Nonalcoholic fatty liver disease (NAFLD) is common worldwide and closely associated with metabolic dysfunction. NAFLD leads to a higher risk of development of severe liver diseases, such as nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC). To date, no pharmacotherapy targeting NAFLD has received general approval. Adlay is a plant that has been used as traditional herbal medicine in Asia and is a promising candidate to solve this global issue. We have established a mouse model of NAFLD by feeding a high-fat diet (HFD) for 10 weeks. Here, ethanolic or water extracts of adlay seed (ASE and ASW, respectively), mixed with HFD, were fed to the mice for 10 weeks. The ASE and ASW treatment ameliorated hyperglycemia and improved the glucose tolerance and insulin resistance in the HFD mice. Hyperlipidemia in HFD mice was prevented by the ASE and ASW diet. In addition, the ASE and ASW supplementation attenuated hepatic steatosis and inflammation, improved liver function, and caused no harm to the kidneys. Moreover, the mechanism of the effect of ASE and ASW on inhibiting hepatic lipogenesis and inducing fatty acid β-oxidation was certified by the simulated human fatty liver cell model. Our study showed the regulatory potential of the extracts of adlay seeds for alleviating NAFLD, as well as related liver and metabolic diseases.

1. Introduction

The liver is a vital organ that plays a major role in the metabolic processes of carbohydrates, proteins, and lipids, and a properly functioning liver is essential to health. Liver diseases caused by metabolic dysfunction lead to serious health problems. Nonalcoholic fatty liver disease (NAFLD), resulting from metabolic disorders, is considered to be a leading cause of abnormal liver function [1, 2]. Several studies have indicated that NAFLD may develop into the end stage of liver disease and HCC; it can no longer be regarded as a trivial disease but is a risk factor for serious liver disease [3, 4]. The global prevalence of NAFLD in the general population has been estimated to be about 30% and has doubled in the past two decades [5, 6], a major concern for human health. Insulin resistance, resulting from long-term, sustained hyperglycemia, leading to impaired insulin-stimulated glucose utilization and glycogen synthesis, is the key pathogenic feature of metabolic syndrome and is now
2. Materials and Methods

2.1. Preparation of Adlay Seed Extracts. Adlay plants (Coix lacryma-jobi L. var. ma-yuen Stapf) were grown in the TaiChung District Agricultural Research and Extension Station, TaiChung, Taiwan. Whole adlay grains were ground into 20 mesh powder and extracted with 70% ethanol. The ethanolic extract of adlay seeds (ASE; Coixtreme™, A.T.P. Station, Taichung, Taiwan. Whole adlay grains were grinded into 20mesh powder and extracting with water using an ultrasonic bath for 30 minutes at 50°C (Branson Co.). Then, the ASE and ASW were stored at −20°C as functional materials.

2.2. Animals. Five-week-old male C57BL/6j mice were purchased from the National Laboratory Animal Center, Taiwan, and maintained in a temperature-controlled room on a 12h light-dark cycle at the Animal Center of the National Yang-Ming University, Taiwan. They were housed and had free access to food and drinking water. Mice fed with a standard diet and adapted to the environment for 1 week were subsequently divided randomly into four groups. The ND group (n = 6) continued on the normal diet, whereas the other three groups (n = 6 per group) were switched to the high-fat diet (HFD group, 494 kcal/100 g, 45% energy as fat; TestDiet Inc., USA), the HFD mixed with a 1% (weight for weight) ethanolic extract of adlay seeds (1% ASE group), and the HFD mixed with a 3% (weight for weight) water extract of adlay seeds (3% ASW group) for 10 weeks. Food consumption and weight gain were measured daily and weekly, respectively. All mice were sacrificed at the end of the experimental period. Serum samples, liver tissue, and epididymis adipose tissue were harvested for further analysis. The experimental protocol was approved by the Animal Research Committee of the National Yang-Ming University (IACUC no. 1070213), and all procedures followed the Guide for the Care and Use of Laboratory Animals (NIH publication, 85-23, revised 1996) and the guidelines of the Animal Welfare Act, Taiwan.

