Research Article

Effect of Alkaloids from Nelumbinis Plumula against Insulin Resistance of High-Fat Diet-Induced Nonalcoholic Fatty Liver Disease in Mice

Yong Xie,1,2 Yi Zhang,2 Zebin Guo,2 Hongliang Zeng,2 and Baodong Zheng2

1College of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, China
2College of Food Science and Technology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

Correspondence should be addressed to Baodong Zheng; zbdfst@163.com

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This study aimed to investigate the effects of total alkaloids from Nelumbinis Plumula (NPA) on insulin resistance (IR) of high-fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD). Rats were fed with HFD for 8 weeks to induce NAFLD. Then, the effect of NPA on ameliorating IR in HFD-induced NAFLD was evaluated. Fasting serum insulin was determined using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer’s protocol. Some inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were determined using ELISA kits to assess the inflammatory burden in rats. The results showed that HFD could induce a significant increase in blood glucose and IR in rats. However, rats treated with NPA (400 or 600 mg/kg) showed improved IR and reduction in serum inflammatory cytokines TNF-α and IL-6. Further investigation indicated that NPA could inhibit IR by restoring the insulin receptor substrate-1 (IRS-1) and suppressing the expression of c-Jun N-terminal kinase (JNK) phosphorylation. The present results supported the view that the pathogenesis of NAFLD was complex with inflammation, together with increasing serum glucose and IR. Also, JNK and IRS phosphorylation were suggested for their involvement in the modulating of IR during NAFLD progression. Therefore, NPA may serve as a potential natural remedy against IR in NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of hepatic steatosis not due to excess alcohol consumption. NAFLD has become a serious medical problem and an increasing threat to public health. It is estimated that approximately 20%–40% of the people in various countries have excess fat accumulation in the liver [1]. NAFLD encompasses a spectrum of disorders ranging from hepatic steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, or hepatocellular carcinoma [2]. Despite its severity and high prevalence, currently no pharmacological agent is available for treating NAFLD. Many factors including lipid metabolic disturbance, insulin resistance (IR), oxidative stress, and mitochondrial dysfunction are known to cause the occurrence and aggregation of NAFLD [3].

According to the prevailing theory of NAFLD pathogenesis, IR and lipid dysregulation are the “first hit” toward NAFLD, which may trigger following “hits” spontaneously such as oxidative stress, inflammation, necrosis, apoptosis, and fibrosis [4]. Therefore, targeting steatosis and IR in the early stage of NAFLD is extremely important for the retardation of NAFLD in both basic research and clinical trials. Although the initial accumulation of fat in the liver can occur through several mechanisms, most previously done research implicates IR as the key mechanism in primary NAFLD, leading to hepatic steatosis [5]. IR is associated with an increase in peripheral lipolysis, uptake of fatty acids by the liver, and synthesis of triglycerides, which promote the accumulation of hepatic fat [6]. Nowadays, the methods for treating NAFLD involve rational diet, exercise, and medicines including metformin,
statins, and fibrates. However, these drugs have some adverse
effects or contraindications, and no consensus exists on
the most effective drug therapies [7]. Recently, emerging
evidence suggests the possible use of herbal derivatives in
treating NAFLD, such as green tea [8], garlic [9], resveratrol
[10], and coffee [11] in both preclinical studies and clinical
trials.

Lotus seeds have been widely consumed by people in
Asia. The therapeutic hepatoprotective activity of ethanolic
extracts of edible lotus leaves was studied, indicating that
the hepatoprotective activity of lotus leaf extract was comparable
with that of a standard treatment comprising silymarin, a
known hepatoprotective drug [12]. NP is the germ of the
lotus seeds, which tastes bitter and generally can be removed.

A previous research demonstrated the protective effect of
total alkaloid and alkaloid compounds including liensinine,
 isoliensinine, or neferine from NP against oxidative stress
induced by tertiary-butyl hydroperoxide, suggesting their
involvement in the cytoprotective effect on oxidative stress
in liver [13]. Furthermore, total alkaloid and alkaloid com-
 pounds from NP displayed a significant cytoprotective effect
against hepatic steatosis (data not reported). However, no
papers have reported on the total alkaloid from NP against
NAFLD.

