Potentials of Chenpi on Metabolic Syndrome: A Review

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ABSTRACT

Objectives: Metabolic Syndrome (MetS) is strongly related with central obesity, hypertriglyceridemia, low high-density lipoprotein-cholesterol (HDL-C), hyperglycemia, and hypertension. This study reviewed the potential of Chenpi in treatment of MetS through amelioration of correlated diseases, such as diabetes mellitus, hyperlipidemia, obesity, hepatic steatosis, and inflammation.

Methods: Six electronic databases (Oriental Medicine Advanced Searching Integrated System (OASIS), Korean Traditional Knowledge Portal, Korea Institute of Oriental Medicine (KIOM), Research Information Sharing Service (RISS), PubMed, and Embase) were used to search for in vitro, in vivo, and clinical research that discusses the potential effects of Chenpi (Citrus unshiu Markovich, Citrus reticulata Blanco) on diabetes mellitus, hyperlipidemia, obesity, hepatic steatosis, and inflammation.

Results: This review suggests the potential of Chenpi as a candidate for the treatment of metabolic syndrome through improvement of correlated diabetes, hyperlipidemia, obesity, hepatic steatosis, and inflammation. However, comparison of the results of each study was limited by a lack of quantification of the experimental materials.

Key words: Chenpi, Citrus unshiu peel, Citrus reticulata Blanco peel, metabolic syndrome, diabetes

I. Introduction

Metabolic syndrome (MetS), also named as syndrome X, can be diagnosed when more than three symptoms exist out of following five pathological conditions: central obesity, hypertriglyceridemia, low high density lipoprotein-cholesterol (HDL-C), hyperglycemia or hypertension (HTN). As sedentary lifestyle and over-nutrition became more common in many developed countries, the MetS has become epidemic. The global prevalence of MetS is hard to measure, but it can be estimated by the incidence of obesity and type 2 diabetes (T2DM). Years between 1998 and 2007, the prevalence of MetS in Korean population had recorded 31.3%, and the most common pathological condition was...

The diagnosis of MetS leads to increased episodes of cardiovascular diseases (CVD), including stroke and myocardial infarction. The risk factors that would provoke vulnerability to CVD include age, gender, family history of CVD, T2DM, insulin resistance, hyperlipidemia (HL), HTN, obesity and non-alcoholic fatty liver (NAFLD). Owing to multiple pathologic conditions, medicines targeting each of them are prescribed, such as hypoglycemic agents, lipid-lowering agents, anti-hypertensive agents, anti-platelet drugs, and weight-loss agents. Increased risks of atherosclerotic cardiovascular disease (ASCVD) are consequences of cholesterol level imbalance. Statins are used to reduce low density lipoprotein cholesterol (LDL-C) and total cholesterol levels, but adverse effects including musculoskeletal pain, gastrointestinal events, headache, and elevation of blood glucose and glycohemoglobin levels are present. Also, as CVD is the most common risk factor of mortality in diabetes, biguanide (metformin) is commonly prescribed to MetS patients with T2DM. However, biguanides may induce vitamin B12 deficiency, gastrointestinal disease, hemolytic anemia, hyperlactatemia, and metabolic acidosis. Therefore, many attempts have been done to find effective therapy of MetS with less side effects.

With growing demand for natural products in MetS treatment, there had been several herbal agents reviewed about their MetS modulating effects. Some of the herbs include Satureja Species, silymarin, Saffron and Ginger.

Chenpi, the peel of Citrus unshiu Markovich (CMP) or Citrus reticulata Blanco (CBP), has traditionally been used to treat indigestion, improve bronchial conditions, and blood circulation in east Asia. It contains various phytochemicals such as hesperidin, naringin and nobiletin. Among them, hesperidin, which has largest proportion, has been proven to have anti-inflammatory, neuroprotective, anti-cancer, anti-diabetic, antihypertensive and antioxidant and CVD risk lowering effects. Although several reviews about each single flavonoids are present, there are none about the herbal plant itself. As Chenpi is comprised of various phytochemicals, the effect should be differentiated from that of single constituents.

In aspects of MetS, diverse values of Chenpi have been discovered until now. These values are summarized as follows: 1. Anti-diabetic effect, 2. Lipid-lowering effect, 3. Anti-obesity effect, 4. Anti-hepatic steatosis activity, and 5. Anti-inflammatory effect.

II. Method

A. Selection of database and searching method

research papers.

In domestic and English search, the keyword of 'metabolic syndrome', 'syndrome X', 'diabetes', 'D.M.', 'diabetes mellitus', 'hyperglycemia', 'hyperlipidemia', 'dyslipidemia', 'lipid', 'obesity', 'NAFLD', 'fatty liver', 'hepatic steatosis', 'inflammation', 'Citrus unshiu Markovich pericarpium', 'Citrus unshiu Markovich peel', 'Citrus reticulatae pericarpium', 'Citrus reticulata Blanco peel', 'Citrus reticulata blanco pericarpium' were included. We combined these keywords regarding characteristic of each database. The searching procedure was done between 2021.07.01-2021.07.04.

B. Selection and exclusion

1. Selection criteria
a) In vitro/in vivo experimental study
b) Clinical study on patients with hypertension, type 2 diabetes, hyperlipidemia, obesity, or hepatic steatosis
c) Exclusive study on freeze-dried powder, water extract, ethanol extract or fermented product of Citrus unshiu Markovich peel or Citrus reticulata Blanco peel
d) Study including measurements indicating treatment effects about hypertension, type 2 diabetes, hyperlipidemia, obesity, or hepatic steatosis
e) Study written in Korean or English

2. Exclusion criteria
a) Study using combined herbal treatments
b) Study not including measurements indicating treatment effects about hypertension, type 2 diabetes, hyperlipidemia, obesity, or hepatic steatosis

C. Material selection and study analysis

We collected literatures searched in domestic and English databases, and excluded duplicating literatures based on study title, published year and authors. First step of screening was conducted based on title and abstract, following selection and exclusion criteria. Second step of screening was conducted through examining full text of previous screened records. In sequence, clinical application possibility of Chenpi was judged, and the final literature selection and analysis was conducted.

D. Data extraction

On our study, full texts of finally selected literatures were analyzed. Data about study methods, experimental model(species, number), interventions of treatment and control group (including dose and duration), positive control, measurements and results were extracted. Based on extracted data, characteristics of each literature were discussed.

III. Result

A. Literature screening

At first, 85 literatures were searched, and 50 literatures were selected after exclusion of 35 duplicating literatures. On first step of screening, titles and abstracts were examined following selection and exclusion criteria, and 14 literatures which were not related to Chenpi and MetS were excluded. On second screening, investigation of full text removed 17 literatures using treatments combined with other herbs, flavonoids or written in other language than Korean or English. As a final outcome, 19 literatures were selected on our study (Fig. 1).
Fig. 1. PRISMA flow diagram for process or literature research.

B. Literature analyzing

1. Characteristics of the selected literatures

Among 19 selected literatures, there were 7 in vitro, 8 in vivo, 2 in vitro and in vivo, and 2 clinical researches. 11 were published in Korea, 3 in United States, 3 in international journals, and 1 of each in Switzerland, India and Japan.

2. Analysis of experimental models

Among 9 in vitro studies, RIN-m5f β-cells, HIT-15 cells, OP9 cells, HepG2 cells. Human embryonic kidney 293 cells and NIT-1 (murine pancreatic β-cells) were used in 1 of each study. L6 myotubes in were used in 2 studies and 3T3-L1 cells were used in 3 studies. Among 10 in vivo studies, C57BL/KsJ-db/db mice and Wistar-Hannover GALAS rats were used in 1 of each study. 3 studies selected C57BL/6 mice and 6 studies selected Sprague Dawely rats. In 2 clinical researches, patient with hypertriglyceridemia and obesity (BMI > 23 kg/m²) were chosen.