2.3. Blood Glucose, Serum Insulin, Intraperitoneal Glucose Tolerance Test, and Homeostasis Model Assessment of Insulin Resistance Index. The analysis of blood glucose, serum insulin, intraperitoneal glucose tolerance test, and the homeostasis model assessment of insulin resistance index were performed as described previously [19].

2.4. Biochemical Characterization. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), creatinine (CRE), and blood urea nitrogen (BUN) were measured using enzymatic assay kits with a FUJII DRI-CHEM analyzer (Fujifilm, Tokyo, Japan). The low-density lipoprotein-cholesterol (LDLC) level was calculated as $TC - (HDLC + TG/5)$ [20].

2.5. Triglyceride and Cholesterol Analysis of Liver Tissue. For hepatic triglyceride and cholesterol determinations, the methods were performed as described previously [19].

2.6. Cell Line. HuS-E/2 cells, kindly provided by Kunitada Shimotohno (Kyoto University, Japan), were grown as described previously [21]. To generate fatty liver disease cell model, HuS-E/2 cells at 70% confluence were incubated with 0.1 mM oleic acid (OA) for 18 h.
2.7. Antibodies and Western Blot Analysis. Antibodies against AMPK, ACC, pACC (Ser79), and tubulin were obtained from GeneTex. The anti-pAMPK (Thr172) antibodies were from Cell Signaling. The horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG antibodies were from Abcam. Western blot analysis was performed as previously described [19].

2.8. Quantitative Real-Time Polymerase Chain Reaction. In brief, total mRNA of mice liver tissues or HuS-E/2 cells were extracted from TRZOL reagent and reverse transcribed into cDNA by using a Deoxy RT kit (Yeastern Biotech.). All qPCR reactions were performed with SYBR Green PCR Master Mix (Applied Biosystems). The fluorescent signal data were processed using StepOne software. The primers used were described previously [19].

2.9. Statistical Analysis. Data obtained from all experiments are shown as means ± SEM. All the differences were assessed for significance using the one-way ANOVA followed by Dunnett’s honest significant difference post hoc tests. Asterisks indicate that the values differed significantly from the control (*p < 0.05; **p < 0.01; ***p < 0.001; #p < 0.05; ##p < 0.01; and ###p < 0.001).

3. Results

3.1. ASE and ASW Improved Glucose Hemostasis in HFD Mice. To determine the effect of ASE and ASW on NAFLD, we used an established HFD-induced mouse model of NAFLD, produced by feeding an HFD, and the HFD mice were treated with ASE or ASW for 10 weeks (Figure 1(a)). The body weight gain and the adipose tissue weight of the HFD group were significantly greater than the ND group (Table 1). This suggests that the HFD mice had a tendency to develop metabolic complications, which fit the main characteristics of central obesity. The amount of food consumed by the mice did not differ significantly among the groups. Interestingly, the liver weights of 3% ASW mice were significantly lower than those of HFD mice after 10 weeks of treatment (Table 1). Moreover, the amount of lipid droplet accumulated in the hepatocytes of HFD mice, compared with ND mice, while supplemen-

3.2. ASE and ASW Attenuated Hyperlipidemia in HFD Mice. Variation of the lipid composition of the serum is one of the features of metabolic syndrome [22]. Therefore, serum TG, TC, HDLC, and LDLC levels were monitored to evaluate the effect of ASE and ASW on lipid metabolism. We found that feeding HFD for 10 weeks significantly increased the levels of serum TG, TC, and LDLC, leading mice to hyperlipidemia. Interestingly, the increase in the level of serum TG was significantly prevented in the 1% ASE group (Figure 2(a)), and the increases in the levels of serum TC and LDLC were prevented significantly in the 1% ASE and 3% ASW groups (Figures 2(b) and 2(d)). The level of serum HDLC did not differ significantly from the HFD group after ASE or ASW treatment (Figure 2(c)). These results indicate that intervention with ASE or ASW inhibited hyperlipidemia in HFD mice.

