Summary

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Title	**Studies on the preventive effects and molecular mechanisms of vine tea polyphenol on metabolic syndrome** **（藤茶ポリフェノールによるメタボリックシンドロームの予防効果及び分子機構に関する研究）**

Key word（**Vine tea**）（**Polyphenol**）（**Metabolic syndrome**）（**Gut microbiome**）（**Redox states**）

Chapter 1 Introduction

Ampelopsis grosedentata, addressed as “vine tea”, is distributing in the southwest of China with high polyphenol contents. Vine tea has been used as herb medicine for hundreds of years in China, it is not belonging to traditional *Camellia* tea plant variety, but usually been used to prepare tea-like beverages for improving health. The manufacturing process of vine tea is similar to green tea. The tender stems and leaves are pan-fired immediately after plucking, dried leaves and tender were brewed with freshly boiled water, it is slightly bitter with a strong sweet flavor aftertaste. Vine tea is accepted by most local people who drink vine tea when they have a sore throat or common cold. Previous study has reported that vine tea contained an exceptionally high amount of polyphenol, this special property indicated that vine tea is a valuable resource and has the potential for developing for a plant sources polyphenol. Although daily intake of vine tea has been showed beneficial effect on human health, but the molecular mechanisms remained unclear. Therefore, the present study aimed to investigate the preventive effects and molecular mechanisms of vine tea on metabolic syndrome.

Chapter 2 Antioxidant properties of a traditional vine tea, *Ampelopsis grossedentata*

Chapter 2 was aimed to investigate the chemical and antioxidant properties of vine tea from three principle produce area in China, including Guizhou, Hunan and Guangxi province. Different solvents were used to find an efficient extraction method to obtain highest polyphenols from vine tea, and then determine major compounds in vine tea by HPLC. Then, antioxidant capacity of vine tea and its major compounds were investigated by using *in vitro* and culture cell methods, focusing on the effect on the expression of Nrf2/Keap1-mediated antioxidant enzymes. Firstly, Vine tea from three major production area in China were extracted to optimize the extraction condition by investigating the extraction solvents, solvent concentration, extraction time and temperature, and result showed the highest polyphenol yield from vine tea was extracted by 70% ethanol at 70°C for 40 min with ultrasonic treatments. The major compound in vine tea polyphenols (VTP) was determined as a dihydromyricetin (DMY) by HPLC, and the content was estimated as 21.42%, 20.17%, 16.47% of dry weight basis from Hunan, Guizhou and Guangxi products, respectively. The antioxidant activities were investigated *in vitro* and in culture hepatic cells. VTP and DMY showed stronger DPPH scavenging ability and higher ORAC *in vitro*. Moreover, VTP and DMY enhanced the level of Nrf2 and reduced the level of Keap1, sequentially, increasing the level of downstream antioxidant enzyme, NQO1. These data demonstrated that vine tea and its major compound DMY might exert the antioxidant activity in culture cells by activating Nrf2/Keap1 pathway.

Chapter 3 Ameliorative effects and molecular mechanisms of vine tea polyphenol on Western diet-induced NAFLD

Non-alcoholic fatty liver disease (NAFLD) is worldwide prevalent, metabolic syndrome associated disease. In this study, we administrated mice with VTP to investigate the preventive effect on Western diet (WD)-induced NAFLD. Male C57BL/6N mice were fed either a normal diet (ND) or WD with or without VTP for 12 weeks. Results revealed that VTP supplementation decreased serum levels of cholesterol and triglyceride, and reduced accumulation of hepatic lipid droplets caused by WD. Molecular data revealed that VTP enhanced fatty acid oxidation by reactivating WD-suppressed phosphorylation of AMP-activated protein kinaseα (AMPKα) and the expressions of peroxisome proliferator-activated receptor alpha (PPARα), carnitine palmitoyl transferase IA (CPT1A) and cytochrome P450, family 4, subfamily a1 (CYP4A1). Meanwhile, VTP inhibited hepatic lipogenesis by reducing WD-enhanced the level of mature sterol regulatory element-binding protein 1 (SREBP1) and fatty acid synthase (FAS). Moreover, VTP activated nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-mediated expressions of hemeoxygenase-1 (HO-1) and quinone oxidoreductase (NQO1), and reduced hepatic TBARS level to prevent hepatic oxidative stress. On the other hand, VTP also increased intestinal zonula occludens-1 (ZO-1) expression and relative abundance of gut *Akkermansia*, and reduced the ratio of *Firmicutes/Bacteroidetes*. Thus, VTP might prevent WD-induced NAFLD by balancing fatty acids oxidation and lipogenesis, hepatic oxidative stress, and gut microbiome at least. These results suggested vine tea, containing high content of bioactive compound dihydromyricetin, is a potential food resource to prevent NAFLD.

