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Research article

Effects of *Citrus depressa* Hayata juice on high-fat diet-induced obesity in HBV transgenic mice

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ABSTRACT

The present study investigated the potential anti-obesity properties of *Citrus depressa* Hayata (CDH) juice in HBV transgenic mice, as well as the impact of fermentation on the effectiveness of the juice. The results revealed that fermentation increased the levels of polyphenols and hesperidin in CDH juice. The animal study demonstrated that both juices were effective in mitigating the weight gain induced by a high-fat diet by correcting metabolic parameter imbalances, reducing hepatic lipid accumulation, and reversing hepatic immune suppression. Furthermore, fermented juice exhibited superior efficacy in managing body weight and inhibiting the expansion of white adipose tissue (WAT). Fermented juice significantly enhanced adiponectin production and PPARγ expression in WAT, while also reducing hypertrophy. This study offers valuable insights into the potential role of CDH juices in combating obesity associated with high fat consumption and underscores the promise of CDH juice as a functional beverage.

1. Introduction

Chronic hepatitis B virus (HBV) infection continues to pose a significant global health challenge. A comprehensive international study revealed that 296 million individuals were living with chronic HBV infection and HBV-related ailments resulted in the deaths of 555,000 people globally in 2019 [1]. Regions such as Asia and sub-Saharan Africa have the highest prevalence rates, ranging from 5 %

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to 10 % [2]. In general, chronic HBV patients might exhibit weight loss or a leaner appearance in certain populations, implying a lower prevalence of obesity compared to normal individuals, substantial prevalence rates are observed in various regions. In China, rates reach up to 29.9 % [3], while in Taiwan, they range from 28.9 % to 42 % [4,5] and in Korea, they range from 30.7 % to 38.9 % [6,7]. Additionally, high waist circumference prevalence stands at 29.3 % in the USA [8]. Hence, a lean appearance in HBV carriers may not universally apply to all HBV patients worldwide. Obesity is still an alarming alarmingly increasing global health issue. A retrospective analysis (from 1990 to 2019) emphasized the escalating trend for the burden of hepatitis B linked to high BMI, indicating a potential association between higher BMI and the impact of HBV [9]. More recent studies elucidated the pivotal role of obesity and metabolic syndrome in the progression from a healthy HBV carrier state to liver cancer and liver-related mortality [10–12]. A large cohort study using data from the Korea National Health Insurance Service highlighted a direct, dose-response correlation between body mass index (BMI) and the risk of liver cancer. Specifically, severely obese men had a hazard ratio (HR) of 1.22 for liver cancer, while the association was even more pronounced for females, with severely obese women having a higher hazard ratio of 1.46 [6]. Another population-based study conducted in Taiwan with approximately 24,000 subjects demonstrated that obesity was linked to a 1.36-fold higher risk of hepatocellular carcinoma among HBV-infected individuals [13]. The causes of obesity in HBV patients have not yet been conclusively determined. HBV itself does not induce obesity; instead, it showed an inverse association with obesity in HBV carrier [14]. Regarding obesity, the urbanized lifestyle, characterized by low energy expenditure and high-fat processed food consumption, appears to contribute. Mitigating high-fat diet-associated obesity in HBV patients seems beneficial for their overall health.

Citrus depressa Hayata (CDH) is a small-fruited citrus cultivar that is distributed from the south-western regions of the Japanese islands to Taiwan [15]. The ripe fruits of CDH are typically harvested during the winter season [16]. Most of the harvest is processed into food products, such as juice, which has an abundance of bioactive compounds including ascorbic acid and polyphenols [17]. Polyphenolic compounds in citrus, predominantly flavonoids, encompass naringenin, naringin, hesperidin and methoxylated flavones (PMFs) like nobiletin, recognized as primary components with anti-HBV properties [18–26]. These polyphenolic compounds also have demonstrated benefits in treating obesity, cardiovascular diseases, metabolic syndrome, and exhibiting antiviral activities [27,28]. In a study focusing on HBV, nobiletin—a flavonoid compound derived from *Citrus depressa* — was found to inhibit the production of hepatitis B surface antigen (HBsAg) and suppress hepatitis B virus replication in mice [22]. Considering their impacts on obesity in vivo animal experiemnts, the extract of CDH peel inhibited weight gain, improved biochemical parameters, and reduced the expression of lipogenesis-related genes or enzymes in high-fat diet (HFD)-fed mice [19,29,30]. The important bioactive flavonoid compound in CDH, hesperidin, has been suggested as a therapeutic agent for obesity and metabolic syndrome due to its effect on lipid metabolism and adipokine production [26]. Hesperidin-enriched dietary supplements can significantly improve obesity symptoms such as postprandial hyperglycemia and hyperlipidemia [31]. In addition, hesperidin has been observed to induce a significant decrease in triacylglycerol content in preadipocytes [32]. Although an extract of CDH peal mixed with the diet can inhibit weight gain in mice, the bio-effect of CDH juice has never been investigated. According to these studies, CDH juice can be a good candidate for preventing obesity in HBV carrier. However, the effect of CDH juice in HBV carrier has never been investigated.

