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Recent advances of rodent models for alcoholic liver disease in evaluating healthy effects of food bioactive ingredients

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ABSTRACT: The development of rodent models that accurately reflect the pathogenesis of alcoholic liver disease (ALD) in humans is crucial for evaluating the nutritional intervention of food bioactive ingredients in ALD. Although various models have been employed to establish ALD models over the past few decades, most successful cases are associated with high mortality rates, operational difficulties, and incompatibility formation mechanism compared to human ALD. However, the ALD models established by oral administration that simulate human drinking behavior often fail to induce significant liver damage. Therefore, it is imperative to explore simple and effective modes of oral administration for establishing ALD models consistent with the pathophysiological process of human ALD. Herein, we summarized the pathogenesis of ALD and discussed several issues related to construct ALD models with rodents (mainly mice and rats) by oral administration, including animal selection, animal feeding, alcohol intervention, and evaluation criteria. The purpose of this review is to provide a standardized and efficient formula for ALD modeling, so as to facilitate efficacy evaluation and mechanism analysis of food bioactive ingredients in ALD.

Keywords: alcoholic liver disease, acute, chronic, rodent model

1. Introduction

Alcohol is a substance with addictive potential that has been used for centuries as a recreational substance in beverages around the world [1]. Approximately 90% of the alcohol consumed by the body is metabolized in the liver [2]. This process can activate large amounts of oxygen free radicals and seriously disrupt the normal physiological function of liver cells, leading to alcoholic liver disease (ALD). Fortunately, the intake of bioactive ingredients in food plays a vital role in preventing ALD [3]. However, the mechanism of most food bioactive ingredients for the prevention of ALD is still unclear. Therefore, it is imperative to comprehensively evaluate the preventive effects and mechanisms of various food bioactive ingredients on ALD.

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Animal experiments have always been recognized as an important means to explore the treatment of various diseases. A stable, reliable, and repeatable ALD model is the prerequisite for carrying out relevant experimental work. At present, the more mature ALD modeling methods are oral administration and intraperitoneal injection. In order to ensure the success rate of the ALD model, intraperitoneal injection will be adopted to assist. However, this approach has the disadvantages of poor stability and high mortality, which is highly incompatible with the pathogenesis of ALD in humans [4]. Therefore, it is of great significance to construct an animal model that conforms to the characteristics of human ALD in the process of studying the effect and mechanism of food components on ALD and screening suitable food functional components [5].

An ideal ALD animal model should include the entire spectrum of ALD symptoms, encompassing aberrant animal behavior, disrupted biochemical markers, and substantial liver tissue damage [6]. In the past decades, numerous rodent models have been established to investigate ALD, yielding substantial advancements in the understanding of ALD's pathogenesis and pathology [6]. In this review, we describe the formation mechanism of ALD, focus on a series of issues related to animal selection, animal feeding, alcohol intervention, and evaluation standard during the construction of different kinds of ALD models (including acute and chronic) by oral administration (Fig. 1). Additionally, the review extensively outlines several widely employed examples in constructing ALD models using rodents, primarily focusing on mice and rats, hoping to provide technical supports for the ALD model construction in food active ingredient related studies.

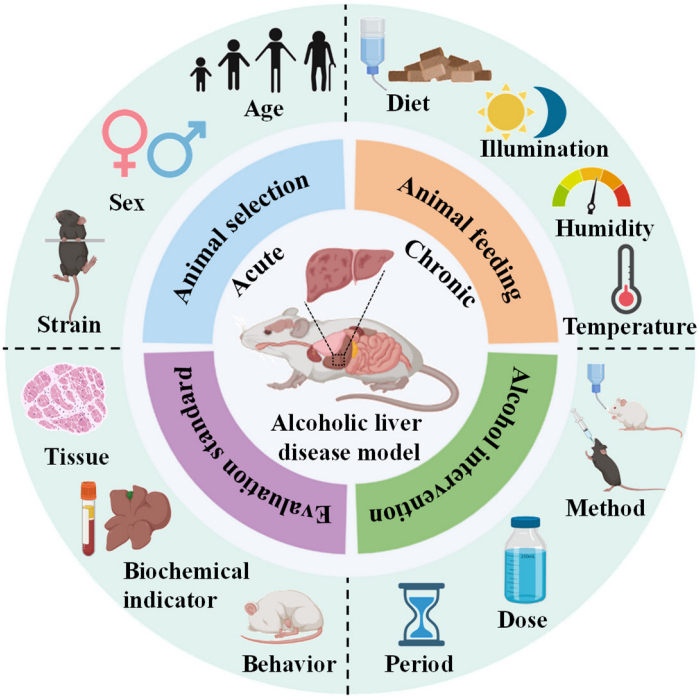

Fig. 1 Several critical aspects during the construction of alcoholic liver disease (ALD) models, including animal selection, animal feeding, alcohol intervention, and evaluation standard. Firstly, the strain, sex, and age of rodents should be considered in animal selection. Then, it is important to keep the environment with constant illumination, temperature, and humidity. If necessary, different diets can be used to induce the formation of ALD. The method, dose, and period should be strictly controlled to ensure the success of model construction in terms of alcohol intervention. Finally, the successful establishment of ALD model was judges by animal behavior, biochemical index, and liver histopathology.

2. Formation mechanism

Alcohol, a polar molecular substance, is absorbed mainly in the stomach (about 22%) and intestine (about 75%) after being orally ingested. After absorption, the alcohol is primarily accumulated within the liver via the circulatory system, where approximately 95% of it undergoes metabolic processes [7]. Within hepatocytes, the metabolism of alcohol yields acetaldehyde, primarily facilitated by alcohol dehydrogenases (ADHs), cytochrome P450 family 2 member E1 (CYP2E1), and catalase. Since the alcohol metabolites trigger oxidative stress, lipid accumulation, and inflammation, these metabolic pathways play a pivotal role in contributing to the formation of ALD. These factors collectively influence liver cells and hepatic stellate cells, ultimately contributing to the onset of ALD [8].

