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Current anti-diabetes mechanisms and clinical trials using *Morus alba* L.

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Abstract *Backgrounds:* Diabetes mellitus, especially type 2 diabetes, with its fast-rising prevalence, has become a global epidemic. Mulberry (*Morus alba* L.) leaf has been known to have hypoglycemic effects since ancient times. In Asia mulberry leaf is used as tea to complement the treatment of diabetes mellitus. The methods by which mulberry leaf affects the body and its mechanism when combined with chemical agents have been studied extensively.
Conclusions: We summarize the possible mechanisms of the anti-diabetic effects of mulberry leaf based on extraction procedures, *in vitro* and *in vivo* experiments, and clinical trials. We also discuss the hypothesis that crosstalk and "critical nodes" may be useful for a deeper molecular understanding of the treatment and prevention of diabetes with mulberry leaf.

Introduction

Diabetes mellitus is a disease that affects the body's regulation of blood glucose, the main symptoms of which include thirst, frequent urination, hunger, and weight loss. Diabetes, especially type 2 diabetes metillus (T2DM), is increasingly prevalent worldwide according to the WHO, calling for a monitoring of diabetes rates and researches into cost-effective treatments to slow its rise.

As for the medicinal interventions, certain traditional plants have been proved to retard the development of diabetic mice induced by streptozotocin (STZ).[1] Traditional Chinese herbs have been shown to have beneficial effects over Western pharmaceutical medications in treating diabetes, including duration effect, moderate hypoglycemic effects, and fewer side effects.[2]

In China, mulberry (*Morus alba* L.) leaf has been used to treat various illnesses since ancient times. Its use was first recorded in *Divine Husbandman's Classic of Materia Medica* (*Shennong Bencao Jing*; 200–220 CE), which is the earliest materia medica in China. The herb was described as being

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able to eliminate cold and heat in the body, promote perspiration, and have detoxifying properties.[3] With the wider use of mulberry leaf, more properties and effects were recorded in *Comprehensive Outline of the Materia Medica* (*Bencao Gangmu*; 1590), an extensive summary of Chinese herbal pharmacology. The author, Li Shizhen, indicated that brewed mulberry tea can treat wasting and thirsting (*xiao ke*) syndrome. It is now recognized that diabetes falls under this syndrome.

Pharmacological experiments have shown that the active compounds in mulberry leaf with hypoglycemic effects including alkaloids, flavonoids, polysaccharides, amino acids, simple phenylpropanoids, and phenols.[4] In this brief review, we present the mechanisms of the anti-diabetic effects of mulberry leaf from perspectives of extracts, their compounds, molecular mechanisms, and clinical trials. We hope that this manuscript provides a summary for future research on mulberry leaf as an herbal supplement for T2DM.

Extraction conditions resulting in the best anti-diabetic effects *in vitro* and *in vivo*

In ancient China, dried mulberry leaf was brewed in hot water to make a tea for treating diabetes. Modern extraction methods are now able to maximize treatment hypoglycemic effects with increased glucose uptake attributed to various factors during extraction, such as brewing time, concentration of organic solution, and extraction solution temperature.

Water extracts

Chen et al[5] compared two extracts from mulberry leaf on STZ-induced diabetic mice, and found that hot water extracts exhibited more potent hypoglycemic effects than ethanol or n-butanol fractions. Attila et al[6] investigated the dichloromethane-soluble fraction from the hot water extract of mulberry leaf had the potentiality to exert anti-diabetic activity on the 3T3-L1 adipocytes. Powder of dried mulberry leaf was given to diabetic rats by gavage, showing no traces of lenticular opacity in diabetic-mulberry group and no significant differences of blood glucose in normal group.[7] When a mixture of mulberry leaf and mulberry tea was steeped in hot water (98°C) for 3—5 min, a stronger inhibitory effect on α-glucosidase was observed in a Caco-2 cell culture experiment, compared with other extraction conditions.[8]

Ethanolic and other extracts

Aside from the water extract, the ethanol extract of mulberry leaf is effective at treating diabetes in STZ-induced rats,[9,10] So is the acetone extract, however, it is not so well as the ethanolic.[11,12] From these studies, it is clear that the mulberry extraction method plays a crucial role in preventing diabetes, and each extract has different hypoglycemic effects.

