Ameliorating effects of *Inonotus obliquus* on high fat diet-induced obese rats

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Lab Note

According to the data from WHO, there will be 2.16 billion overweight and 1.12 billion obese individuals in the world by the year 2030 [1], which raises the risk of multiple diseases, such as hypertension, diabetes, cardiovascular disease, and several types of cancer [2,3]. Obesity is viewed as a metabolic disorder, which is mainly induced by high energy intake. In mammals, excess energy is primarily stored as triglyceride (TG) in adipose tissue. Over the past decades, numerous molecules that regulate the process of adipogenesis have been well documented. Peroxisome proliferator-activated receptor γ (PPAR-γ) is required for the accumulation of adipose tissue and contributes to obesity [4]. Sterol regulatory element binding protein (SREBP) has been identified as a family of transcription factors that activate the entire program of cholesterol and fatty acid synthesis [5]. Glyceraldehydes 3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT) are widely expressed in mammalian tissues and catalyze the first and final step in TG synthesis, respectively [6]. Thus, these molecules may be great targets for the evaluation of obesity and lipid metabolism.

Recently, Sun et al. [7] have shown that the serum levels of lipids in the diabetic mice were significantly decreased after 3 weeks’ treatment with the dry matter of culture broth of *Inonotus obliquus*. Lee and Hyun [8] demonstrated that the water extracts from *I. obliquus* reduced the fat weight of high-fat diet fed obese mice. Thus, what we are further interested in is how can *I. obliquus* extracts affect the lipid metabolism and what is the possible mechanism underlying this anti-obesity effect. In the present study, we built a high-fat diet-induced rat model, and compared alterations of serum biochemical index, lipid accumulation in the liver, and expressions of some lipid-related genes among the normal, obese, and *I. obliquus* extract treated obese rats.

*Inonotus obliquus* was collected from Greater Khingan Mountains, Heilongjiang Province of China, and identified by Professor Xueqian Wu (Zhejiang Academy of Medical Science). The air-dried and powered sample (1.0 kg) was extracted with boiling water (30 l) for 3 h and concentrated. The supernatant was evaporated *in vacuo* at 45°C, followed by 95% ethanol (v/v) extraction to get crude extract. Then, the extracts were dispersed in double-distilled water and dried *in vacuo* and lyophilized to powders (yield 3.6%), and the powders were stored at −40°C until for use.

Twenty-four male Wistar rats (~120 g in weight) were purchased from the China National Laboratory Animal Resource Center (Shanghai, China). Rats were housed under a 12:12 h light–dark cycle and constant temperature (22 ± 1°C). Animals were adapted to laboratory lighting and feeding condition for 1 week before experiment. Water was available *ad libitum*, while food was provided only in the dark period. After adaptation, rats were randomly divided into three groups (n = 8). The control group (CON) was given normal commercial diet (M01-F; Shanghai Slac Laboratory Animal Co., Ltd, Shanghai, China) and treated with vehicle (reverse osmosis water). The other two groups were fed with high-fat diet and treated with either vehicle (HD) or 1000 mg/kg *I. obliquus* extract (HD-IOE). The high-fat diet consists of 18% fat, 20% carbohydrate, 3% egg, and 59% normal diet. The HD and HD-IOE rats were given intragastrically 15 min before food every day. During the experiment, the body weight and food intake of each rat were measured once and twice per week, respectively. At the end of the experiment, all rats were sacrificed under anesthesia by an intraperitoneal injection of sodium pentobarbital. Blood was collected from the axillary vessels and centrifuged at 6000 g for 5 min at 4°C, and the plasma was stored at −80°C until use. The serum free fatty acids (FFA), TG, and total cholesterol (TC) were determined by their kits (Nanjing Jianchen Institute of Biotechnology, Nanjing, China) respectively. Liver and epididymal fat tissues were quickly removed, weighed, frozen immediately in liquid nitrogen, and kept at −80°C for further use. Total RNA from the liver and epididymal fat tissues was isolated using Trizol reagent (TaKaRa, Dalian, China), and cDNA was synthesized using a reverse transcriptase kit (Toyobo, Tokyo, Japan). All experiments were performed according to the international ethical standards and approved by the Research Committee of the Zhejiang University of Technology.

