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Original Article

Disease chemopreventive effects and molecular mechanisms of mulberry leaf extract

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Recently, there has been increasing research on mulberry (Morus alba L.) leaves due to their wide range of reported biological properties. Mulberry leaves contain many nutritional components including flavonoids, which are known to be powerful polyphenols and antioxidants. Several *in vitro* and *in vivo* studies have documented the chemopreventive effects of mulberry leaf extract (MLE) and mulberry leaf polyphenol extract (MLPE) including anti-atherosclerotic, anti-diabetic, anti-obesity and hepatoprotective effects. These function to regulate cell proliferation, cell death, cell differentiation, and metabolism through modulation of signal transduction, gene expression, protein function, and enzyme activity. Although the efficacies of MLE and MLPE in chemoprevention and clinical trials require further investigation, they provide great promise for improving human health. This review highlights the recent published data on MLE and MLPE in terms of chemopreventive potential and underlying mechanisms.

Keywords: mulberry leaf extract, polyphenol, anti-atherosclerosis, anti-diabetes, anti-obesity, hepatoprotection

Introduction

Mulberry trees (Morus alba L.; Moraceae family) are extensively cultivated in Asian countries (e.g., China, Korea, Japan, and Taiwan). Mulberry leaves possess various phytochemical constituents, such as flavonoids and phenolic acid[1]. It has been reported that mulberry leaves contain significant levels of protein with good amino acid profile, carbohydrates, fats, minerals, fibers, metabolizable energy and vitamins such as β-carotene and ascorbic acid[2]. Mulberry leaves are eaten by silkworms, but are also fit for human consumption. In Taiwan, mulberry leaf preparations are commonly used in commercial beverages (mulberry tea) and health foods. Mulberry leaves have many applications in traditional medicine due to their anti-hyperglycemia, anti-hyperlipidemia, anti-hypertension and fat neutralizing properties[3-5]. In recent studies, water extracts of mulberry leaves have been shown to exhibit a variety of biological functions, including antioxidant, anti-inflammatory, anti-atherosclerotic, anti-diabetic, anti-obesity, anti-tumor, and anti-hypertensive actions[6-8]. Our previous study indicated that mulberry leaf extract (MLE) has anti-

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cholesteremic[9], cardiovascular and hepatoprotective properties[10,11]. Furthermore, recent evidence has demonstrated several entirely distinct, new functions such as anti-cancer stem cell[12], anti-platelet[13], anti-osteoporotic[14], anti-aging[15], and neuroprotective[16] properties for the dried powder, water extract, and ethanol extract of mulberry leaves. Altogether, increasing evidence suggests a beneficial role of mulberry leaves in disease chemoprevention, although the underlying mechanisms remain to be elucidated.

In recent years, much effort has been dedicated to developing new therapies for various human diseases. Among the potential chemopreventive agents, phytochemicals are considered natural products amenable to long-term use with limited side effects. In this review, we focus on the mulberry leaf and discuss the mechanisms of its chemopreventive effects.

Mulberry leaf extract and atherosclerosis

In *in vitro* studies, MLE has been shown to effectively inhibit vascular smooth muscle cell (VSMC) proliferation and migration, through up-regulation of p53, inhibition of cyclin-dependent kinase (cdk), and suppression of small GTPase and protein kinase B (PKB, also known as Akt) nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, thereby inhibiting the development of atherosclerosis[10,17]. MLE and its polyphenolic extracts (MLPE) also influence low-density lipoprotein (LDL) oxidation, macrophage activation, foam cell formation, and protein expressions of related signals. Both MLE and MLPE reduce the expressions of scavenger receptors, CD36 and SR-A, as well as their upstream transcription factor, peroxisome proliferator-activated receptor γ (PPARγ), indicating molecular regulation of oxidative LDL (ox-LDL) uptake[18]. In addition, dietary mulberry leaf has shown anti-atherogenic effects in certain animal models. Mulberry leaf and its butanol extracts inhibited the oxidative modification of rabbit and human LDL[19], and attenuated atherosclerosis in LDL receptor- and apo E-deficient mice[20]. The anti-atherosclerotic effects of MLE and MLPE were further confirmed by a high cholesterol-fed rabbit model to evaluate the clinical use of mulberry leaf for atherosclerosis. In addition to improvement in liver function, the atheroma burden and levels of serum cholesterol, triglycerides, and LDL are significantly reduced after MLE treatment[9]. MLE and MLPE improve endothelial function, inhibit proliferation and migration of aortic VSMCs, and reduce atheromas in the vascular wall. Taken together, these studies imply that mulberry leaf is a potential atherosclerosis chemopreventive agent. There are four possible mechanisms of action: (i) inhibition of LDL oxidation, (ii) reduction of foam cell formation, (iii) inhibition of VSMC migration, and (iv) repression of VSMC proliferation (Figure 1).