Mechanisms of the anti-diabetes effects of mulberry leaf *in vivo*

After confirming that crude extracts from mulberry leaf have hypoglycemic effects, additional extracts are purified for mechanistic studies. In these studies, various extracts from mulberry leaf are separated with reagents under different conditions, and active constituents are detected and enriched. Here we summarize these mechanistic studies with respect to their extracts.

Inhibition of α-glucosidase

Alkaloids

Total alkaloids from mulberry leaf have hypoglycemic effects in streptozotocin- (STZ-) induced diabetic mice.[13] Some alkaloids in mulberry leaf are potent inhibitors of mammalian digestive glycosidases.[14] These mulberry alkaloids, especially 1-deoxynojirimycin (DNJ), decrease α-glucosidase activity by competitive inhibition through binding to the enzyme active site to mimic natural substrates.[15]

DNJ-enriched mulberry extract may be useful in controlling postprandial hyperglycemia in pre-diabetic or mild diabetic individuals.[16] Oral administration of 0.24% DNJ and its derivatives was found to inhibit absorption of sucrose and polysaccharides in human and rat intestinal tissue samples.[17] Other investigations on the pharmacokinetics and bioavailability of DNJ showed that oral mulberry DNJ administered to rats is absorbed intact from the gastrointestinal tract, diffuses into the liver, and is then excreted with a short half-life.[18,19] Thus, studies such as the aforementioned on the metabolism of DNJ help lay the foundation for developing mulberry leaf as a dietary supplement for diabetes. As such, extracting the highest DNJ content as possible from mulberry is a high priority. In studies that investigated extraction of DNJ, when temperature was sustained at 98°C for 400 s, 95% of DNJ in dry mulberry tea was extracted[20] and water extraction versus extraction using different concentrations of ethanol produced varying contents of DNJ.[21] Other studies are exploring ways to increase production of DNJ during fermentation of mulberry leaf, such as inoculating the fermentation broth with the fungus ganoderma (*Ganoderma lucidum*).[22]

Flavonoids

Flavonoids from mulberry leaf also have *in vitro* inhibitory effects on α-glucosidase activity and can suppress blood glucose level after oral administration of starch and sucrose in Kunming mice.[23] Two flavonoids (isoquercitrin and astragalin) were shown to inhibit α-glucosidase as for the laboratory experiment.[24] Furthermore, chlorogenic acid (a phenylpropanoid) and rutin were found to play an important role in preventing diabetes, but not isoquercitrin.[25]

Polysaccharides

Polysaccharides from mulberry leaf have been shown to decrease blood glucose level, improve glucose tolerance,

and increase the hepatic glycogen content, thus regulating glucose metabolism in diabetic mice and promoting insulin secretion in normal rats.[26] Polysaccharides from mulberry leaf have also been found to inhibit α-glucosidase activity.[27] The highly-purified polysaccharides α-arabinose, α-xylose, α-glucose, α-rhamnose, and α-mannose that were extracted from mulberry leaf were able to induce regeneration of pancreatic β-cells, which were then able to effectively secreted insulin and reduce liver fat accumulation in diabetic rats.[28] Glycosides and their aglycons that were isolated from a water extract of mulberry leaf were found to have potential type 2 anti-diabetic activity.[29]

While the inhibition of α-glucosidase plays a crucial role in the anti-diabetes effect of mulberry leaf, an individual compound cannot elucidate metabolic mechanisms, thus molecular mechanisms need investigating.

Gluconeogenesis and glycolysis

The rate of hepatic glycogenolysis decreases after an overnight fast, such that an increase in blood glucose in type 2 diabetics is caused by gluconeogenesis.[30] Under similar conditions in diabetic rats, mulberry leaf was shown to decrease the activities of key hepatic gluconeogenic enzymes, glucose-6-phosphatase (G-6-ase), fructose 1,6-diphosphatase (FDPase), and phosphoenolpyruvate carboxy kinase (PEPCK).[31] Using real time-PCR analysis, DNJ plus polysaccharide (HDP) was found to up/down-regulate glucokinase (GCK), PEPCK, and G-6-ase.[18]

All carbohydrates undergo glycolysis, but this process is more active in healthy persons than in diabetics.[32] The glycolytic enzymes hexokinase, operating partial inhibition, phosphofructokinase-1, the important control point, and pyruvate kinase are involved in gluconeogenesis.[33] However, mulberry leaf extract did not significantly alter the activities of hexokinase and phosphofructokinase-1, while glucokinase did in STZ-induced diabetic rats.[34]

Results from studies such as those, it appears mulberry leaf likely suppresses gluconeogenesis and promotes glycolysis. Although some studies focused on gluconeogenesis and glycogenolysis, their results are insufficient to explain why mulberry leaf could have an anti-diabetic effect, and how it affects each step in gluconeogenesis and glycogenolysis.