Therefore, the aim of the present study was to investigate
the effects of NPA on ameliorating IR in high-fat diet (HFD-
) induced nonalcoholic fatty liver disease in mice. And the
possible mechanisms were also studied.

2. Materials and Methods

2.1. Reagents. HFD, composed of 5% lactalbumin, 10%
cholesterol, 20% lard oil, and 1% yolk powder, was obtained
from Research Diets, Inc. Serum blood glucose, free fatty
acid, alanine amino transferase (ALT) and aspartate amino-
transferase (AST) detection kits, and bicinchoninic acid pro-
tein assay kit were obtained from Nanjing Jiancheng Institute
of Biotechnology (Nanjing, China). Phosphorylated insulin
receptor substrate 1 (IRS-1) and c-Jun N-terminal kinase
(JNK) antibody were purchased from Sigma Chemicals Co.
The insulin enzyme immunoassay kit was purchased from
Puer Biotechnology Co., Ltd. (Beijing, China). Enzyme-
linked immunosorbent assay (ELISA) kits for tumor necrosis
factor alpha (TNF-α) and interleukin-6 (IL-6) were supplied
by Glory Science Co. Ltd. (TX, USA).

2.2. Preparation of Total Alkaloid from NP. NP was purchased
from Fujian Xin Zi Jin Curative Co. Ltd. (Fuzhou, Fujian,
China). NPA was prepared as described previously [13].
Briefly, the peel powder was extracted with 80% ethanol.
Then, the extraction was centrifuged at 1500 g for 15 min,
and the supernatant was collected. Next, 5% HCl solution
was used to immerse the extract, followed by the addition
of NaOH to regulate pH to 9-10. The crude sample was then
extracted with chloroform using liquid-liquid separation. The
collected soluble fraction from chloroform was evaporated to
dryness and called NPA. The alkaloid composition of NPA
was also reported in previous studies [13, 14], which indicated
that 3 alkaloid components of liensinine, isoliensinine, and
neferine make up the majority in NPA.

2.3. Animals. Male Wistar rats (180–200 g) were provided
by the Experimental Animal Center of Fujian University of
Traditional Chinese Medicine (Fuzhou, China). The animals
were housed in a room with a controlled temperature (21 ±
3°C), relative humidity (65%–70%), and 12 h light/dark cycles.
They were given ad libitum access to water. After adaptation
for 1 week, the rats were used for the experiment approved by
the ethical committee and the Laboratory Animal Center of
Fujian University of Traditional Chinese Medicine.

The rats were randomly divided into six groups (n =
10). Group I (normal control) was given distilled water
(10 mL/kg body weight) and normal feeding. Groups II–VI
were administered with HFD feeding for 8 weeks to induce
NAFLD in the animal. Among them, Group II was designed
as the model control group. Groups III–V were administered
with NPA (200, 400, and 600 mg/kg, resp., dissolved in
water) for another 4 weeks. Group VI was given compound
methylthionine and choline bitartrate tablets as positive control
medicine. At the end of the experiment, blood samples of all
rats were obtained via the abdominal vein under anesthesia
after an overnight fasting. Liver sample from each rat was
collected for molecular measurement and stored at −80°C for
the assays.

2.4. Serum Biochemistry. Serum was obtained from blood
after centrifugation (2500 × g, 10 min). The serum blood
glucose, free fatty acid, ALT, and AST levels of rats were
detected using corresponding detection kits according to the
manufacturer’s protocols.

2.5. Assessment of the Level of Serum Insulin and IR. Fasting
serum insulin was determined using ELISA kit for insulin fol-
lowing the manufacturer’s protocol. Using an ELISA reader,
the absorbance of the reaction product was read at 450 nm.
The homeostasis model assessment of IR (HOMA-IR)
was calculated as [fasting glucose (mmol/L) × fasting
serum insulin (mU/L)/22.5] [15].

2.6. Determination of Serum Levels of TNF-α and IL-6 Using
ELISA Kits. Some inflammatory cytokines were determined
to assess the inflammatory burden in rats. An ELISA kit for
TNF-α or interleukin-6 (IL-6) was employed for estimating
the serum levels of these parameters. The assays were per-
formed in accordance with the manufacturer’s protocol.

