HSD GEE_230628 AM

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The anti-obesity effects of *Gastrodia elata* extract on obese mice induced by high starch diet through the suppression of SREBP-1 and chREBP

Abstract

Background: Traditional Korean medicine frequently employs the herbal remedy *Gastrodia elata* (GE). The aim of the study was to investigate the effects of GE on obese mice developed by a high starch diet (HSD).

Methods: Using an HSD, we generated obesity in C57BL/6 J mice. For the following five weeks, the mice received oral doses of 100, 200, and 500 mg/kg of *Gastrodia elata* extract (GEE). At the completion of the experiment, we assessed body weight, liver and fat weight, blood biochemical parameters, and gene expression that is associated with obesity.

Results: The overall body weight of animals was dramatically lowered via GEE oral treatment. In comparison to the HSD group, the serum and liver TG levels were considerably lower in the GEE-treated group. Additionally, regional fatty tissues and liver weight markedly dropped in the GEE-treated batch in comparison to the HSD batch. We observed considerably lower levels of PPAR-γ, C/EBP-α, and chREBP mRNA expression level in the GEE-treated group in addition to lower levels of SREBP-1, ACC, and FAS protein expression.

Conclusion: According to the findings, GEE exerts anti-obesity benefits in the mouse model of obesity caused by HSD. The study has important implications for how GEE generally works, even if the number of experimental animals used was small and the pharmacological effects applied to mice rather than people, who have different constitutions.

Keywords: *Gastrodia elata*, Anti-obesity, High starch diet, SREBP-1, chREBP

Introduction

Since its discovery in 1980, non-alcoholic fatty liver disease (NAFLD) has shared pathological characteristics with hepatic disease caused by alcoholics, with the exception of alcohol usage. The cause of this illness still hasn't fully been understood despite of forty years of research. The prevalence of sufferers of NAFLD is rising quickly, and greater than a quarter of the world's population suffers from it. The main contributors to NAFLD include a lack of physical activity and a diet loaded with of sugar and cholesterol. Consumers frequently substitute high-sugar diets, primarily starchy carbs, for foods high in fat in order to lower calorie intake and achieve satiety in order to treat or prevent NAFLD. Yet, compared to investigations on fat consumption, there are significantly fewer investigations that focus on the impact of dietary carbs on liver function with NAFLD.

There are 3 phases to the origins of carbs eating in human history: (1) As rice became a staple food, it gradually took the place of foods like animal flesh, fruit, and vegetables that were acquired through prehistoric hunter-gatherer lifestyles. (2) The carbohydrate became a widely available consumer good thanks to the large production of sugar cane. Beginning to arrive were cookies, fruit preserves, and packaged meals. The manufacturing of carbohydrates encouraged consumers to switch from natural foods to processed ones and to consume more carbs. (3) Following the Second World War, sugar syrup and other ultra-processed carbs became available all over the place, while the cost of unrefined carbs fell sharply (Kroemer et al., 2018). After that time forward, usage of sugars has spread across society and became essential to daily life.

Three substances, oxygen, hydrogen, and carbon make up sugars. Following ingestion, sugars breakdown into smaller molecules including cellulose, lactose, and monosaccharides (such as fructose and glucose). These less complex types of sugars have comparable constituents in common, yet they perform somewhat differently and go through distinct processes of metabolism in the human system. Fructose, glucose, sucrose, and other sugars are examples of mono- and disaccharides. The two basic types of polysaccharides are cellulose and starch. Based on their structural differences, starchy sugars are commonly classified as resistant starch, amylose, and amylopectin. Amylopectin has the greatest impact on postprandial blood sugar

within starchy carbs then resistant starch and amylose. The majority of the processed starchy foods which are frequently consumed in everyday circumstances, like steamed buns, white noodles, and white rice, contain a substance called amylopectin the "culprit" behind the sudden rise in blood sugar levels following dinners. A whole-grain food has a less impact on weight gain and widespread inflammation than a food high in processed grain has on insulin sensitivity or the composition of the gut flora (Roager et al., 2019).