Fermentation is a common method used since prehistory to preserve and develop the flavor of food. In the present era, it has emerged as a bioprocessing strategy to augment health-promoting properties, including increasing phenolic content and enhancing its nutritive value [33,34]. Fruit juices containing abundant nutrients are considered good vehicles for *Lactobacillus* bacteria [35]. During *Lactobacillus* fermentation, macromolecular compounds may convert to smaller compounds with higher bioactivity, which improves the nutritional quality of vegetable and fruit products [36]. Several studies have shown a significant increase in total polyphenol content and antioxidant activity in fermented fruit juices [35,37–39]. Moreover, the fermentation of fertile orange citrus pomace causes a surge in antioxidative efficacy and polyphenol content, notably elevating the hesperidin content, which undergoes a several-fold increase across varying conditions [40]. In light of these findings, it is possible that the fermentation of CDH juice could potentially mirror this increase in polyphenols and hesperidin content, offering a substantial benefit for managing obesity caused by HFDs.

Based on these studies, we anticipate that CDH juice would be effective against obesity in HBV carriers, and that the fermented juice would show higher efficacy than the unfermented juice. To test the anti-obesity effect of CDH juice in HBV-infected organisms, the HBV transgenic mouse model was employed, and the mice were administered daily with fermented or unfermented CDH juices. Liver tissue, white adipose tissue (WAT), and serum samples were collected. The anti-obesity effect was evaluated by observing the alterations in body weight, metabolic parameters, liver health, and WAT expansion.

2. Materials and methods

2.1. Preparation of CDH juice and fermentation

CDH from Pingtung County, Taiwan, was purchased (JIAN MAO BIOTECH CO, Kaohsiung, Taiwan). The fruit underwent a process involving surface washing, followed by air-drying. Subsequently, juices were extracted using a mechanical juice extraction method. The juice was then inoculated with *Lactobacillus* bacteria and yeast at room temperature, undergoing a fermentation process for 14 days. After fermentation, the liquid was filtered, sterilized, and stored at room temperature.

2.2. Total polyphenol and hesperidin contents and SOD-like activity determination

The total polyphenol contents of CDH juices and its fermentation formula were analyzed by SGS Taiwan Ltd., Taipei, Taiwan, using spectrophotometric method (AOAC 952.03). The hesperidin content of two aforementioned products were determined by Jackson

International Standard Inspection Co., Ltd, Tainan, Taiwan using high-performance liquid chromatography (HPLC) equipped with the photo diode array detector (PDA) [41,42]. The SOD-like activity of two aforementioned citrus juices was analyzed and determined by SGS Taiwan Ltd., Taipei, Taiwan, using ultra-weak chemiluminescence method.

2.3. Nutritional facts

The both products of nutrition contents were analyzed by JTTEC SERVICE CORPORATION, Taipei, Taiwan and the experiments were performed through a variety of certified methods. Typical analyses include: Protein was determined by analysis of total nitrogen, either by Semimicro-Kjeldahl methods (CNS 5035 (1986));

Total fat was analyzed by the ether extraction equipped with Soxhlet apparatus methods of test for crude fat in food (CNS 5036 (1984)); Total sugars, normally by a liquid chromatography technique, such as HPLC (CNS 12634 (2006)); Moisture (water) and crude ash (total inorganic matter) were measured by national standard methods (CNS 5033 (1984), CNS 5034 (1984)).

2.4. Animals and diets

Chronic HBV infection is a persistent state of HBV presence. Clinically, it is defined as the continuous detection of HBsAg in the serum for at least 6 months following acute infection. Since mice cannot be directly infected with HBV, a model of transgenic mice expressing HBV genes has been developed to simulate HBV carriers, a model of transgenic mice expressing HBV genes has been developed to simulate HBV carriers [43]. Female 8-week-old C57bl/6J HBV transgenic mice were generously provided by Dr. Wen-Lung Ma (Sex Hormonal Research Center, China Medical University, Taichung, Taiwan). These transgenic mice carrying the whole HBV genome have been established using the methods described previously [44]. The expression of HBV was confirmed in each mouse after birth. All experimental protocols followed animal care guidelines and were approved by the Institutional Animal Care and Use Committees at China Medical University (IACUC NO. 2021-398). The mice were housed in a specific pathogen-free room with controlled temperature and humidity (25 °C, 70 % humidity), under a 12-h light/dark cycle, and were given unrestricted access to food and water. To induce obesity, the mice were fed either HFD (58Y1; TestDiet, New Brunswick, NJ, USA) (containing 60 % of calories from fat) or normal diet (ND) (5001; LabDiet, Richmond, IN, USA). The mice were then randomly divided into five groups, each consisting of eight animals (n = 8): ND group, Mice were fed with ND and orally treated with distilled water (20 mL/kg, by gavage); HFD group, Mice were fed with HFD and orally treated with distilled water (20 mL/kg); HFD + J group, Mice were fed with HFD and orally treated with unfermented CDH juice (diluted 5 times in distilled water, 20 mL/kg); HFD + FJ(L) group, Mice were fed with HFD and orally treated with fermented CDH juice (diluted 10 times in distilled water, 20 mL/kg); HFD + FJ(H) group, Mice were fed with HFD and orally treated with fermented CDH juice (diluted 5 times in distilled water, 20 mL/kg). The dosages of juice administered in the treatments were 4 mL/kg in the HFD + J group, 2 mL/kg in the HFD + FJ(L) group, and 4 mL/kg in the HFD + FJ(H) group, respectively. Body weight and food intake were monitored throughout the experiment. After 12 weeks, mice were anesthetized with 2 % isoflurane (Darien, IN, USA), and samples of blood, liver tissue, and perigonadal fat tissue were collected.