AMPK signaling

AMP-activated protein kinase (AMPK) is a regulator of intracellular and systemic energy metabolism. Research has found that AMPK can be targeted in the treatment of diabetes and metabolic syndrome.[35] One study found that a hot water extract of mulberry leaf could activate skeletal muscle AMPK signaling, as shown by increased uptake of 3-O-methyl-D-glucose transport and was also involved in phosphorylation of Akt substrate of 160 kDa (AS160) and acetyl-CoA carboxylase, without any energy changes.[36]

Anti-apoptosis of β-cells

Increased β-cell apoptosis leads to β-cell loss and results in onset of type 2 diabetes.[37] Apoptosis is a fundamental mechanism for homeostasis and a form of programed cell death, with 2 large protein families involved in the process: the caspase family and the B-cell lymphoma 2 (Bcl-2) proteins. These two protein families are linked with the development of insulin resistance,[38] with the increased ratio of Bax/Bcl-2 and caspase-3 showing an association with diabetes.[39] Polysaccharides from mulberry leaf have been shown to elevate the Bcl-2/Bax ratio to protect pancreatic islet cells from apoptosis.[40] However, the Bcl-2 family is divided into 3 subgroups,[41] thus further study is needed to determine which proteins are altered when mulberry leaf is administered.

PI3K/Akt signaling

The glucose transporter GLUT4 is an insulin-sensitive transporter that can facilitate a decreased plasma glucose level. GLUT4 is translocated to the plasma membrane via the lipid kinase phosphatidylinositol 3-kinase (PI3K) signaling pathway.[42] Additionally, pancreatic duodenal homeobox-1 (PDX-1) is a regulator of beta cell identity and function in pancreatic islets. Glucose-simulated PDX-1 DNA-binding activity is inhibited by inhibitors of PI3K, such as wortmannin,[43] which was used to validate the hypoglycemic mechanism of mulberry leaf. Therefore, mulberry leaf extract might mediate hyperglycemia by increasing glucose uptake via the PI3K pathway and translocation of GLUT4 to the plasma membrane, with gallic acid the specific compound that mediates this effect.[44] In addition, polysaccharides from mulberry leaf have been shown to up-regulate PDX-1, insulin-1, and insulin-2 expressions in the pancreas.[18]

The serine/threonine protein kinase Akt is a major target downstream of PI3K, and PI3K and Akt appear to activate islet β-cells. Thus, islet-cell damage caused by oxidative stress may be attenuated via the PI3K—Akt pathway.[45] PTP1B is a phospho-tyrosine protein phosphatase that negatively modulates insulin sensitivity and is a substrate for Akt.[46] Polysaccharides from mulberry leaf have been shown to inhibit PTP1B, activate the PI3K—Akt pathway, and reduce oxidative stress resulting in anti-diabetic effects.[47]

The early studies cited in the text have illustrated that polysaccharides from mulberry leaf could activate PI3K/Akt pathway and elevate Bcl-2/Bax ratio to protect pancreatic islet cell from apoptosis. There are some important facts to support our hypothesis: 1) Akt has direct effects on regulating apoptosis, and there also exists connections between PI3-kinase pathways and control of apoptosis[48]; 2) PI3K/Akt pathway inhibits Bax translocation and could promote survival[49]; Akt possibly promote cell survival through inactivation of Bad with phosphorylation and up-regulation of Bcl-2.[48,50,51]

Thus we hypothesize that polysaccharides from mulberry leaf initiate Akt in PI3K/Akt pathway, which could inhibit Bax translocation and phosphorylate and up-

regulate of Bcl-2, but as for which isoform of Akt exerts such function needs the research.