A common achlorophyllous orchid known as *Gastrodia elata* (family Orchidaceae) is highly prized for its medicinal and culinary properties. *Gastrodia elata* is mostly found in Oceania, Southeast Asia, and East Asia [Chen et al., 1999; Liu et al., 2015]. In Asian nations like China, Korea, and Japan, its tuber (mature rhizome) is a well-liked medicinal remedy [Ahn et al., 2007; Tang et al., 1992]. Additionally, the root has been consumed as foodstuff and is frequently prepared in soups. Extracts of *G. elata* or its compounds that are active have pharmacological and health-improving properties, such as neuroprotective and anticancer actions [Liu et al., 2018; Heo et al., 2007; Park et al., 2015; Xian et al., 2016]. This medication has been used extensively to treat paralysis, dizziness, epilepsy, and hypertension, particularly in Asia [Ojemann et al., 2006; Xiong et al., 2013]. In this region, *G. elata* has also been used to address cognitive impairments and prevent neurodegeneration, vascular dementia and Parkinson's disease (Liu et al., 2018; Xian et al., 2016; Shi et al., 2020).

A variety of phenolic molecules, organic acids and sterols have been found in G. elata through earlier phytochemical examinations. These substances possess a range of health-improving actions, including anticonvulsant, neuroprotective, anti-inflammatory, and antioxidant properties [Ojemann et al., 2006; Lee et al., 2006; Huang et al., 2006; Jung et al., 2007]. It has been shown, for instance, that a number of phenolic compounds, including vanillin, gastrodin, and gastrodigenin, protect neuronal cells and enhance neurological function by minimizing damage brought on by oxidative stress and inflammatory responses [Jang et al., 2015].

However, there hasn't been any research done on how Gastrodia elata extract (GEE) affects obesity brought on by a high starch diet. As a result, we looked into GEE's anti-obesity effects in a mouse model.

Materials and methods

Plant Material preparation of crude GE extract

1 fold of dried GE rhizome has been boiled for 6 hours at 90 degrees Celsius in 15 folds of distilled water to produce GEE. The filter paper was used to filter the final product to produce the crude extract. The remaining rhizome was then mixed with an additional 15 folds of distilled water; the combination was once more boiled for 6 hours at 90°C in order to obtain the additional extract. The two extracts were combined, concentrated under vacuum pressure, and the resulting product was spray-dried before being stored until use. The yield was 30.2%.

Mouse model of HSD-induced obesity

Male C57BL/6 N mice aged 8 weeks (20 ± 2 g) were bought from Iksan, Korea's Orient Bio, Inc. In this study, 40 perfectly normal mice have been utilized as the study's test subjects. These animals spent a week in a lab environment before the study properly began. Following this, mice (n = 8) were randomized into any of the five distinct groups: the 22% low starch-diet group (LSD), the 56.87% high starch-diet group (HSD), and the GEE-treated groups (GEE 100, 200, or 500 mg/kg p.o.). Both water and food were available to the mice at all times. All mice other than those in the LSD group were given an HSD. The GEE-treated groups received GEE orally, whereas the LSD and HSD groups received physiological saline as a treatment. Animals were kept in a 12/12-hour-dark environment with a humidity level of 50 ± 5% and a temperature of 22 ± 2 °C. On a weekly basis, records of food consumption and body weight were kept. Animals were starved for 12 hours after all treatments finished before being given ether anesthesia. After the mice were euthanized, blood was taken using a heart puncture. The specimens of blood have been spun at 2,000 x g for fifteen minutes at 4˚C after being allowed to clot for 30 minutes at room temperature. Laboratory animal care and use is governed by the Ethical Committee of Womkwang University (reg. no. WKU21-84) gave its approval to all procedures, which followed the guidelines provided by the National Institutes of Health.

Biochemical analysis

The appropriate test kits were used to determine the serum and liver TG levels in accordance with the manufacturer's recommendations.