3. Analysis of interventions

16 literatures selected Citrus unshiu Markovich peel, and 3 selected Citrus reticulata Blanco peel as an intervention. There were various extraction methods of Chenpi. 4 literatures used water extraction, 10 used ethanol extraction, and 2 chose both water and ethanol extraction. Chenpi powder, Chenpi juice and 1% albedo TDF extracted by method of Prosky were also chosen. Furthermore, 6 literatures contained positive control groups. Rosiglitazone 0.001 g/100 g diet, estradiol (E2) 10 μg/kg, simvastatin 0.05 mg/mL, simvastatin 0.04 mg/kg, resveratrol 0.1% and
insulin 1 μM were used.

C. Results

1. Diabetes Mellitus

   Diabetes is a worldwide health problem, with the estimated prevalence of 6.4%, globally. The total predicted increase in prevalence of T2DM from 2010 to 2030 is 54%, with estimated annual growth of 2.2%, which almost doubles the current annual global adult population. Insulin resistance (IR) is frequently related to central obesity and CVD. Accordingly, visceral adipocytes secrete adipokines, which are the inflammatory cytokines, and they are thought to be closely related to the pathological conditions of MetS. The effect of Chenpi on DM is analyzed below (Table 1).

### Table 1. Summary of studies on Chenpi in Diabetes Mellitus

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Method</th>
<th>Dose and duration</th>
<th>Experimental model</th>
<th>Control and intervention groups</th>
<th>Positive control</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park. 2011&lt;sup&gt;15&lt;/sup&gt;</td>
<td>FCMPE and CMPE in vivo</td>
<td>CMPE or 0.05% of total diet for 10 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) Normal diet (n=8)</td>
<td>none</td>
<td>↓ Serum glucose level in FCMPE 0.1%, 0.5% and CMPE 0.5% group (p&lt;0.05)</td>
</tr>
<tr>
<td>Park. 2013&lt;sup&gt;16&lt;/sup&gt;</td>
<td>in vitro</td>
<td>CMPE 2 μg per 100 g diet for 6 weeks</td>
<td>C57BL/6J-db/db mice</td>
<td>1) Control (n=10)</td>
<td>none</td>
<td>↑ Cell viability in all groups</td>
</tr>
<tr>
<td>Park. 2017&lt;sup&gt;17&lt;/sup&gt;</td>
<td>in vitro</td>
<td>CMPE 1%, FCMPE 0.3% or FCMPE 1% for 13 weeks (ad libitum)</td>
<td>HFD</td>
<td>1) LFD (n=8)</td>
<td>none</td>
<td>↓ Fasting glucose level (p&lt;0.05)</td>
</tr>
<tr>
<td>Kim. 2018&lt;sup&gt;18&lt;/sup&gt;</td>
<td>in vitro</td>
<td>CMPW 50, 100, 200, 500 μg/ml</td>
<td>L6 myoblast cells</td>
<td>1) Control</td>
<td>↑ IRS-1, PI3K and GLUT4 mRNA expression in all groups, dose-dependently</td>
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↑: Increase, ↓: Decrease
<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Treatment</th>
<th>Conditions</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Kim, 2019&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Human embryonic kidney 293 cells</td>
<td>CMPW 100, 200, 500 μg/mL</td>
<td>1) Control 2) CMPW 100 μg/mL 3) CMPW 200 μg/mL 4) CMPW 500 μg/mL</td>
<td>↓ Kv2.1 channel currents but had no effect on Kv2.2 channel currents</td>
</tr>
<tr>
<td>Kwak, 2020&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NIT-1 (murine pancreatic β-cell)</td>
<td>CMPW</td>
<td>none</td>
<td>↑ insulin secretion from NIT-1 cells (p&lt;0.01)</td>
</tr>
<tr>
<td>Ke, 2020&lt;sup&gt;4&lt;/sup&gt;</td>
<td>C57BL/6J mice</td>
<td>CBPE 0.2% or 0.5% for 10 weeks</td>
<td>1) ND (n=8) 2) HFD (n=8) 3) HFD+0.1% resveratrol (n=8) 4) HFD+0.2% CBPE (n=8) 5) HFD+0.5% CBPE (n=8)</td>
<td>0.1% resveratrol ↓ AUC value of GTT in both groups (p&lt;0.05)</td>
</tr>
<tr>
<td>Kwak, 2020&lt;sup&gt;4&lt;/sup&gt;</td>
<td>HIT-T15 cells</td>
<td>CMPW 100/200/300 μg/mL</td>
<td>none</td>
<td>↑ insulin secretion in β cell (p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>L6 myotubes</td>
<td>CMPW 100/200/300 μg/mL</td>
<td>1) Normal control 2) Insulin 3) Insulin+CMPW 100 μg/mL 4) Insulin+CMPW 200 μg/mL 5) Insulin+CMPW 300 μg/mL</td>
<td>Insulin 10 nM. ↑ Glucose uptake in myotubes (p&lt;0.05)</td>
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<td>↓ fasting blood glucose level in both groups (p&lt;0.05).</td>
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<td>↓ HbA1c level in both groups (p&lt;0.05). ↑ Serum insulin level in both groups (p&lt;0.05). ↓ liver and kidney size in both groups ↓ liver and kidney weight in both groups (p&lt;0.05)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ liver and kidney weight in both groups (p&lt;0.05)</td>
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- **a) Glucose-lowering effect**
  - (a.1) In vivo
    - A number of in vivo studies have shown that Chenpi has glucose-lowering effect. Fasting blood
glucose (FBS) level was significantly reduced by CMP ethanol extract (CMPE) 2 g per 100 g diet administered to diabetic mice (p<0.05)\(^{35}\). CMP water extract (CMPW) 200 or 300 mg/kg to STZ induced diabetic mice (p<0.05)\(^{38}\). They had tendency of being more effective dose-dependently. Bioconversed Chenpi was also studied for its anti-diabetic effects. Ethanol extract of CMP fermented by Aspergillus niger (FCMPE) 0.3%, 1% and CMPE 1% were fed ad libitum to HFD mice, and FBS was significantly lowered only in FCMPE groups (p<0.05)\(^{39}\). Also, FCMPE bioconversed by Aureobasidium pullulans and CMPE 0.1% or 0.5% applied to HFD rats and reduction of serum glucose was observed in all FCMPE groups and 0.5% CMPE group (p<0.05)\(^{39}\).

By fermentation, naringin was bioconversed into naringenin. CMP water extract (CMPE) had glucose reducing effect, as well. Even though it had no significant effect in α-glucosidase inhibition, CMPW 100, 200 and 300 mg/kg administration significantly lowered glucose level in OSTT and OGTT (p<0.05). This result suggests the possibility of other hypoglycemic pathway existence, excluding α-glucosidase inhibition.

b) Insulin-stimulating effect

(b.1) In vitro

Insulin secretion stimulating effect was also tested. In vitro studies revealed insulin-promoting effect of Chenpi. CMPW 100, 200 and 300 mg/kg was applied to 15 mM glucose processed RIN-m5F β-cells, and elevation of insulin secretion in β cells was monitored (p<0.05)\(^{39}\). Aureobasidium pullulans bioconversed FCMPE and CMPE 0.01, 0.025 and 0.1 mg/mL applied to deoxyribose processed Hamster Islet Transformed−Triquarine resistant clone 15 cell (HT−T15 cell, pancreatic beta cell) stimulated insulin secretion in all groups\(^{39}\). In this study, FCMPE performed better effect than CMPE, especially in high dose.

