Regulation of lipid disorders by ethanol extracts from Zingiber zerumbet in high-fat diet-induced rats

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A B S T R A C T

The aim of this study was to investigate the antihyperlipidaemic effects of the ethanol extract of Zingiber zerumbet (L) Smith (EEZZ). After being fed a high-fat diet (HFD) for 2 weeks, rats were dosed orally with EEZZ (100, 200 or 300 mg/kg) or fenofibrate (100 mg/kg) once daily for 8 weeks. EEZZ (300 mg/kg/day) produced effects similar to fenofibrate in reducing body weight gain, visceral fat-pad weights and plasma lipid levels. EEZZ caused reductions in hepatic triglyceride and cholesterol content, and lowered hepatic lipid droplet accumulation and the size of epididymal adipocytes. HFD-induced reductions in the hepatic proteins of peroxisome proliferator-activated receptor (PPAR) α, acyl-CoA oxidase (ACO) and cytochrome P450 isoform 4A1 (CYP4A1) were reversed by EEZZ. These results suggest that EEZZ reduced the accumulation of visceral fat and improved hyperlipidaemia in HFD-fed rats by increasing fatty acid oxidation, an effect which is likely to be mediated via up-regulation of hepatic PPARα.

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1. Introduction

Obesity is a common chronic disorder of carbohydrate and fat metabolism, characterised by excessive fat deposition in adipose tissue and other internal organs, such as liver, heart, skeletal muscle, and pancreatic islets (McCarthy, 2010). Obesity remains a major global public health issue because of its increasing prevalence, which cuts across issues of sex, age-group, ethnicity, or race (McCarthy, 2010). Obesity alone can induce all the symptoms of metabolic syndrome, which is associated with many additional health problems, including increased risk of insulin resistance, non-alcoholic fatty liver, atherosclerosis, degenerative disorders, such as dementia, some immune-mediated disorders, such as asthma, and certain cancers (Tilg & Moschen, 2006). Pharmacological approaches to weight control have become an overriding priority (Padwal & Majumdar, 2007). Current trends for obesity management involve multiple pharmacological strategies, including blocking nutrient absorption, modulating fat metabolism, regulating adipose signals, and modulating the satiety centre. However, these approaches have been associated with several serious adverse effects in the clinic, including adverse gastrointestinal effects and significant unfavourable cardiovascular effects (Padwal & Majumdar, 2007). As a result, a much safer therapeutic approach is necessary.

Zingiberaceae is widely distributed throughout the tropics, particularly in Southeast Asia (Afzal, Al-Hadidi, Menon, Pesek, & Dhani, 2001). Zingiber zerumbet (L) Smith (EEZZ), commonly known as the pinecone or shampoo ginger, is a perennial, tuberous root herb plant that has gained much interest from scientists all over the world because of its high medicinal values. This herb plant has been cultivated for thousands of years as a spice and also for medicinal purposes, i.e. as a cure for headaches, swelling, colds, ulcers, sores and loss of appetite, nausea and even menstrual discomfort, and has been introduced to many parts of the world as a rich source of compounds of phytomedicinal interest (White, 2007). The rhizome of Z. zerumbet has been used to treat various ailments in Asian and Arabic traditional medicine since ancient times (Altman & Marcussen, 2001; Jaganath & Ng, 2000). It is used in local traditional medicine as a cure for swelling, sores and loss of appetite. The juice of boiled Z. zerumbet rhizomes has been used for the treatment of worm infestation in children. Z. zerumbet has been shown to inhibit prostaglandinduced paw oedema, a commonly used acute inflammatory reaction and the efficacy is equivalent to the nonsteroidal anti-inflammatory drug, mefenamic acid (Sulaiman et al., 2010). The ethanol extracts of Z. zerumbet exhibit significant antipyretic activity in brewer’s yeast-induced pyrexia in rats (Somchit, Nor Shahida, & Nur Shukriyah, 2002). Z. zerumbet is also used in herbal medicinal for the treatment of rheumatological conditions and muscular discomfort (Langner, Greifenberg, &
Diet-induced obesity in rodents has been used as a model to investigate the interactions between the environment and genetics. Rats fed a high-fat diet (HFD) become obese and show distinctive visceral adiposity, dyslipidaemia, hyperinsulinaemia, and hepatic steatosis, which are typical of human obesity (Sclafani & Springer, 1976). Therefore, this study investigated the effects of Z. zerumbet on body fat and lipid profiles in rats with diet-induced obesity and sought possible mechanisms of action.