2.7. Western Blotting Assay. Western blotting assay for detect-
ing phosphorylation of JNK or IRS-1 in liver tissue was
determined according to the method described by Li et
al. [16] with some modification. Briefly, total protein was
obtained from the livers. Then, it was denatured, resolved
with sodium sulfate poly acrylamide gel electrophoresis,
and transferred onto a polyvinylidene difluoride membrane
(PVDF). After blocking, the membranes were individually
incubated overnight with mouse anti-JNK (1:700 dilution),
mouse anti-p-JNK (1: 700 dilution), mouse anti-IRS-1 (1:700
diabetes, or mouse anti-P-IRS-1 (1:700 dilution), respectively, followed by washing and incubation with secondary antibodies to show the resulting bands. Parallel blotting of β-actin was used as the internal control.

2.8. Statistical Analysis. All values for each group were given as mean and standard deviation. The data were analyzed using one-way analysis of variance coupled with Duncan post hoc multiple range test at 95% confidence level using SPSS (version 16.0, Chicago, USA).

3. Results

3.1. Effects of NPA on Body Weight of Rats. Before the start of the experiment, the average body weight of each group was similar. As showed in Table 1, after feeding with HFD for 8 weeks, the body weights of rats in the NAFLD group significantly increased compared with the control group, indicating that HFD had a significant influence on body weight. However, after oral administration of NPA (400 and 600 mg/kg) for 4 weeks, the body weight of rats significantly reduced compared with the NAFLD group. Therefore, the consumption of NPA could significantly reduce the body weight of NAFLD rats.

3.2. Serum AST and ALT Activities. Serum levels of amino transferases were indexed for general hepatic injury. To correlate hepatic injury with NAFLD, serum ALT and AST measurements were conducted in each group of rats. As shown in Figure 1, the progression of NAFLD significantly induced the higher levels of both serum ALT and AST compared with the control rats. However, the levels of ALT and AST significantly decreased in a dose-dependent manner after NPA treatment, and the hepatoprotective effects produced by NPA at the dose of 600 mg/kg reach the highest as well as the positive control group. Therefore, NPA treatment potently alleviated hepatic injury by reducing the levels of aminotransferases.

3.3. Effects of NPA on Serum Blood Glucose and Insulin Levels. As noted, IR and glucose metabolism dysfunction are typical clinical symptoms of NAFLD and metabolic syndrome [17]. The levels of blood glucose are given in Table 2. Eight weeks of daily ingestion of HCD contributed to the insulin resistance of the model group, which exhibited a significant increase in fasting blood glucose (FBG) compared with the control group. However, after NPA exposure, the concentrations of FBG in blood significantly decreased in a dose-dependent manner compared with the model group. These findings indicated that NPA had obvious glucose-lowering effects against NAFLD. The fasting serum insulin level increased significantly in association with the elevated FBG in the model group; however, NPA intake at higher dose significantly decreased the fasting insulin level in Groups IV-V compared with the NAFLD group.

3.4. HOMA-IR. A high-fat, cholesterol-rich diet induced a significant increase of IR in the NAFLD model group.

### Table 1: Body weight changes of rats during NAFLD development.

| Group              | Body weight at week 0 (P value) (g) | Body weight at week 12 (P value) (g) |
|--------------------|-------------------------------------|--------------------------------------|
| Control            | 185.3 ± 6.2<sup>a</sup>             | 310.2 ± 19.1<sup>b</sup>             |
| NAFLD              | 188.9 ± 5.1<sup>c</sup>             | 372.3 ± 22.6<sup>c</sup>             |
| NAFLD + NPA (L)    | 190.3 ± 9.2<sup>a</sup>             | 362.0 ± 17.6<sup>bc</sup>            |
| NAFLD + NPA (M)    | 181.5 ± 5.6<sup>a</sup>             | 347.3 ± 22.5<sup>b</sup>             |
| NAFLD + NPA (H)    | 180.2 ± 4.7<sup>a</sup>             | 338.8 ± 31.6<sup>cd</sup>            |
| Positive control   | 184.1 ± 5.4<sup>a</sup>             | 322.5 ± 19.2<sup>bc</sup>            |

Values are means ± SD, n = 10. Control group means the normal group of rats; NAFLD group means the model group of rats; NAFLD + NPA (L) group means the NAFLD model of rats which were administrated with 200 mg/kg NPA; NAFLD + NPA (M) group means the NAFLD model of rats which were administrated with 400 mg/kg NPA; NAFLD + NPA (H) group means the NAFLD model of rats which were administrated with 600 mg/kg NPA. Different letters (a, b, c, or d) in the same column represent significant differences between the treated groups (P < 0.05).