General voltage-dependent K+ (Kv) channels has been proved to improve insulin secretion, membrane depolarization and Ca2+ influx in a glucose-dependent manner\(^{40−43}\). Among them, Kv2.1 and Kv2.2 are enriched in the pancreatic islets, and Kv2.1 is enhanced in pancreatic β-cells\(^{44}\). Kv2.1 modulates insulin secretion by affecting β-cells, and Kv2.2 regulates somatostatin release by affecting δ-cells. CMPW 100, 200, 500 µg/mL application to Human embryonic kidney 293 cells\(^{29}\) inhibited Kv2.1 channel currents in a dose-dependent manner. However, none of them had effect on Kv2.2 channel current. Also, CMP application to NIT-1 (murine pancreatic β-cell) resulted in significant increase of insulin secretion (p<0.01). Therefore, selective inhibition of Kv2.1 without cross-inhibition of Kv2.2 would exhibit increase of insulin secretion without concern of adverse effects.

(b.2) In vivo

CMPE 2 g per 100 g diet fed to diabetic mice exhibited increase in serum insulin/glucagon ratio (p<0.05)\(^{35}\). CMPW 200 and 300 mg/kg were fed to STZ induced diabetic mice, and serum insulin was on the rise in both groups, dose-dependently (p<0.05)\(^{38}\).

c) Glucose-regulating effect

(c.1) In vitro

In vitro study of CMPW 100, 200 and 300 mg/kg applied to L6 myotubes performed elevation of glucose uptake in myotubes (p<0.05)\(^{39}\).

(c.2) In vivo

In vivo study of CMPE 2 g per 100 g diet administration to diabetic mice demonstrated reduction of hepatic glycogen content (p<0.05)\(^{35}\).

(d) Glycosylated Hemoglobin, Type A1C (HbA1c) reducing effect

(d.1) In vivo
CMPW 200 and 300 mg/kg were fed to STZ-induced diabetic mice, and it resulted in decrease of HbA1c index (p<0.05).

e) Amelioration of glucose tolerance

(e.l) In vivo

In HFD mice, CBP ethanol extract (CBPE) 0.2% and 0.5% induced decrease in Area Under the Receiver Operating Characteristic Curve (AUC) value of glucose tolerance test (GTT) (p<0.05) in both groups. On a study conducted in 2017, fermented Chenpi showed decrease in AUC of Intraperitoneal Glucose Tolerance Test (IPGTT). Among them, only 0.3% FCMPE has significant effect, suggesting that fermented Chenpi has better effect than normal Chenpi, and it's efficient in low dose.

f) Inhibition of gluconeogenesis

(f.l) In vitro

Protein kinase B (Akt) is one of signaling pathways that increases glucose uptake in cells. When insulin combines to insulin receptor, phosphorylation of insulin receptor substrate-1 (IRS-1) and activation of phosphatidylinositol 3-kinase regulatory (PI3KR) results in phosphorylation of Akt. Phosphorylated Akt transports glucose into intracellular space, by bumping transporter 4 (GLUT4) to membrane cell. As well, GLUT4 is a glucose transporter that is activated by insulin, and related influx of glucose into cells. In L6 myotubes, CMPW 50, 100, 200, 500 μg/ml up-regulated IRS-1, PI3KR and GLUT4 mRNA expression in all groups, dose-dependently. Akt mRNA expression in CMPW 100, 200 and 500 μg/ml groups were also increased, dose-dependently.

(f.2) In vivo

There are several experiments about mRNA expression regulation related to glucose metabolism. Hepatic phosphoenol pyruvate carboxykinase (PEPCK) is an enzyme related to hepatic gluconeogenesis, which is activated in diabetic state, leading to insulin resistance. CMPW 2 g per 100 g diet applied to diabetic mice suppressed activity of hepatic PEPCK. Another study revealed activation of glucokinase (Gk), glucose transporter protein type 2 (Glut2) and suppression of glucose6phosphatase (G6pase) by Chenpi and fermented Chenpi medication to HFD mice. Gk phosphorylates glucose to glucose-6-phosphate, and its activation leads to glycolysis and glycogen synthesis. Glut2 is an enzyme found in liver that transports glucose and G6pase is a gluconeogenic enzyme. Up-regulation of GK and Glut2 mRNA expression were superior in FCMPE groups, and down-regulation of G6pase mRNA expression was superior in CMPE group (p<0.05). Regulation of these mRNA expressions implies anti-diabetic effect of Chenpi, presenting similar property of all types of Chenpi.

g) Pancreatic beta cell protecting effect

(g.l) In vitro

Administration of FCMPE and CMPE 0.01, 0.05 and 0.1 mg/mL to deoxyribose processed HIT-T15 cell increased cell viability in all groups. It suggests that Chenpi ameliorates DM complications.

(h) Ameliorating effects in DM complication

(h.l) In vitro

In vitro study about CMPW 200 and 300 mg/kg administration to STZ induced diabetic mice reduced liver and kidney size, and lowered weight of them (p<0.05) in both groups. It suggests that Chenpi ameliorates DM complications.

2. Hyperlipidemia

The increase of apo B-containing lipoproteins is a major risk factor of coronary artery disease. The effect of Chenpi on hyperlipidemia (HL) is analyzed below (Table 2).
Table 2. Summary of studies on Chenp in Hyperlipidemia