Western blot analysis

In order to extract protein from tissue, RIPA buffer was utilized. A Bradford assay kit (Bio-Rad Laboratories, Hercules, California) was used to measure the protein concentration. Electrophoresis on polyacrylamide gels with sodium dodecyl sulfate at a concentration of 8–12%

separated exactly same amounts of protein, which were subsequently moved to Polyvinylidene difluoride (PVDF) membranes. The primary antibody was incubated on the blot for the overnight period at 4 °C after blocking solution (5% skim milk) was added to the blot for 1 hour at room temperature. After being washed 3 times with Tween 20/Tris-buffered saline (T/TBS), blots were incubated with a secondary antibody coupled to horseradish peroxidase for two hours at room temperature. T/TBS was used once again to wash the blots three times before being developed using enhanced chemiluminescence solution and the Imaging system (Tokyo, Japan: AE-9300 Ez-capture MG/AE-9160 Ez-capture ST) was used to capture images of the protein bands.

Quantiative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from liver tissue in line with the manufacturer's recommendations to analyze the genes linked with adipogenesis. A kit for cDNA synthesis from TaKaRa in Shiga, Japan was used to make complementary DNA (cDNA). According to the instructions provided by the manufacturer, qRT-PCR was carried out using a TaKaRa SYBR Green I qPCR kit from Shiga, Japan. The following were the gene-specific primers for the genes in liver tissues that are linked to obesity: Forward 5′–CGTCCCGTAGACAAAATGGT–3′ and reverse 5′– TTGATGGCAACAATCTCCAC –3′ for mouse GAPDH, forward 5′– GTACTGTCGGTTTCAGAAGTGCC–3′ and reverse 5′–ATCTCCGCCAACAGCTTCTCCT–3′ for mouse PPAR-γ, forward 5′–GCGAGCACGAGACGTCTATAGA–3′ and reverse 5′– GCCAGGAACTCGTCGTTGAA–3′ for mouse C/EBP-α, and forward 5′– GCTTTGACCAGATGCGGGACA–3′ and reverse 5′–AGTGCTGAGTTGGCGAAGGGA–3′ for mouse chREBP. The expression levels of each group were expressed as fold changes in comparison to the HFD group, and relative expression levels were standardized to mouse Gapdh expression.

Statistical evaluation

Version 26.0 of the SPSS Software Package (IBM, NY, USA) was used for the statistical analysis, which included Duncan's multiple comparison evaluation. The data were provided as mean standard deviation (SD), with $p < 0.05$ being the significance level.

Results

Every week, the body weights of the animals were recorded. Comparing the mice fed with HSD and LSD at the end of the fifth week, it was clear that the HSD-fed mice had significantly more

visible body fat. GEE was administered orally at doses of 100, 200, or 500 mg/kg, resulting in a decrease in body weight compared to the HSD group (Fig. 1).

After the fifth week, the HSD-fed mice showed considerably more visible increases in liver and fat mass as compared to the LSD-fed mice. The liver and fat mass were decreased when GEE was given orally at a dose of 500 mg/kg in contrast to the HSD group (Fig. 2).

In comparison to the LSD group, the HSD group's serum TG concentrations were noticeably higher. In mice given an HSD, the level of serum TG was 125.84± 8.68 mg/dL; however, when given 500 mg/kg of GEE, the serum TG level was lowered to 103.36 ± 9.32 mg/dL (Fig. 3A). The HSD group had significantly greater liver TG levels than the LSD group. When 500 mg/kg of GEE was administered to mice, the liver's TG level dropped to 23.03 ± 2.16 mg/dL from 28.56± 2.91 mg/dL in HSD-treated mice (Fig. 3B).

SREBP-1, ACC, and FAS levels of protein expression were higher in the livers of the HSD group compared to the LSD group and decreased in the groups that had undergone GEE treatment (Fig. 4). When GEE was administered, the hepatic PPAR-γ, C/EBP-α, and chREBP mRNA expression levels was noticeably suppressed. Comparing the HSD group to the LSD group, these levels were up-regulated (Fig. 5).