2.5. Measurement of metabolic parameters in serum

Blood samples were collected from the abdominal vein and were then transferred to serum separation tubes. After centrifugation at 3000 rpm for 20 min at 4 °C, serum samples were obtained. The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TCHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG) were assessed using a blood biochemical analyzer (AU480, Beckman Coulter, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

The levels of TNF-α, IL-6 and IL-10 in tissue were detected using ELISA kits from R & D Systems (Minneapolis, MN, USA), while the levels of leptin and adiponectin in serum were measured using ELISA kit from Crystal Chem (Village, IL, USA). The absorbance was measured at 450 nm using a spectrophotometer (SPECTROstar Nano, BMG LABTECH, Ortenberg, Germany).

2.7. Histological evaluation

A histological examination was performed to assess liver injury, liver steatosis, and adipose tissue morphology. In brief, liver and adipose tissues were fixed in a 10 % phosphate-buffered formalin solution at room temperature. The fixed samples were then dehydrated using graded alcohol and embedded in paraffin. Thin sections of 5 μm were obtained from each tissue and stained with hematoxylin and eosin (H&E) for evaluating tissue morphology, as well as with oil red for assessing lipid accumulation [45]. Bright-field images were captured using a tissue scanner system (Tissue FAXS Flou, TissueGnostics, CA, USA) at 40 × magnification. Adipose tissue sections stained with H&E were analyzed for size determination which was quantified using BioTek Gen5 Software (Biotek, Winooski, VT, USA). In brief, the frozen liver sections were washed with propylene glycol for 5 min at room temperature, stained with heated Oil Red solution for 10 min, and counterstained with hematoxylin for 1 min. The quantification of the oil red stain was performed using StrataQuestso 7.1 software (Tissue FAXS Flou, TissueGnostics, CA, USA).

2.8. Western blotting

PPAR-γ protein expression was detected. The total protein was extracted from fat pad tissues by using the Minute Total Protein Extraction Kit (Invent Biotechnologies, Plymouth, MN, USA). The 30 μg total lysate protein were separated by a 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred at 100 V for 1 h onto a nitrocellulose membrane. After blocking with 5 % nonfat dry milk, the membrane was incubated with PPAR- γ antibody (1:1000 dilution for overnight; Sigma, MO, USA) or β-actin antibody (1:10000; Sigma) and then a secondary antibody. After adding HRP substrates, the expression was detected using a CCD camera (ChemiDoc, Bio-Rad, CA, USA) and quantified using the software Image Lab 5.2.1 (Bio-Rad).

2.9. Statistical analysis

The data are presented as mean ± standard deviation (SD). Statistical significance differences between groups were analyzed using an unpaired *t*-test through the GraphPad Prism 6 program (GraphPad Software Inc., San Diego, CA, USA). A *p*-value lower than 0.05 was considered statistically significant.

3. Results

3.1. The characteristics of unfermented and fermented CDH juices

The nutritional information for both juices is presented in Table 1. Calories and nutritional content, including protein, saturated fat, carbohydrates, and sugars, were found to be higher in the fermented juices than in the unfermented juices. The total polyphenol content of the fermented CDH juices (1702 μg/mL) was notably 1.98 times higher than that of the unfermented juices (861 μg/mL). Furthermore, the fermented juices had a higher hesperidin content (1752.16 μg/mL) in contrast to the unfermented juices (508.80 μg/mL) (Table 2). These findings strongly suggest that the fermentation process led to the production of a higher quantity of polyphenolic compounds. However, both juices showed similar SOD-like activity.

3.2. Effects of unfermented and fermented CDH juices on body weight gain and metabolic parameters in HBV mice with HFD-induced obesity

To clarify the efficacy of CDH in inhibiting obesity in HBV carriers and to determine whether fermentation can augment these effects, both unfermented juice and fermented juice were orally administered to HFD-fed HBV mice for a duration of 12 weeks. Body weight and food intake were recorded. The results showed that in HBV mice, both unfermented juice and fermented juice effectively inhibited the weight gain caused by HFD. In addition, the treatment with fermented juice at a lower dosage (2 mL/kg/daily) had a superior inhibitory effect on weight gain compared to the treatment with unfermented juice at a higher dosage (4 mL/kg/daily) (Fig. 1A). This suggests that fermentation enhances the anti-obesity potential of CDH juice. In this model, although HFD significantly increased the body weight gain, there were no significant differences on energy intake between the normal diet-fed group and every HFD-fed groups (Fig. 1B). The result was attributed to the fact that mice in the HFD-fed groups consume a smaller quantity of food compared to those on a normal diet. Despite the similar overall energy intakes, there is a significant distinction in nutrient composition. In the HFD-fed groups, 60 % of the energy was derived from fat which can cause metabolic syndrome. CDH juice-treated groups did not decrease the levels of energy intake in HFD-fed mice (Fig. 1B), implying that the anti-obesity effect of CDH juice was not influenced by affecting energy intake.