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Method</th>
<th>Dose and duration</th>
<th>Experimental model</th>
<th>Control and intervention groups</th>
<th>Positive control</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park, 2011&lt;sup&gt;4&lt;/sup&gt;</td>
<td>CMPE 48.5 mg/kg (0.1%) or 241.9 mg/kg (0.5%) for 10 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND (n=8) 2) HFD (n=8) 3) HFD+CMPE 0.1% (n=8) 4) HFD+CMPE 0.5% (n=8)</td>
<td>none</td>
<td>↓ Serum TC, LDL-C level in all groups (p&lt;0.05) ↓ Serum TG level in 0.5% group (p&lt;0.05) ↓ Al in 0.5% group (p&lt;0.05) ↑ Serum HDL-C level (p&lt;0.05)</td>
<td></td>
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<tr>
<td>Lee, 2011&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Clinical CMPE 1,200 mg for 8 weeks</td>
<td>1) Placebo (n=46) 2) CMPE 1,200 mg (n=45)</td>
<td>none</td>
<td>↓ Serum TG level (p&lt;0.05) ↑ Serum TC. ↑ serum HDL-C level</td>
<td></td>
<td></td>
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<tr>
<td>Park, 2011&lt;sup&gt;6&lt;/sup&gt;</td>
<td>FCMPE or CMPE, 0.1% or 0.05% of total diet for 10 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) Normal diet (n=8) 2) HFD (n=8) 3) HFD+CMPE 0.1% (n=8) 4) HFD+CMPE 0.5% (n=8) 5) HFD+FCMPE 0.1% (n=8) 6) HFD+FCMPE 0.5% (n=8)</td>
<td>none</td>
<td>↓ Serum TC level in all groups (p&lt;0.05) ↓ Serum LDL-C level in CMPE 0.1% and FCMPE 0.5% group (p&lt;0.05) ↓ Serum TG level in CMPE 0.5%. FCMPE 0.1% and 0.5% group (p&lt;0.05) ↑ Serum HDL-C level in CMPE 0.1% 0.5% and FCMPE 0.5% group (p&lt;0.05) ↓ Al in CMPE 0.5%. FCMPE 0.1% and 0.5% group (p&lt;0.05)</td>
<td></td>
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<tr>
<td>Iwata, 2012&lt;sup&gt;7&lt;/sup&gt;</td>
<td>1% albedo TDF extracted by method of Prosky</td>
<td>Water-Hamster GALAS rat</td>
<td>1) Control (n=6) 2) 1% albedo TDF (n=6)</td>
<td>none</td>
<td>↓ Serum TG level (p&lt;0.05)</td>
<td></td>
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<tr>
<td>Park, 2013&lt;sup&gt;8&lt;/sup&gt;</td>
<td>CMPE 2 g per 100 g diet for 6 weeks</td>
<td>CS/BLK/Cj- Cd/d/db mice</td>
<td>1) Control (n=10) 2) CMPE 2 g/100 g diet (n=10) 3) Rosiglitazone 0.001 g/100 g diet (n=10)</td>
<td>none</td>
<td>↓ Serum TG level (p&lt;0.05)</td>
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<tr>
<td>Jung, 2013&lt;sup&gt;9&lt;/sup&gt;</td>
<td>CMPE 100 or 300 mg/kg, twice daily for 8 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND (n=10) 2) HFD (n=10) 3) HFD+CMPE 100 mg/kg (n=10) 4) HFD+CMPE 300 mg/kg (n=10)</td>
<td>none</td>
<td>↓ Serum TC level in 300 mg/kg group (p&lt;0.001) ↓ Serum TG level in 100 and 300 mg/kg group (p&lt;0.001)</td>
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<tr>
<td>Lim, 2014&lt;sup&gt;10&lt;/sup&gt;</td>
<td>CMPE 30, 100 or 300 mg/kg for 8 weeks</td>
<td>OVX Sprague Dawley rat</td>
<td>1) Sham (n=12) 2) OVX (n=12) 3) OVX+22.10 μg/kg (n=12) 4) OVX+CMPE 30 mg/kg (n=12) 5) OVX+CMPE 100 mg/kg (n=12) 6) OVX+CMPE 300 mg/kg (n=12)</td>
<td>E2 10 μg/kg</td>
<td>↓ Serum TC, TG, LDL-C level (p&lt;0.05) ↓ Serum HDL-C level (p&lt;0.05) ↓ CRI, Al in 300 mg/kg group (p&lt;0.01)</td>
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<tr>
<td>Cho, 2014&lt;sup&gt;11&lt;/sup&gt;</td>
<td>CMP powder, 5%, 10% or 15%, for 4 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND 2) HFD 3) HFD+CM 5% 4) HFD+CM 10% 5) HFD+CM 15%</td>
<td>none</td>
<td>↓ Serum LDL-C level in all groups 10% group (p&lt;0.05) ↑ Serum HDL-C level in 15% group (p&lt;0.05) ↑ HDL/TG ratio (p&lt;0.05) ↓ Al in 10% and 15% group (p&lt;0.05)</td>
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</table>
a) Serum triglyceride, cholesterol reducing effect

Low serum HDL and elevated serum TG level are convincing markers of cardiovascular diseases, and causes an increase of serum LDL level, resulting in increased cardiovascular risk\(^2\).

(a.1) In vivo

Various in vivo studies have been done to test hypolipidemic effects in HFD rats. Medication of CMPE 48 mg/kg (0.1%) and 241 mg/kg (0.5%) significantly reduced serum total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) and elevated high density lipoprotein cholesterol (HDL-C) level (p<0.05)\(^{24}\).

Administration of Aureobasidium pullulans fermented CMPE and CMPE 0.1%, 0.5% induced reduction of serum TG, TC, LDL-C and elevation of HDL-C level (p<0.05)\(^{39}\). Aspergillus niger fermented CMPE 0.3% and 1% reduced serum TC level (p<0.05)\(^{24}\). CMPE 100 and 300 mg/kg were applied to HFD Sprague Dawley mice twice daily for 8 weeks\(^{32}\). Serum TC level was significantly lowered in 300 mg/kg group (p<0.05), and serum TC level in 100 (p<0.05) and 300 (p<0.001) mg/kg group. CBPE 0.2% and 0.5% lowered serum TG, LDL-C level in both groups (p<0.05), and TC level in 0.5% group (p<0.05)\(^{36}\). In 2020, 0.2% and 0.5% of CBPE were fed to
HFD C57BL/6 mice, for 10 weeks. Resultingly, decrease of LDL-C and TG in both groups, and TC in 0.5% group were observed, all significantly (p<0.05). CBPW 1.04 g/kg reduced serum TG, TC, LDL-C level (p<0.05) and elevated HDL-C level (p<0.05). As well, the efficacy of CMP powder in dyslipidemia was discovered. Freeze-dried CMP, each 5%, 10%, 15% of HFD diet, were fed to Sprague Dawley mice for 4 weeks. Serum LDL-C was reduced in 10% group (p<0.05) and serum HDL-C was increased in 15% group (p<0.05). In both 10% and 15% groups, HDL/TC ratio was elevated, and AI was lowered, all significantly (p<0.05).

In diabetic mice, CMPE 2 g per 100 g diet significantly lowered serum TG level (p<0.05), even though there were no change in plasma TC and free fatty acid level. In OVX rats, apply of CMPE 30, 100 and 300 mg/kg resulted in decrease of TG, TC, LDL-C level and increase of HDL-C level (p<0.05).

(a.2) Clinical research

Clinical research also has been done on patients with 170 mg/dL-250 mg/dL of TC level. CMPE 1200 mg medicated to 91 patients with 170 mg/dL-250 mg/dL of TC level resulted in significant reduction of serum TG level (p<0.05) and noticeable change in serum TC and HDL-C level. Besides, there was no evidence of hepatotoxicity, as no significant differences in serum GOT, GPT, γ-glutamyl transferase (γ-GT) level were noted between CMPE and placebo groups. CMP juice extract 18 mg lowered serum TC (p<0.0001) and LDL-C level (p=0.011) in 118 adult patients with body mass index (BMI) over 23 kg/m². The hypolipidemic effect of albedo, the white part of CMP, has also been tested. Albedo, which contains arabinose, galactose, xylose, and glucose, was extracted as total dietary fiber (TDF) by the method of Prosky. Wistar-Hannover GALAS rats were fed freely with diet containing 4% cellulose and 1% TDF, and their serum TG was significantly reduced (p<0.05).

b) Alleviation of coronary artery risk index and atherogenic index

(b.1) In vivo

Currently, atherogenic index (AI), a ratio of serum lipid concentrations, has been recommended as a biomarker for cardiovascular diseases and atherosclerosis. Coronary artery risk index (CRI), is also related with MetS.

Four in vivo studies have shown significant alleviation of AI. CMPE 241.9 mg/kg (0.5%) of non-fermented, and CMP powder 10% of each led to significant reduction of AI (p<0.05) in HFD rats. In other study, Aureobasidium pullulans fermented Chenpi has been revealed to be more effective than non-fermented Chenpi in lowering AI. In HFD rats, FCMPE 0.1% and 0.5% and CMPE 0.5% of total diet significantly lowered AI (p<0.05), showing that only FCMPE has significant risk-lowering effect in same dose (0.1% of total diet). Also, study about effect of Chenpi CRI and AI has been conducted. In OVX rats, CMPE 300 mg/kg significantly decreased CRI and AI (p<0.01). OVX rat, a model of postmenopausal symptoms, has shown bone loss caused by estrogen deficiency and lipid metabolic disturbance. It is known that CMP extract has both plasma and hepatic lipid-lowering effect through inhibition of 3-Hydroxy-3-Methyl Glutaryl-Coenzyme A (HMG-CoA) reductase activity.