3.3. ASE and ASW Alleviated Hepatic Steatosis and Hepatic Inflammation in HFD Mice. The liver, as a central regulator of lipid homeostasis, is an essential organ in lipid metabolism [23]. An imbalance of lipid metabolism is related to metabolic dysfunctions and may precipitate the retention of fat within the liver and the subsequent development of NAFLD [24]. We investigated the effect of administration of ASE or ASW on treatment of NAFLD in HFD mice. Feeding HFD for 10 weeks resulted in a significantly (31.4%) higher hepatic TG content than the ND group, while supplementation with 1% ASE or 3% ASW led to only 19.3% and 41% higher hepatic TG content than the ND group, respectively (Figure 3(a)), indicating that ASE and ASW significantly prevented lipid deposition in HFD mouse livers. There was no difference among the groups in the amount of hepatic cholesterol (Figure 3(b)). H&E staining revealed that a significant amount of lipid droplet accumulated in the hepatocytes of HFD mice, compared with ND mice, but this symptom of NAFLD was prevented by ASE or ASW supplementation for 10 weeks (Figure 3(c)). Moreover, to examine the effect of ASE and ASW on hepatic steatosis in the HFD mice liver, we identified the changes of genes associated with fatty acid synthesis and β-oxidation. The expression of genes involved in de novo lipogenesis in hepatocytes, peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element-binding protein 1 (SREBP1), and fatty acid synthase (FAS) was substantially higher in the HFD group than the ND group, while all the genes were expressed at greatly lower levels after treatment with ASE and ASW, compared with the HFD group (Figures 3(d)–3(f)). The expression of transcription factors, peroxisome proliferator-activated receptor alpha (PPARα), and peroxisome proliferator-activated receptor delta (PPARδ), associated to fatty acid β-oxidation, was markedly increased by treatment with ASE and ASW, compared with the HFD group (Figures 3(g) and 3(h)). The data indicate that ASE and ASW treatment leads to upregulation of fatty acid oxidation and downregulation of fatty acid synthesis, resulting in the ame-

4. Discussion

Inflammation is a central feature of NAFLD, with evidence that liver steatosis is associated with the inflammatory process [25]. TNF-α and interleukin 6 (IL-6) are known to play significant roles in the development of NAFLD. We measured the TNF-α and IL-6 levels in liver tissue of the high-fat diet group and the normal diet group after 10 weeks of treatment (Figure 4). The results showed that both TNF-α and IL-6 in the liver tissue of the high-fat diet group were higher than those of the normal diet group,
Figure 1: Effect of ASE and ASW on glucose metabolism, glucose tolerance, and insulin resistance in C57BL/6J mice fed by HFD. (a) The experimental approach. Six-week-old male C57BL/6 mice were divided into four groups and fed normal diet (ND), high-fat diet (HFD), 1% ASE mixed with HFD, and 3% ASW mixed with HFD for 10 weeks. At 16 weeks of age, the follow-up analysis was conducted. (b) Blood glucose levels after 16 h of fasting. (c) Curve of normalized IPGTT. Data are normalized by subtracting the baseline value (fasting blood glucose) from the measured value in each mouse. (d) Area under the curve (AUC) of blood glucose in the IPGTT assay. (e) Serum insulin levels after 16 h of fasting. (f) The HOMA-IR index calculated using fasting blood glucose and insulin levels. Data are shown as means ± SEM (n = 6 in each group). HFD vs. ND, ASE, or ASW, ### p < 0.001 in increased level. HFD vs. ND, ASE, or ASW, ** p < 0.01; *** p < 0.001, in decreased level.

Table 1: Biological parameters of mice after 10 weeks of treatment.

| Parameter                  | HFD       | HFD-1% ASE | HFD-3% ASW | ND       |
|----------------------------|-----------|------------|------------|----------|
| Body weight (g)            | 35.90 ± 1.04 | 37.40 ± 2.72 | 34.78 ± 1.73 | 26.85 ± 0.72*** |
| Food intake (g)            | 3.92 ± 0.68     | 3.95 ± 0.90     | 3.99 ± 2.66     | 3.59 ± 0.49     |
| Adipose tissue weight (g)  | 2.16 ± 0.16     | 2.02 ± 0.44     | 2.11 ± 0.23     | 0.33 ± 0.07***  |
| Liver weight (g)           | 1.11 ± 0.05     | 1.18 ± 0.15     | 1.02 ± 0.06**   | 0.93 ± 0.07***  |

