction because they will stop consuming alcohol when the acetaldehyde level in the blood is extremely high. However, previous studies demonstrated that rats could display alcohol deprivation effects, and this suggests that their bodies could imitate the human body (23–25).

Besides compiling the articles, we were interested in seeing animals other than rats used in hepatoprotective evaluation models. Large animals have also been used in the models, including baboons and monkeys. Studies on ethanol toxicity in baboons showed that baboons metabolize alcohol similar to humans (53). Other articles stated that if a baboon consumes ethanol at a mild level, ADH isozyme levels could adapt and possibly have a protective effect, similar to humans (22,54,55). Considering that, baboons are probably a good choice in an ethanol-induced liver injury model. Investigation of ethanol toxicity in monkeys showed that 6.2 ± 0.3 gram per kg body weight of oral administration of ethanol displays no signs of hepatotoxicity or fibrosis; therefore, they differ from baboons (56). The higher ethanol metabolism in monkeys might be the reason behind the absence of hepatotoxicity since the same result was reported in another study where monkeys were administered ethanol (57).

**3 Results and Discussion**

**Table 1. Summarized data of animal models.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Animal used</th>
<th>Administration route</th>
<th>Ethanol concentration</th>
<th>Dose</th>
<th>Duration of ethanol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute ethanol-induced liver injury model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Kunming mice</td>
<td>Intrapерitoneal</td>
<td>50%</td>
<td>14 mL/kg</td>
<td>Once</td>
<td>(26)</td>
</tr>
<tr>
<td>2</td>
<td>C57BL/6 mice</td>
<td>Oral</td>
<td>56%</td>
<td>13 g/kg</td>
<td>14 days</td>
<td>(27)</td>
</tr>
<tr>
<td>3</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>Not available</td>
<td>5 g/kg</td>
<td>Once</td>
<td>(28)</td>
</tr>
<tr>
<td>4</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>Not available</td>
<td>6 g/kg</td>
<td>Once</td>
<td>(29)</td>
</tr>
<tr>
<td>5</td>
<td>C57BL/6 mice</td>
<td>Oral</td>
<td>50%</td>
<td>5 g/kg</td>
<td>Once</td>
<td>(30)</td>
</tr>
<tr>
<td>6</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>Not available</td>
<td>7 mL/kg</td>
<td>5 times every 12 hours</td>
<td>(31)</td>
</tr>
<tr>
<td>7</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>56%</td>
<td>1.8 mL/100 g</td>
<td>2 times</td>
<td>(32)</td>
</tr>
<tr>
<td>8</td>
<td>Kunming mice</td>
<td>Oral</td>
<td>Not available</td>
<td>5 g/kg</td>
<td>3 times every 12 hours</td>
<td>(33)</td>
</tr>
<tr>
<td>9</td>
<td>C57BL/6 mice</td>
<td>Oral</td>
<td>Not available</td>
<td>6 g/kg</td>
<td>Once</td>
<td>(34)</td>
</tr>
<tr>
<td>10</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>50%</td>
<td>12 mL/kg</td>
<td>8 days</td>
<td>(35)</td>
</tr>
<tr>
<td>11</td>
<td>Kunming mice</td>
<td>Oral</td>
<td>50%</td>
<td>12 mL/kg</td>
<td>3 times each 12 hours</td>
<td>(36)</td>
</tr>
<tr>
<td>12</td>
<td>Kunming mice</td>
<td>Oral</td>
<td>50%</td>
<td>10 mL/kg</td>
<td>3 days</td>
<td>(37)</td>
</tr>
<tr>
<td><strong>Chronic ethanol-induced liver injury model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Wistar rats</td>
<td>Intrapерitoneal</td>
<td>15%</td>
<td>3 g/kg</td>
<td>15 days</td>
<td>(38)</td>
</tr>
<tr>
<td>2</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>50%</td>
<td>0.5 mL/100 g</td>
<td>4 weeks</td>
<td>(39)</td>
</tr>
<tr>
<td>3</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>40%</td>
<td>4 g/kg</td>
<td>3 weeks</td>
<td>(40)</td>
</tr>
<tr>
<td>4</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>56%</td>
<td>0.8–1.5 mL/100 g</td>
<td>8 weeks</td>
<td>(41)</td>
</tr>
<tr>
<td>5</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>20% w/v</td>
<td>5 g/kg</td>
<td>21 days</td>
<td>(42)</td>
</tr>
<tr>
<td>6</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>56% v/v</td>
<td>10 mL/kg</td>
<td>9 weeks</td>
<td>(43)</td>
</tr>
<tr>
<td>7</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>28.5%</td>
<td>3 ml/100 g</td>
<td>30 days</td>
<td>(44)</td>
</tr>
<tr>
<td>8</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>10%</td>
<td>Not available</td>
<td>4 weeks</td>
<td>(45)</td>
</tr>
<tr>
<td>9</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>25%</td>
<td>1 mL</td>
<td>21 days</td>
<td>(46)</td>
</tr>
<tr>
<td>10</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>70%</td>
<td>10 mL/kg</td>
<td>30 days</td>
<td>(47)</td>
</tr>
<tr>
<td>11</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>50%</td>
<td>4 g/kg</td>
<td>90 days</td>
<td>(48)</td>
</tr>
<tr>
<td>12</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>50%</td>
<td>4 g/kg</td>
<td>90 days</td>
<td>(49)</td>
</tr>
<tr>
<td>13</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>Not available</td>
<td>3 g/kg</td>
<td>4 weeks</td>
<td>(50)</td>
</tr>
<tr>
<td>14</td>
<td>Wistar rats</td>
<td>Oral</td>
<td>50%</td>
<td>5 mL/kg</td>
<td>4 weeks</td>
<td>(51)</td>
</tr>
<tr>
<td><strong>Lieber–DeCarli model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sprague–Dawley rats</td>
<td>Oral</td>
<td>6.7% v/v</td>
<td>Not available</td>
<td>28 days</td>
<td>(52)</td>
</tr>
</tbody>
</table>

