Myrciaria Cauliflora Extracts Attenuating Hyperlipidemia and Obesity in Vivo

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Abstract:

Obesity, the major reason causing the metabolic syndrome (MetS), leads to abnormal lipid metabolism, and also causes hyperlipidemia, morbidity and mortality in type 2 diabetes, cardiovascular and other diseases. These diseases often have increased insulin levels resulting from an insulin resistance lead to blood lipid abnormalities. Previously studies indicate that the polyphenols and anthocyanins ingredients have antioxidant, anti-arteriosclerosis, hypertension and diabetes suppression, hypolipidemic and tumor suppressor role. Therefore, this experiment will explore the preventive effect of extract from Myrciariacauliflora (MCE) for the high-fat diet-induced obesity and dyslipidemia. In this study, we investigated the chemopreventive potential of the water extract from Myrciariacauliflora (MCE) on the obesity male Wistar rat induced by lard oil for 10 weeks. The Serum biochemical parameters, body weight, were monitored every two weeks. After 10 weeks, the rats were sacrificed and the liver was assayed by HE stain and western blot. The results showed that dietary administration of MCE (0.5, 1, 1.5%) reduced the body weight of rat, serum biochemical parameters such as TG, TC, HDL-C, LDL-C, AST, ALT and lipid droplets in liver sections. Furthermore, MCE can also decrease the expression of lipid synthesis-related proteins by western blotting. These
findings demonstrated that MCE prevented high-fat diet-induced high cholesterol and excess body fat, suggesting that MCE has a potentially protective effect in obesity-related and cardiovascular diseases.

**Keywords:** *Myrciaria cauliflora* extract (MCE), hyperlipidemia, obesity, inflammation

**Introduction:**

Obesity, characterized by an abnormal excess of white adipose tissue, is an important public health problem worldwide. It is known to be a major determinant of metabolic syndrome associated with co-morbidities and increased risk for the development of lipotoxicity, diabetes, cardiovascular disease, cancer, and nonalcoholic fatty liver disease (NAFLD)[1]. Among them, lipotoxicity results from the accumulation of lipid intermediates in non-adipose tissue, leading to liver dysfunction and cell death and NAFLD is defined as hepatic fat accumulation greater than five percent of liver weight in the absence of excessive alcoholic intake [2]. About 20–25% patients with NAFLD remain asymptomatic progress to develop a more severe chronic hepatic inflammatory disease, defined as non-alcoholic steatohepatitis (NASH) a condition associated with liver fibrosis, cirrhosis and HCC[3, 4]. Despite prevalence of NAFLD, effective therapy for NAFLD is not fully established.

In obese individuals, the hypertrophy of adipocytes predisposes to immune cell infiltration, which produces proinflammatory cytokines (for instance, TNF-α and IL-6), then induces the altered secretion of adipokines such as adiponeectin and leptin in adipose tissue [5]. Altered expression of proinflammatory factors acts reciprocally on adipocytes, perpetuating white adipose tissue (WAT) inflammation and dysfunction. Unfortunately, how WAT inflammation is triggered is not completely understood, but suggested lipotoxicity involved [6].

AMP-activated protein kinase (AMPK) is a major regulator of energy homeostasis and nutrient metabolism. It is known to regulate fatty acid metabolism, protein synthesis, and glucose uptake [7]. AMPK activation in the liver shuts down the cholesterol and TG synthesis by reducing the activation of sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FAS). In addition, Liver AMPK activation also promotes fatty acid β-oxidation by regulating acetyl-Co A carboxylase (ACC) and carnitine palmitoyltransferase-1 (CPT-1) activity [8, 9]. Further, AMPK inhibits glycerol-3-phosphate acyltransferase (GPAT), an integral rate-limiting enzyme in triglyceride accumulation to stop the triglyceride synthesis [10].