2. Materials and methods

2.1. Plant material and extraction

Z. zerumbet rhizomes were purchased from a local market in Dongshan, Dongshan Dist. (Tainan City, Taiwan) during October 2010. Macropscopic and microscopic examinations, as well as thin-layer chromatography and high-performance liquid chromatography, were used to confirm the authenticity of the plant material provided (this analysis was performed by Dr. Hong T.Y., Department of Biotechnology, Collage of Pharmacy and Health Care, Tajen University). Random amplified polymorphic DNA analysis of the Z. zerumbet rhizomes supplied was also performed, to identify DNA polymorphisms. The voucher specimen (Lot No. 201010188) has been deposited in our laboratory. Pulverised Z. zerumbet rhizomes (5 kg) were added to 10L of 95% ethanol at room temperature for 7 days, and were occasionally shaken. The ethanol extract of Z. zerumbet rhizomes (EEZZ) was evaporated to dryness under reduced pressure, for the total elimination of alcohol, followed by lyophilisation, yielding approximately 575 g of dry residue (w/w yield: 11.5%). EEZZ was kept at –20°C until use and suspended in distilled water. The samples were then analysed using the LC/MS/MS protocol described below.

2.2. LC–MS/MS system

Chromatographic separation was performed using an HPLC apparatus equipped with two Micropumps Series 200 (PerkinElmer, Waltham, MA), a UV/Vis series 200 detector (PerkinElmer) at 280 nm, and a Prodigy ODS3 100A column (250 × 4.6 mm, particle size 5 μm; Phenomenex, Torrance, CA). The eluents were (A) 0.2% formic acid in water, and (B) acetonitrile/methanol (60:40, v/v). The following gradient program was used: 20–30% B (6 min), 30–40% B (10 min), 40–50% B (8 min), 50–90% B (8 min), 90% B (3 min), 90–20% B (3 min), at a constant flow of 0.8 mL/min. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer. Three 20-μL injections were performed for each sample. MS and MS/MS analyses of EEZZ were performed on an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA), equipped with a TurbolonSpray source and working in negative ion mode. The analyses were performed using the following settings: drying gas (air), 400 °C; capillary voltage (IS), 4000 V; nebuliser gas (air), 12 (arbitrary units); curtain gas (N₂), 14 (arbitrary units); and collision gas (N₂), 4 (arbitrary units). To optimise the declustering potential, focus potential, and collision energy for each compound, standard solutions (10 μg/mL) were infused directly into the mass spectrometer at a constant flow rate of 5 μL/min using a model 11 syringe pump (Harvard Apparatus, Holliston, MA). Kaempferol (purity ≥ 97.0%, Sigma–Aldrich, Inc., Saint Louis, MO) or zerumbone (purity ≥ 98.0%, Sigma–Aldrich, Inc.) at concentrations of 25–200 μg/mL were used to construct the standard curve. The retention times of kaempferol and zerumbone were 7.19 and 8.47 min, respectively. The linearity of the peak area (y) vs. concentration (x, μg/mL) curve for kaempferol and zerumbone was used to calculate the contents of the main components in EEZZ.