### Table 2: Effect of NPA on serum levels of glucose, insulin, and insulin sensitivity index in all groups of rats.

| Group              | FBG (P value) (mmol/L) | Insulin (P value) (mU/L) | HOMA-IR (P value) |
|--------------------|------------------------|--------------------------|-------------------|
| Control            | 3.41 ± 0.37<sup>a</sup>| 21.15 ± 3.26<sup>d</sup>| 3.23 ± 0.75<sup>a</sup> |
| NAFLD              | 4.61 ± 0.52<sup>c</sup>| 37.57 ± 3.21<sup>c</sup>| 7.65 ± 1.34<sup>c</sup> |
| NAFLD (200 mg/kg)  | 4.34 ± 0.49<sup>bc</sup>| 35.89 ± 5.18<sup>bc</sup>| 7.07 ± 1.45<sup>c</sup> |
| NAFLD (400 mg/kg)  | 4.12 ± 0.28<sup>b</sup>| 30.69 ± 3.86<sup>b</sup>| 5.60 ± 0.61<sup>b</sup> |
| NAFLD (600 mg/kg)  | 3.73 ± 0.27<sup>c</sup>| 26.71 ± 3.11<sup>c</sup>| 4.53 ± 0.36<sup>c</sup> |
| Positive control   | 3.65 ± 0.13<sup>c</sup>| 25.58 ± 3.26<sup>e</sup>| 4.16 ± 0.57<sup>c</sup> |

Different letters (a, b, c, or d) in the same column represent significant differences between the treated groups (P < 0.05).
animals compared with the control group, as indicated by the high HOMA-IR. NPA administration in Groups IV-V could decrease their HOMA-IR level compared with the model group, reaching the control range (Table 2).

3.5. Effects of NPA on the Level of Serum Free Fatty Acid. Our results showed that the NAFLD model rats could exhibit a much higher level of free fatty acid (FFT) compared with the control group (Figure 2). However, treating the NAFLD model with NPA at a concentration of 400 or 600 mg/kg could significantly inhibit the FFT level in serum, suggesting its significant effect on alleviating steatosis in rats.

3.6. Inhibitory Effects of NPA on Key Inflammatory Mediators of TNF-α and IL-6. Rats fed with HFD for 8 weeks showed a significant increase in serum TNF-α and IL-6 levels compared with the rats in the control group. Treatment with NPA (400 or 600 mg/kg) significantly decreased these high levels compared with the control group. Notably, NPA at a higher dose could decrease the serum IL-6 level to a much greater extent, which was comparable with the positive control group (Figures 3(a) and 3(b)).

3.7. JNK Phosphorylation Was Associated with IR. The hypothesis that phosphorylated JNK is a crucial mediator for IR was tested. The results showed that JNK phosphorylation significantly increased in the NAFLD rats compared with the normal group. However, JNK phosphorylation in liver tissue could be significantly reversed by NPA treatment. Thus, increased JNK phosphorylation might explain the increased IR (Figure 4(a)).

3.8. Improvement in NAFLD by NPA Was Partially Attributed to the Modulation of IRS-1 Phosphorylation. IRS phosphorylation was strongly impaired in the liver of rats with NAFLD. It is well established that the insulin signaling pathway is impaired in obese objects as a result of decreased IRS phosphorylation [18]. In the present NAFLD model, western blot results showed that hepatic tissue levels of phosphorylated IRS-1 were inhibited by the induction of NAFLD. However, co-treatment with NPA completely restored the levels of these proteins (Figure 4(c)), indicating the basis of improving IR in NAFLD.

4. Discussion

The aim of the present study was to evaluate the hepatoprotective effect of NPA by ameliorating IR in a model of HFD-induced NAFLD. HFD is well known to increase body weight and induce IR, in addition to an inflammatory and oxidative burden in rodent models [19]. In the present study, high-fat, high-glucose diet was administered to rats to induce the occurrence of NAFLD. The aforementioned findings confirmed that 8 weeks of HFD feeding in rats resulted in increments in body weight, adiposity index, systemic IR, and inflammatory markers. HFD feeding could also increase serum FFA, causing the overloads of FFA in hepatocytes and promoting lipogenesis.