(a) ND, normal diet; HFD, high-fat diet; ASE, ethanolic extract of adlay seeds; ASW, water extract of adlay seeds. (b) All data are shown as means ± SEM, n = 8 per group. Data of different groups were compared with the corresponding data from HFD-fed mice. Differences were examined for statistical significance using Student’s t-test. (c) HFD vs. adlay extracts and ND: ** p < 0.01 and *** p < 0.001 indicated decreased level.
indicating the high-fat diet induced liver inflammation, while treating with 1% ASE or 3% ASW significantly decreased the inflammatory factors TNF-α and IL-6, preventing the liver in an inflamed state (Figures 3(i) and 3(j)). Taken together, treatment with ASE or ASW prevented hepatic steatosis in HFD mice.

3.4. ASE and ASW Improved Liver Function in HFD Mice. Excess lipid accumulation in the liver has a strong association with insulin resistance and low-grade hepatic inflammation, which may cause NAFLD to develop to the more severe form, NASH [25]. Here, we found that the serum GOT and GPT levels of HFD mice were significantly higher than those of ND mice, showing that the liver was in an injured state and that was significantly prevented after 10 weeks of feeding 1% ASE or 3% ASW (Figures 4(a) and 4(b)). Besides, neither the group which fed HFD nor the groups which fed ASE or ASW experienced a significant change in the levels of serum creatinine (CRE) and blood urea nitrogen (BUN), which are commonly used as markers of kidney function in the clinic (Figures 4(c) and 4(d)). These data indicate that ASE and ASW supplementation improved liver function during HFD-induced NAFLD development.

3.5. ASE and ASW Inhibited the Hepatic de novo Lipogenesis Pathway and Induced Fatty Acid β-Oxidation in a Human Fatty Liver Cell Model. An increase in intrahepatic TG (IHTG) content is the hallmark characteristic of NAFLD. In order to determine the mechanism of the effect of ASE and ASW, hepatic de novo lipogenesis-related proteins and gene expression were examined in a human fatty liver cell model by using an immortalized human primary hepatocyte, HuS-E/2 cells [26]. AMPK plays a crucial role in regulation of fat metabolism in the liver, and the activation of AMPK phosphorylates its downstream target enzyme, ACC, by phosphorylation at Ser-79 [27]. We found that treatment with ASE or ASW significantly increased both pAMPK and pACC, respectively (Figures 5(a) and 5(b)). Also, treatment of OA led to higher gene expression of fatty acid synthase (FAS) and sterol regulatory element-binding protein-1c (SREBP-1c) than that of NT control, while treatment with ASE or ASW significantly lowered both FAS and SREBP-1c gene expressions, which are involved in fatty acid synthesis (Figures 5(c) and 5(d)). In addition, the gene expression of peroxisome proliferator-activated receptor alpha (PPARα) and peroxisome proliferator-activated receptor delta (PPARδ) in the OA treatment was lower than that of the NT control, while treatment of ASE and ASW significantly increased PPARα and PPARδ gene expressions, associated to fatty acid β-oxidation (Figures 5(e) and 5(f)). These data reveal that ASE and ASW inhibited the hepatic de novo lipogenesis pathway and promoted the fatty acid β-oxidation pathway.

4. Discussion

As one of the most common liver diseases, with increasing prevalence worldwide, NAFLD is a progressive pathological
Figure 3: Continued.
Figure 3: Effect of ASE and ASW on hepatic steatosis and inflammation in the livers of C57BL/6J mice fed by HFD. (a) Changes in hepatic TG. (b) Changes in hepatic TC. (c) Hematoxylin and eosin staining of transverse liver sections (original magnification ×200). (d–f) The hepatic de novo lipogenesis-related gene expression. (d) PPARγ. (e) SREBP-1c. (f) FAS. (g and h) The hepatic β-oxidation-related gene expression. (g) PPARα. (h) PPARδ. (i) Hepatic inflammation factor, TNF-α. (j) Hepatic inflammation factor, IL-6. Data are shown as means ± SEM (n = 6 in each group). HFD vs. ASE or ASW, ∗∗∗p < 0.001; ∗∗p < 0.01.