The outcomes were reduction of TC, TG, LDL-C (p<0.05) and elevation of HDL-C (p<0.05) level. Also, coronary artery risk index (CRI) and atherogenic index (AI) were lowered (p<0.01). This study has
confirmed risk lowering effect of CVDs, which are the major complications of MetS.

c) Reduction of apolipoprotein B-100

(c.1) In vivo

Dyslipidemia is one of the common insulin resistance complications of metabolic syndrome. It is characterized by elevated atherogenic lipid and lipoprotein profile, especially hepatic very low-density lipoprotein (VLDL) overproduction. Elevated hepatic VLDL secretion leads to increased plasma apolipoprotein B100 (apoB-100)–containing lipoprotein.

d) Regulation of mRNA expressions related to serum lipid metabolism

(d.1) In vivo

Aspergillus niger fermented Chenpi also was discovered to have hypolipidemic effects by modulating mRNA expressions involved with lipid-metabolism. CMPE 1%, FCMPE 0.3% and FCMPE 1% were applied to HFD mice for 13 weeks. As a result, significant suppressions of those mRNA expressions were observed only in fermented Chenpi groups. Sterol regulatory element binding protein 1c (Srebp1c) mRNA expression in both FCMPE groups, fatty acid synthase (Fas) mRNA expression in 0.3% FCMPE group, and acetyl CoA carboxylase (Acc), carnitine palmitoyl transferase 1 (Cpt1) mRNA expression in 1% FCMPE group were all significantly reduced (p<0.05). These mRNAs are all related to hepatic lipogenesis and fatty acid oxidation. Also, expressions of sterol regulatory element binding protein 2 (Srebp2), HMG CoA reductase (Hmgr) and proprotein convertase subtilisin/kexin type 9 (Pcsk9), which are related to hepatic cholesterol homeostasis, were significantly reduced in FCMPE 0.3% and 1% group (p<0.05).

e) Regulation of biomarkers related to plasma lipid metabolism

(e.1) In vivo

CPW reversed abnormal changes in biomarkers, related to uric acid and hypotaurine metabolism, fatty acid biosynthesis and arginine and proline metabolism. 5-L-Glutamyl-taurine, an intermediate product of taurine metabolism, which is related to oxidative stress reaction was significantly decreased after CPW treatment (p<0.05). Cis-4-Octenedioic acid and 2-octenedioic acid, the unsaturated fatty acids which increase in abnormal fatty acid metabolism situation, was also reduced by CPW (p<0.05). Additionally, 5-Aminopentanoic acid, a lysine degradation product, was decreased significantly (p<0.05).

3. Obesity

The overweight and obesity are the risk factors of HL, HTN, IR and T2DM. Worldwide overweight currency is estimated about 2 billion, and one-third of them satisfies the criteria of obesity. Since World Health Organization (WHO) has defined obesity as a global epidemic in 1996, the demand for effective treatments raised, as well as herbal therapy. The effect of Chenpi on obesity is analyzed below (Table 3).
<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Method</th>
<th>Dose and duration</th>
<th>Experimental model</th>
<th>Control and intervention groups</th>
<th>Positive control</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>CMPE 4.6 mg/kg (0.1%) or 241.9 mg/kg (0.5%) for 10 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND (n=8) 2) HFD (n=8) 3) HFD + CMPE 0.1% (n=8) 4) HFD + CMPE 0.5% (n=8)</td>
<td>none</td>
<td>↓FER* in 0.5% group (p&lt;0.05) ↓Weight of visceral adipose tissue in 0.5% group (p&lt;0.05)</td>
</tr>
<tr>
<td>Park 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>FCMPE, CMPE 0.1% and 0.5% of total diet for 10 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) Normal diet (n=8) 2) HFD (n=8) 3) HFD + CMPE 0.1% (n=8) 4) HFD + CMPE 0.5% (n=8) 5) HFD + FCMPE 0.1% (n=8) 6) HFD + FCMPE 0.5% (n=8)</td>
<td>none</td>
<td>↓Body weight and FER in FCMPE 0.1%, 0.5% and CMPE 0.1% group (p&lt;0.05) ↓Weight of visceral adipose tissue in FCMPE 0.5% group (p&lt;0.05)</td>
</tr>
<tr>
<td>Park 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vitro</td>
<td>FCMPE, CMPE and FCB 25 or 50 μg/mL</td>
<td>3T3-L1 cell</td>
<td>1) Adipocyte 2) FCMPE 25 μg/mL 3) CMPE 50 μg/mL 4) CMPE 25 μg/mL 5) CMPE 50 μg/mL 6) FCB 25 μg/mL 7) FCB 50 μg/mL</td>
<td>none</td>
<td>↓Adipogenesis in all groups</td>
</tr>
<tr>
<td>Iwata 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>1% albedo TDF extracted by method of Prosky</td>
<td>Sprague-Dawley</td>
<td>1) Control (n=6) 2) 1% albedo TDF (n=6)</td>
<td>none</td>
<td>↓Weight of the esophagus content (p&lt;0.05) ↓Inhibition of pancreatic lipase activity (p&lt;0.05) ↓Lipid content of feces (p&lt;0.05)</td>
</tr>
<tr>
<td>Park 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>CMPE 2 g per 100 g diet for 6 weeks</td>
<td>C57BL/6j-db/db mice</td>
<td>1) Control (n=10) 2) CMPE 2 g/100 g diet (n=10) 3) Rosiglitazone 0.001 g/100 g diet (n=10)</td>
<td>rosiglitazone (0.001g/100g diet)</td>
<td>↓Body weight gain (p&lt;0.05) ↓FER (p&lt;0.05) ↓Total WAT weight (p&lt;0.05) and epididymal WAT ↑plasma adiponectin level</td>
</tr>
<tr>
<td>Jung 2013&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>CMPE 100, 330 mg/kg, twice daily for 8 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND (n=10) 2) HFD (n=10) 3) HFD + CMPE 100 mg/kg (n=10) 4) HFD + CMPE 300 mg/kg (n=10)</td>
<td>none</td>
<td>↓Body weight in 300 mg/kg group (p&lt;0.001)</td>
</tr>
<tr>
<td>Cho 2014&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vivo</td>
<td>CMP powder, 5%, 10%, 15%, for 4 weeks</td>
<td>HFD Sprague Dawley rat</td>
<td>1) ND 2) HFD 3) HFD + CMPE 5% 4) HFD + CMPE 10% 5) HFD + CMPE 15%</td>
<td>none</td>
<td>↓Body weight in 15% group (p&lt;0.05) ↓FER in 10% and 15% group (p&lt;0.05) ↓Liver, kidney, testis weight in 10% and 15% group (p&lt;0.05)</td>
</tr>
<tr>
<td>Choi 2014&lt;sup&gt;13&lt;/sup&gt;</td>
<td>in vitro</td>
<td>CMPW 100 μg/ml</td>
<td>OP9 cell</td>
<td>1) Normal (pre-adipocyte) 2) Control (adipocyte) 3) CMPW 100 μg/ml 4) CMPW 100 μg/ml</td>
<td>none</td>
<td>↓Lipid accumulation (p&lt;0.001) ↓Protein expression of PPARγ2 ↓Protein expression of Adiponectin</td>
</tr>
<tr>
<td>Lim. in vitro</td>
<td>CMPC. CMPE. 0.5 mg/mL each</td>
<td>3T3-L1 cell</td>
<td>(CMPC)</td>
<td>CMPE 0.5 mg/mL</td>
<td>Sinetrol 0.05 mg/mL</td>
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<tr>
<td>CMPE 0.5 mg/mL</td>
<td>SREBP1 diet, 2018</td>
<td>1) Preadipocyte</td>
<td>2) Adipocyte</td>
<td>3) Sinetrol 0.05 mg/mL</td>
<td>4) CMPE 0.5 mg/mL</td>
<td>5) CMPE 0.5 mg/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parkinson in vivo</th>
<th>CMPE 1% or FCMPE 0.3% or FCMPE 1% for 13 weeks (ad libitum)</th>
<th>CMPE 1%</th>
<th>CMPE 0.5%</th>
<th>CMPE 0.5%</th>
<th>(CMPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) LFD (n=8)</td>
<td>2) HFD (n=8)</td>
<td>3) HFD + CMPE 1% (n=8)</td>
<td>4) HFD + FCMPE 0.3% (n=8)</td>
<td>5) HFD + FCMPE 1% (n=8)</td>
<td></td>
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<tr>
<td>↓ Adipocyte differentiation (p&lt;0.05)</td>
<td>↓ mRNA levels of C/EBPα, PPARγ, SRBEP1 (p&lt;0.05)</td>
<td>↓ Protein expression of C/EBPα and PPARγ (p&lt;0.05)</td>
<td>↑ glycerol secretion (p&lt;0.05)</td>
<td>↓ mRNA levels of PPARγ (p&lt;0.05)</td>
<td>↑ glycerol secretion (p&lt;0.05)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kang, 2018*</th>
<th>Clinical research</th>
<th>CMPE juice extract</th>
<th>138 adult patients BMI 23 (Female: 88; Male: 50)</th>
<th>ND (n=8)</th>
<th>0.1% naringenin</th>
<th>None</th>
<th>↓ Weight. BMI (p&lt;0.001)</th>
<th>↓ Waist circumference (p&lt;0.002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ND (n=8)</td>
<td>2) HFD (n=8)</td>
<td>3) HFD + CMPE 1% (n=8)</td>
<td>4) HFD + FCMPE 1% (n=8)</td>
<td>5) HFD + FCMPE 1% (n=8)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Zeng, 2020*</th>
<th>In vivo</th>
<th>CBPW 1.04 g/kg for 4 weeks</th>
<th>Sprague-Dawley rat</th>
<th>ND (n=8)</th>
<th>HFD (n=8)</th>
<th>1.04 mg/kg simvastatin</th>
<th>Weight (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ND (n=8)</td>
<td>2) HFD (n=8)</td>
<td>3) HFD + 104 mg/kg simvastatin (n=8)</td>
<td>4) HFD + CBPW 104 g/kg (n=8)</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ke, 2020*</th>
<th>In vivo</th>
<th>CBPE 0.2% and 0.5% for 10 weeks</th>
<th>CMPE 1%</th>
<th>ND (n=8)</th>
<th>HFD (n=8)</th>
<th>0.1% naringenin</th>
<th>Weight in both groups (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ND (n=8)</td>
<td>2) HFD (n=8)</td>
<td>3) HFD + 0.1% naringenin (n=8)</td>
<td>4) HFD + 0.2% CBPE (n=8)</td>
<td>5) HFD + 0.5% CBPE (n=8)</td>
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* FER: food efficiency ratio: body weight gain / food intake for 4 weeks