Chronic heavy alcohol consumption induces oxidative stress in hepatocytes, which stands as a primary contributor to the development ALD. Alcohol triggers an up-regulation of CYP2E1, leading to the enhanced metabolism of alcohol and generating significant quantities of acetaldehyde [9]. Acetaldehyde induces mitochondrial dysfunction, including the decrease of adenosine triphosphate (ATP) through the respiratory chain, the increase of ROS production, and the decrease of ALDH activity. Additionally, this process is accompanied by endoplasmic reticulum stress and apoptosis.

Alcohol consumption disrupts lipid metabolism by inactivating enzymes related to β-oxidation and impeding the utilization of free fatty acids through a range of mechanisms. This disruption stands as a prevalent characteristic of ALD. Firstly, the inhibition of adenosine 5’-monophosphate (AMP)-activated protein kinase (AMPK) is a primary effect of alcohol, thereby inhibiting lipid metabolism [10]. The impaired AMPK activity could contribute to lipid accumulation, as it mediates the synthesis of acetyl-CoA carboxylase (ACC), consequently attenuating the function of carnitine palmitoyl transferase-1 (CPT-1). Subsequently, β-oxidation was further prevented by the inhibitory effect of ethanol on peroxisome proliferator-activated receptor-alpha (PPARα) activity. Furthermore, alcohol consumption can lead to the up-regulation of sterol-regulatory element binding proteins 1c (SREBP1c) expression in the liver, which facilitates the expression of genes involved lipid synthesis [11].

Liver inflammation is often associated with ALD. Ethanol metabolism in hepatic Kupffer cells produces reactive oxygen species (ROS), prompting the generation of significant quantities of proinflammatory cytokines, including IL-1β and TNF-α. These cytokines, in turn, activate natural killer T cells, macrophages, neutrophils, and various downstream immune responses [12]. The inflammatory cascade is synchronized with the activation of NF-κB-inducing kinase/IκB kinase/IκB-α pathway induced by ROS [13]. Moreover, prolonged chronic alcohol consumption can contribute to a 'leaky' intestine, allowing the translocation of endotoxins like lipopolysaccharide (LPS) to the portal vein and liver. LPS can combine with diverse toll-like receptors (TLRs), initiating the synthesis and release of cytokines and inflammatory factors, such as platelet-derived growth factor (PDGF), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α). This process further stimulates the accumulation of neutrophils and macrophages, which ultimately leads to hepatic inflammation [14].

3. Model type

The initial phase of constructing an ALD model involves selecting the specific type of model to be employed. In prior research, ALD models have typically been categorized into acute and chronic ALD models [15]. Most acute ALD refers to the toxic pathological damage to the liver caused by short-term heavy drinking. In experimental settings, acute ALD models are commonly established by simulating the ingestion of high doses of alcohol solution within a short time, mimicking human behavior. The acute ALD model, known for its relatively simple procedure and short modeling cycle, is primarily applied in the context of preventing and treating acute ALD-related conditions. Nonetheless, inducing significant liver damage through short-term alcohol intervention can prove challenging, occasionally even making liver lesions difficult to detect. In contrast, the chronic ALD model encompasses liver pathology resulting from prolonged alcohol consumption. Experimental chronic ALD models typically replicate cumulative drinking patterns observed in humans and find extensive application in chronic ALD research. Compared to the acute ALD model, this model has a longer modeling period and a higher success rate [5]. Furthermore, certain chronic ALD models are developed to replicate the combination of long-term low-dose and short-term high-dose alcohol consumption observed in humans, which represents the most prevalent pattern of alcohol abuse among human populations. ALD induced through this approach closely mirrors the disease progression observed in human ALD, showing more serious liver damage. However, high-dose alcohol intake during modeling can easily lead to higher mortality in rodents. Consequently, when constructing an ALD model, it's crucial to comprehensively assess factors such as applicability, complexity, benefits, and drawbacks of different models. This assessment helps in selecting the most suitable model type. Herein, some rodent models used for the previous research on ALD are summarized in Table 1 for readers to reference [5].

Table 1. Rodent models were used for the research of ALD.

Type	Strain	Sex	Method	Dose	Period	Advantage and disadvantage	Reference
Acute	C57BL/6	Male	Single gavage	56% (*v/v*) 8 g/kg/bw	2 h	Easy to perform	[16]
	ICR	Male	Single gavage	50% (*v/v*) 12 mL/kg/bw	1 h	Affordable	[17]
	BALB/c	Male	Single gavage	50% (*v/v*) 12 mL/kg/bw	12 h	Low mortality rate	[18]
	ICR	Male	Multiple gavages	50% (*v/v*) 12 mL/kg/bw	12 h×3	Short period	[19]
	Kunming	Male	Multiple gavages	50% (*v/v*) 8 mL/kg/bw	6 h×4	Mild elevation of liver injury Low success rate	[20]
	C57BL/6	Male	High-fat diet & single gavage	60% kcal% fat 31.25% (*v/v*) 5g/kg/bw	3 d 9 h	Easy to perform Costly Low mortality rate Short period	[21]
	CRAMP KO	Male	High-fat diet & single gavage of alcohol solution	42% kcal% fat 31.25% (*v/v*) 5g/kg/bw	10 wk 6 h	Moderate elevation of liver injury Low success rate Easy to perform Costly	[22]
	ICR	Male	High-fat diet & single gavage	60% kcal% fat 31.25% (*v/v*) 5g/kg/bw	12 wk 9 h	Low mortality rate Long period Marked elevation of liver injury High success rate	[23]
Chronic	C57BL/6	Male	Lieber-DeCarli liquid diet	4% (*w/v*)	4 wk	Easy to perform	[24]
	C57BL/6	Male	Lieber-DeCarli liquid diet	1% − 4% (*w/v*) 4% (*w/v*)	1 wk 6 wk	Costly Low mortality rate	[25]
	C57BL/6	Male	Lieber-DeCarli liquid diet	1.6% − 5% (*w/v*) 5% (*w/v*)	6 d 24 d	Long period Marked elevation of liver injury	[26]
	C57BL/6	Male	Lieber-DeCarli liquid diet	1% − 5% (*w/v*) 5% (*w/v*)	5 d 6 wk	High success rate	[27]
	C57BL/6	Male	Lieber-DeCarli liquid diet	1%, 2% (*v/v*) 4%, 5%, 6% (*v/v*)	2 d each 1 wk each	The most common form of alcohol abuse in humans	[28]
	Wister	Male	Alcohol solution beverage	20% (*v/v*)	4 wk	Easy to perform Affordable Low mortality rate Long period Moderate elevation of liver injury Low success rate The most common form of alcohol abuse in humans	[29]
	C57BL/6	Male	High-fructose diet & alcohol solution beverage	60% (kcal%) fructose 20% (*v/v*)	18 wk 10 wk (9th-18 th)	Easy to perform Costly Low mortality rate Long period Marked elevation of liver injury	[30]