2.3. Animal models and treatment protocols

Male Wistar rats, 8 weeks of age, were obtained from the National Laboratory Animal Center (Taipei, Taiwan). They were maintained in a temperature-controlled room (25 ± 1 °C) on a 12:12 h light–dark cycle (lights on at 06:00 h) in the animal centre (Tajen University, Ping Tung Shien, Taiwan). Food and water were available ad libitum. Regular rat chow diet (RCD, #D12450B, Research Diets, New Brunswick, NJ) with 20 kcal% protein, 70 kcal% carbohydrate, and 10 kcal% fat from lard was used as the maintenance and control diet. A purified ingredient HFD with 20 kcal% protein, 35 kcal% carbohydrate and 45 kcal% fat primarily from lard (#D12451, Research Diets) was used to induce a rapid increase in body weight (BW) and obesity (Van Heek et al., 1997). The caloric density of the control diet was 3.85 kcal/g; that of the HFD was 4.73 kcal/g. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. These studies were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Tajen University (Approval number: IACUC 99-16; Approval date: September 9, 2010).

After being fed a HFD for 2 weeks, rats were dosed by oral gavage once per day for 8 weeks with EEZZ doses of 100, 200, and 300 mg/kg in a volume of 2 mL/kg distilled water. Another group of HFD-fed rats was treated orally for 8 weeks with 100 mg/kg/day fenofibrate (purity ≥ 99.0%; Sigma–Aldrich). The dose of fenofibrate was based on studies with long term fenofibrate treatment in rats (Zhou et al., 2008). A vehicle-control group of HFD-fed and RCD-fed rats was treated with 1.5 mL/kg distilled water only over the same treatment period. Eight weeks after treatment with EEZZ or fenofibrate (total diet-fed period was 10 weeks), HFD-fed rats were weighed and anaesthetised with sodium pentobarbital (30 mg/kg) administered intraperitoneally (i.p.), and blood samples were collected from the lateral tail vein. Feed and water were supplied ad libitum throughout the 10-week experimental period. Samples were centrifuged at 2000g for 10 min at 4°C. The plasma was then removed and placed into aliquots for the respective analytical determinations. After blood was collected, the liver and visceral and subcutaneous white adipose tissues (WAT) were removed, rinsed with physiological saline, weighed, and immediately stored at –70°C. Each experimental group in the study contained eight rats.

2.4. Biochemical parameter analysis

Diagnostic kits for the measurement of plasma glucose, total cholesterol (TC), and triglycerides (TG) were purchased from Cayman Chemical Company (Ann Arbor, MI). The diagnostic kit to determine plasma and hepatic levels of high density lipoprotein cholesterol (HDL-C) was purchased from Bio-Quant Diagnostics (San Diego, CA), and low density lipoprotein cholesterol (LDL-C) levels were calculated using Friedewald’s equation (Friedewald et al., 1972). Plasma free fatty acid (FFA) levels were determined using an FFA-quantification kit obtained from Abcam plc (Boston, MA). All samples were analysed in triplicate. Atherogenic index (AI) and coronary risk index (CRI) were calculated as LDL-C/HDL-C and TC/HDL-C, respectively (Abbott et al., 1988; Shanmugasundaram et al., 1986).
2.5. Extraction of hepatic lipids

A section of each liver was collected for lipid content analysis. The liver (1.25 g) was homogenised with chloroform/methanol (1:2, 3.75 mL) and then chloroform (1.25 mL) and distilled water (1.25 mL) were added to the homogenate and mixed well. After centrifugation (1500g for 10 min), the lower clear organic phase solution was transferred into a new glass tube and then lyophilised. The lyophilised powders were dissolved in chloroform/methanol (1:2) as the hepatic lipid extracts and stored at −20 °C for fewer than 3 days (Folch, Lees, & Sloane-Stanley, 1957).