a) Reduction of food efficiency ratio (FER)
   (a1) In vivo
   CMPE 0.5% (24.1 mg/kg) applied to HFD rats and CMPE 2 g per 100 g diet to diabetic mice induced significant decrease of FER (p<0.05). Especially in CMPE 2 g group the other hand, FER was significantly increased in ROG group by 2.9-fold, suggesting greater weight gain efficiency. CMAP powder 10% and 15% also reduced FER (p<0.05). Acrelobacterium pullulans fermented CMPE and CMPE 0.1%, 0.5% each were administered to HFD rats and FER was significantly reduced (p<0.05), except for CMPE 0.5% group. As well, CMPE fermented by Aspergillus niger, which was found to contain naringenin, was administered to HFD mice. CMPE 0.3%, 1% and CMPE 1% all lowered FER in HFD mice (p<0.05).

b) Reduction of body weight, body mass index and waist circumference
   (b1) In vivo
   Significant decrease in body weight gain was observed in CMPE 2 g per 100 g diet fed diabetic mice (p<0.05), whereas those in rosiglitazone (RGZ) group were markedly increased compared to both
the control and CMPE groups\(^5\). As well, body weight was significantly reduced by CMPE 300 \(\mu\)g/kg twice daily administration \((p<0.001)^{32}\). Daily feeding of CMP powder 15\% had significant bodyweight reducing effect in HFD rats \((p<0.05)^{30}\). Weight lowering effect of fermented Chenpi at HFD rats also have been proved. Aureobasidium pullulans fermented CMPE and CMPE 0.1\%, 0.5\% each were fed, and body weight of FCMPE 0.1\%, 0.5\% and CMPE 0.1\% were significantly reduced \((p<0.05)^{29}\). Especially FCMPE 0.5\% group, which was the most effective, lost more than 40 g compared to control group. Aspergillus niger fermented CMPE 0.3\%, 1\% and CMPE 1\% were applied, and both doses of FCMPE significantly reduced body weight \((p<0.05)^{28}\). A clinical research about CMP also has been conducted. CMP juice extract administration to 118 adult patients of BMI over 23 kg/m\(^2\) resulted significant loss of body weight \((p<0.0001)^{40}\). Several in vivo studies about CBP are also presented. CBPW 1.04 g/kg \((p<0.05)^{38}\), CBPE 0.2\% and 0.5\% \((p<0.05)^{37}\) led to significant body weight reduction in HFD rats.

(b.2) Clinical research

A clinical research, 4 weeks application of CMP juice extract to 118 adult patients of BMI over 23 kg/m\(^2\) resulted in reduction of BMI \((p<0.0001)\) and waist circumference \((p=0.0002)^{40}\).

c) Reduction of visceral adipose tissue

(c.1) In vivo

CMPE 0.5\%\(^{26}\) and Aureobasidium pullulans fermented CMPE 0.5\%\(^{26}\) significantly reduced weight of visceral adipose tissue \((p<0.05)\). In addition, Aspergillus niger fermented CMPE 0.3\% and 1\% lowered epididymal fat weight and adipocyte size of epididymal fat \((p<0.05)\) in HFD rats\(^{24}\). In diabetic mice, CMPE 2 g per 100 g diet significantly reduced total white adipose tissue (WAT) weight \((p<0.05)\) and epididymal WAT weight\(^{25}\). Also in this case, total WAT weight of RGZ group was markedly increased compared to both the control and CMPE group. CMP powder 10\% and 15\% lowered liver, kidney and testis weight of HFD fed rats \((p<0.05)^{30}\).

d) Suppression of TG biosynthesis

(d.1) In vitro

Glycerol 3-phosphate dehydrogenase (GPDH) uses NAD as a coenzyme, and transfers dihydroxyacetone phosphate into glycerol-3-phosphate\(^{63,64}\). It is usually activated in case of differentiation of preadipocyte into adipocyte. GPDH activity seems to be elevated in adipose tissue of obese subjects\(^5\). CMPE, Aureobasidium pullulans fermented CMPE and fermented citrus peel extract broth powder (FCB) 25 mg/mL suppressed GPDH activity in 3T3-L1 cell\(^9\).

e) Suppression of lipid accumulation

(e.1) In vitro

CMPE, Aureobasidium pullulans fermented CMPE and FCB 25 mg/mL lowered TG content in 3T3-L1 cell\(^9\). In 2014 study, CMPW and Citrus Unshiu Pericarpium Immaturus water extract (CMPIW), each 100 \(\mu\)g/ml, were administered to OP9 cell\(^2\). As a result, both significantly inhibited lipid accumulation and CMPIW was found out to be more effective than CMPW \((p<0.01)\).

f) Stimulation of lipid excretion through feces

(f.1) In vivo

Fibrous element of Chenpi also has shown anti-obesitic effect. 1\% albedo total dietary fiber (TDF), extracted by method of Prosky, was fed to Wistar–Hannover GALAS rats. As a result, weight of the cecum content and lipid content of feces were significantly reduced \((p<0.05)^{31}\).
g) Suppressing adipogenesis

(1) In vitro

In 3T3-L1 cells, CMPE, Aureobasidium pullulans fermented CMPE and FCB 25, 50 mg/mL suppressed adipogenesis39.

h) Regulation of lipid metabolism

(1) In vitro

Adiponectin, which promotes insulin activity, is suppressed by inflammatory cytokines such as IL-6, tumor necrosis factor α (TNFα) and IFN-γ66. Decrease of adiponectin level was observed in hepatic steatosis and T2DM model (OP9 cell)67,68.