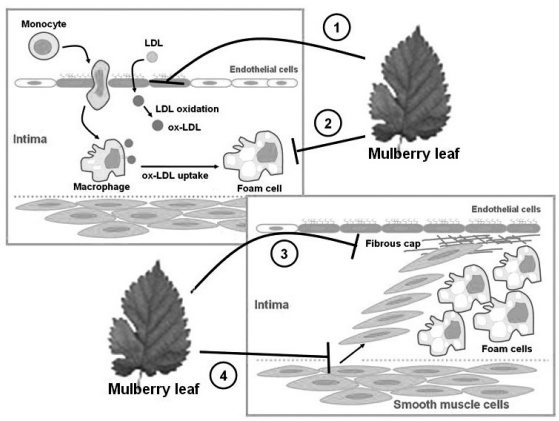

Figure.1. Various steps involved in the development of atherosclerosis and their suppression by mulberry leaf.

Mulberry leaf extract and diabetes

Several studies have investigated various alkaloids, flavonoids and phytochemicals in mulberry leaves that exhibit anti-diabetic effects. These effects include inhibition of α-glycosidase, sucrase and maltase enzyme activities[21,22], reduction in carbohydrate metabolism, lowering of blood glucose levels[22], prevention of lipid peroxidation[23], improvement of dyslipidemia, especially hypercholesterolemia[24], and inhibition of LDL cholesterol oxidation[25]. Kimura et al. found that 1-deoxynojirimycin (DNJ), an alkaloid in white mulberry leaf, lowers non-fasting blood glucose in humans[26]. The effects of mulberry leaf on rat models of type 2 diabetes were investigated in 2010. The results showed

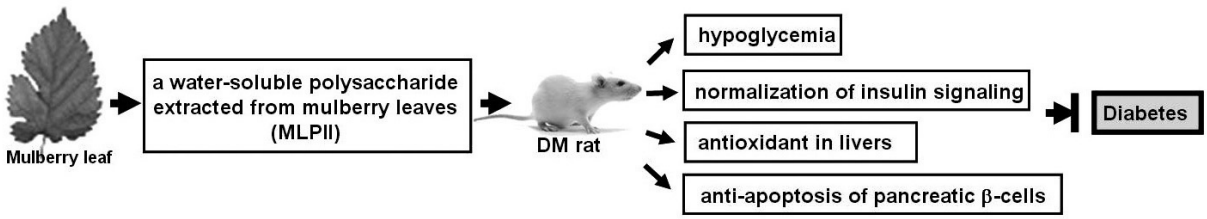

Figure.2. Anti-diabetic effects of a water-soluble polysaccharide extracted from mulberry leaves (MLPII) in rat model.

that a water-soluble polysaccharide extracted from mulberry leaves, designated MLPII, plays a significant role in ameliorating hepatic glucose metabolism by lowering blood glucose and increasing glucokinase activity in liver[27,28]. MLPII treatment also effectively normalized insulin signaling by inhibiting the expression of protein tyrosine phosphatase 1B (PTP1B), activating the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and mitigating oxidative stress in the livers of rats with type 2 diabetes induced by high fat diet and streptozotocin[29]. These findings of blood glucose lowering activity were in accordance with the results of a study by Naowaboot et al. (2012), which showed that anti-hyperglycemic action of MLE is mediated by increased glucose uptake via the activation of PI3K signaling pathway and translocation of glucose transporter 4 to the plasma membrane[30]. Moreover, MLPII treatment results in a marked recovery of pathological changes in the pancreatic islets and in the numbers of pancreatic β-cells[31]. Zhang et al. also reported that MLPII plays a critical role in protecting pancreatic islet cells from apoptosis via elevation of apoptosis-regulatory protein Bcl-2/Bax ratio, and amelioration of insulin secretory capacity of pancreatic β-cells via restoration of pancreatic duodenal homeobox-1 (PDX-1) nuclear localization and expression levels in diabetic rats[32]. From these studies, we conclude that MLPII has anti-diabetic effects in rats with experimental diabetes mellitus (DM) through hypoglycemia, normalization of insulin signaling, antioxidant activity in liver, and inhibition of apoptosis of pancreatic β-cells (Figure 2). To summarize, mulberry leaf is worthy of further investigation as an adjunct therapy for diabetes or metabolic syndromes.