2.6. Hepatic pathological evaluation

Small pieces of hepatic tissues taken from experimental animals were fixed in 10% neutral formalin, dehydrated with alcohol, embedded in paraffin, and sectioned to a mean thickness of 2.6. Hepatic pathological evaluation were few sections (Folch, Lees, & Sloane-Stanley, 1957). The lyophilised powders were dissolved in chloroform/methanol (1:2) as the hepatic lipid extracts and stored at −20 °C for fewer than 3 days (Folch, Lees, & Sloane-Stanley, 1957).

2.7. Preparation of hepatic fractions

Hepatic fractions were prepared as described previously (Park et al., 2009). To prepare nuclear fractions, hepatic tissue was homogenised with ice-cold lysis buffer containing 5 mM Tris–HCl (pH 7.5), 2 mM MgCl₂, 15 mM CaCl₂, and 1.5 M sucrose, 0.1 M dithiothreitol (DTT), and protease inhibitor cocktail. After centrifugation (10,500g for 20 min at 4 °C), the pellet was suspended in extraction buffer containing 20 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (pH 7.9), 1.5 mM MgCl₂, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, 0.1 M DTT, and protease inhibitor cocktail. The mixture was placed on ice for 30 min. The nuclear fraction was prepared by centrifugation at 20,500g for 5 min at 4 °C. The post-nuclear fraction was extracted from the liver of each rat as described below. In brief, hepatic tissue was homogenised with ice-cold lysis buffer (pH 7.4) containing 137 mM NaCl, 20 mM Tris–HCl, 1% Tween 20, 10% glycerol, 1 mM phenylmethylsulphonyl fluoride (PMSF), and protease inhibitor cocktail solution in DMEM. The homogenate was then centrifuged at 2000g for 10 min at 4 °C. The protein concentration of each fraction was determined using a commercial kit (Bio-Rad Laboratories, Hercules, CA).

2.8. Western blot analyses

For the determination of peroxisome proliferators activated receptor (PPAR), 30 mg protein of each nuclear fraction were resolved using 8% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS–PAGE). Separated proteins were transferred electrophoretically to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies to PPARα (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or β-actin (Santa Cruz Biotechnology, Inc.) overnight at 4 °C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP-conjugated secondary antibody for 1.5 h at room temperature. The blots were stripped with Restore Western Blot Stripping Buffer (CANDOR Bioscience GmbH, Wangen, Germany) for 15 min and incubated with the antibodies. In addition, 30 mg protein from each post-nuclear fraction for acyl-CoA oxidase (ACO; Santa Cruz Biotechnology, Inc) and cytochrome P450 isofrom 4A1 (CYP4A1; Santa Cruz Biotechnology, Inc.) were subjected to 10% SDS–PAGE. Each antigen–antibody complex was visualised using ECL Western Blotting Detection Reagents and detected by chemiluminescence with LAS-1000 plus (Fujifilm, Tokyo, Japan). Band densities were determined using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to β-actin. The mean value for samples from the vehicle-treated RCD-fed group on each immunoblot, expressed in densitometry units, was adjusted to a value of 1.0. All experimental sample values were then expressed relative to this adjusted mean value. A total of five independent experiments were carried out for statistical analysis.

2.9. Statistical analysis

Data are expressed as the mean ± standard deviation (SD) for each group of animals, at the number indicated in tables. Statistical analysis was performed with one-way analysis of variance (ANOVA). The Dunnett range post-hoc comparisons were used to determine the source of significant differences where appropriate. A p value <0.05 was considered statistically significant.

3. Results

3.1. Quantitative analysis

The standard curves for kaempferol and zerumbone are

\begin{align*}
y &= 189.64x + 268.41 \quad (r^2 = 0.9995) \\
y &= 188.1x + 333.68 \quad (r^2 = 0.9991),
\end{align*}

respectively. The chromatogram of the sample solution is shown in Fig. 1. The contents of kaempferol and zerumbone in EZZZ were 94.6 ± 0.18 and 164.5 ± 0.23 μg/g, respectively.