PPARγ, a transcription factor which is strongly expressed in adipose tissue, is involved in adipogenesis differentiation, carbohydrate, and lipid metabolism69. CMPIW and CMPIW down regulated the protein expression of peroxisome-proliferator activated receptor γ2 (PPARγ2) and Adiponectin22. In addition, CMPI was found to have better lipid-lowering and PPARγ2 suppressing effect than CMPIW. CMPIW 10 μg/mL suppressed protein expression of PPARγ2 in OP9 cell69. Cytolase is a compound of glycosidases removed from Aspergillus niger, which biocorverted CMP into gycoside forms. In 3T3-L1 cells, Citrus unshiu with cytolase (CMPC) 0.5 mg/mL significantly reduced mRNA levels of CCAAT/Enhancer-binding Protein α (C/EBPα), SREBP1, protein expression of C/EBPα and PPARγ (p<0.05)27. It also significantly suppressed adipocyte differentiation (p<0.05), showing better effect than that of Sinetrol positive control group. In all CMPC, CMPI and CMPIW 0.5 mg/mL groups, mRNA level of PPARγ was reduced, and glycerol secretion, which is involved in lipolysis, was increased (p<0.05).

(2) In vivo

Anti-obesitic activity related to lipase activity of Chenpi dietary fiber was evaluated. 1% albedo TDF extracted by method of Prosky significantly suppressed inhibition of pancreatic lipase activity in Wistar-Hannover GALAS rat (p<0.05)31. Also, CMPE 2 g per 100 g diet fed to diabetic mice for 6 weeks increased plasma adiponectin level25.

4. Hepatic steatosis

IR has strong correlation to hepatic steatosis, a previous phase of NAFLD70. Resistance to insulin activity on hepatic gluconeogenesis leads to an excessive lipid accumulation in the liver71. Susceptibility of IR and type 2 DM in patients with NAFLD has been studied23. The effect of Chenpi on hepatic steatosis is analyzed below (Table 4).
| Cho. 2014\(^{a}\) in vivo | CMP powder for 4 weeks | HFD Sprague Dawley rat | 1) ND  
2) HFD  
3) HFD+CMP  
4) HFD+CMP 5%  
5) HFD+CMP 10%  
6) HFD+CMP 15% | none | ↓ Hepatic total lipid level in 10% and 15% group (p<0.05)  
↓ Hepatic TC level in 10%, 15% group (p<0.05)  
↓ Hepatic TG level in 10% group (p<0.05)  
↓ Hepatic lipid level accumulation |
| Lim. 2014\(^{b}\) in vivo | CMPE 30, 100 or 300 mg/kg for 8 weeks | OVX-Sprague Dawley rat | 1) Sham (n=12)  
2) OVX (n=12)  
3) OVX+E2 10 μg/kg (n=12)  
4) OVX+CMPE 100 mg/kg (n=12)  
5) OVX+CMPE 300 mg/kg (n=12)  
6) E2 10 μg/kg | none | ↓ Serum GOT, GPT level (p<0.05)  
↓ Hepatic TC, TG level in 100, 300 mg/kg group (p<0.05)  
↓ Hepatic fatty deposition in hepatocytes in 300 mg/kg group |
| Park 2017\(^{c}\) in vivo | CMPE 1%, FCMP 0.3% or FCMP 1% for 13 weeks | C57BL/6J mice | 1) LFD (n=8)  
2) HFD (n=8)  
3) HFD+CMPE 0.3% (n=8)  
4) HFD+CMPE 1% (n=8)  
5) HFD+CMPE 1% (n=8) | none | ↓ Hepatic lipid accumulation in FCMP 0.3% and 1% group (p<0.05) |
| Kwak 2020\(^{d}\) in vivo | CMPW 200 or 300 mg/kg for 4 weeks | diabetics C57BL/6J mice  
induced with STZ | 1) Diabetic mice (n=6)  
2) Diabetic mice+CMPW 200 mg/kg (n=6)  
3) Diabetic mice+CMPW 300 mg/kg (n=6) | none | ↓ Liver and kidney weight (p<0.05) and size |
| Ke. 2020\(^{e}\) in vivo | CBPE 0.2% and 0.5% for 10 weeks | C57BL/6J mice | 1) ND (n=8)  
2) HFD (n=8)  
3) HFD+0.1% resveratrol (n=8)  
4) HFD+0.2% CBPE (n=8)  
5) HFD+0.5% CBPE (n=8) | none | ↓ Lobe structure micro steatosis and excessive lipid droplet accumulation in the liver, in both groups  
↓ NAS in 0.5% group (p<0.05)  
↓ Hepatic TG level in both groups (p<0.05)  
↓ Hepatic TC level in both groups (p<0.05)  
↓ Hepatic MDA level in both groups (p<0.05)  
↓ Hepatic MDA level and hepatic LPO in 0.5% group (p<0.05)  
↑ Hepatic GR level in both groups (p<0.05)  
↑ mRNA expressions of Nrf2 in both groups (p<0.05)  
↑ mRNA expressions of Prdx and NQ-01 in 0.5% group (p<0.05) |


a) Reduction of liver weight and size  
(a1) In vivo  
CMPE 2 g per 100 g diet fed to diabetic mice significantly reduced liver weight (p<0.05, p<0.01)
each) and size\(^3\). CMPW 200 and 300 mg/kg significantly decreased liver and kidney weight (p<0.05) and size in STZ induced diabetic mice\(^3\).

b) Suppression of hepatic lipid accumulation

(b.1) In vivo

Various studies have shown reduction of hepatic lipid accumulation in liver. It was shown at CMPE 300 mg/kg in OVX rats\(^2\). Particularly, CMPE 2 g per 100 g diet administration to diabetic mice lowered hepatic lipid levels were in comparison to those in RGZ 0.001 g per 100 g diet group, which were conversely increased\(^2\). Similar results were shown at CMP powder 5%, 10%, 15% and Aspergillus niger fermented CMPE 0.3%, CMPE 1% (p<0.05)\(^3\) in HFD mice. CBPE 0.2%, 0.5% in HFD mice lowered lobule structure, micro steatosis and excessive lipid droplet accumulation in the liver, in both groups\(^3\). Also, 10% and 15% CMP powder in HFD rats resulted in reduction of hepatic total lipid level (p<0.05)\(^3\).

c) Reduction of hepatic lipid levels

(c.1) In vivo

CMPE 2 g per 100 g diet lowered hepatic TG, TC and FFA level in diabetic mice (p<0.05)\(^5\). Freeze dried Chenpi also had lipid lowering effect. Hepatic TC level was reduced by 10%, 15% freeze dried CMP medication, and hepatic TG level was reduced by 10% CMP powder medication\(^5\). In OVX rats, CMPE 100 and 300 mg/kg administration induced decreased of hepatic TC and TG level (p<0.05)\(^3\). In HFD mice CBPE 0.2%, 0.5% all reduced hepatic TG level significantly (p<0.05)\(^7\). In addition, CBPE 0.5% apply lowered hepatic TC level (p<0.05)\(^7\).

d) Suppression of hepatic lipid synthesizing enzyme activity

(d.1) In vivo

CMPE 2 g per 100 g diet fed to diabetic mice resulted in down regulation of hepatic lipid regulating enzyme activities and mRNA expression\(^5\). Hepatic FAS, malic enzyme (ME) activities, which implies fatty acid synthesis, were significantly down-regulated, even compared to that of RGZ group (p<0.05). Activity of phosphatidate phosphohydrolase (PAP), related to TG synthesis, was down-regulated (p<0.05), and reverse effect was shown in RGZ group. The rate-limiting enzyme in cholesterol synthesis, mRNA level of HMGR, was significantly lowered (p<0.05). Also, hepatic CMPT mRNA expression and fatty acid β-oxidation were significantly up-regulated (p<0.05).