As metabolic dysfunction is closely associated with obesity and obesity-related diseases, the metabolic parameters in serum were evaluated. Lipid metabolic parameters, including TCHOL, HDL, LDL, and TG levels, showed an increase due to the consumption of HFD. Both unfermented juice and fermented juice mitigated these increases induced by the HFD (Fig. 2A–D). No significant differences were observed between unfermented juice and fermented juice.

Table 1
Nutrition facts per 100 mL.

	CDH juice	Fermented juice
Calories	26.6 Kcal	35.9 Kcal
Protein	0.5 g	0.8 g
Total fat	1.0 g	0.7 g
Saturated fat	0.00 g	0.07 g
Trans fat	0.00 g	0.00 g
Total carbohydrate	3.9 g	6.6 g
Total sugars	0.7 g	3.3 g
Sodium	7.1 mg	15.3 mg
Moisture	95.0 g	91.6 g
Ash	1.1 g	0.3 g

Table 2
Total polyphenol content, hesperidin content, and SOK-like activity.

	CDH juice	Fermented CDH juice
Polyphenol	861 μg/mL	1702 μg/mL
Hesperidin	508.80 μg/mL	1752.16 μg/mL
SOD-like activity	25340.88 Unit/mL	28096.31 Unit/mL

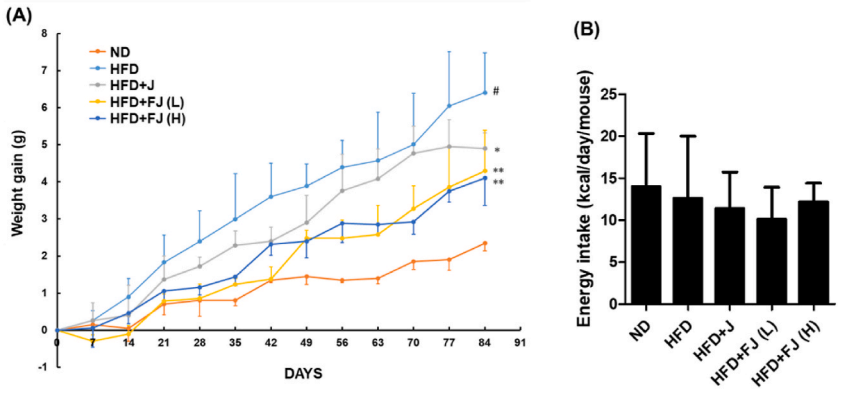

Fig. 1. Effects CDH juices on weight gain and energy intake in HFD-fed HBV mice. Mice were fed with HFD and daily given orally with water (HFD group), unfermented CDH juice 4 mL/kg (HFD + J group), fermented CDH juice 2 mL/kg (HFD + FJ (L) group), fermented CDH juice 4 mL/kg (HFD + FJ (H) group) for 12 weeks. Control mice were fed with normal diet (ND group). (A) Weight gain; (B) Energy intake. Data are presented as mean ± SD (n = 8–10). #denotes a significant difference between HFD group compared to ND group ($P < 0.05$); *denotes a significant difference between juice treating groups compared to the HFD group (*$P < 0.05$; **P < 0.01).

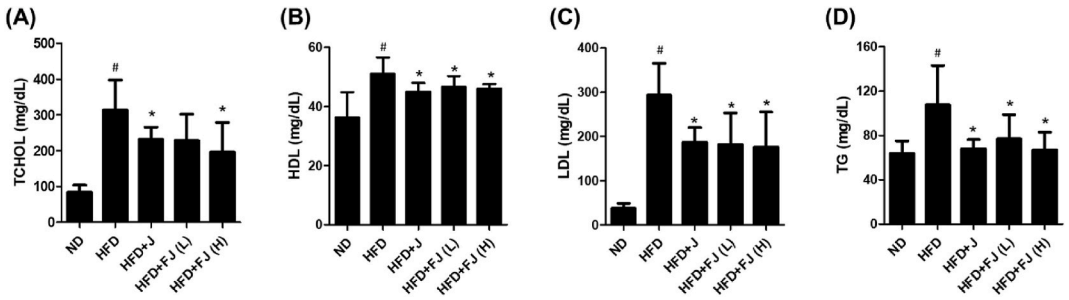

Fig. 2. Effects CDH juices on metabolic parameters. (A) Serum TCHOL level; (B) Serum HDL level; (C) Serum LDL level; (D) Serum TG level. Data are presented as mean ± SD (n = 5). #denotes a significant difference between HFD group compared to ND group ($P < 0.05$); *denotes a significant difference between juice-treating groups compared to the HFD group ($P < 0.05$).