3.2. Effects of treatment on body weight (BW) and food intake

The BWs of HFD-fed rats in the drug-treated and vehicle-treated groups were monitored over the 8 week treatment period (Table 1). At the end of treatment, the BW of EZZZ-treated HFD-fed rats (n = 8) was significantly lower than that of rats in the vehicle-treated group (n = 8). EZZZ significantly suppressed BW gain in HFD-fed rats at both the moderate (200 mg/kg/day; n = 8) and high doses (300 mg/kg/day; n = 8). Similar results were seen in HFD-fed rats treated with fenofibrate (100 mg/kg/day; n = 8; Table 1). No significant differences in daily food intake were observed among the groups over the experimental period, despite the slightly higher water intake observed in the vehicle-treated HFD-fed group, as compared to the others (n = 8; Table 1).

3.3. Effects of treatment on fat pad weight

The weights of fat pads from HFD-fed rats in the drug-treated and vehicle-treated groups were assessed after the treatment period (Table 1). Epididymal WAT, perirenal WAT, mesenteric WAT, and inguinal fat pads were lower in fenofibrate-treated rats (n = 8) than in their vehicle-treated counterparts. Similarly, a significant (p < 0.05) reduction in fat-pad weight was seen in fenofibrate-treated rats (n = 8).

3.4. Effect of treatment on plasma lipids

The HFD caused elevated concentrations of plasma TC, TG, and LDL-C. The moderate (200 mg/kg/day) and high doses (300 mg/kg/day) of EZZZ significantly reduced plasma total TC levels (23.8%...
and 31.4% reduction, respectively) compared with vehicle-treated, HFD-fed rats (Table 2). All doses of EEZZ decreased plasma TG levels in HFD-fed rats (n = 8; Table 2). The low (100 mg/kg/day), moderate, and high doses of EEZZ significantly reduced plasma LDL-C levels (25.1%, 35.8%, and 60.1% reductions, respectively; Table 2). Plasma TC, TG, and LDL-C concentrations were reduced significantly by 38.5%, 49.8%, and 60.9%, respectively, in fenofibrate-treated, HFD-fed rats compared with vehicle-treated, HFD-fed rats (n = 8; Table 2).

The plasma concentration of HDL-C in HFD-fed rats was reduced to 62.5% of the level in the RCD-fed group (n = 8; Table 2). After 8 weeks of treatment with EEZZ (300 mg/kg/day; n = 8) or fenofibrate (n = 8), the plasma HDL-C concentration in HFD-fed rats was elevated to nearly that of the RCD-fed rats. Plasma FFAs were significantly higher in vehicle-treated, HFD-fed rats, compared to RCD-fed rats (Table 2). The plasma FFA level was reduced by 38.3% in HFD-fed rats treated with 300 mg/kg per day EEZZ,

Fig. 1. LC–MS/MS chromatogram for kaempferol and zerumbone in EEZZ sample.
EEZZ or fenofibrate was dissolved in distilled water for oral administration at the desired doses in a volume of 2 mL/kg once a day into HFD-fed rats. The vehicle (distilled water) used to dissolve the tested medications was given at the same volume. Values (mean ± SD) were obtained from each group of eight animals in each group after 8 weeks of the experimental period.

Table 2
Changes on body weight, food intake and fat-pad weight in HFD-fed rats receiving 8-week treatment with EEZZ or fenofibrate.