Discussion

The majority of animals, including humans, rely on starch as a source of nutrition because of its unique flavor and low cost. Even some of them choose to eat a lot of starchy meals. According to studies (Lee et al., 2011; Vilà et al., 2014; Feng et al., 2015), chronic consumption of high-starch diets has been linked to a range of diseases, including obesity, non-alcoholic fatty liver disease (NAFLD) and hyperlipidemia.

Hoekstra et al. (2013) state that the liver performs vital physiological tasks such nutrition metabolism, detoxification, and absorption in the animal body. Thus, liver dysfunction from an excess of nutrients is a simple consequence. According to research done on rats, a high-fat diet led to severe steatosis, enlarged hepatocytes, and a markedly greater rate of apoptosis (Bede et al., 2020). Panchal et al. (2011) and Ip et al. (2014) found that rats on a high-carb diet were more likely to grow fat, suffer hepatitis, and experience oxidative stress in their livers. These ailments can damage the liver and potentially result in liver cancer. Consequently, consuming too many carbohydrates is bad for the liver.

A study by Greenberg and Obin (2006), an imbalance between the consumption and use of energy is what leads to obesity, and the extra energy builds up as TG in fat tissue. To test GEE's ability to prevent obesity, we utilized mice that had been made obese by HSD. We verified that a 5-week GEE therapy reduced body weight increase without altering calorie intake Which is consistent with earlier research (Gao et al. 2023). Additionally, eWAT (epididymal white adipose tissue) weights in GEE plus HSD fed mice were considerably lower than those in the HSD fed group. These findings imply that GEE treatment affects body weight growth reduction and is probably connected to decreased eWATs weight. Additionally, our findings showed that mice given HSD and a GEE supplement had considerably lower serum and liver TG levels. These findings imply that GEE may lessen hyperlipidemia brought on by HSD.

This study shows that an HSD can worsen NAFLD by enhancing the influx of fatty acids into the liver, which is brought on by the enhanced expression of PPAR-γ, C/EBP-α, chREBP, and SREBP-1 mediated molecular pathways.

The levels of the lipogenic enzymes Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), as well as their transcription factors carbohydrate-responsive element binding protein (ChREBP) (Dentin et al., 2005) and sterol regulatory element binding protein 1 (SREBP1), are all influenced by a diet high in carbohydrates, according to research (Bray et al., 2004; Dentin et al., 2005). Based on studies, ACC, FAS, SREBP1, and ChREBP levels rise on a carbohydrate-rich diet compared to fasting levels (Dentin et al., 2004).

In order to comprehend the mechanism underlying the decrease in body fat brought on by GEE, we looked at the levels of expression of genes and proteins linked to adipogenesis in liver tissue in HSD-induced obese mice.

Vidal-Puig et al. (1996) claim that PPAR-γ is a transcription factor that is ligand-activated and mediates the expression of genes specific to fat. It also drives adipocyte differentiation and adipogenesis.

The late phases of adipogenesis also result in the production of the protein C/EBP, which controls adipocyte differentiation by collaborating with PPAR to promoting differentiation (Farmer, 2005).

SREBP-1, which promotes the expression and synthesis of PPAR-ligands, can also activate PPAR-γ. A transcription factor called SREBP-1 regulate the synthesis of fatty acids, cholesterol,

and LDL receptors while also activating the genes and proteins involved in lipogenesis in the liver, including Acetyl-CoA carboxylase and (ACC) FAS (Farmer, 2005).

GEE medications prevent adipogenesis and lipid accumulation in HSD-induced adipose tissues, as evidenced by the decreased mRNA expression of PPAR-γ, C/EBP-α, and chREBP. Our research also revealed that GEE therapy significantly reduced the levels of adipogenesis-linked protein expression in the liver tissue of obese HSD-fed mice, including SREBP-1c, ACC, and FAS.

Conclusion

In conclusion, the GEE dose-dependently inhibited the major transcription factors and markedly reduced WATs weight and body weight gain in HSD-induced obese mice. This shows that the GEE may prevent the obesity caused by a high-starch diet. As a result of these findings, GEE may represent a promising herbal treatment for obesity.

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