3.3. Effects of unfermented and fermented CDH juices on liver injury, inflammation, and lipid accumulation

Liver steatosis, defined as lipid accumulation in hepatocytes, is frequently linked to HFD-associated obesity. This condition often progresses to non-alcoholic fatty liver disease (NAFLD), which can lead to severe liver damage and cirrhosis. To evaluate the effect of unfermented and fermented CDH juices on HFD-associated liver impact in HBV mice, various parameters such as liver injury indicators, liver weight, lipid accumulation, and cytokine production were assessed. Fig. 3A shows that HFD slightly increased the level of the liver injury indicator AST in the serum, which rose from 44.8 ± 5.07 to 64.00 ± 17.00 U/L. Both unfermented and fermented CDH juices at a dose of 4 mL/kg significantly reversed this increase caused by the HFD. Interestingly, the HFD did not elevate the levels of another liver injury indicator, ALT, and did not result in increased liver weight (Fig. 3B and C). Previous studies have consistently reported that 10–12 weeks of HFD treatment induced significant increases in AST and ALT levels and liver weight gain in wild-type mice [46–49]. In contrast, we found that HFD did not exert the same significant impact, as seen in wild type mice in previous studies, on the liver in HBV mice.

Histological staining with H&E showed massive microsteatosis (small vacuoles) and a few instances of macrosteatosis (large vacuoles) in the liver tissue of the HFD group. Both unfermented and fermented CDH juices reduced the steatosis in mice fed HFD (Fig. 3D). There were no signs of cell degeneration, necrosis, or inflammatory cell infiltration in these liver tissues in each group, consistent with the data showing mild liver injury indicators. However, the distribution of Kupffer cells in the livers was significantly

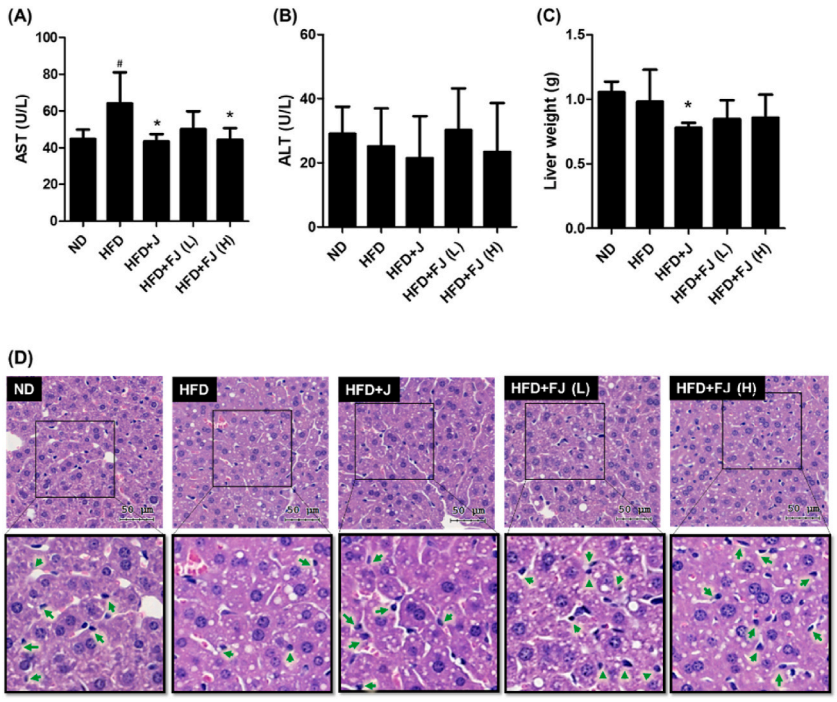

Fig. 3. Effects CDH juices on liver injury. (A) Serum AST level; (B) Serum ALT level; (C) Liver weight; (D) Histological H&E staining (x40). Massive microsteatosis (small vacuole); macrosteatosis (large vacuole); Kupffer cells (green arrow). Data are presented as mean ± SD (n = 5). # denotes a significant difference between HFD group compared to ND group ($P < 0.05$); * denotes a significant difference between juice-treating groups compared to the HFD group ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reduced in HFD-fed mice. Unfermented and fermented CDH juices attenuated the impact of the HFD on Kupffer cells (Fig. 3D). Oil Red O staining of liver tissues highlighted the presence of lipid droplets within the liver, which appeared as red particles [50]. The HFD group showed a significant accumulation of lipid droplets, while both unfermented and fermented CDH juices attenuated this lipid