<table>
<thead>
<tr>
<th>Variable (s)</th>
<th>RCD-fed</th>
<th>HFD-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>EEZZ (mg/kg/day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Initial BW (g/rat)</td>
<td>180.8 ± 8.6</td>
<td>181.5 ± 7.3</td>
</tr>
<tr>
<td>BW gain (g/rat)</td>
<td>18.2 ± 2.8</td>
<td>52.8 ± 6.7</td>
</tr>
<tr>
<td>Food intake (g/rat/day)</td>
<td>208 ± 7.4</td>
<td>212 ± 7.2</td>
</tr>
<tr>
<td>Water intake (ml/rat/day)</td>
<td>69.1 ± 8.2</td>
<td>91.7 ± 7.7</td>
</tr>
<tr>
<td>Epididymal WAT (mg/100 g BW)</td>
<td>297.5 ± 15.8</td>
<td>417.8 ± 21.1</td>
</tr>
<tr>
<td>Perirenal WAT (mg/100 g BW)</td>
<td>169.4 ± 11.4</td>
<td>261.2 ± 14.1</td>
</tr>
<tr>
<td>Mesenteric WAT (mg/100 g BW)</td>
<td>127.2 ± 9.4</td>
<td>189.8 ± 12.4</td>
</tr>
<tr>
<td>Inguinal WAT (mg/100 g BW)</td>
<td>143.6 ± 10.7</td>
<td>221.4 ± 13.9</td>
</tr>
</tbody>
</table>

Changes in the plasma lipids, hepatic lipids, atherogenic index (AI) and coronary artery index (CRI) in HFD-fed rats receiving 8-week treatment with EEZZ or fenofibrate.

<table>
<thead>
<tr>
<th>Variable (s)</th>
<th>RCD-fed</th>
<th>HFD-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>EEZZ (mg/kg/day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Plasma TC (mg/dL)</td>
<td>80.1 ± 6.4</td>
<td>143.3 ± 13.5</td>
</tr>
<tr>
<td>Plasma TG (mg/dL)</td>
<td>59.6 ± 6.9</td>
<td>110.2 ± 6.0</td>
</tr>
<tr>
<td>Plasma LDL-C (mg/dL)</td>
<td>34.9 ± 3.5</td>
<td>110.2 ± 6.0</td>
</tr>
<tr>
<td>Plasma HDL-C (mg/dL)</td>
<td>45.3 ± 4.1</td>
<td>28.3 ± 2.7</td>
</tr>
<tr>
<td>Plasma FFAs (mg/dL)</td>
<td>29.4 ± 3.1</td>
<td>63.1 ± 4.7</td>
</tr>
<tr>
<td>AI</td>
<td>0.8 ± 0.2</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>CRI</td>
<td>1.8 ± 0.2</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Hepatic TC (μmol/g liver)</td>
<td>17.9 ± 3.4</td>
<td>55.2 ± 2.9</td>
</tr>
<tr>
<td>Hepatic TG (μmol/g liver)</td>
<td>12.6 ± 2.5</td>
<td>28.1 ± 1.9</td>
</tr>
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</table>

EEZZ or fenofibrate was dissolved in distilled water for oral administration at the desired doses in a volume of 2 mL/kg once a day into HFD-fed rats. The vehicle (distilled water) used to dissolve the tested medications was given at the same volume. Values (mean ± SD) were obtained from each group of eight animals in each group after 8 weeks of the experimental period.

- p < 0.05 compared to the values of vehicle-treated RCD-fed rats in each group, respectively.
- p < 0.01 compared to the values of vehicle-treated RCD-fed rats in each group, respectively.
- p < 0.05 compared to the values of vehicle-treated HFD-fed rats in each group, respectively.
- p < 0.01 compared to the values of vehicle-treated HFD-fed rats in each group, respectively.

3.5. Effect of treatment on hepatic lipids

The hepatic TC level was significantly higher in HFD-fed rats than in rats from the RCD-fed group. The hepatic TC levels were reduced by 25.6% in HFD-fed rats treated with 300 mg/kg/day EEZZ (n = 8; Table 2). Similarly, EEZZ treatment (300 mg/kg/day) also produced a significant reduction in hepatic TG concentration, to 64.7% of that in vehicle-treated, HFD-fed rats (n = 8; Table 2). Hepatic TC and TG levels were significantly reduced (by 33.2% and 48.1%, respectively) in fenofibrate-treated rats compared with vehicle-treated, HFD-fed rats (n = 8; Table 2).