Several studies have shown that natural ingredients in plants, especially polyphenols and anthocyanins, have insulin sensitizing effects as well as hypolipidemic and antioxidant potential and are effective in preventing hypercholesterolemia by suppressing LDL oxidation [11, 12]. Natural plant extracts seem to have multiple modes of action in lipid metabolism and inflammation. Jaboticaba (*Myrciaria cauliflora*) is a native fruit with interesting nutritional properties from the Brazilian Atlantic Forest, and is rich in minerals, fibers, and phenolics, especially polyphenols and anthocyanins and could improve insulin resistance in rat in a diet-induced obesity model[13, 14]. We have determined that Myriciariacauliflora extracts (MCE) attenuated diabetic nephropathy via the Ras signaling and suppression of oxidative stress and inflammation in streptozotocin-nicotinamide (STZ/NA) mice.
Hui Pei Huang et al, Myrciaria Cauliflora Extracts Attenuating Hyperlipidemia and Obesity in Vivo

fed a high-fat diet [15, 16]. Here, we want to demonstrate the Role of *Myrciaria cauliflora* Attenuating hyperlipidemia and obesity *in vivo*.

**Materials and Methods:**

**Preparation of MCE:**

*M. cauliflora* Berg fruit was purchased from the Modern Garden Jabuticaba Co. Ltd, Changhua, Taiwan. 100 g lyophilized fruit were macerated and stirred with 100 mL water, and the juice was then filtered and centrifuged (10,000g, 15 min). The MCE was obtained after being filtered and concentrated under reduced pressure at 30°C and stored at -20°C before use.

**Animals and Diet:**

All procedures involving animals were approved by the guidelines of IACUC (Institutional Animal Care and Use Committee) of CSMU (Animal Center of Chung Shan Medical University). The rat license of the laboratory (no. 1204) was issued by the CSMU. Six-week-old male Wistar rats were individually housed at a constant temperature of 24 °C, with a 12-h light/dark cycle, and allowed to take food and water ad libitum for 1 week after arrival. Animals were randomly divided into 5 groups (n-10) and fed the respective experimental diets for 10 weeks: a normal diet (control, 5% fat (corn oil), w/w), high-fat diet (HFD, 50% fat (5% corn oil and 15% lard) or HFD with three different concentration of MCE (0.5, 1.0, 1.5%, w/w).

**Liver pathological Analysis:**

Liver parts were collected from the sacrificed and immersed in neutral buffered formalin 10% for at least 24 h. Fixed tissues were processed routinely for paraffin embedding, and 4-µm sections were stained with Haematoxylin and Eosin (H&E) for general histological architecture. Stained areas were viewed using an optical microscope with a magnifying power of 100X or 200X.

**Blood biochemical and Liver Lipid Content parameters assay:**

After 10 weeks of MCE treatment, blood samples were collected after 4 h of food deprivation. Blood samples were centrifuged at 3000 g for 20 min, and serum was collected. Plasma-lipid concentrations were determined using commercially available kits. Total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol, alanine amino transferase (ALT), aspartate amino transferase (AST), were measured using Beckman Coulter AU680 chemistry analyzer (Beckman Coulter, Inc. Brea, CA, USA).

**Quantification of TG and TC in the liver:**

0.1 g of liver was weight and homogenized with chloroform/methanol (v/v: 2/1) thoroughly. After centrifugation (1,500g for 10 min), the lower clear lipid extraction was transferred into a new glass tube and lyophilized. The lyophilized powder was dissolved in isopropanol as the liver lipid extractand stored at -20 °C. The liver TG and TC in the lipid extracts were measured by enzymatic colorimetric methods using commercial kits(HUMAN, Wiesbaden, Germany).

**Elisa:**

Serum specimen were collected, adiponectin (Rat Adiponectin ELISA Kit, ERA2500-1, AssayPro, St. Charles, Missouri, USA), leptin (MOUSE/RAT Leptin ELISA KIT, SK00050-08, Aviscera Bioscience, Santa Clara, CA, USA), TNF-α (Rat Tumor Necrosis Factor-α ELISA Kit, ERT2010-1, AssayPro, St. Charles, Missouri, USA) and IL-6 (RAT...
IL-6 ELISA KIT, SK00110-02, Aviscera Bioscience, Santa Clara, CA, USA) levels were measured using enzyme-linked immunosorbent assays according to the manufacturer’s protocol.