As for the current researches, crosstalk with signaling cascades and the network among the pathways has been introduced and the "critical nodes" are shown to define the junctions.[52] The study has demonstrated the significant crosstalk between the AMPK and PI3K/Akt in embryonic cells,[53] and the mammalian target of rapamycin complex 1 (mTORC2) has been identified as a site of signaling crosstalk and it has used to study the molecular mechanism of metformin.[54]

In the domain of physiopathology, AMPK enhances insulin sensitivity involved in direct regulation of PI3K and stimulates PI3K/Akt.[55] As for the mulberry leaf, since mulberry hot water extract could increase the phosphorylation of AS160, however, without significant change in Akt in skeletal muscle cell. Two possible interpretations might elucidate this result: 1) The active compounds in the extracts under different extraction condition could make a difference; 2) Researches have showed that the primary role Akt2 is highlighted in adipocytes on insulin responsiveness and the isoforms of Akt have their own structures and functions,[52] may be deeper researches will fulfill the finding that extracts from mulberry leaf could have relations with crosstalk between AMPK and PI3K/Akt pathways.

Peroxisome proliferator-activated receptors (PPARs)

The peroxisome proliferator-activated receptors (PPARs) are important in insulin resistance, hyperlipidemia, and diabetes.[56] Water extract of mulberry leaf was found to increase insulin sensitivity and attenuate hyperglycemia through up-regulation of PPAR expression in diabetic mice.[57] 3T3-1L can be used to study the development of insulin resistance because of a reduced GLUT4 pool.[58] It is found that PPARγ agonists have been found to strengthen basal and insulin-stimulated glucose uptake in 3T3-L1 adipocytes.[59] Mulberry leaf extract was shown to significantly increase secretion of adiponectin in 3T3-L1 and enhanced the expression of PPARγ. Therefore, the anti-diabetic mechanism of mulberry leaf may be attributable to stimulation of adipogenic activity and adiponectin secretion.[60]

Mechanisms under investigation

Mulberry leaf continues to be investigated for its hypoglycemic mechanism. For example, the anti-diabetic function of mulberry leaf is correlated with anti-inflammatory reactions.[61] Furthermore, Attenuation of insulin resistance may contribute to regulation of the genes in the c-Jun amino-terminal kinase (JNK) signaling pathway.[62] As inflammation is closely associated with metabolic disorder and the JNK pathway is an inflammatory pathway, in the light of it, one hypothesis can be assumed that mulberry leaf might be anti-diabetes through anti-inflammation.

Clinical trials

It is important to conduct studies with diabetic patients and healthy volunteers to understand how natural products such as mulberry leaf work in the body. This is because botanical products have many pharmacologically-active constituents that can interact with each other as well as with pharmaceutical drugs.[63]

Thirty-day treatment with mulberry powder was found to significantly lower fasting blood glucose compared with glibenclamide.[64] Other studies have found that treatment with mulberry tea can cause malabsorption of carbohydrates.[65,66]

Several clinical trials have focused on the enriched compounds of mulberry leaf. To evaluate the effect of mulberry leaf extract with enriched DNJ on postprandial hyperglycemia, parameters, such as serum 1,5-anhydrogluctol concentration, can be used as a sign for positive improvement of impaired glucose metabolism.[67] In contrast, significant differences in postprandial hyperglycemia can be analyzed alone.[68] Results of a clinical trial that combined dietary control, exercise, and the total alkali fraction from mulberry leaf showed a reduction of blood glucose, regulation of lipids, and fewer side effects compared with acarbose treatment.[69] Mulberry tea has also been shown to suppress postprandial hyperglycemia after 90 min of its consumption.[70]

While the aforementioned studies have shown that mulberry leaf decreases blood glucose, regulates glucose and lipid metabolism, has few adverse effects, and may exert a wider treatment range than pharmaceuticals. There is a lack of research focusing on the safety of mulberry leaf. Therefore, investigations are needed on the absorption, distribution, metabolism, and excretion of compounds in mulberry.[14]

Discussion and conclusion

The anti-diabetes mechanism of mulberry leaf remains incompletely understood at the molecular level. However, active compounds in mulberry leaf likely affect crosstalk among the signal transduction pathways and networks. A single pathway cannot explain the hypoglycemic effect of mulberry leaf. Therefore, the crosstalk between AMPK and PI3K/Akt may be worth exploring for the anti-diabetic effects of mulberry leaf compounds.

More extensive clinical research should also be conducted on mulberry leaf's potentiality as a hypoglycemic agent. For example, well-designed randomized controlled trials, the gold standard for evaluating whether an intervention is effective, should be performed to validate and confirm findings of molecular studies.

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