NAFLD is defined histologically as the presence of lipid droplets in more than 5% of hepatocytes, not caused by excessive alcohol consumption, drugs, or viruses. Numerous studies have demonstrated a correlation between NAFLD and IR [20]. IR represents the main link among obesity, metabolic syndrome, and liver disease with fat accumulation, that is, NAFLD [21]. Studies have also shown that hepatic steatosis may induce hepatic IR [22]. The present research investigated the effect of NPA on the metabolic alterations in the glucose level and IR in HFD-induced NAFLD. Feeding the rats with HFD resulted in dramatic increases in the FBG, FFA, and IR. The clustering of these metabolic risk factors was identified as “IR syndrome” [23].

The causes for IR during NAFLD development are numerous. It is well known that infusions of triglyceride emulsions to rapidly increase FFA levels would cause a decrease in insulin sensitivity [24]. Therefore, controlling IR, as well as associated glucose metabolism and lipid accumulation, is the first but most important step toward the therapy of NAFLD.
Fat accumulation and hepatocellular injury can lead to nonalcoholic steatohepatitis and advanced fibrosis [25]. The adipocytes are no longer considered passive cells that store excessive triglycerides but are instead considered active cells that regulate the energy balance and secrete the proinflammatory cytokines IL-6 and TNF-α [26]. TNF-α is an important mediator of IR due to its ability to influence the tyrosine kinase activity of the insulin receptor [27]. In the present study, the serum level of TNF-α and IL-6 increased after HFD feeding. This suggested that multiple immunomodulatory factors could contribute to the chronic inflammatory condition and hepatocytes injury observed in NAFLD. It is assumed that inflammatory factors may be mediators of IR because the plasma levels of both markers are correlated negatively with insulin sensitivity [28]. Excessive fat accumulation in hepatocytes, regardless of its cause, tends to induce the activation of nuclear factor-κB, which is the key regulator of inflammation [29]. Subsequently, the expression of inflammatory cytokines TNF-α and IL-6 may lead to infiltration of neutrophils and inflammation in liver injury.

IR is the major abnormality in NAFLD with metabolic syndrome. Impaired IRS-1 phosphorylation is responsible for reduced insulin signaling and impaired downstream PI3K/Akt signal transduction [30]. It is well established that the insulin signaling pathway is impaired in obese patients as a result of decreased IRS phosphorylation [18]. The pathways by which increased visceral adiposity leads to IR are not fully understood. Some authors suggest that lipolysis induced by TNF-α and IL-6, resulting in the inhibition of IRS, may represent a major mechanism [31]. Thus, it could be considered that the restoration of IRS-1 might be beneficial for the amelioration of NAFLD. The present results showed that insulin hypersecretion was prevalent in the group of NAFLD model rats, which significantly decreased on administering NPA. Considering the survival-promoting role of IRS-1 in several hepatic diseases, particularly NAFLD,
Figure 4: Effect of NPA on phosphorylation of JNK (a) and IRS-1 (c) in the hepatic tissue of NAFLD rats analyzed by western blot. Phosphorylation levels were evaluated by the band strength of P-JNK/JNK or P-IRS1/IRS (b and d). Liver and adipose tissue protein blots were normalized to endogenous β-actin. Different letters (A, B, or C) upon each column represent significant differences between the treated groups (P < 0.05).

Moreover, cotreatment with NPA could regulate IR through restoring phosphorylation of IRS-1 and alleviating phosphorylation of JNK in liver tissue, suggesting its involvement in modulating IR by protein phosphorylation during NAFLD progression. Further investigations are needed to ensure the safety, benefits, and utility of NPA in patients with NAFLD.