**Western blotting:**

Cells were solubilized with Radio immuno precipitation assay buffer (RIPA buffer). Cell lysates (50μg of protein) were separated by electrophoresis on 8−12% SDS poly acryl amide gels and transferred to nitrocellulose membranes (Millipore, Bedford, MA, USA). The membranes were incubated with Tris-buffered saline containing 1% (w/v) nonfat milk and 0.1% (v/v) Tween-20 for 1 h to block non-specific binding, washed with Tween-20 for 30 min, incubated with the appropriate primary antibody for 2 h, incubated with horseradish peroxidase conjugated second antibody (Sigma, St. Louis, MO) for 1 h, developed using enhanced chemiluminescence (ECL, Millipore, Bedford, MA), and analyzed by densitometry using Alpha-Imager Series 2200 software. The results were representative of at least three independent experiments.

**Statistical analysis:**

Results are reported as mean±standard deviation (SD) of three independent experiment. Statistical comparisons were evaluated by one-way analysis of variance (ANOVA). A P value less than 0.05 was considered to be statistically significant.

**Results:**

**MCE decreased lipid accumulation in liver and adipose tissue in HFD-fed rat:**

The accumulation of hepatic lipid with a HFD is a most important cause of NAFLD. After 10 weeks of fat feeding, fat mass from peripheral of kidney, testicles and intestine of rats were measured and obesity rate calculated by the ratio of fat mass and body weight was showed in Fig 1A. Treatment with MCE diminished the increase the obesity rate of HFD-fed group. Histo pathological examination of liver tissues showed a normal intact structure of hepatic lobules, round shape of liver cells, central location of nuclei, and normal structure of portal area around hepatic lobules in the control group (C). However, the structural disorder of hepatic lobules, dilation of portal area-central vein, fatty degeneration of liver cells within hepatic lobules, visible cavitation within the cytoplasm were observed in HFD group (Fig. 1B). Compared with HFD group, a relatively normal structure of hepatic lobules, significantly improved fatty degeneration of liver cells in hepatic lobules and less visible cavitation in liver was noted in HFD+MCE group, indicating that MCE appeared to decrease HFD-induced lipid accumulation in liver and adipose tissue.
MCE lowered the plasma lipids levels and improved the blood biochemical in HFD-fed rat:

Whether there were any changes in serum lipid levels after MCE treatment was investigated next. The levels of plasma TG, TC and HDL/LDL in control and HFD groups of rats at 10 weeks after treatment are reported in Fig. 2(A, B, C). In comparison with control group, HFD caused a marked increase in plasma concentrations of TG, and TC level and decreased the HDL/LDL ratio but normalized by MCE supplementation. A significant increase in the serum marker enzymes AST and ALT could be observed in HFD-fed rat(Fig. 2D, E). However, exposure of HFD-fed rat to MCE resulted in a marked reduction in AST and ALT activities.

MCE improved adipokines and inflammatory cytokines of serum in HFD-fed rat:

Adipokines such as adiponectin, leptin, TNF-α and IL-6 play a major role in the regulation of the inflammatory response in adipose tissue during the development of obesity[17].HFD-induced obesity is also known to be strongly associated with the levels of leptin and adiponectin, both of which are secreted from adipose tissue. Circulating plasma levels of adiponectin was decreased and leptin was increased in HFD-fed rat compared to control group (Fig. 3A, B). Treatment with MCE restored plasma adiponect-
Hui Pei Huang et al., Myrciaria Cauliflora Extracts Attenuating Hyperlipidemia and Obesity in Vivo

tin and 1.5% MCE could reduce the leptin concent-
tration to normal level. Further, the higher adiponec-
tin expression was observed in the 1.5 MCE treated
HFD-fed group.
Furthermore, we evaluated TNF-α and IL-6 protein
levels in the serum of untreated obese mice or obese
mice treated with MCE. A high-fat diet increased
TNF-α and IL-6 levels whereas MCE treatment re-
duced TNF-α and IL-6 expression (Fig. 3C, D).