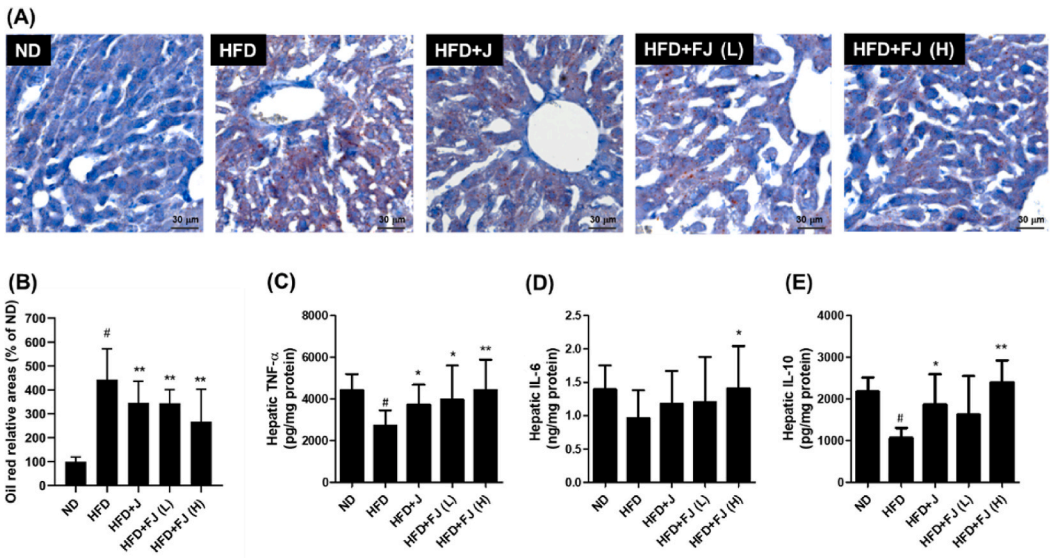

Fig. 4. Effects of CDH juices on lipid accumulation and cytokine production in liver. (A) Oil Red O staining (x40); (B) Lipid area; (C) Liver TNF-α level; (D) IL-6 level; (E) IL-10 level. Data are presented as mean ± SD (n = 8). #Significant difference between HFD and ND group ($P < 0.05$). *Significant difference between HFD and juice treating groups (*$P < 0.05$; **$P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

accumulation in liver tissues (Fig. 4A and B).

Cytokine production is closely linked to liver steatosis and is subsequently associated with chronic liver pathologies. Both TNFα and IL-6 play critical roles in the pathogenesis of NAFLD [51,52]. It is noteworthy that in our study, the HFD did not induce hepatic TNFα and IL-6 production (Fig. 4C and D). Instead, the HFD inhibited their production in liver tissues of HBV mice. Both unfermented and fermented CDH juices reversed this inhibition, restoring the levels to those of the control group (Fig. 4C and D). Furthermore, we examined the anti-inflammatory cytokine IL-10. The data showed that the HFD also led to reduced IL-10 production in the liver, which was restored by both juices (Fig. 4E). This phenomenon is consistent with the results of Kupffer cell distribution, which is the primary source of cytokine production. It appears that the HFD may induce an immunosuppressive effect in the liver of HBV mice, and CDH juices may counteract this suppression.

3.4. Effects of unfermented and fermented CDH juices on WAT expansion and adipocytokine production

White adipose tissue expansion is a primary characteristic of obesity and predominantly regulates energy metabolism within the body. We measured the weights of perigonadal fat pads and found a significant increase in the HFD group. Interestingly, only the group treated with 4 mL/kg of fermented CDH juice exhibited a significant inhibition of this increase (Fig. 5A). Histological staining with H&E of these fat pad tissues showed that adipocyte sizes were significantly smaller in HFD-fed mice treated with fermented CDH juice compared to those in the HFD-only group. However, unfermented juice failed to significantly inhibit the hypertrophy induced by the HFD (Fig. 5B and C).

Adipokines secreted by WAT play critical roles in the regulation of whole-body energy homeostasis and inflammation [53]. This makes them a potential target for the treatment of obesity. Leptin and adiponectin are two dominant adipokines studied in obesity research. Typically, obese individuals have higher serum leptin levels and lower adiponectin levels compared to normal-weight individuals. Our study showed that the increase in leptin levels and the decrease in adiponectin levels caused by the HFD were attenuated by both fermented and unfermented CDH juices (Fig. 6A and B). Adiponectin is a downstream target gene of PPAR-γ [54]. In the present study, PPARγ expression was lower in the HFD group, and was upregulated in the fermented juice treatment compared to the control group (Fig. 6C). These results suggest that fermented CDH juice has a more pronounced effect on WAT compared to unfermented juice, which may contribute to its inhibition of weight gain.