Chapter 4 Vine tea dihydromyricetin improves fatty acid oxidation and redox signaling to prevent NAFLD development

Fatty acid β-oxidation is the major source of adenosine triphosphate(ATP) in liver, especially in fasting condition while glucose utilization is limited, fatty acid become the principal source of energy. The first step of fatty acid oxidation is the transport of fatty acyl-CoA to the mitochondria matrix through the carnitine system, Then, fatty acid β-oxidation involves the progressive removal of two-carbon units, from the carboxyl end of the fatty acyl-CoA substrate in a series of four reactions that act sequentially and repeatedly. Each turn of the cycle generates FADH2 and NADH in addition to acetyl-CoA and an acyl chain that is two carbons shorter than the original. Thus, fatty acid oxidation is additionally regulated by the energetic balance of the cellular NAD+/NADH ratio, which serving as the determinant of the cellular energetic status; excess NADH inhibits β-hydroxyacyl-CoA dehydrogenase, limiting the conversion of l-α-hydroxyacyl-CoA to α-ketoacyl-CoA, the third step in the β-oxidation loop.

In the previous study, we have demonstrated that VTP increased fatty acid oxidation in WD induced NAFLD, and this enhancement is associated with AMPK activation. In chapter 3, administration of 1% VTP and 0.6% DMY were performed in the same mouse model to identify whether the effect of VTP is due to DMY, and investigate the effect of DMY on fatty acid oxidation and intracellular redox states. The results showed both VTP and DMY supplementation reduced serum and hepatic cholesterol and triglyceride accumulation. Molecular analysis revealed that both VTP and DMY facilitated fatty acid β-oxidation and inhibited endogenous cholesterol synthesis, which were associated with AMPK phosphorylation. Phosphorylated AMPK induced mitochondria biogenesis and resulted in an enhancement of mitochondria fatty acid consumption. Meanwhile, phosphorylated AMPK

inhibited the expression of hepatic HMG-CoA reductase to reduce cholesterol biosynthesis. Furthermore, cellular analysis showed that AMPK activated by both DMY and VTP was related to cellular nicotinamide adenine dinucleotide (NAD+) levels, both DMY and VTP increased NAD+ salvage pathway and declined NAD+ consumption enzyme expressions to finally result in a NAD+ boosted effect, which induced AMPK phosphorylation. Therefore, DMY acted as bioactive compound of VTP and targeted AMPK signaling pathway to facilitated fatty acid β-oxidation and inhibited endogenous cholesterol synthesis.

Chapter 5 Conclusion

Vine tea, contains high amount of polyphenol, has showed the preventive effects on obesity and obesity associated metabolic syndrome in present study. The main bioactive compound of vine tea polyphenol was DMY, DMY targeted AMPK mediate lipid metabolism, increased fatty acid oxidation and decreased endogenous cholesterol biosynthesis, the AMPK activation and the effect on lipid metabolism are associated with intracellular proton donor NAD+ and NADH balance. Although we have found the mechanisms of DMY on lipid metabolism regulation, however, whether the effect of DMY on intracellular redox states is due to its “antioxidant property”, the capability of proton seizure or loss, still need further investigation. In summary, these findings provide new insight for understanding the molecular mechanisms of VTP on the prevention of metabolic syndrome, and suggested that vine tea has the potential as a functional food resource for preventing against metabolic syndrome.