Figure 3. Effect of MCE on fat-relative and in-
flammatory factors in serum in control and HFD
rats. The rats were fed with HFD and MCE for 10
weeks, then the serum adiponectin (A), leptin (B),
TNF-α (C) and IL-6 (D) were measured. *, P<0.05,
significant difference compared with control
group. #, P<0.05 significant difference compared
with HFD group, determined by Student’s t test.

Inhibitory effect of MCE on lipid content of liver
in HFD-fed rat:
MCE could lower the plasma lipids levels and im-
prove the blood biochemical in HFD-fed rat. Next,
the effect of MCE on lipid content of liver was ex-
amined. As expected, MCE significantly lessened TG
and total cholesterol levels of liver induced by
HFD in a dose-dependent manner (Fig. 4A, B), in-
dicating the inhibitory efficacy of MCE on lipid
accumulation of liver.

Figure 4. Effect of MCE on total cholesterol and
triglyceride of liver in control and HFD rats. To-
tal cholesterol (A) and triglyceride (B) of liver were
tested after treatment MCE with HFD-induced rats.
The results showed that total cholesterol and tri-
glyceride of liver were both increase in HFD group
compared with control group, and MCE treatment
groups (1% and 1.5%) had significant decreased.
*P<0.005 significant difference compared with co-
ntrol group, #P<0.05, significant difference com-
pared with HFD group, determined by Student’s t
test.

Effect of MCE on lipid synthesis-related proteins
and hepatic inflammation in liver of HFD-fed
rat:
AMPK is a latent regulator of metabolism of lipid
accumulation by up-regulation of fatty acid oxida-
tion, TG synthesis, and inflammatory cytokines[18,
19]. The HFD did not modify the expression of
AMPK in the liver as judged by the results of
Western blotting (Fig. 5). To investigate the effect of the HFD on the activity of the AMPK, we also analyzed the expression level of pAMPK (the phosphorylated active form of AMPK) and observed MCE decreased pAMPK expression induced by HFD. In addition, to clarify the downstream of AMPK activation, the effects of MCE on the expression of lipid metabolism-related proteins were evaluated, such as CPT1 and PPAR-α. As shown in Fig. 6, high fat diet indeed affected PPAR-α, and CPT1 expression. However, treatment with MCE induced both proteins expression. MCE caused AMPK phosphorylation showed that MCE inhibited HFD-induced hepatic lipid accumulation via AMPK signaling.

Figure 5. Effect of MCE in lipid synthesis-related proteins on HFD rats. The rats were fed with high fat diet and MCE 10 weeks and liver tissue were collected. Lipid synthesis-related proteins (CPT1 and PPAR-α) expression was detected by Western blotting. And the levels of the proteins were subsequently quantitated by densitometric analysis with that of control being 100%. Data are represented as the mean ± SD for the three experiments. *P<0.05 and **p<0.001, significant difference compared with control group. #P<0.05 and ##P<0.001 significant difference compared with HFD group, determined by Student’s t test.

Figure 6. Effect of MCE in phosphorylation of AMPK. After rats fed with high fat diet and MCE 10 weeks, triglyceride phosphorylation of AMPK protein in liver tissue were test by western blot. The levels of the proteins were subsequently quantitated by densitometric analysis with that of control being 100%. Data are represented as the mean ± SD for the three experiments. *P<0.05, significant difference compared with control group. #P<0.05 and ##P<0.001 significant difference compared with HFD group, determined by Student’s t test.
AMPK phosphorylation stimulates fatty acid oxidation via down-regulating the expression of the transcription factor SREBP 1 and suppresses TG synthesis through GPAT expression. As showed in Fig. 7, the protein level of SREBP 1 and GPAT increased significantly in the HFD group compared with control group. However, MCE down-regulated SREBP 1 and GPAT levels, indicating the effect of MCE on inhibiting lipid synthesis.