Figure 4: Effect of ASE and ASW on the serum levels of hepatic steatosis-related markers in C57BL/6J mice fed by HFD. (a and b) The serum levels of the hepatic lipotoxicity markers GOT and GPT. (c and d) The serum levels of the kidney toxicity markers CRE and BUN. Data are shown as means ± SEM (n = 6 in each group). HFD vs. ASE or ASW, ∗p < 0.05; ∗∗p < 0.01.
Figure 5: Continued.
adlay seeds may provide a novel strategy with great potential for the treatment of NAFLD.

One of the common comorbidities of NAFLD is dyslipidemia [36], and studies have shown that NAFLD and dyslipidemia are strongly related [37, 38]. To determine the effect of extracts of adlay seeds on mice with diet-induced metabolic dysfunction, we mixed 1% ASE or 3% ASW, respectively, with HFD as the feed for mice for 10 weeks. We found that treatment with either ASE or ASW for 10 weeks prevented the higher levels of fasting blood glucose, insulin, glucose tolerance, and HOMA-IR caused by feeding HFD to mice. Furthermore, dyslipidemia was observed in the HFD mice, and supplementation with ASE or ASW significantly ameliorated the increases in the concentrations of serum TC and LDL-C, and treatment with ASE significantly prevented increases in the concentrations of serum TG. Recent studies investigated NAFLD, as well as atherosclerosis, resulting from hypercholesterolemia [39, 40]. Our result showed that ASE and ASE improved hypercholesterolemia of HFD mice, which is consistent with a previous study [17], suggesting the potential treatment effect on not only NAFLD but also atherosclerosis.

NAFLD is thought to be a leading cause of abnormal liver function [1, 2]. We confirmed that feeding HFD for 10 weeks indeed induced the development of early lesions of NAFLD in the livers of mice, while the supplementation with ASE or ASW significantly prevented increases not only in the levels of hepatic TG but also the accumulation of lipid droplets within hepatocytes. Lipotoxicity is a harmful effect of lipid accumulation in non-adipose tissue, leading to liver inflammation and fibrosis [41]. To investigate the hepatic inflammation, we tested the crucial inflammatory factors,
TNF-α and IL-6, as well as the clinical parameters, GOT and GPT, as markers of liver injury [42]. Increased gene expression of TNF-α and IL-6 and the levels of serum GOT and GPT by feeding HFD for 10 weeks were significantly attenuated by ASE or ASW supplementation, indicating improvement preventive effect of ASE and ASW on hepatic inflammation and injury. In addition, no significant differences in serum CRE and BUN were observed among the groups, showing no influence of ASE and ASW on kidney function.

We also verified the mechanism of the effect of ASE and ASW. In our study, we used the HuS-E/2 cell line, which was derived from human primary hepatocytes and has been shown to be phenotypically and functionally similar to human primary hepatocytes [21, 43]. To unveil the underlying mechanism of the effect of ASE and ASW on ameliorating NAFLD in high-fat diet-induced mice, we simulated a high-fat environment in the HuS-E/2 cell line and treated with different doses of ASE or ASW. Our results showed ASE and ASW significantly increased the protein expression of pAMPK and pACC. One of the identified AMPK targets is ACC, playing a role in the control of fatty acid metabolism via the regulation of malonyl-CoA synthesis. By phosphorylation to inhibit ACC and lowering the concentration of its reaction product malonyl-CoA, AMPK activation is expected to coordinate the partitioning of fatty acids between oxidative and biosynthetic pathways by increasing fatty acid oxidation capacity and inhibiting de novo lipogenesis [44]. Also, treatment with ASE or ASW significantly decreased the gene expression of FAS and SREBP-1c and significantly increased the gene expression of PPARα and PPARδ. SREBP-1c activates other lipogenic genes, such as ACC and FAS, and prevents fatty acids from β-oxidation [45]. PPARα and δ are correlated to mitochondrial fatty acid β-oxidation and lead fatty acid to go into the mitochondrial and to be metabolized [46].

Despite the results in our study which indicate the prophylactic effect of adlay seed extracts on improving NAFLD, there was still limitation in our study. NAFLD with hepatic necroinflammation and faster progression to fibrosis may develop into NASH, and even to HCC [47]. Whether ASE and ASW have therapeutic effect on NASH should be further investigated.