https://doi.org/10.1051/bioconf/20214107007
Another animal used for studying alcoholic liver disease is guinea pigs (Cavia porcellus). In this study, the pigs were administered 4 g/kg of 50% ethanol orally, and the duration of the treatment was 90 days (58). Guinea pigs metabolize alcohol similar to humans in several aspects, including an equal redox status of mitochondrial and cytosolic NAD/NADH system; a similar subcellular distribution of a critical enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase, between mitochondria and cytosol; similar activities of several essential enzymes of carbohydrate metabolism and ketogenesis. Thus, guinea pigs provide a better animal model for studying alcoholic hypoglycemia and alcoholic ketoacidosis (58–60). The zebrafish larva model has recently been developed and shows high sensitivity, bridging in vitro cell-based models and in vivo mammalian models (61–63).

3.1 Animal Model of Alcoholic Liver Injury
Ethanol could be administered both intraperitoneally and orally in one or multiple doses. Both routes of administration have been used by many laboratories to study the pathogenesis of the alcoholic liver disease. Table 1 shows that the oral administration is the most frequently used. This route can mimic how humans consume alcohol. According to the dose of ethanol and duration of treatment, there are several animal models: the acute ethanol-induced liver injury model, chronic ethanol-induced liver injury model, Lieber–DeCarli model, and Tsukamoto-French model. However, the studies we evaluated in this review used only the acute ethanol-induced liver injury model, chronic ethanol-induced liver injury model, and Lieber–DeCarli model.

3.2 Acute Ethanol-Induced Liver Injury Model
The oral route is the most frequently used in the acute ethanol-induced liver injury model. Only one study used the intraperitoneal route. 50% ethanol was used in both mice and rats. Ethanol doses in mice vary from 5 to 14 ml/kg body weight, while, in rats, they vary from 5 to 18 ml/kg body weight. Generally, the used doses in rats are 4–6 g ethanol/kg body weight (64).