**Figure 7. Effect of MCE in triglyceride synthesis-related proteins.** After rats were fed with high fat diet and MCE 10 weeks, TG synthesis-related proteins of liver tissue were test by western blotting. The levels of the proteins were subsequently quantitated by densitometric analysis with that of control being 100 %. Data are represented as the mean ± SD for the three experiments. *P<0.05 and **p<0.001, significant difference compared with control group. *P<0.05 and ###P<0.001 significant difference compared with HFD group, determined by Student’s t test.

The safeguarded action of MCE on inflammation was further examined. The results presented in Fig. 8 showed MCE prevented HFD-induced proinflammatory cytokines secretion such as IL-6 and TNF-α.

**Figure 8. Effect of MCE in inflammatory-related proteins.** After rats were treated indicated, inflammatory-related protein of liver tissue were test by western blot. The levels of the proteins were subsequently quantitated by densitometric analysis with that of control being 100 %. Data are represented as the mean ± SD for the three experiments. *, P<0.05 significant difference compared with control group. #, P<0.05 significant difference compared with HFD group. ##, determined by Student’s t test.
**Discussion:**

This study aims to assess the effect of the extract from plant on the modulation of hepatic steatosis and inflammation in obesity. Our data confirmed that MCE could improve liver function by activating AMPK to regulate lipogenesis, β-oxidation and inflammation (Fig. 9).

![Figure 9. The summary of MCE mediated the possible mechanisms involved in HFD-induced lipogenesis, obesity and inflammation.](image)

Hepatic lipid accumulation is accompanied by excessive cumulation of cholesterol and triglycerides. It occurs in conditions of elevated dietary fat, obesity, and decreased metabolic function because of decreased liver function. Visceral obesity is recognized as an alert for insulin resistance and as a prerequisite for the diagnosis of the metabolic syndrome [20]. Administration of the MCE appeared to stabilize the increased values in lipid profiles, and reversed the incidence of steatosis, that had been observed in the HFD groups. These changes were associated with improvements in liver function, serum TC, TG, AST, and ALT levels that control fat metabolism during exposure to HFD. Another reason for observed improvements was the regulation of the HE stain of liver; a return to a normal state was observed following the treatment MCE with the high fat diet animals.

The major function of the adipocyte is to store and release energy in the form of triglyceride during excess food consumption and starved periods, respectively. However, adipose tissue is not a passive site of energy storage, it is also an endocrine organ producing several adipokines (like adiponectin and leptin) and cytokine mediators (IL-6 and TNF-α) with many biological activities[21]. Development and complications of obesity causes numerous adipokines, hormones and cytokines takenplace[22]. Adipokines, when imbalanced, will orchestrate a proinflammatory and insulin-resistant state that further leads to the pathogenesis of NAFLD and progresses to NASH[23]. Many studies suggest that plant extractshave beneficial effectson improving the development of obesity and its complications including obesity-related liver disease[24-27]. As expected, MCE also has a great benefit on the health on normalization the adipokines and cytokines levels of HFD animal.

Obesity causes leptin resistance in the hypothalamus accompanied by hepatic steatosis in the liver. Leptin resistance could down-regulate the phosphorylation of the leptin-mediated signals, such as STAT3, JAK, and AMPK [28]. When leptin re-
sistance occur, the level of serum leptin and the feed efficiency ratio was increased [29].

**Conclusion:**

In this study, we prove MCE suppressed the leptin level and enhanced the activation of AMPK protein firstly. These results show that MCE may be used to improve the leptin resistance caused by obesity. However, other possible mechanisms like STAT3 regulated by central nervous system and leptin receptor involved JAK will further examine in primary cultured cortical neuron cells pre-treated with MCE in vitro.

**Conflicts of Nterest:**

The authors declare that they have no competing interests.

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