5. Conclusions

In conclusion, our study indicated that ASE and ASW ameliorated the symptoms of hyperglycemia and impaired glucose tolerance, hyperlipidemia, hepatic steatosis, and hepatic inflammation via inhibiting hepatic de novo lipogenesis and promoting fatty acid β-oxidation. These results demonstrated the potential therapeutic effect of ASE and ASW on NAFLD and diet-induced metabolic dysfunction.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

References

[1] P. Angulo, “Nonalcoholic fatty liver disease,” *New England Journal of Medicine*, vol. 346, no. 16, pp. 1221–1231, 2002.
[2] J. M. Clark, F. L. Brancati, and A. M. Diehl, “Nonalcoholic fatty liver disease,” *Gastroenterology*, vol. 122, no. 6, pp. 1649–1657, 2002.
[3] D. Issa and N. Alkhouri, “Nonalcoholic fatty liver disease and hepatocellular carcinoma: new insights on presentation and natural history,” *HepatoBiliary Surgery and Nutrition*, vol. 6, no. 6, pp. 401–403, 2017.
[4] F. Kanwal, J. R. Kramer, S. Mapakshi et al., “Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease,” *Gastroenterology*, vol. 155, no. 6, pp. 1828–1837, 2018.
[5] C. D. Williams, J. Stengel, M. I. Asike et al., “Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasonography and blood biopsy: a prospective study,” *Gastroenterology*, vol. 140, no. 1, pp. 124–131, 2011.
[6] Z. M. Younossi, A. B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, and M. Wymer, “Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes,” *Hepatology*, vol. 64, no. 1, pp. 73–84, 2016.
[7] G. Pagano, G. Pacini, G. Musso et al., “Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association,” *Hepatology*, vol. 35, no. 2, pp. 367–372, 2002.
[8] S. Polyzos, J. Kountouras, and C. Zavos, “Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipokines,” *Current Molecular Medicine*, vol. 9, no. 3, pp. 299–314, 2009.
[9] H. Tilg and A. R. Moschen, “Insulin resistance, inflammation, and non-alcoholic fatty liver disease,” *Trends in Endocrinology and Metabolism*, vol. 19, no. 10, pp. 371–379, 2009.
[10] M. Mota, B. A. Banini, S. C. Cazanave, and A. J. Sanyal, “Molecular mechanisms of lipotoxicity and gluco-toxicity in nonalcoholic fatty liver disease,” *Metabolism*, vol. 65, no. 8, pp. 1049–1061, 2016.
[11] N. Chalasani, Z. Younossi, J. E. Lavine et al., “The diagnosis and management of non-alcoholic fatty liver disease: practice...
Evidence-Based Complementary and Alternative Medicine

guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association," *Hepatology*, vol. 55, no. 6, pp. 2005–2023, 2012.

[12] C.-C. Kuo, H.-H. Chen, and W. Chiang, "Adlay (薏苡 yì yǐ: "soft-shelled job’s tears"); the seeds of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf is a potential cancer chemopreventive agent toward multistage carcinogenesis processes," *Journal of Traditional and Complementary Medicine*, vol. 2, no. 4, pp. 267–275, 2012.

[13] D.-W. Huang, C.-P. Chung, Y.-H. Kuo, Y.-L. Lin, and W. Chiang, "Identification of compounds in adlay (*Coix lachryma-jobi* L. var. *ma-yuen*Stapf) seed hull extracts that inhibit lipopolysaccharide-induced inflammation in RAW 264.7 macrophages," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 22, pp. 10651–10657, 2009.

[14] L. Xu, L. Chen, B. Ali et al., "Impact of germination on nutritional and physicochemical properties of adlay seed (*Coix lachryma-jobi* L.)," *Food Chemistry*, vol. 229, pp. 312–318, 2017.

[15] M. Takahashi, C. Konno, and H. Hikino, "Isolation and hypoglycemic activity of coxains A, B and C, glycans of *Coix lachryma-jobi* var. *ma-yuen* Seeds1," *Planta Medica*, vol. 52, no. 1, pp. 64-65, 1986.