3.6. Morphological changes in hepatocytes

HFD-fed rats showed significantly greater hepatic lipid accumulation than RCD-fed animals (panels A and B of Fig. 2). HFD-fed rats treated with 300 mg/kg/day EEZZ showed considerably lower hepatic lipid accumulation than their vehicle-treated counterparts (panels B and C of Fig. 2). The extent of hepatic lipid accumulation after 8 weeks in fenofibrate-treated, HFD-fed rats was similar to that in RCD-fed rats (panels A and D of Fig. 2). The pathological grading of hepatic steatosis in HFD-fed rats was 3.1 ± 0.4, which was reduced to 1.9 ± 0.6 and 1.3 ± 0.3 after receiving an 8 week treatment with EEZZ (300 mg/kg/day) or fenofibrate, respectively.

3.7. Protein expressions of PPARα, ACO and CYP4A in hepatic tissues

Hepatic PPARα protein expression in HFD-fed rats was lower than that in RCD-fed animals, but was elevated significantly by treatment with EEZZ (300 mg/kg/day) (Fig. 3). In addition, the hepatic expression levels of ACO and CYP4A proteins in HFD-fed rats were markedly lower than in RCD-fed rats, but were significantly elevated in EEZZ-treated (300 mg/kg/day) HFD-fed rats (Fig. 3). Similar results were seen in rats treated with fenofibrate (Fig. 3).
4. Discussion

Although the public enthusiasm for herbal remedies is growing around the world as well as in the Far East, the lack of scientific analysis published on the herbs makes it difficult to determine both the validity of their effects and their mechanisms of action. Due to the limited data concerning the efficacy of herbs and mechanistic explanation of their effects, more detailed herbal studies are demanded. High-energy diets are used widely in nutritional experiments as a strategy to induce overweight conditions and fat deposition in animals (Madsen et al., 2010). In this study, BW loss in HFD-fed rats was accompanied by a depletion of body fat stores, since treatment with EEZZ also significantly reduced the weight of the visceral and subcutaneous WAT, compared with that of vehicle-treated HFD-fed rats. This suggests that EEZZ suppresses the HFD-induced increase in adipose tissue mass and BW gain and that it may inhibit lipid accumulation in adipose tissue in particular.

Obesity, especially abdominal obesity, is associated with dyslipidaemia, characterised by elevated TG and reduced HDL-C concentrations (Lavie, Milani, & O’Keefe, 2011). TGs are involved in the ectopic accumulation of lipid stores in the liver and are associated with a number of diseases, such as metabolic syndrome and type 2 diabetes (Paccaud, Schlüter-Fasmeyer, Wietlisbach, & Bovet, 2000). High TC levels increase the risk of developing coronary heart disease, and high levels of LDL-C are a risk factor for coronary heart disease, while high HDL-C is helpful in transporting excess cholesterol to the liver for excretion in the bile (Malloy & Kane, 2001). As a result, HDL-C levels are inversely related to coronary heart disease risk (Toth & Davidson, 2010). Similar to fenofibrate treatment, the oral administration of EEZZ significantly lowered plasma TC, TG, and LDL-C levels in rats with HFD-induced obesity. Thus, EEZZ may be of benefit to patients with hypercholesterolaemia and hypertriglyceridaemia.

The effect of EEZZ on the atherogenic and coronary artery risk indices is also notable. The ratio of total cholesterol to HDL-C (i.e., the atherogenic index) and the ratio of LDL-C to HDL-C (i.e., the coronary artery index) are strong and reliable indicators of whether or not cholesterol is deposited into tissues or metabolised and excreted (Ansell, Watson, Fogelman, Navab, & Fonarow, 2005). The results of this study show that treatment with EEZZ or fenofibrate causes profound reductions in the atherogenic and coronary indices in experimental hyperlipidaemic rats. This strongly suggests that EEZZ has therapeutic potential for the management of obesity and hyperlipidaemia, and for the prevention of atherogenic cardiovascular diseases.