					High success rate	
BALB/c	Male	Multiple gavages	50% (*v/v*) 2.4 g/kg/bw 50% (*v/v*) 4 g/kg/bw	4 wk 2 wk		[31]
BALB/c	Female	Multiple gavages	52% (*v/v*) 3.8 g/kg/bw	4 wk		[32]
ICR	Male	Multiple gavages	56% (*v/v*) 6 mL/kg/bw	5 wk	Difficult to perform	[33]
Kunming	Male	Multiple gavages	56% (*v/v*) 10 mL/kg/bw	20 d	Affordable High mortality rate	[34]
ICR	Female	Multiple gavages	56% (*v/v*) 10 mL/kg/bw	4 wk	Long period	[35]
Sprague-Dawley	Male	Multiple gavages	30% (*v/v*) 10 mL/kg/bw 40% (*v/v*) 10 mL/kg/bw 50% (*v/v*) 10 mL/kg/bw	2 d 2 d 6 d	Moderate elevation of liver injury High success rate The most common form of alcohol abuse in humans	[36]
Kunming	Male	Multiple gavages	50% (*v/v*) 5 mL/kg/bw	6 wk (Twice a day)		[37]
Wister	Male	Multiple gavages	56% (*v/v*) 8 mL/kg/bw increased by 1 mL every other week	8 wk		[38]
C57BL/6	Male	Alcohol solution beverage & single gavage	5% (*w/v*) 35% (*v/v*) 5 g/kg/bw	10 d 12 h	Easy to perform Affordable Low mortality rate Long period	[39]
C57BL/6	Male	Alcohol solution beverage & single gavage	5% (*w/v*) 20% (*v/v*) 5 g/kg/bw	10 d 9 h	Moderate elevation of liver injury Low success rate The most common form of alcohol abuse in humans	[40]
C57BL/6	Male	Lieber-DeCarli liquid diet & single gavage	5% (*w/v*) 50% (*v/v*) 5 g/kg/bw	10 d 12 h	Easy to perform Affordable High mortality rate Long period Marked elevation of liver injury High success rate The most common form of alcohol abuse in humans	[41]
Kunming	Male	Lieber-DeCarli liquid diet & multiple gavages	5% (*w/v*) 50% (*v/v*) 5 mL/kg/bw (once a week)	8 wk	Difficult to perform Costly Low mortality rate Long period Marked elevation of liver injury High success rate The most common form of alcohol abuse in humans	[42]
Sprague-Dawley	Male	Lieber-DeCarli liquid diet & multiple gavages	1.25% (*w/v*) 1.67% (*w/v*) 2.5% (*w/v*) 5% (*w/v*) 32% (*v/v*) 7.5 mL	1 d 1 d 2 d 4 wk 12 h ×3		[43]

"h" means hours, "d" means days, "wk" means weeks, "×" means the number of times.

4. Animal selection

4.1 Strain

The differences between animal strains should be taken into account when selecting experimental animals for the construction of ALD models. Different rodent strains exhibit varying levels of alcohol sensitivity, making the selection of the appropriate strain a pivotal factor in successfully establishing ALD models. Almost all the available strains of rat (e.g. Sprague-Dawley, Wistar, Long-Evans, Fischer-344, Brown Norway, Lewis) and mouse (e.g. C57BL/6, BALB/c, ICR, 129S1/SvlmJ, C3H, Kunming, CRAMP KO) have been documented for ALD studies [44]. Among these strains, Wistar, Sprague-Dawley, ICR, C57BL/6, and BALB/c exhibit greater sensitivity to alcohol (Fig. 2a). Ordinarily, rodents show an aversion to alcohol and do not actively ingest it. However, the C57BL/6 strain is notable for its preference for alcohol and is more inclined to actively consume it compared to other strains. Moreover, this strain exhibits a lower mortality rate during the modeling process [45]. Therefore, when constructing an ALD model that closely mimics human alcohol consumption, prioritizing the selection of C57BL/6 mice is recommended, followed by considering the other four aforementioned strains as secondary options.

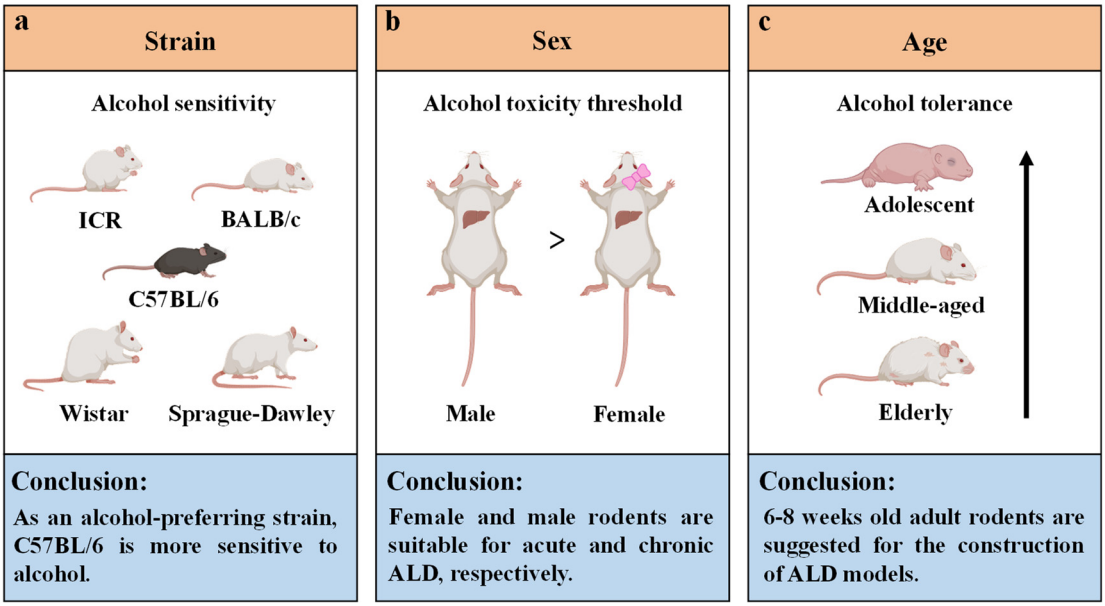

Fig. 2 The factors affecting rodent selection. The (a) strain, (b) sex, and (c) age.