[16] Y. Park, H. Suzuki, Y. S. Lee, S. Hayakawa, and S. Wada, "Effect of coix on plasma, liver, and fecal lipid components in the rat fed on lard- or soybean oil-cholesterol diet," *Biomedical Chemistry and Metabolic Biology*, vol. 39, pp. 11–17, 1998.

[17] L. Wang, J. Sun, Q. Yi, X. Wang, and X. Ju, "Protective effect of polyphenols extract of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) on hypercholesterolemia-induced oxidative stress in rats," *Molecules*, vol. 17, no. 8, pp. 8886–8897, 2012.

[18] Y. Kondo, K. Nakajima, S. Nozoe, and S. Suzuki, "Isolation of ovulatory-active substances from crops of Job’s tears (*Coix lachryma-jobi* L. var. *ma-yuen*Stapf)," *Chemical & Pharmaceutical Bulletin*, vol. 36, no. 8, pp. 3147–3152, 1988.

[19] H. Chiang, J. C. Lee, H. C. Huang, H. Huang, H. K. Liu, and C. Huang, "Delayed intervention with a novel SGLT2 inhibitor NG1001 suppresses diet-induced metabolic dysfunction and non-alcoholic fatty liver disease in mice," *British Journal of Pharmacology*, vol. 177, no. 2, pp. 239–253, 2019.

[20] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, vol. 18, no. 6, pp. 499–502, 1972.

[21] H. H. Aly, K. Watashi, M. Hijikata et al., "Serum-derived hepatitis C virus infectivity in interferon regulatory factor-7-suppressed human primary hepatocytes," *Journal of Hepatology*, vol. 46, no. 1, pp. 26–36, 2007.

[22] M. Canals, D. Figueroa, C. Alfaro et al., "Effects of diet and water supply on energy intake and water loss in a mygalomorphic spider in a fluctuating environment of the central Andes," *Journal of Insect Physiology*, vol. 57, no. 11, pp. 1489–1494, 2011.

[23] P. Nguyen, V. Leray, M. Diez et al., "Liver lipid metabolism," *Journal of Animal Physiology and Animal Nutrition*, vol. 92, no. 3, pp. 272–283, 2008.

[24] E. Fabbrini and F. Magkos, "Hepatic steatosis as a marker of metabolic dysfunction," *Nutrients*, vol. 7, no. 6, pp. 4995–5019, 2015.

[25] Q. Liu, S. Bengmark, and S. Qu, "The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD)," *Lipids in Health and Disease*, vol. 9, no. 1, p. 42, 2010.

[26] H. K. Liu, T. M. Hung, H. C. Huang et al., "Bai-Hu-Jia-Ren-Shen-Tang decoction reduces fatty liver by activating AMP-activated protein kinase in vitro and in vivo," *Evidence Based Complementary and Alternative Medicine*, vol. 2015, Article ID 651734, 11 pages, 2015.

[27] D. G. Hardie, "AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 10, pp. 774–785, 2013.

[28] B. J. Perumpail, M. A. Khan, E. R. Yoo, G. Cholankeril, D. Kim, and A. Ahmed, "Clinical epidemiology and disease burden of nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 23, no. 47, pp. 8263–8276, 2017.

[29] P.-X. Wang, Y.-X. Ji, X.-J. Zhang et al., "Targeting CASP8 and FADD-like apoptosis regulator ameliorates nonalcoholic steatohepatitis in mice and nonhuman primates," *Nature Medicine*, vol. 23, no. 4, pp. 439–449, 2017.

[30] A.-j. Hu, S. Zhao, H. Liang, T.-q. Qiu, and G. Chen, "Ultraso-assisted supercritical fluid extraction of oil and coenzyme from adlay seed," *Ultrasonics Sonochimistry*, vol. 14, no. 2, pp. 219–224, 2007.

[31] H. C. Lu, P. L. Jiang, L. R. Hsu, C. L. Chyan, and J. T. Tzen, "Characterization of oil bodies in adlay (*Coix lachryma-jobi* L)," *Bioscience, Biotechnology and Biochemistry*, vol. 74, no. 9, pp. 1841–1847, 2007.