Mulberry leaf extract and obesity

Naowaboot et al. first demonstrated that MLE stimulates adipogenesis and adiponectin secretion in murine 3T3-L1 adipocytes. The stimulatory effects of MLE on adipocyte proliferation and differentiation likely occur through upregulation of adipogenic transcription factors, including CCAAT/enhancer-binding protein alpha (C/EBPα) and PPARγ, and downstream gene expression. Such effects of MLE on adiponectin secretion and adipocyte activity may account for the anti-obesity effects of mulberry leaf, at least in part[33]. In addition, MLE has beneficial effects on obesity-related fatty liver disease through regulation of hepatic lipid metabolism, fibrosis, and antioxidant defense system. MLE supplementation might be a potential therapeutic approach for obesity-related diseases including non-alcoholic fatty liver disease (NAFLD)[34]. In recent studies, anti-adipogenic effects of MLE and MLPE on lipid accumulation in 3T3-L1 cells and obesity in mice fed a high fat diet (HFD) have been investigated[35,36]. Both MLE and MLPE efficiently suppress the protein expressions of PPARγ and sterol regulatory element-binding proteins (SREBP) 1c, as well as their downstream lipogenic targets adipocyte-specific fatty acid-binding protein (A-FABP) and fatty acid synthase (FAS). They also increase phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) *in vivo* and *in vitro*. Therefore, MLPE inhibits hepatic lipogenesis through attenuation of SREBP1/FAS/triglyceride pathway and SREBP2/HMG-CoA reductase (HMGCR)/total cholesterol pathway. MLPE also reduces peripheral lipid accumulation by inducing mature adipocyte apoptosis and suppressing adipocyte differentiation

(Figure 3). Furthermore, orally administered MLE significantly reduces body weight gain and lipid accumulation in the liver, as well as serum/hepatic triglyceride and total cholesterol levels[36]. In summary, mulberry leaf may be a promising dietary supplement or functional food to reduce the risk of obesity and obesity-related metabolic disorders.

Mulberry leaf extract and hepatoprotection

A protective effect of MLPE against long-chain oleic acid-induced hepatic lipid accumulation in human hepatocytes HepG2 has been reported. MLPE can regulate hepatic lipid accumulation through induction of PPARα expression, which is required for fatty acid oxidation, and inhibition of numerous lipogenic enzymes, such as FAS, acetyl-CoA carboxylase (ACC), HMGCR and associated-lipogenic transcriptional factors (SREBP1 and SREBP2). Therefore, mulberry leaf should be developed as a potential therapeutic treatment for fatty liver and NAFLD[37]. The findings of this study are presented in Figure 4. Furthermore, Zou et al. evaluated the effects of MLPE on hyperglycemia-induced oxidative stress and mitochondrial membrane potential (ΔΨm) in HepG2 cells. Treatment with MLPE led to significant decreases in the levels of ΔΨm, reactive oxidant species (ROS), malondialdehyde (MDA) and NF-κB activation in high glucose-induced liver oxidative damage[38]. Taken together, mulberry leaf exerts hepatoprotective effect via antioxidant and anti-inflammatory properties, inhibiting lipogenesis and promoting hepatic lipid clearance.