4.2 Sex

Sex plays a significant role in developing ALD, both epidemiological and zoological research achievements have shown that liver injury tends to progress more swiftly in women, with women also exhibiting a lower threshold for alcohol toxicity compared to men (Fig. 2b) [46]. While women are more susceptible to initial injury after alcohol consumption, they demonstrate a lower likelihood of progressing to cirrhosis and end-stage hepatocellular carcinoma. Gao et al. [6] have indicated that short-term heavy alcohol consumption renders female C57BL/6 mice more susceptible to ALD, whereas liver damage in male C57BL/6 mice is more likely to arise from long-term consumption of smaller alcohol amounts over a span

of 12 weeks. These findings underscore the significant role of animal sex in the success of ALD-related modeling research. Consequently, when constructing acute ALD models, preference should be given to female rodents, while male rodents are more suitable for the construction of chronic ALD models.

4.3 Age

Animal age has a great influence on the establishment of ALD models. In general, the alcohol tolerance of rodents decreases with age. Previous results have shown that adolescent rodents are less sensitive to sedative-hypnotic, motor impairment, and cognitive impairment caused by alcohol. This phenomenon may lead to heightened acute alcohol tolerance and increased alcohol consumption when using adolescent rodents for ALD (Fig. 2c) [47]. In contrast, aging livers are more susceptible to the effects of ALD. Middle-aged and elderly rodents are more sensitive to alcohol, which is susceptible to liver tissue damage and fibrosis [48]. In addition, the high sensitivity of middle-aged and elderly rodents to alcohol is also a major cause of increased mortality during ALD modeling. Therefore, in order to improve the success rate of ALD model and reduce mortality, it is generally recommended to select adult mice or rats at 6-8 weeks of age for the construction of ALD models [49].

5. Animal feeding

5.1 Environment

The environment is considered to be one of the most easily neglected factors during the modeling of ALD. It is believed that factors such as illumination, temperature, humidity, and pathogens can directly affect the physical condition of rodents during modeling. Altering these factors unfavorably can directly influence rodent survival rates, subsequently affecting experimental outcomes. Firstly, hypothermia is the main factor causing the death of rodents during the construction of ALD model [6]. Therefore, the room temperature should not be too low during modeling, which is kept at 21-25 ℃ [50]. Next, excessive alcohol consumption can promote the urination in rodents, which leads to wet bedding and accelerated hypothermia in mice. Therefore, the indoor humidity is generally maintained in the range of 40% − 60% [51], and bedding must keep dry at all times. Synchronously, alcohol metabolism is affected by circadian rhythm factors. The blood alcohol content is higher during the physiological day, and the rate of alcohol metabolism slows down at this moment. Therefore, a stable circadian rhythm in rodents is essential during ALD modeling [52]. Generally, the rodents are usually placed in a room with a cycle of 12 h each light/dark [53]. In addition, the cage should be cleaned and sterilized regularly to prevent the invasion of pathogens. For example, bacteria that are susceptible to rodents such as *Shigella* and *Pasteurella* can also affect the construction of ALD models. In conclusion, it is necessary to pay attention to the illumination, temperature, humidity, and hygiene of the environment momently and make adjustments to ensure the success of the experiment during the process of model animal rearing.

5.2 Diet

The structure of animal diet is also a crucial factor in the formation of ALD. It has been shown that a diet with high fats and carbohydrates elevates the susceptibility of rodents to ALD [54, 55]. More serious liver damage could be formed by the oral administration of high concentration alcohol based on a high fat and carbohydrate diet [56]. The diet for ALD model animals can be divided into solid and liquid fodder. The 45%, 60% (kcal%) high-fat solid fodder (Table 2) and 60% (kcal%) high-fructose solid fodder (Table 3) are commonly used during modeling. The liquid fodder also refers to the Lieber-DeCarli liquid diet, which is designed by Lieber et al. [57] to mitigate rodents' natural aversion to alcohol. The Lieber-DeCarli liquid diet is an isocalorically-controlled (0.6-1.0) kcal/mL liquid diet while modifying specific components to cater to distinct groups and experimental goals [5]. The Lieber-DeCarli liquid diet used for control group rodents usually consists of fat (mainly olive oil and corn oil), protein (mainly methionine and cysteine), and carbohydrate (mainly dextrin and maltose). In contrast, the Lieber-DeCarli liquid diet for model group rodents is usually supplemented with an alcohol fraction equivalent to 28% or 36% of total calories instead of the carbohydrate in the diet (Table 4,) [42]. Therefore, the proportion of fat and carbohydrate should be adjusted appropriately to ensure that the ALD model can be successfully constructed.

Table 2. High-fat solid fodder formula for rodents during modeling.

	Normal		45% high-fat		60% high-fat	
	Mass ratio (%)	Caloric ratio (%)	Mass ratio (%)	Caloric ratio (%)	Mass ratio (%)	Caloric ratio (%)
Fat	18.80	20.54	22.51	19.54	23.25	18.14
Protein	5.20	12.79	24.15	47.18	34.55	60.65
Carbohydrate	61.00	66.67	38.34	33.29	27.20	21.22

Table 3. High-fructose solid fodder formula for rodents during modeling.

	Normal		60% high-fructose	
	Mass ratio (%)	Caloric ratio (%)	Mass ratio (%)	Caloric ratio (%)
Protein	19.2	20	19.2	20
Starch	8.5	8.9	33.2	34.5
Sucrose	0.9	1	34.1	35.5
Fructose	57.8	60.1	0	0
Fat	4.3	10	4.3	10

Table 4. Lieber-DeCarli liquid diet formula for rodents during modeling.