[32] D. Normile, "Asian medicine: the new face of traditional Chinese medicine," *Science*, vol. 299, no. 5604, pp. 188–190, 2003.

[33] J.-H. Woo, L. Dapeng, L. Dapeng et al., "Coix seed extract, a commonly used treatment for cancer in China, inhibits NFkB and protein kinase C signaling," *Cancer Biology & Therapy*, vol. 6, no. 12, pp. 2005–2011, 2007.

[34] Y.-P. Zhan, X.-E. Huang, J. Cao et al., "Clinical safety and efficacy of kanlaita (coix seed oil) injection combined with chemotherapy in treating patients with gastric cancer," *Asian Pacific Journal of Cancer Prevention*, vol. 13, no. 10, pp. 5319–5321, 2012.

[35] D. T. Ha, T. Nam Trung, N. Bich Thu et al., "Adlay seed extract (*Coix lachryma-jobi* L.) decreased adipocyte differentiation and increased glucose uptake in 3T3-L1 cells," *Journal of Medicinal Food*, vol. 13, no. 6, pp. 1331–1339, 2010.

[36] A. M. Diehl and C. Day, "Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis," *New England Journal of Medicine*, vol. 377, no. 21, pp. 2063–2072, 2017.

[37] A. Kotronen, J. Westerbacka, R. Bergholm, K. H. Pietilainen, and H. Yki-Järvinen, "Liver fat in the metabolic syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 9, pp. 3490–3497, 2007.

[38] E. K. Speliotes, J. M. Massaro, U. Hoffmann et al., "Fatty liver diseases, American college of gastroenterology, and the guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association," *Hepatology*, vol. 55, no. 6, pp. 2005–2023, 2012.

[39] R. Kleemann, L. Verschuren, M. J. van Erk et al., "Athero-sclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis," *Genome Biology*, vol. 8, no. 9, p. R200, 2007.

[40] M. n. Tous, N. I. Ferre, J. Camps, F. Riu, and J. Joven, "Feeding apolipoprotein E-knockout mice with cholesterol and fat enriched diets may be a model of non-alcoholic steatohepatitis," *Molecular and Cellular Biochemistry*, vol. 268, no. 1-2, pp. 53–58, 2005.
[41] N. Anderson and J. Borlak, “Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis,” Pharmacological Reviews, vol. 60, no. 3, pp. 311–357, 2008.

[42] S. Sharma, K. Khalili, and G. C. Nguyen, “Non-invasive diagnosis of advanced fibrosis and cirrhosis,” World Journal of Gastroenterology, vol. 20, no. 45, pp. 16820–16830, 2014.

[43] H.-C. Huang, C.-C. Chen, W.-C. Chang, M.-H. Tao, and C. Huang, “Entry of hepatitis B virus into immortalized human primary hepatocytes by clathrin-dependent endocytosis,” Journal of Virology, vol. 86, no. 17, pp. 9443–9453, 2012.

[44] M. Foretz, P. C. Even, and B. Viollet, “AMPK activation reduces hepatic lipid content by increasing fat oxidation in vivo,” International Journal of Molecular Sciences, vol. 19, no. 9, 2018.

[45] J. C. Fraulob, V. Souza-Mello, M. B. Aguila, and C. A. Mandarim-de-Lacerda, “Beneficial effects of rosuvastatin on insulin resistance, adiposity, inflammatory markers and non-alcoholic fatty liver disease in mice fed on a high-fat diet,” Clinical Science, vol. 123, no. 4, pp. 259–270, 2012.

[46] G. Serviddio, A. M. Giudetti, F. Bellanti et al., “Oxidation of hepatic carnitine palmitoyl transferase-I (CPT-I) impairs fatty acid beta-oxidation in rats fed a methionine-choline deficient diet,” PLoS One, vol. 6, no. 9, Article ID e24084, 2011.

[47] V. W.-S. Wong, L. A. Adams, V. de Ledinghen, G. L.-H. Wong, and S. Sookoian, “Noninvasive biomarkers in NALFD and NASH-current progress and future promise,” Nature Reviews Gastroenterology & Hepatology, vol. 15, no. 8, pp. 461–478, 2018.