4. Discussion

This is the first study to present the bioactive effects of CDH juice supplements and to reveal the anti-obesity effect of fermented CDH juice in HBV mice. Previous studies showed the effects of CDH by applying peel extracts to obese mice [19,55,56]; these extracts required more processing to prepare and to modify their taste. The present study demonstrated that CDH juice also exhibits anti-obesity effects, suggesting that this simple CDH juice may serve as a functional beverage to improve health. In addition, this study highlighted that fermentation offers significant benefits in enhancing the content of bioactive compounds in CDH juice and improving its efficacy. Polyphenols have long been recognized as the primary components in citrus responsible for exerting anti-obesity effects by regulating lipid metabolism and inhibiting inflammation. These actions involve the inhibition of intracellular TG, fat accumulation, and lipogenesis, as well as regulating adipokine production [57–59]. Among these polyphenols, hesperidin, a dominant flavonoid found in citrus, shows significant anti-obesity activity. Hesperidin regulates lipid metabolism and glucose metabolism [26] and also mitigates inflammation and oxidative damage induced by hyperlipidemia [60]. Increasing the content of these components may enhance the anti-obesity effect. Consistent with the results observed for fermented orange citrus pomace [40], our study showed that

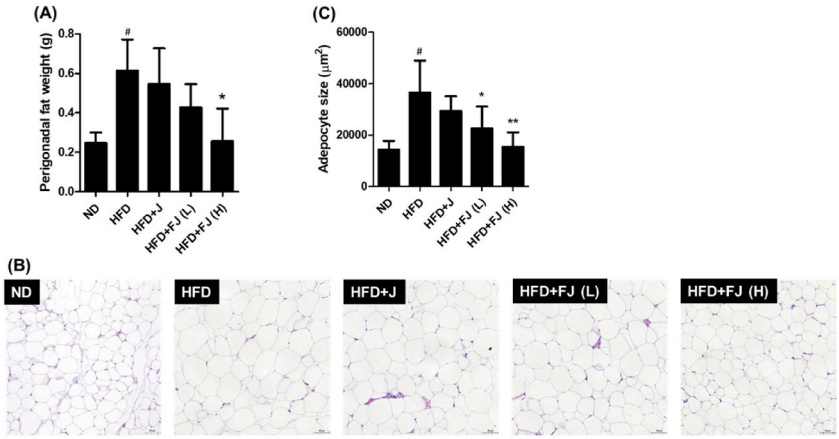

Fig. 5. Effects of CDH juices on WAT expansion and hypertrophy. (A) Perigonadal fat pads weight; (B) WAT H&E staining (x40); (C) Adipocyte size. Data are presented as mean ± SD (n = 8). #Significant difference between HFD and ND group ($P < 0.05$). Significant difference between HFD and juice treating groups (*$P < 0.05$; **$P < 0.01$).

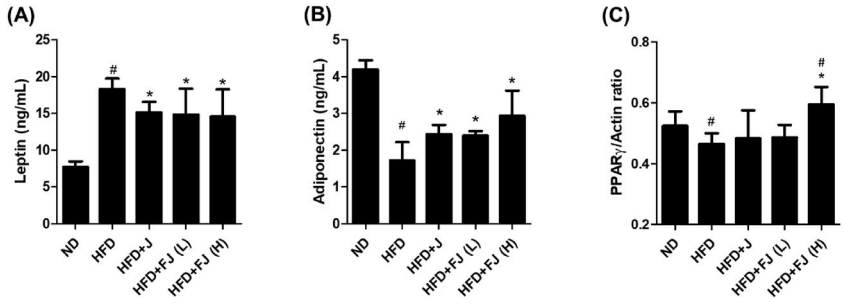

Fig. 6. Effects of CDH juices on adipokine production and PPARγ protein expression. (A) Serum leptin level; (B) Serum adiponectin level; (C) PPARγ expression in WAT. Data are presented as mean ± SD (n = 5). #Significant difference between HFD or HFD + FJ (H) and ND group ($P < 0.05$). *Significant difference between HFD and juice treating groups ($P < 0.05$).

fermentation increased the polyphenol and hesperidin content in CDH juice by about 2 times and 3 times, respectively. Our efficacy study demonstrated that while both fermented and unfermented juices mitigated body weight gain and altered the lipid profile induced by the HFD in HBV mice, the fermented juice showed superior anti-obesity properties, particularly in reducing WAT expansion. We suggest that daily CDH juice intake could be of benefit for preventing obesity in HBV carriers. In addition, fermentation transformed CDH juice into a more functionally effective beverage with anti-obesity properties.

Cirrhosis and liver cancer are the major threats to people with chronic HBV infection [61]. Although first-line nucleoside analogue therapy is effective in suppressing HBV replication, the risk of liver cancer in patients with chronic HBV is still high [62]. Avoidance of obesity could be of benefit to reduce the risk of liver cancer in chronic HBV patients. In the present study, to investigate the potential anti-obesity effect of CDH juice on HBV-infected mice, we used HBV transgenic mice that were fed an HFD for 12 weeks to simulate the process of chronic HBV patients developing obesity. Interestingly, in this particular model, we observed that liver steatosis and liver damage were not as severe as in wild-type mice. One of the liver injury indicators, the AST level, remained low and the other, the ALT level, did not increase. Although lipid accumulation significantly increased due to the HFD, it was not severe enough to induce an increase in liver weight or lead to liver cell necrosis. This observation is consistent with the lower prevalence of NAFLD among chronic HBV patients, as previously reported [63,64]. In addition, it was surprising to note that the HFD led to a decrease in the number of Kupffer cells and a reduction in cytokine production in HBV mice in the present study. In contrast, in wild type mice, HFD leads to Kupffer cell activation and cytokine production, which promotes inflammation, fatty acid synthesis, and lipid accumulation in the liver and accelerates the pathogenesis of nonalcoholic steatohepatitis [65]. However, whether an HFD leads to the elimination of Kupffer cells and subsequently induces immunosuppression in HBV-infected livers, along with the associated consequences, has never been explored. More studies are needed to investigate these effects in the future. The present study showed that both fermented and unfermented CDH juice restored Kupffer cell expression and cytokine production in the liver of HFD-fed mice. These juices helped to avoid the immunosuppression caused by the HFD, which could be beneficial in maintaining liver health in HBV carriers.