Competing Interests
The authors wish to confirm that there are no known competing interests associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References
[1] J. A. López-Velázquez, K. V. Silva-Vidal, G. Ponciano-Rodríguez et al., "The prevalence of nonalcoholic fatty liver disease in the Americas," Annals of Hepatology, vol. 13, no. 2, pp. 166–178, 2014.
[2] B. P. Mulhall, J. P. Ong, and Z. M. Younossi, "Non-alcoholic fatty liver disease: an overview," Journal of Gastroenterology and Hepatology, vol. 17, no. 11, pp. 1136–1143, 2002.
[3] M. Charlton, “Nonalcoholic fatty liver disease: a review of current understanding and future impact,” Clinical Gastroenterology and Hepatology, vol. 2, no. 12, pp. 1048–1058, 2004.

[4] S. A. Polyzos, J. Kountouras, and C. Zavos, “Nonalcoholic fatty liver disease: the pathogenic roles of insulin resistance and adipocytokines,” Current Molecular Medicine, vol. 9, no. 3, pp. 299–314, 2009.

[5] S. A. Zaitone, B. M. Barakat, S. E. Bilasy, M. S. Fawzy, E. Z. Abdelaziz, and N. E. Farag, “Protective effect of boswellic acids versus pioglitazone in a rat model of diet-induced non-alcoholic fatty liver disease: influence on insulin resistance and energy expenditure,” Naunyn-Schmiedeberg’s Archives of Pharmacology, vol. 388, no. 6, pp. 587–600, 2015.

[6] K. L. Kopec and D. Burns, “Nonalcoholic fatty liver disease: a review of the spectrum of disease, diagnosis, and therapy,” Nutrition in Clinical Practice, vol. 26, no. 5, pp. 565–576, 2011.

[7] B. W. Smith and L. A. Adams, “Non-alcoholic fatty liver disease,” Critical Reviews in Laboratory Sciences, vol. 48, no. 3, pp. 97–113, 2011.

[8] C. Masterjohn and R. S. Bruno, “Therapeutic potential of green tea in nonalcoholic fatty liver disease,” Nutrition Reviews, vol. 70, no. 1, pp. 41–56, 2012.

[9] J. Xiao, Y. P. Ching, E. C. Liong, A. A. Nanji, M. L. Fung, S. A. Zaitone, B. M. Barakat, S. E. Bilasy, M. S. Fawzy, E. Z. Abdelaziz, and N. E. Farag, “Protective effect of boswellic acids versus pioglitazone in a rat model of diet-induced non-alcoholic fatty liver disease: influence on insulin resistance and energy expenditure,” Naunyn-Schmiedeberg’s Archives of Pharmacology, vol. 388, no. 6, pp. 587–600, 2015.

[10] S. Polyzos, J. Kountouras, and C. Zavos, “Nonalcoholic fatty liver disease: influence on insulin resistance and energy expenditure,” Naunyn-Schmiedeberg’s Archives of Pharmacology, vol. 388, no. 6, pp. 587–600, 2015.

[11] G. M. Trovato, G. F. Martines, F. M. Trovato, and D. Catalano, “Regular coffee: a magic bullet or a naked gun? Regular coffee but not espresso drinking is protective against fibrosis in NAFLD,” Journal of Hepatology, vol. 58, no. 6, pp. 1264–1265, 2013.

[12] B. Huang, X. Fan, J. He, J. Tong, J. Tian, and Y. Wang, “Hepato-protective and antioxidant activity of ethanolic extracts of edible lotus (Nelumbo nucifera Gaertn. leaves),” Food Chemistry, vol. 120, no. 3, pp. 873–878, 2010.

[13] Y. Xie, Y. Zhang, L.-T. Zhang, S.-X. Zeng, Z.-B. Guo, and B.-D. Zheng, “Protective effects of alkaloid compounds from Nelumbinis Plumula on tert-butyl hydroperoxide-induced oxidative stress,” Molecules, vol. 18, no. 9, pp. 10285–10300, 2013.

[14] W. Liu, H.-J. Wang, N. Si, and B.-L. Bian, “Study on preparation technology of plumum nelumbinis alkaloid dripping pill and determination of content by HPLC,” Journal of Chinese Medicine Medicine, vol. 32, no. 7, pp. 581–584, 2007.

[15] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner, “Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man,” Diabetologia, vol. 28, no. 7, pp. 412–419, 1985.

[16] Y. Li, L. Liu, B. Wang et al., “Impairment of reproductive function in a male rat model of non-alcoholic fatty liver disease and beneficial effect of N-3 fatty acid supplementation,” Toxicology Letters, vol. 222, no. 2, pp. 224–232, 2013.