	Normal	28% Lieber-DeCarli liquid diet	36% Lieber-DeCarli liquid diet
Fat (%)	35	35	35
Protein (%)	18	18	18
Carbohydrate (%)	47	19	11
Ethanol (%)	0	28	36

6. Alcohol intervention

6.1 Method

Currently, several oral administration methods have been developed for constructing ALD models, with the most frequently utilized being the free-drinking method and the gavage method. The free-drinking method is the practice of inducing ALD by allowing rodents to drink alcohol solution freely (Fig. 3a).

Although the process of human alcohol consumption is recreated by this method, it is difficult to control the alcohol intake of rodents due to their inherent aversion to alcohol and their strong ability to metabolize alcohol. Hence, this method tends to cause less liver damage [58]. To counterbalance the inherent low alcohol intake, the Lieber-DeCarli liquid alcohol diet is frequently employed alongside the free-drinking method for modeling. This approach effectively triggers chronic ALD by facilitating substantial alcohol consumption in rodents [49]. The gavage method involves inducing ALD by directly administering alcohol solution, at a specific concentration, into the stomach of rodents using a gavage apparatus (Fig. 3b). Compared with the free-drinking method, the alcohol intake of rodents during the modeling period can be ensured maximumly by this method, which is widely used to construct acute and chronic ALD models. However, there are high requirements for the operator, and the essentials of gavage administration must be mastered to avoid asphyxiation and tissue contusion in rodents. In addition to using either alcohol intervention method individually, a combination of free-drinking and gavage methods can also be used to construct ALD models. Therefore, it is necessary to comprehensively consider factors such as the type of ALD model and the proficiency of the operator to select an appropriate alcohol intervention method.

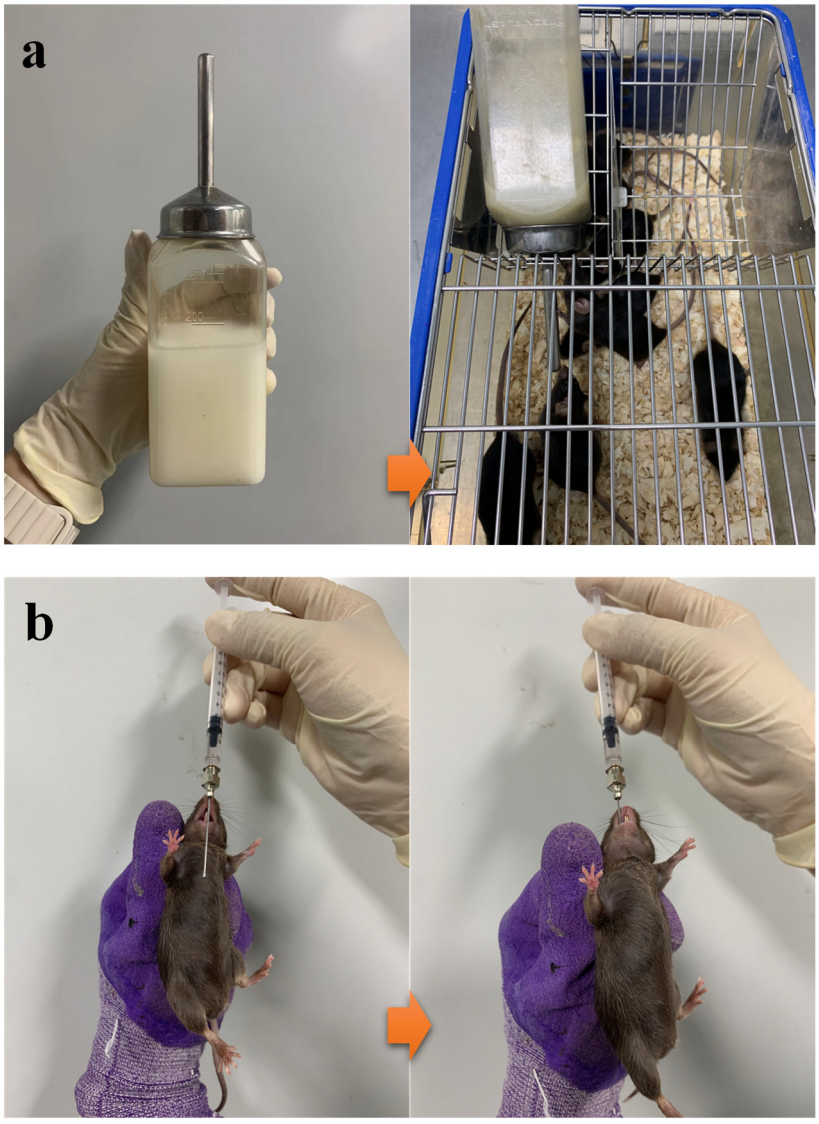

Fig. 3 The alcohol intervention of (a) free-drinking, and (b) gavage method.

6.2 Dose

Appropriate alcohol intake should be one of the most important factors during the establishment of ALD. The alcohol intervention dose should be comprehensively considered according to the type of ALD model, alcohol intervention method, and difference in rodents. It is well known that there are great differences in the tolerance of different animals to alcohol. Therefore, it is necessary to make a certain conversion when considering the alcohol intervention dose of different rodents according to the body surface area. The dose conversion of alcohol intervention in rodents follows the formula below:

$$A*Km_A=B*Km_B \quad (1)$$

where A and B represent the alcohol intervention dose of two different animals, and different animals have different Km factors. The human and common rodent body weight, body surface area, and Km factor are shown in the Table 5. In acute ALD models, mice are usually given 12 mL/kg body weight of 50% alcohol solution (high-dose) at one time to induce liver injury [18]. However, for some strains with high alcohol tolerance, such as C57BL/6, the alcohol concentration can be increased to 56% (*v/v*).