Due to the ability of EEZZ to reduce serum levels of TGs and total cholesterol, as well as adipose tissue mass and BW gain-functions similar to those of PPARα activation, we hypothesised that the actions of EEZZ are related to the regulation of hepatic expression of PPARα-target genes involved in lipid metabolism. In hepatocytes and other tissues (e.g., heart), ligand- (natural long-chain fatty acids) activated PPARα binds to peroxisome proliferator response elements of DNA and increases the transcription of genes encoding enzymes involved in fatty acid oxidation and lipoprotein metabolism (Mandard, Müller, & Kersten, 2004). The outcome is an increase in hepatic fatty acid oxidation and ketogenesis, decreased lipid levels in tissues, and protection against lipotoxicity. We found that EEZZ-treated HFD-fed rats had significantly higher hepatic PPARα protein, and that the effect of EEZZ was equivalent to that of fenofibrate. It appears that EEZZ regulates the lipid-profile in HFD-fed rats through an increase in hepatic PPARα levels. The discovery of EEZZ as a PPARα activator may offer the promise of a novel class of anti-diabetic drug.

Although reductions in the lipogenic activity and in dietary lipid absorption have been suggested as causes of the reduced liver lipid content, an increased capacity of peroxisomal β-oxidation (ACO)
and microsomal ω-oxidation (CYP4A) of fatty acids could also be contributing factors (Mandard, Müller, & Kersten, 2004). Elevated hepatic protein levels of ACO and CYP4A imply an enhanced fatty acid oxidation in peroxisomes and microsomes of EEZZ-treated HFD-fed rats. These results support the contention that EEZZ, by directly or indirectly activating PPARα, can up-regulate the expression of PPARα downstream genes, which may lead to enhanced hepatic fatty acid oxidation and reduced TG content.

_Z. zerumbet_ rhizomes have been the subject of extensive chemical investigations because of their high medicinal values. Various reports have been published regarding the phytochemical content of the plant. Not only has zerumbone been reported as the predominant compound in EEZZ; a considerable content of flavonoids such as kaempferol were found in this medicinal plant (Dae, Han, Park, Jhon, & Seo, 2004; Dung, Chinh, Rang, & Leclercq, 1993). Several polyphenols have been shown to exert an effect on lipid catabolism, glucose transport, the insulin receptor function and PPARs activation, all of which play essential roles in obesity (Crozier, Jaganath, & Clifford, 2009). It has been reported that kaempferol has an ameliorating effect on dyslipidaemia through an increase in hepatic PPARα levels (Chang, Tzeng, Liou, Chang, & Liu, 2011). Although immunomodulatory and anti-inflammatory effects have been demonstrated, the action of zerumbone on lipid regulation remains uncertain (Keong et al., 2010; Sulaiman et al., 2010). Kaempferol seems to play a principal role in lipid modulation of EEZZ. Further studies aimed at isolating and characterising the antiobesity and antihyperlipidaemic phytoprinciples in EEZZ are under way.

Due to different fat/lipid metabolism in humans and rats, the results from rat studies cannot be generalised to human. The placebo-controlled human studies are required to find the usability of EEZZ in human cholesterol/lipid/obesity indications. Also safety testing should be taken with the chronic consumption of large doses of this plant, especially in pregnant women, children, old people, and people with kidney diseases.

5. Conclusion

The results of this study show that EEZZ suppresses BW gain and body fat accumulation by increasing fatty acid oxidation, an effect which is likely mediated via up-regulation of PPARα in the

![Figure 3](image-url)
liver of HFD-fed rats. We suggest that supplemental treatment with EEZZ may prevent or improve obesity, by modulating lipid metabolism and preventing metabolic syndrome, a representative, lifestyle-related cluster of diseases caused by an excessively HFD.

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References