Transaminase serum is a biochemical marker used in assessing liver functions. The study with the highest serum alanine transaminase (ALT) (389.20%) levels used 12 mL/kg body weight 50% ethanol (35), while the study with the highest serum aspartate transaminase (AST) (366.67%) used the highest dose and concentration of ethanol (18 mL/kg body weight 56% ethanol) (32). Both studies used rats (Wistar and Sprague–Dawley rat) and used a relatively high dose of ethanol. However, high serum ALT levels are not always followed by elevation of serum AST. Serum ALT is a more specific parameter of liver damage because AST is found in different tissues, including the heart, brain, and skeletal muscles (65). Glutathione S-transferase alpha 1 (GSTA1) can be used as a biomarker in the acute ethanol-induced liver injury model. It can be detected at a low level during the early stage of acute hepatic injury. Furthermore, GSTA1 is a more sensitive and accurate indicator than ALT (26).

Liver sections of rats treated with acute ethanol administration showed mild histopathological alterations such as a disordered arrangement of hepatic cells, inflammatory cell infiltration, cavitation in hepatocytes, and necrosis. Rats experienced steatosis in only two studies.

3.3 Chronic Ethanol-Induced Liver Injury Model
The chronic ethanol-induced liver injury model is associated with a long period of excessive ethanol consumption. Several studies using the chronic ethanol-induced liver injury model were conducted. The highest ethanol dose was 10 ml/kg body weight with a concentration of 70%. The study was conducted for 30 days resulting in elevating serum ALT and AST levels by 109.94% and 94.22%, respectively (47).

A study that had the highest serum ALT and AST levels was conducted using a lower dose but with more prolonged treatment (three weeks) (48). Chronic ethanol consumption can promote the formation of hepatic steatosis—the early symptom of alcoholic liver disease. Studies (41) and (51) used methods that caused severe steatosis and abundant infiltrated inflammatory cells. All studies conducted using this model showed no fibrosis on liver morphology.

3.4 Lieber–DeCarli Model
At first, the Lieber–DeCarli model was developed to outrun the liver injury of the ethanol drinking model. In this model, the animal is given a diet that consists of protein, fat, carbohydrate, and fiber to imitate the clinical situation of heavy alcohol consumption in humans. However, this original formula has been developed with three variant diets: regular diet, low-fat diet, and high protein-content diet. The low-fat diet is used to study the effect of ethanol in minimized hepatic fat accumulation. The high protein diet is used to study conditions requiring high protein consumption, such as gestation and lactation (66).

Only one study used this model. A study conducted by (52) used the original Lieber–DeCarli liquid diet as the control diet and used an ethanol concentration of 6.7% (v/v). ALT and AST serum levels increased mildly compared with those in the acute ethanol-induced liver injury model.

Lieber–DeCarli liquid diet was administered for 4–12 weeks. The Lieber–DeCarli diet model produces fatty liver, and metabolic tolerance and lesion beyond steatosis are rare. Although the ethanol in the drinking water model (oral) showed similar results, no fibrosis was present, and this model is considered more appropriate for studying the early stages of alcoholic liver diseases (67).

4 Conclusion
Rodents are more preferred for studying alcoholic liver diseases, and Wistar rats are the most frequently
used. All studies conducted using rodents showed significant elevation of serum ALT and AST. Alterations in the histopathological analysis depend on the ethanol dose and duration of the treatment. Severe steatosis is shown in both chronic ethanol-induced liver injury and Lieber–DeCarli models. However, fibrosis remains undetected in all models.

**Authors’ Contributions**

HDR was involved in data collection, drafting, writing, and revising the article. TH and RM conceptualized the idea and supervised and critically revised the article.

**References**

25. Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R. A comparative study on alcohol-prefering rat lines: effects of deprivation...
and stress phases on voluntary alcohol intake. 


49. Prathibha P, Rejitha S, Harikrishnan R, Das SS, Abhilash PA, Indira M. Additive effect of alpha-


51. Rezaee Khorasany A, Razavi M, Taghiabadi E, Tabatabaei Yazdy A, Hosseinizadeh H. Effect of crocin, an active saffron constituent, on ethanol toxicity in the rat: histopathological and biochemical studies. Iranian Journal of Basic Medical Sciences [Internet]. 2020 ;23(1).