For the construction of chronic ALD models, a low dose of long-term administration is generally used. In the establishment of the chronic ALD model by the free-drinking method, there is no significant difference in the concentration of alcohol given to rats and mice for free-drinking. Typically, Lieber-DeCarli liquid diet is dissolved in 4% − 6% (*v/v*) alcohol for rodents, and the consumption of alcohol is roughly estimated to be about (10 − 20) g/kg body weight everyday [59]. In addition, rodents are usually given the 10% − 15% alcohol solution when establishing chronic ALD models by free-drinking [30]. In the construction of a chronic ALD model by gavage, the dose of alcohol intervention is different for the heavier Sprague-Dawley and Wister rats than BALB/c and C57BL/6 mice. In general, the mice and rats are gavaged at a dose of (2-6) g/kg and (1-3) g/kg body weight, respectively. Collectively, the type of ALD model, alcohol intervention method, and difference in rodents should be considered roundly in the modeling process to select the appropriate alcohol intervention dose.

Table 5. Body weight, body surface area, and Km factor of human and common rodents.

Animals	Body weight (kg)	Body surface area (m^2)	Km factor
Human	60	1.6	37
Mouse	0.02	0.007	3
Rat	0.15	0.025	6

6.3 Period

The duration of the modeling process also varies based on the type of ALD model being employed. Acute ALD is a liver disease caused by a high-dose intake of alcohol over a short period, which is essentially an oxidative stress response [60]. After (1 − 2) h of alcohol intake, the peak of alcohol concentration in the blood can be easily reached, and excess reactive oxygen species are generated through metabolism to induce oxidative damage. Rodents are sacrificed after a single ingestion of 12 mL/kg body weight of 50% alcohol for 12 h, and obvious liver damage can be found by histopathological examination [18]. It is generally considered that modeling of chronic ALD is a long-term process, with symptoms typically

appearing after alcohol intake for 3 − 4 weeks. Because of the difference in the alcohol intervention dose, early symptoms of ALD can also be detected within 1 − 2 weeks, a month, or even beyond. Therefore, multiple factors, such as ALD model type, alcohol intervention method and dose, need to be considered in the process of modeling. Furthermore, real-time monitoring of clinical symptoms of ALD in rodents is essential to adjust the duration of oral administration accordingly and achieve the intended objectives.

7. Evaluation standard

7.1 Behavior

Numerous studies have revealed that rodents display diverse levels of abnormal behavior following alcohol ingestion. In the case of acute alcohol injury, abnormal behavior in rodents could be clearly observed after 10 min of high-dose alcohol intake. Rodents also exhibit a wobbly gait, bloodshot eyes, rapid breathing, and increased righting reflex recovery time, which returns to normal in about 0.5-2 h [61]. During the later stages of chronic ALD modeling, rodents were observed to have lethargy, reduced activity, dull fur, slow-growing or even decreased weight, soft stools, and other phenomena. However, relying solely on visual symptoms is insufficient to determine the presence of ALD in rodents, Comprehensive assessments are necessary, encompassing biochemical indicators and liver histopathological examinations, to make informed judgments.

7.2 Biochemical indicator

Biochemical indicators play a crucial role in determining the presence of ALD in rodents. Typically, ALD rodents exhibit pronounced abnormalities in biochemical indicators present in both serum and liver tissues. When compared to healthy rodents, the levels of enzymes that can reflect liver function, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), acetaldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), and γ-glutamyl transpeptidase (γ-GT), tend to be elevated [62]. ALT and AST are often regarded as key indicators reflecting the degree of liver damage, which are also used to determine the success of modeling [63]. In terms of the content of endogenous lipid level markers, the rodents with ALD mainly showed an increase in total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) [64]. In addition, the abnormal levels of relevant markers reflecting the extent of oxidative damage are found in rodents with ALD, such as decreased levels of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) and increased levels of malonaldehyde (MDA) [65]. In studies of ALD, various frequently measured biochemical indicators can be selected to holistically assess the success of modeling.

7.3 Tissue

The observation of liver tissue is the most direct way to determine whether a rodent has ALD. Normally, a combination of hematoxylin and eosin (H&E) is used to stain the liver tissue to analyze the liver histomorphology of rodents affected by ALD. Characteristic histological changes (Fig. 4) are observed in most livers of rodents with ALD, including cell ballooning, steatosis, lobular inflammation, fibrosis, and

cirrhosis [66]. Ballooning is the most frequently observed histomorphology in rodents with acute ALD. In addition, steatosis and lobular inflammation can be found in the vast majority of rodents with chronic ALD. As the disease advances, fibrosis and cirrhosis are detected in the livers of a minority of rodents with severe ALD. Therefore, the success of the ALD model can be determined based on the histopathological examination of the liver.