Administration of 0.2% and 0.5% CBPE to HFD mice proved anti-hepatic oxidative stress effect\(^8\). In both groups, serum malondialdehyde (MDA) level was lowered (p<0.05) and hepatic glutathione reductase (GR) level was elevated (p<0.05). And in 0.5% group, hepatic MDA, and hepatic lipid peroxidation (LPO) level were reduced (p<0.05). Lastly, it upregulated hepatic mRNA expression of nuclear factor – like 2 (Nfr2) signaling genes and ameliorated inflammatory cytokines. Up-regulations of nuclear factor erythroid 2-related factor 2 (Nfr2) mRNA expression in both groups (p<0.05) and peroxiredoxin (Prdx) and Nicotinamide Adenine Dinucleotide Phosphate Hydrogen quinone oxidoreductase (NQ-O1) mRNA expressions in 0.5% group (p<0.05) were observed.

e) Reduction of non-alcoholic fatty liver activity score

(e.1) In vivo

0.5% CBPE fed to HFD mice confirmed significantly lowered NAFLD activity score (NAS) (p<0.05).

5. Inflammation

Reports have proved the relation of inflammation in the pathogenesis of MetS associated disorder.
T2DM\(^7\). In addition, it is known that chronic inflammation is closely related to T2DM and MetS\(^7\). The effect of Chenpi on inflammation is analyzed below (Table 5).

### Table 5. Summary of studies on Chenpi in Inflammation

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Method</th>
<th>Dose and duration</th>
<th>Experimental model</th>
<th>Control and intervention groups</th>
<th>Positive control</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park 2013(^6) in vivo</td>
<td>CMPE 2 g/100 g diet for 6 weeks</td>
<td>5BL/6-db/db mice</td>
<td>1) Control (n=10) 2) CMPE 2 g/100 g diet (n=10) 3) Rosiglitazone 0.001 g/100 g diet (n=10)</td>
<td>Rosiglitazone (0.001 g/100 g diet)</td>
<td>↓Serum IL-6, IFN-γ level (p&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td>Ke. 2020(^8) in vivo</td>
<td>CBPE 0.2% and 0.5% for 10 weeks</td>
<td>C57BL/6 mice</td>
<td>1) ND (n=8) 2) HFD (n=8) 3) HFD +0.1% resveratrol (n=8) 4) HFD +0.2% CBPE (n=8) 5) HFD +0.5% CBPE (n=8)</td>
<td>0.1% resveratrol</td>
<td>↓TNF-α, IL-6 and IL-1β mRNA levels in both groups (p&lt;0.05)</td>
<td></td>
</tr>
</tbody>
</table>

CMPE : Citrus unshiu Marcovich ethanol extract, db/db : diabetic, IL-6 : interleukin 6, TNF-α : tumor necrosis factor α, IFN-γ : Interferon-γ, MCP-1 : monocyte chemoattractant protein 1, IL-10 : interleukin 10, CBPE : Citrus reticulata Blanco pericarpium ethanol extract, HFD : high fat diet, LFD : low fat diet, ND : normal diet

a) Reduction of tumor necrosis factor and interferon level

(a.1) In vivo

CMPE 2 g per 100 g diet was fed to diabetic mice, for 6 weeks, and RGZ was selected as positive control\(^6\). In conclusion, reduction of inflammatory biomarkers in blood and liver were observed, which indicate that CMPE attenuated diabetes-induced inflammatory responses. Plasma interleukin (IL-6), tumor necrosis factor-α (TNF-α) and IFN-γ level were significantly decreased (p<0.05). In addition, adiponectin (plasma anti-inflammatory) and IL-10 level were increased but RGZ group lowered them. Elevation of plasma proinflammatory cytokines such as interleukin-6 (IL-6), TNF-α and interferon (IFN)-γ\(^7\) was linked to insulin resistance, leading to NAFLD\(^7\). CMPE 2 g per 100 g applied to HFD mice reduced plasma MCP-1 level and hepatic MCP-1 mRNA expression. However, RGZ group did not alter this pro-inflammatory marker in plasma and liver.

**IV. Conclusion and Discussion**

Recently, various in vitro, in vivo, and clinical studies have substantiated that Chenpi has beneficial effects against T2DM, HL, obesity, hepatic steatosis, and inflammation, resulting in amelioration of MetS.
According to previous discussion in this article, Chenpi has effect on T2DM through glucose lowering, insulin secretion stimulation, glucose regulation, gluconeogenesis suppression, beta cell protection and anti-inflammatory activity. It exerts effect on HL through plasma lipid regulation, apolipoprotein reduction, plasma lipid metabolism regulation and preventing atherosclerosis. In aspects of obesity, Chenpi exerts decrease of FER, body weight, BMI, visceral adipose tissue. TG bio synthesis, lipid accumulation and adipogenesis. Lipid metabolism regulation and lipid excretion through feces are also included. Chenpi has also been substantiated hepatic steatosis ameliorating effect, by reduction of liver weight and size, hepatic lipid accumulation, hepatic lipid level, NAFLD activity score and suppression of hepatic lipid synthesizing activity.

Chenpi has effect of regulating qi (理氣), dissolving abscesses (散結), drying dampness (燥濕), relieving hiccup (止嘔), suppressing cough (止嗽), ameliorating diarrhea (止瀉), resolving phlegm (化痰), removing food stagnation (消食), eliminating phlegm (消痰), increasing appetite (開胃), relieving strangury (通淋). It has been used in various symptoms, comprising vomiting, hiccup, lost of appetite, cough, sputum and dyspepsia. It is included in various decoctions for digestive symptoms and invigorants. Gamibojungki-tang, gamiyukguna-tang, yukgunja-tang, gamiyijn-tang, gamiyukwhanjeonggi-san had been reported for their anti-obesitic effects. Also, bojungkei-tang for its hypolipidemic effect, sopyun-tang, gamiyukmijhwang-tang for their hypoglycemic effect, and saenggangeobbi-tang for its anti-fatty liver effect had been reported. All mentioned herbal decoctions contains Chenpi as common herbal medicine. It implies possibility of Chenpi as treatment of various metabolic diseases. However, lack of clinical or reviewing researches so far built limits to prove the efficacy of Chenpi. Therefore, our research targeted the potentials of Chenpi for MetS treatment. Chenpi appears to be potential treatment of chronic metabolic disorders, as hypoglycemic, hypolipidemic, anti-obesitic, fatty liver-ameliorating and anti-inflammatory effects were broadly studied. On the basis of our research, we look forward to continuous studies and to prove applicability of Chenpi as MetS treatment, in clinical use.

There were some limitations on discussing effects of Chenpi on our research. First, every dose of Chenpi used in experiments were not quantified, so it was not enough to compare exact effect with same amount of experimental material. Second, there were some studies about effect of bioconverted Chenpis, claiming that anti-diabetic, anti-hyperlipidemic and anti-obesitic effect would be reinforced by bioconversion. Though, bioconverted flavonoids were not standardized due to all different mycotoxins used in fermentation, resulting in inexact comparison. Finally, more clinical studies about efficacy of Chenpi in above-mentioned conditions (diabetes, hyperlipidemia, obesity, hepatic steatosis and inflammation) should be required to provide evidence of clinical use.

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