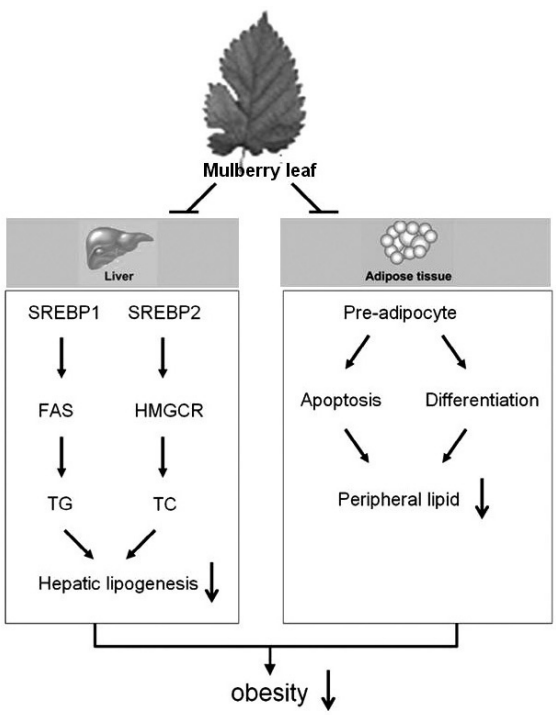

Figure.3. Schematic model of mulberry leaf amelioration of obesity via inhibition of hepatic lipogenesis and reduction in peripheral lipid accumulation.

Future perspectives

Several of the studies establishing the potential of mulberry leaf were carried out in animals. Further testing of mulberry leaf in humans is required to confirm these observations. How mulberry leaf produces its disease chemopreventive effects is not fully understood. They are likely mediated in part through regulation of cell proliferation, cell death, cell differentiation, and metabolism. Future studies are also needed to understand if single, pure component of MLE or MLPE has chemopreventive activity and to reveal its possible mechanisms of action. It is quite likely that mulberry leaf exerts its effects through mechanisms other than the ones discussed here. Over a dozen different cellular proteins and enzymes are regulated by mulberry leaf. High-throughput ligand interacting technology can reveal more molecular targets of mulberry leaf. Microarray gene chip technology may in the future indicate which genes are regulated by mulberry leaf. In addition, the dosage is not like that of pure chemical directly producing antioxidant or prooxidant effects towards overt toxicity. MLE, an aqueous extract, not only has the above advantage, but also higher yield ratio (50%) from dried leaves. We suggest that naturally occurring agents such as MLE are potential chemopreventive agents and natural health foods for the management of metabolic disease.

Conclusion

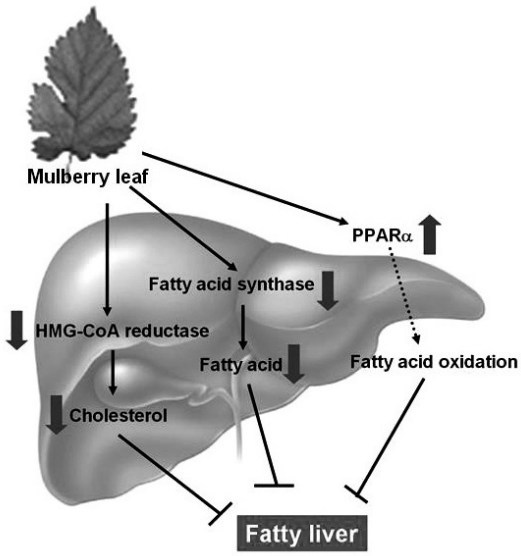

Figure.4. Molecular targets of fatty liver regulated by mulberry leaf.

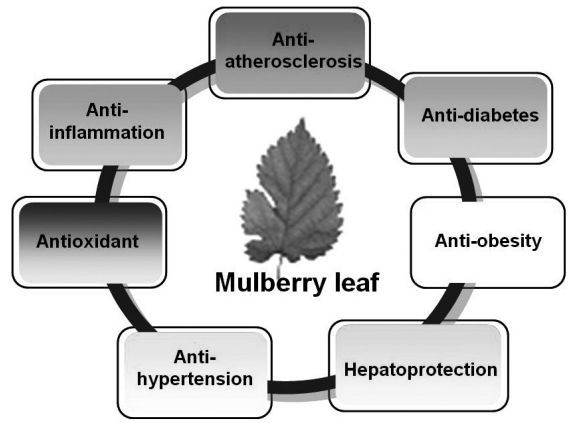

Figure.5. Chemopreventive effects of mulberry leaf extract on various diseases.

It is clear that MLE and MLPE exhibit activities against atherosclerosis, diabetes, obesity, liver disease, and other metabolic disorders (such as hypertension) (Figure 5). The data also demonstrate that the chemopreventive effects of MLE may be due, in part, to the polyphenol functional component of mulberry leaf. In summary, these results reflect the disease chemoprevention potential of mulberry leaf extract.

Acknowledgement

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