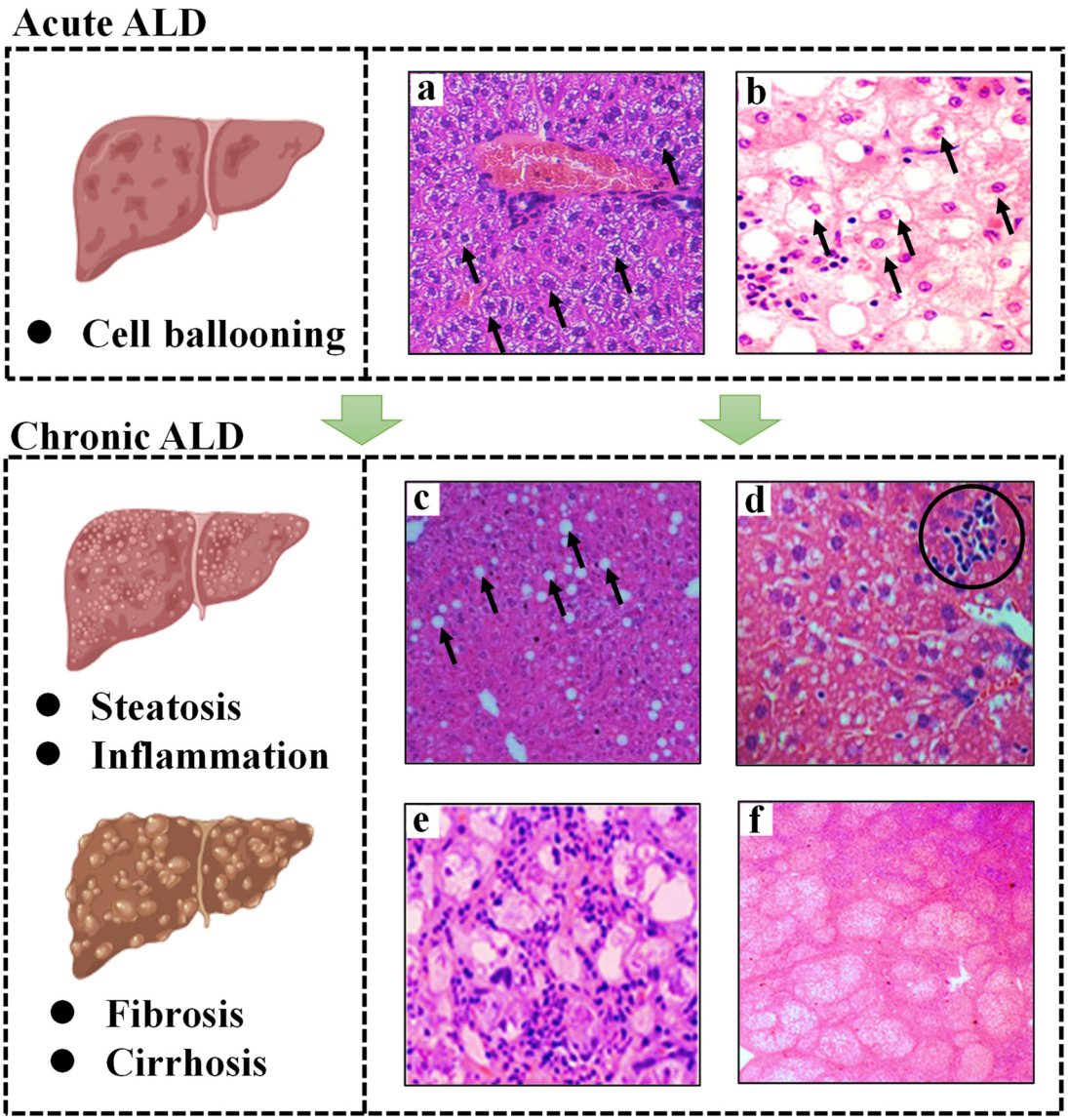

Fig. 4 Liver tissue sections from rodents with ALD. (Copyright, 2018, Wolters Kluwer Health Lnc.) (a) Mild cell ballooning, (b) serious cell ballooning, (c) steatosis, (d) lobular inflammation, (e) fibrosis, and (f) cirrhosis.

8. Modeling methods

Outlined in Fig. 5 are the modeling techniques for both acute and chronic ALD, presented for the reader's reference and adaptable to specific experimental requirements. All experimental procedures and methods are subject to approval by the Experimental Animal Welfare and Ethics Committee.

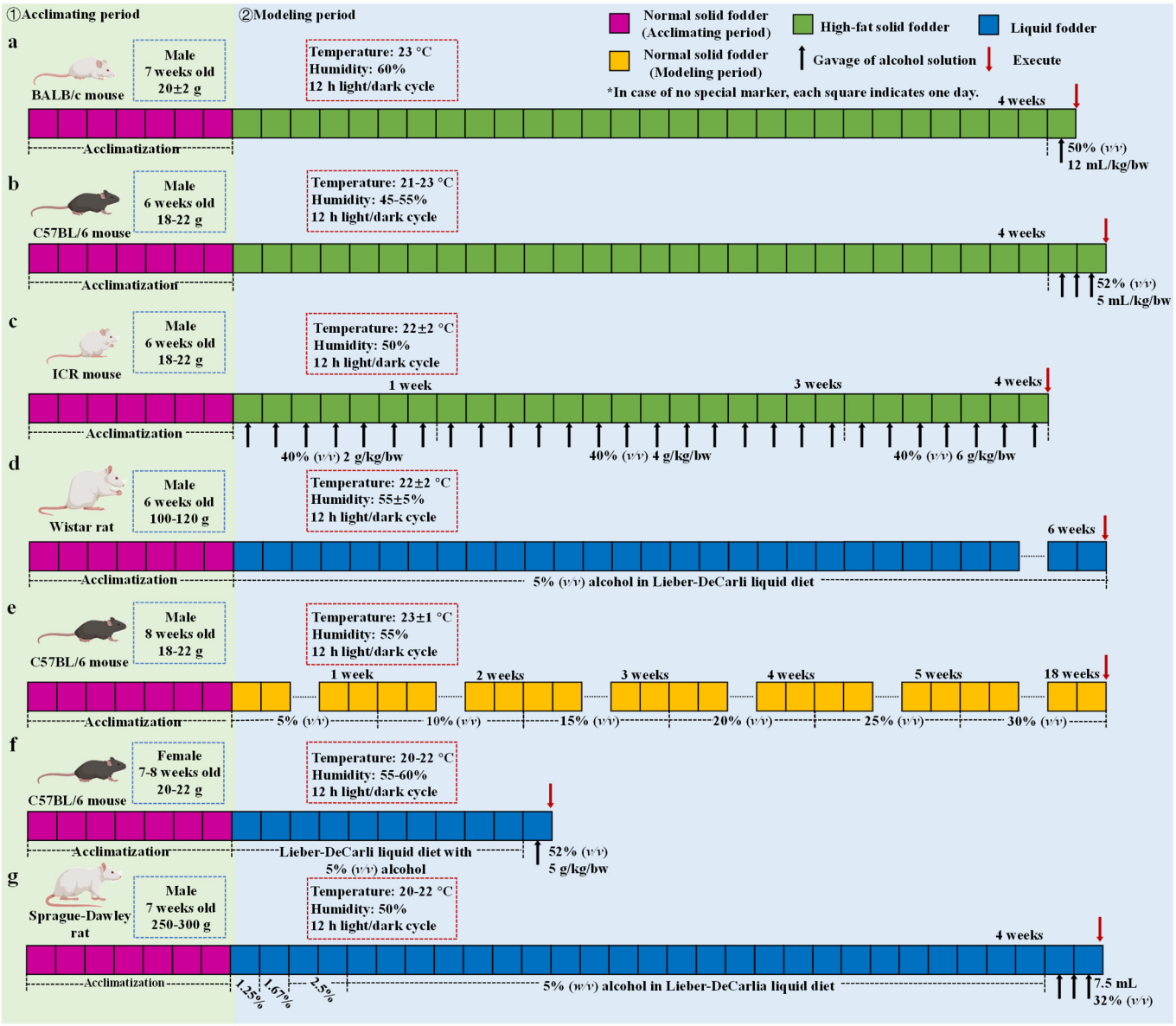

Fig. 5 The establishment of acute and chronic ALD models by the method of (a) single gavage of high-dose alcohol solution, (b) multiple gavages of low-dose alcohol solution, (c) long-term gavage of alcohol solution, (d) long-term free-drinking of alcohol liquid diet, (e) long-term free-drinking of alcohol solution, (f) long-term free-drinking of alcohol liquid diet & single gavage of alcohol solution, and (g) long-term free-drinking of alcohol solution & multiple gavages of alcohol solution.