[17] S. Deivanayagam, B. S. Mohammed, B. E. Vitola et al., “Non-alcoholic fatty liver disease is associated with hepatic and skeletal muscle insulin resistance in overweight adolescents,” The American Journal of Clinical Nutrition, vol. 88, no. 2, pp. 257–262, 2008.

[18] L. J. Goodyear, F. Giorgino, L. A. Sherman, J. Carey, R. J. Smith, and G. L. Dohm, “Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects,” The Journal of Clinical Investigation, vol. 95, no. 5, pp. 2195–2204, 1995.

[19] S. A. Zaitone and S. Essawy, “Addition of a low dose of rimonabant toorlist at therapy decreases weight gain and reduces adiposity in dietary obese rats,” Clinical & Experimental Pharmacology & Physiology, vol. 39, no. 6, pp. 551–5594, 2012.

[20] F. R. Jornayvaz and G. I. Shulman, “Diacylglycerol activation of protein kinase Cε and hepatic insulin resistance,” Cell Metabolism, vol. 15, no. 5, pp. 574–584, 2012.

[21] G. Marchesini, E. Bugianesi, and G. Forlani, “Nonalcoholic fatty liver diseases tea to hepatitis and metabolic syndrome,” Hepatology, vol. 37, no. 4, pp. 917–923, 2003.

[22] E. Bugianesi, A. J. McCullough, and G. Marchesini, “Insulin resistance: a metabolic pathway to chronic liver disease,” Hepatology, vol. 42, no. 5, pp. 987–1000, 2005.

[23] K. Cusi, “Role of insulin resistance and lipotoxicity in nonalcoholic steatohepatitis,” Clinics in Liver Disease, vol. 13, no. 4, pp. 545–563, 2009.

[24] P. Steaehr, O. Hother-Nielsen, B. R. Landau, V. Chandramouli, J. J. Holst, and H. Beck-Nielsen, “Effects of free fatty acids per se on glucose production, gluconeogenesis, and glycolysis,” Diabetes, vol. 52, no. 2, pp. 260–267, 2003.

[25] A. G. Shah, A. Lydecker, K. Murray, B. N. Tetri, M. J. Contos, and A. J. Sanjal, “Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease,” Clinical Gastroenterology & Hepatology, vol. 7, no. 10, pp. 1104–1112, 2009.

[26] A. Schäffler, J. Schölemrich, and C. Büchler, “Mechanisms of disease: adipocytokines and visceral adipose tissue—emerging role in nonalcoholic fatty liver disease,” Nature Clinical Practice Gastroenterology & Hepatology, vol. 2, no. 6, pp. 273–280, 2005.

[27] G. S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M. F. White, and B. M. Spiegelman, “IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity-induced insulin resistance,” Science, vol. 271, no. 5259, pp. 665–668, 1996.

[28] Y. Miyazaki, R. Pipek, L. J. Mandarino, and R. A. DeFronzo, “Tumor necrosis factor α and insulin resistance in obese type 2 diabetic patients,” International Journal of Obesity, vol. 27, no. 1, pp. 88–94, 2003.

[29] S. Joshi-Barve, S. S. Barve, K. Amancherla et al., “Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes,” Hepatology, vol. 46, no. 3, pp. 823–830, 2007.

[30] S. Schenk, M. Saberi, and J. M. Olefsky, “Insulin sensitivity: modulation by nutrients and inflammation,” Journal of Clinical Investigation, vol. 118, no. 9, pp. 2992–3002, 2008.

[31] C. R. Bruce and D. J. Dyck, “Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-α,” American Journal of Physiology—Endocrinology & Metabolism, vol. 287, no. 4, pp. E616–E621, 2004.

[32] L. A. Videla, G. Tapia, R. Rodrigo et al., “Liver NF-κB and AP-1DNA binding in obese patients,” Obesity, vol. 17, no. 5, pp. 973–979, 2009.

[33] G. Tuncman, J. Hirosumi, G. Solinas et al., “Functional in vivo interactions between JNK1 and JNK2 is in obesity and insulin resistance,” Proceedings of the National Academy of Sciences of the United States of America, vol. 132, no. 2, pp. 311–322, 2005.