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As shown in Fig. 1A, the body weights of the HD and HD-IOE groups were significantly increased at the second week compared with that of the CON group. At the ninth week, the significant difference was also observed between the HD and HD-IOE groups. By the end of the experiment, the body weight in the HD-IOE group was 9.2% lower than that in the HD group, but the food intake was found to be similar (Supplementary Table S1). On the other hand, after 12 weeks, no significant difference was found in the relative liver weight among the three groups (Fig. 1A). However, the relative epididymal fat weights in the HD-IOE group were significantly lower than those in the HD group (Fig. 1A), indicating that IOE feeding significantly decreased the relative epididymal fat weight in obesity rats.

The effects of IOE on lipid accumulation in liver were also investigated. As shown in Fig. 1B, the liver in the CON group did not show any significant changes after the experiment; in contrast, obvious lipid accumulation was observed in the liver of the HD group, and this fat accumulation was clearly ameliorated by the administration of IOE for 12 weeks. To confirm this observation, the contents of hepatic TG and TC were further examined as previously described [9]. The hepatic TG and TC levels in the HD group were significantly higher than those in the CON group (Fig. 1B), and the increases were inhibited by IOE treatment. In addition, the serum levels of FFA, TG, and TC were also significantly decreased by IOE treatment (Supplementary Table S2).

To further investigate the possible mechanism underlying the anti-obesity effects of IOE, RT-PCR analysis was applied to determine the expressions of several genes related to lipid metabolism, and the primer sequences used for PCR were shown in Supplementary Table S3. In the liver (Fig. 1C), the mRNA levels of PPAR-γ and Srebp1c in the HD group were significantly higher than those in the CON group. However, the expressions of these genes in the HD-IOE group were significantly decreased when compared with those in the HD group. Moreover, the mRNA level of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR), which is the rate-limiting enzyme for cholesterol synthesis, was significantly up-regulated in the HD group. However, the expression of HMGCoAR in the HD-IOE group was extremely reduced when compared with that in the HD group. Acetyl-CoA carboxylase (Acc) and fatty acid synthase (Fas) are the key enzymes in fatty acid synthesis. As shown in Fig. 1C, the mRNA level of Fas in the HD-IOE group was extremely lower than that in the HD group. In addition, the expression of Acc in the HD-IOE group showed the same tendency without significant difference. As shown in Fig. 1C, Dgat1, Dgat2, and Gpat are the responsible genes for TG synthesis. As expected, the mRNA levels of Dgat1 and Gpat in the HD-IOE group were significantly lower than those in the HD group. The same tendency was also found in the expression of Dgat2 but without the significant difference. As shown in Supplementary Fig. S1A, the expressions of genes involved in fatty acid β-oxidation, such as PPAR-α, carnitine palmitoyltransferase 1 (Cpt1), Cpt2, and medium chainacyl-CoA dehydrogenase (Mcad), were almost not affected in the liver by HD and IOE treatments. With respect to FFA transport pathway, the mRNA levels of some key genes, such as fatty acid transporter (Fat) and fatty acid transport
protein (Fatp), were significantly higher in the HD group than those in the CON group, while IOE feeding significantly decreased the expressions of Fat and Fatp when compared with the HD group (Supplementary Fig. S2).

In the adipose tissue (Fig. 1D), the expressions of lipid metabolic genes were found similar to those in the liver. The mRNA levels of PPAR-γ, Acc, Dgat1, Gpat, and HMGCoAR in the HD group were significantly higher than those in the CON group, whereas the expressions of these genes in the HD-IOE group were significantly decreased when compared with those in the HD group. In addition, the expressions of PPAR-α, Cpt, and Mcad did not change among the treated groups (Supplementary Fig. S1B).

Taken together, the results of the present study demonstrate that the administration of IOE for 12 weeks can obviously control the body weight and attenuate the lipid accumulation accompanied with the decreased levels of FFA, TG, and TC in the serum, liver, and adipose tissue. These effects may be achieved through inhibiting the expressions of several genes involved in lipid synthesis (Supplementary Fig. S3). The extracts of *I. obliquus* may be a potential anti-obesity agent, and more studies are needed to clarify the exact mechanism.

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**Supplementary Data**

Supplementary data is available at ABBS online.

**References**