White adipose tissue serves as the primary site for storing energy in the form of TG. The expansion of WAT, leading to an increase in fat mass, is a critical factor in the development of obesity. In addition, WAT plays a role in influencing glucose and lipid metabolism throughout the body by producing adipokines [66]. One such adipokine is leptin, which suppresses food intake and regulates energy expenditure. Leptin prevents the body from triggering a hunger response when it does not require additional energy. The extent of TG storage in adipocytes is typically correlated with the serum levels of leptin [67]. Another important adipokine is adiponectin, which possesses antidiabetic, anti-inflammatory, and anti-atherosclerotic properties [68]. The expansion of WAT is associated with a reduction in adiponectin secretion [69]. Consequently, some pharmacological interventions aim to increase adiponectin production as a potential treatment or prevention for obesity-related diseases. In the present study, both fermented and unfermented juice treatments did not lead to an increase in leptin levels nor a decrease in food intake among the HBV mice. On the other hand, both juices significantly increased the levels of adiponectin in HFD-fed mice. Compared to leptin, adiponectin seems to play a more important role in the anti-obesity effects of CDH juice. Hypoadiponectin has been suggested to play a key role in the pathogenesis of obesity and related diseases. The administration of adiponectin to obese or diabetic mice can lead to a reduction in body weight and glucose levels, while also enhancing insulin sensitivity [70]. PPARγ is a regulator of adiponectin production in adipocytes [71]. In the present study, HFD decreased PPARγ expression in HBV mice. Only fermented juice significantly increased the level of PPARγ expression, which was consistent with WAT expansion and the mean expression of adiponectin. Hesperidin is a PPARγ inducer and is regarded as a PPARγ agonist [72,73]. It seems that PPARγ−associated adiponectin expression may play an important role, at least in part, in the anti-obesity effect of fermented CDH juice, which contains a high level of hesperidin.

5. Limitation

The juice samples used in this study were from the same production batch. Variations in composition may occur between juice and fermentation liquids produced in different batches, potentially resulting in differences in efficacy. Thus, if this juice is to be used as a health application in the future, their quality control (QC) needs to be examined to ensure consistency with the findings of this study. As this study is based on mouse research, further clinical trials are necessary to determine their effects on humans.

6. Conclusion

In summary, both unfermented and fermented CDH juice showed potential for managing HFD-induced obesity in HBV-infected mice. They were effective in alleviating obesity-related conditions, including weight gain, metabolic dysregulation, hepatic lipid accumulation, and hepatic immune suppression, while fermented juice, which contains higher levels of polyphenols and hesperidin, showed superior efficacy in inhibiting weight gain and WAT expansion. These effects may be attributed to the enhancement of adiponectin production, along with an increase in PPARγ expression in WAT. In conclusion, daily supplementation of CDH juice may reduce HFD-induced obesity and metabolic syndrome in HBV mice. Fermentation may magnify its anti-obesity efficacy through a PPARγ–adiponectin pathway-associated mechanism, likely due to increased polyphenols and hesperidin content in CDH juice. CDH juice, particularly the fermented variety, may serve as a promising functional anti-obesity beverage for chronic HBV carriers.

Ethics statement

All experimental protocols followed animal care guidelines and were approved by the Institutional Animal Care and Use Committees of China Medical University (IACUC NO. 2021-398).

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9. Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Pei-Yi Chu: Writing - review & editing, Writing - original draft, Project administration, Methodology, Formal analysis, Conceptualization. **Chang-Lu Hsu:** Validation, Investigation, Funding acquisition, Conceptualization. **Yen-An Lin:** Methodology, Formal analysis. **Yi-Cheng Pan:** Writing - original draft, Visualization, Software, Formal analysis. **Yun-Hao Dai:** Methodology, Formal analysis. **Ying-Chun Yu:** Methodology, Investigation. **Juan-Cheng Yang:** Investigation, Funding acquisition. **Wen-Lung Ma:** Resources, Investigation. **Yi-Jinn Lillian Chen:** Investigation, Funding acquisition, Formal analysis. **Chia-Lin Lee:** Writing - review & editing, Writing - original draft, Methodology, Formal analysis. **Yang-Chang Wu:** Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this paper, the authors used ChatGTP for grammar checking and English language editing. After using this tool, the authors have reviewed and edited the content as necessary and take full responsibility for the content of the publication.

Declaration of competing 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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