8.1 Acute ALD model

8.1.1 Single gavage of high-dose alcohol solution

The male BALB/c mice (7 weeks old) are chosen for the construction of the ALD model. Mice with an average weight of (20 ± 2) g are selected for the experiment. The mice are acclimated in a specific-pathogen-free room (23 °C), during which the mice can obtain sterile solid food and distilled water freely. The mice are fed with 60% (kcal%) high-fat solid fodder for 4 weeks. After 4 weeks, the mice are given 50% (*v/v*) alcohol solution by gavage at a dose of 12 mL/kg body weight to establish an acute ALD model (Fig. 5a) [18]. The high-fat solid fodder is purchased from Serve Life Science Co., Ltd. (Shanghai, China).

8.1.2 Multiple gavages of low-dose alcohol solution

6-week-old C57BL/6 mice (male) are chosen for establishing acute ALD models. Mice are housed in a clean room with a controlled temperature of (21 − 23) ℃ and humidity of 45% − 55%. The room has alternating light-dark cycles (12:12 h). All mice can freely take the standard feed (normal solid fodder) and distilled water for 7 days to accommodate the experimental conditions, following this, they are transitioned to a 60% (kcal%) high-fat solid diet for 4 weeks. Finally, the mice are administered with 52% (*v/v*) alcohol (5 mL/kg body weight) every 12 h for 3 times (Fig. 5b) [67].

8.2 Chronic ALD model

8.2.1 Long-term gavage of alcohol solution

ICR mice (male, 6 weeks old, 18 g − 22 g) are selected for modeling. After 7 days of acclimatization, mice are orally administered 40% (*v/v*) alcohol solution every day with a weekly increase in the dose (2 g/kg body weight of alcohol in the first week of the experiment, 4 g/kg body weight of alcohol in the second and third week, and 6 g/kg body weight of alcohol in the fourth week, respectively) to establish a chronic ALD model (Fig. 5c) [68]. The mice are given to the high-fat solid fodder during modeling.

8.2.2 Long-term free-drinking of alcohol liquid diet

The 6-week-old Wistar rats (male, 100 g − 120 g) are purchased for the construction of a chronic ALD model. During the first week, rats are free to get standard food and water. After one week of acclimatization, rats in the model group are switched to the Lieber-DeCarli liquid diet with 5% (v/v) alcohol for 6 weeks (Fig. 5d) [69]. The rats in the control group are provided with a control Lieber-DeCarli liquid diet without alcohol. The Lieber-DeCarli liquid diet was fed in the control group, and the proportion of energy provided by fat, protein, and carbohydrate was 35%, 18%, and 47%, respectively. The proportion of energy in the model group provided by fat, protein, carbohydrate, and ethanol was 35%, 18%, 11%, and 36%, respectively. The Lieber-DeCarli liquid diets are purchased from Dyets Inc. (Bethlehem, USA).

8.2.3 Long-term free-drinking of alcohol solution

Male C57BL/6 mice (8 weeks old, 18 g − 22 g) are bought for establishing the chronic ALD model. The mice are housed for 7 days to adapt to the situations, including temperature (25 ℃), humidity (55%), illumination (lights on 7:00 − 19:00), and diet (standard mouse food pellets and tap water *ad libitums*). During the first week of modeling, the mice are allowed to drink distilled water containing 5% (*v/v*) alcohol. Gradually, the alcohol content increases from 5% (*v/v*) to 30% (*v/v*), with a 5% increase each week until week 18 (Fig. 5e) [70].

8.2.4 Long-term free-drinking of alcohol liquid diet & single gavage of alcohol solution

Female mice (C57BL/6, 20 g − 22 g, 7-8 weeks) are used for the construction of a chronic ALD model. After 5 days of a liquid diet for adapting, mice in the model group are fed on the Lieber-DeCarli liquid diet containing 5% (*v/v*) alcohol for 10 days. The control group mice are pair-fed with the liquid diet without alcohol. On day 11, mice are allowed to receive a single dose of 52% (*v/v*) alcohol solution (5 g/kg body weight) by oral gavage in the morning to construct the ALD model (Fig. 5f) [71].

8.2.5 Long-term free-drinking of alcohol solution & multiple gavages of alcohol solution

Sprague-Dawley rats (male, 7 weeks, 250 g − 300 g) are bought for the experimentation. After one week of acclimatization, the rats are transitioned to the Lieber-DeCarli diet. Alcohol is incrementally introduced into the liquid diet, beginning at 1.25% (w/v) on day 1, escalating to 1.67% (w/v) on day 2, further increasing to 2.5% (w/v) on days 3 and 4, and ultimately stabilizing at 5% (w/v) for the subsequent 4 weeks. The control group rats are fed a control liquid diet in which alcohol is replaced by dextrin/maltose to maintain the isocaloric intake. After 4 weeks, alcohol (32% *v/v*, 7.5 mL) is injected through the oral cavity to the stomach at 12-hour intervals to construct the ALD model (Fig. 5g) [43].

9. Conclusion

This review comprehensively addresses a range of topics associated with the establishment of ALD models, elucidating the formation mechanism, considerations in animal selection, approaches to animal feeding, methods of alcohol intervention, and the standards for evaluation. In addition, some construction methods of ALD models were provided, hoping to provide references for researchers. According to the type of ALD model, the alcohol intervention method, alcohol intervention dose, and alcohol intervention period will be different, which requires corresponding adjustment by researchers. Finally, due to the particularity of animal experiments, the dose and period of oral administration should be adjusted according to the observation of animal behavior during modeling to ensure the successful establishment of ALD models.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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